# Optimization of Silkworm Sericin Extraction *Attacus atlas* and *Samia cynthia ricini* Using Response Surface Methodology

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Submitted: January 6, 2022; Revised: March 9, 2022; Accepted: March 24, 2022;
Published: February 28, 2023

**ABSTRACT**

Silk fiber is an organic waste that can pollute the environment due to its solubility in processing wastewater. The extraction from wastewater was carried out to reduce environmental pollution and produce natural bioactive. Therefore, this research aims to produce an extraction method that maximizes the protein yield of *Attacus atlas* (*A. atlas*) and *Samia cynthia ricini* (*S. ricini*) sericin and analyze the characteristics. The method consists of two stages, the optimization of sericin protein extraction with Response Surface Methodology (RSM) and its characterization. The optimization resulted in the optimum extraction of *A. atlas* and *S. ricini* sericin at a concentration of 0.08 and 0.03 N NaOH, temperature of 130.52 °C and 113.20 °C, time of 71.71 and 33.78 minutes with a yield of 17.39±1.24% and 20.24±2.30%. The sericin protein had a molecular weight of 8.99 and 7.08 kDa in *A. atlas* and *S. ricini*. The extraction produces glycine, alanine, and tyrosin for *A. atlas* and glutamic acid, glycine, and alanine for *S. ricini*. Therefore, the sericin extraction formulation did not change the secondary structure protein, as evidenced by the FTIR results.

**Keywords**: *Attacus atlas*; optimization; RSM; *Samia cynthia ricini*; sericin protein

DOI: http://doi.org/10.22146/agritech.71950
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

## INTRODUCTION

Silk fiber is an organic waste that can pollute the environment when disposed without treatment. Liquid waste contains high organic matter, namely Biological Oxygen Demand (BOD) of 8219.6 mg/L (Gulrajani et al., 2008). Furthermore, the cocoon, which is the main material for silk fiber, contains fibroin and sericin proteins. Sericin protein will dissolve in the liquid waste from silk fiber processing, and the extraction can reduce the BOD value by up to 86.7% (Gulrajani et al., 2008). Furthermore, sericin also has very good benefits as a medical biomaterial and skin care.

The protein functions as an adhesive between filaments (fibroin fibers) to maintain the integrity of the cocoon during its formation (Dash et al., 2007; Hoa et al., 2012). B. mori sericin protein consists of 18 amino acids, most of which are strong polar compounds containing hydroxyl, carboxyl, and amino groups (Rajput and Singh, 2015). It can be used in skin care to increase elasticity and reduce wrinkles and premature aging (Padamwar and Pawar, 2004). Sericin protein can also heal wounds (Aramwit and Sangcakul, 2007; Ersel et al., 2016). Aramwit et al. (2009) stated that the protein could induce fibroblast cell proliferation and collagen production.

Extraction from silk fiber processing wastewater has been widely carried out in the mulberry cocoon type (*Bombyx mori*). The non-mulberry cocoon type (*A. atlas*, *Cricula trifenestrata*, *S. ricini*) is still very limited and focuses more on the quality of the silk fiber (fibroin). The protein extraction was previously conducted by Aini (2009), adding 2 g/L (0.05 N) NaOH, 2 cc/L teepol, and 2 g/L neutral soap to boiling *A. atlas* cocoons at 80°C for 2 hours will produce a better character of the silk fiber in terms of fiber length and weight. The strong base NaOH 0.1 N is a solvent for degumming which produces the best fibroin of *C. trifenestrata* (Suriana 2011). Furthermore, the extraction with a combination of temperature, time, and solvent (NaOH) at a certain concentration is important to optimize sericin yields. This is because the protein is divided into sericin A, B, and C, each having different solubility characteristics (Padamwar and Pawar, 2004). Sericin A is in the outermost layer, which is insoluble in hot water and contains 17.5% nitrogen and amino acids such as serine, threonine, glycine, and aspartic acid. Sericin B is the middle layer, on acid hydrolysis, producing the amino acids sericin A and tryptophan, as well as containing 16.8% nitrogen. Sericin C is insoluble in hot water but will dissolve in hot alkalis or acids. Moreover, it yields proline and the amino acid sericin B upon acid hydrolysis, containing sulfur and 16.6% nitrogen.

Besides protecting the environment from organic pollution, extraction from silk fiber processing wastewater has added value by obtaining sericin protein, which has many benefits. Extraction, which consists of the degumming process and protein isolation, has not produced optimal protein. This is because the formulation and extraction procedures only focus on the variables being tested. Therefore, optimizing the extraction process is necessary. In this research, a response surface methodology (RSM) was used to determine a formulation that maximized the yield of sericin protein. It produces an extraction method that maximizes the yield of *A. atlas* and *S. ricini* sericin proteins and analyzes the characteristics.

## MATERIALS AND METHODS

### Material

The material used is *A. atlas* cocoon shells from the Walini Panglejar Purwakarta Tea Plantation, West Java, and *S. ricini* cocoon shells from the Non-Ruminant and Prospective Animals Laboratory (NRSH), Department of Animal Production and Technology, Faculty of Animal Science IPB University Bogor, West Java. Other materials for extraction and characterization include EMSURE Merck NaOH pellets (Darmstadt, Germany), 37% HCl for analysis EMSURE Merck (Darmstadt, Germany), 96% ethanol EMSURE Merck (Darmstadt, Germany), Bovine Serum Albumin (BSA) Sigma Aldrich (Darmstadt, Germany), Merck EMSURE NaCO3 (Darmstadt, Germany), Merck EMSURE potassium sodium tartrate tetrahydrate (Darmstadt, Germany), Merck Folin-Ciocalteau phenol reagent (Darmstadt, Germany), and Merck 96% acetic acid (Darmstadt, Germany).

### Method

### Sample Preparation

Sample preparation was carried out by cleaning intact cocoons from floss (cocoon fibers) and remaining pupae. Cocoon shells from *A. atlas* and *S. ricini* were washed with demineralized water and dried. Furthermore, the dried cocoon shells is ready to be used for degumming.

### Sericin Extraction

Extraction of sericin protein from cocoon shells using the procedure of Wu et al. (2007), modified by adding solvent (NaOH), as well as the isolation process (neutralization with HCl). The process consists of degumming and isolation of sericin protein. Degumming used an alkaline solvent of 0.08 and 0.03 N NaOH for *A. atlas* and *S. ricini* with a ratio of 1:3 between the solution and distilled water. RSM determines temperature and time treatment through *Central Composite Design* (CCD). The degumming solution was cooled at room temperature and filtered through a 450 mesh sieve to separate the silk fibers

from the filtrate. The sericin protein was isolated from the filtrate by neutralizing it with HCl to a neutral pH (7). During the neutralization process, continuous stirring is also carried out to help the HCL and filtrate homogenization process. Furthermore, the neutralized solution was put into the cooler for 30 minutes and centrifuged at 10,000 rpm at 4 °C for 10 minutes. Furthermore, the precipitate formed was washed with 95% ethanol. To speed up the removal of ethanol, evaporation is carried out, the results of which are stored at -20 °C.

**Sericin Extraction Optimization**

RSM consists of ordo 1 and 2, which produces the optimum area and point, respectively. Therefore, the value of the ordo 2 variable can produce the maximum sericin protein in the extraction process. The results of ordo 1 will be used as the center point in 2, and the data is not displayed. The variable is used as the center point in ordo 2, namely a temperature of 115 °C and 95 °C with 40 minutes for *A. atlas* and *S. ricini*.

The RSM experimental design used *Central Composite Design* (CCD) based on (Said et al., 2015), as shown in Table 1. The experiment used two factors ($2_k$), namely temperature ($X_1$) and time ($X_2$) hence α = 1.414. Furthermore, the analysis was processed with Program-R 4.1.2 (Lenth, 2020), and the relationship between response and independent variables is contained in Equation 1.

$$Y = f(X_1, X_2, ..., X_k) + \varepsilon \quad (1)$$

Description: $Y$= Response (sericin protein); $X_i$= independent variable ($i$ = 1, 2, 3,...., k); $\varepsilon$= *error*.

The second ordo analysis (optimum point) uses a second-ordo polynomial model with a quadratic function (Equation 2).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<i} \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

Description: $Y$ = response (sericin protein); $\beta_o$ = constant; $\beta_i$, $\beta_{ii}$, $\beta_{ij}$ = coefficients of the independent variable ($X$); $k$ = number of factors used.

**Sericin Protein Analysis**

**Lowry Analysis**

Protein analysis used the Lowry method (Maehre et al., 2018) at a wavelength of 750 nm with a *Gene Quant* 1300 spectrometer type (Holliston, USA). The working principle is that a reaction occurs between $Cu^{2+}$ and peptide bonds, and phosphomolybdic and phosphotungstic acid reduction by tyrosine and tryptophan can produce a blue color. A spectrometer reads this process at a wavelength of 750 nm and sericin protein levels (mg/ml) results to calculate the yield.

Table 1. Experimental design with Central Composite Design (CCD)

| Variable Code | | Variable | | | |
|---|---|---|---|---|---|
| | | *Attacus atlas* | | *Samia cynthia ricini* | |
| $X_1$ | $X_2$ | Temperature (°C) | Time (minutes) | Temperature (°C) | Time (minutes) |
| -1 | -1 | 110 | 30 | 85 | 25 |
| -1 | 1 | 110 | 50 | 85 | 55 |
| 1 | -1 | 120 | 30 | 105 | 25 |
| 1 | 1 | 120 | 50 | 105 | 55 |
| 0 | 0 | 115 | 40 | 95 | 40 |
| 0 | 0 | 115 | 40 | 95 | 40 |
| 0 | 0 | 115 | 40 | 95 | 40 |
| 0 | 0 | 115 | 40 | 95 | 40 |
| 0 | 0 | 115 | 40 | 95 | 40 |
| -1.414 | 0 | 107.93 | 40 | 107.07 | 40 |
| 1.414 | 0 | 122.07 | 40 | 82.93 | 40 |
| 0 | -1.414 | 115 | 25,86 | 95 | 57.07 |
| 0 | 1.414 | 115 | 54,14 | 95 | 22.93 |

$$Sericin\ protein\ yield\ (\%) = \frac{(sericin\ protein\ weight)}{(cocoon\ shells\ weight)} x\ 100\% \quad (3)$$

**Molecular Weight Characterization**

Molecular weight analysis using *Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis* (SDS PAGE) refers to Laemmli (1970), modified on the gel percentage and range of markers. The buffer gel used was 2 M Tris HCl pH 8.8 and 6.8 in the separating and stacking gel, while the electrode buffer was Tris-glycine with 25 mM Tris and 192 mM glycine. This is conducted at 12.5% and 4% of separating and stacking gel with a length ratio of 5:1.

Electrophoresis Pharmacia Biotech (USA) uses a dual mini kit at a constant voltage of 60 volts and an electric current range of 24 amperes for 4 hours. The marker used was *Fermentas Multicolor Broad Range Protein Ladder* code #SM1841, and the results are displayed in fragments or protein bands. The molecular weight of the target protein was calculated based on the standard curve of the molecular weight.

**Amino Acid Analysis**

Amino acid analysis was conducted using *High-Performance Liquid Chromatography* (HPLC) based on the HPLC protocol (Shimadzu, 2022). The HPLC device was Shimadzu HPLC (Kyoto, Japan) with a Thermo S Ods-Hypersil column (1 ml/min mobile phase flow rate). Meanwhile, amino acid analysis was carried out using the pre-column reaction of amino groups with ortophthaaldehyde (OPA) reagent to form a fluorescing compound. OPA reagent can react with primary amino acids in an alkaline medium containing mercaptoethanol to form a fluorescent compound. Therefore, the detection can be carried out with a fluorescence detector.

The amino acid analysis results with HPLC are displayed in the form of a chromatogram, as calculated by Equation 4.

$$Amino\ acid\ (\mu mol) = \frac{(\text{Sample peak area x standard concentration})}{(\text{Standard peak area})} \quad (4)$$

The percentage of amino acids in the sample is calculated by Equation 5.

$$Amino\ acid\ (\%) = \frac{(\mu mol\ of\ amino\ acid\ x\ DM\ of\ amino\ acids)}{(\mu g\ sample)} x\ 100\% \quad (5)$$

***Fourier Transform Infrared Spectroscopy* (FTIR)**

FTIR sample preparation is conducted by making pellets, and the preparation is carried out by inserting 200 mg of KBr into the mortar before adding 2 mg of the sample. Mixing of KBr and sample should be achieved quickly and mixed homogeneously. Furthermore, the homogeneous mixture is made into pellets using a mini hand press. The pellets should be dry, and it is included in the Bruker FTIR tool (Massachusetts, USA) with a wave number range between 400-3,500 $cm^{-1}$. The infrared absorption of the sample can be displayed in the form of a wavenumber spectrum in $cm^{-1}$.

***Scanning Electron Microscopy* (SEM)**

SEM analysis is used to determine the morphology of fiber and fibroin fractures due to the tensile test. The SEM tool was Carl Zeiss Bruker EVO MA10 (Massachusetts, USA), and in the initial process, fibroin placed on a copper template was thinly coated with gold before being inserted into the SEM tool. The thin gold functions as a *conductive coating* to improve the imaging produced by SEM.

## RESULTS AND DISCUSSIONS

**Non-mulberry silkworm cocoons**

In natural silk, the term cocoon is known as a source of silk fiber produced by silkworms at various times, depending on the species. The *Bombyx mori*, *A. atlas,* and *S. ricini* silkworms produce cocoons for 18-29 days, 35-43 days, and 24-30 days, respectively. The cocoon is the product of silkworm larvae knitting (Atmosoedarjo et al., 2000) as protection during the morphological transformation of the larvae into pupae and moths. Floss is the thin outermost fiber of the cocoon that the larvae use for hanging, while the cocoon skin is the main home for the pupa, a source of silk fiber. The pupae is the transformation phase in the metamorphosis of the larva to the moth, and the diagram of the cocoons of *A. atlas* and *S. ricini* can be seen in Figure 1.

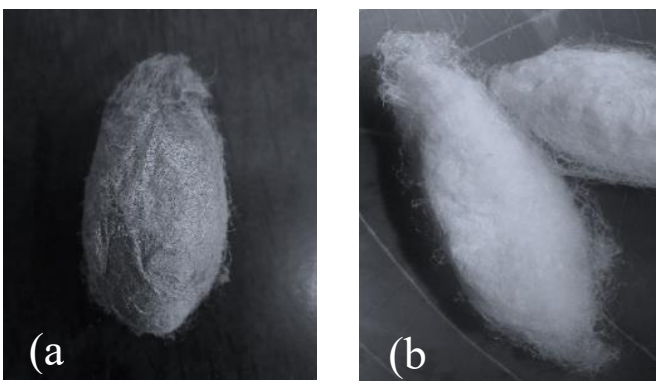


Figure 1. Cocoon *Attacus atlas* (a), and *Samia cynthia ricini* (b)

Table 2. Cocoon weight, cocoon shell, pupa, as well as silkworm floss on *Attacus atlas* and *Samia cynthia ricini* species

| Silkworm species | N | Cocoon weight | Cocoon shell weight | Pupae weight | Floss weight |
|---|---|---|---|---|---|
| | | | (g) | | |
| *Attacus atlas* | 300 | 10.46±1.74 | 1.41±0.84 | 8.81±1.69 | 0.24±0.07 |
| *Samia cynthia ricini* | 95 | 2.20±0.26 | 0.20±0.04 | 1.88±0.24 | 0.13±0.03 |
| *Bombyx mori** | 360 | 1.48±0.09 | 0.11±0.02 | 0.18±0.02 | 2.20±0.26 |

*Estetika and Endrawati (2018), N = number of samples.

Cocoon weight is very important for an application to determine the weight of silk fiber and its protein extracts. The cocoon weight of *A. atlas* ranged between (10.46±1.74 g) and *S. ricini* (2.20±0.26 g). The percentage ratio of the weight of the cocoon, floss, and pupae for species *A. atlas* is 13.50%, 2.27%, 84.23%, and for *S ricini,* it is 8.93%, 5.79%, 85.05%. By knowing these comparisons, protein yield can be predicted, and the complete data regarding cocoon weight can be seen in Table 2.

The sericin protein in the cocoon shell ranges from 20%-30%, while fibroin is around 70%-80% (Gulrajani, 2008). The protein content in the dry matter of *A. atlas, S. ricini*, and *B. mori* cocoon shells was 92.23%, 97.33%, and 94.03 ± 0.38% (Kweon et al., 2012). Therefore, the yield of sericin per shell can be predicted at 0.26-0.39 g, 0.04-0.06 g, and 0.02-0.03 g for *A. atlas, S. ricini*, and *B. mori* cocoon. The yield can be further increased by extracting sericin from floss because the protein content is almost the same in the cocoon shell, judging from its amino acid components (Yamada and Tsubouchi, 2001).

**Sericin Extraction Optimization**

To optimize yield, sericin extraction optimization was carried out using the *Response Surface Methodology* (RSM) method with Program R. In the RSM method, and there are two stages in determining the optimization point, namely ordo 1 and 2. Ordo 1 is an area likely to produce the optimum extract yield. Meanwhile, ordo 2 is the point of the independent variables or factors in the extraction formulation to produce the most optimum yield. Ordo 1 is the independent variable used as the center point in ordo 2. In this research, ordo 1 was generated from the steepest ascent experiment and obtained a central value of 115 °C and 95 °C for *A. atlas* and *S. ricini*. Furthermore, ordo 1 is used as the central value in the CCD experimental design of ordo 2, as seen in Table 3.

The sericin data from Table 3 was processed with the R program and yielded optimum points on the independent variables and equations predicting the yield. The RSM analysis yielded the optimum point for *A. atlas* and *S. ricini* at 130.52 °C and 113.20 °C, 71.71 and 33.78 minutes, and 0.08 N and 0.03 N NaOH solvent, as depicted in the contour of Figure 2.

For the contour of *A. atlas*, the lines are increasingly converging with high values, as shown by arrows, due to the analysis results eigenvalues, namely -0.25 and -1.63. Negative values in both eigenvalues mean that the stationary point can maximize the yield of sericin. Lenth (2020) stated that when the stationary points of the two eigenvalues are positive, the optimization is minimum. Furthermore, the optimization is a saddle point when eigenvalues are positive and negative. The contour of the *S. ricini* analysis shows a greater value on the upper and lower right and left sides, indicated by an arrow solid and dash. These results are by the eigenvalues at the stationary optimization point of 0.88 and -0.45. The optimization point is in the saddle point position, which indicates that the formulation cannot maximize sericin yield. This is because ordo 1 is not the optimum area for sericin yield, hence it is necessary to re-examine the determination of the center point of ordo 1.

The analysis produces an equation function to predict the total yield of sericin. The equation function applies to extraction methods with the same independent variables. The equation does not apply when working with a different extraction method. The following is the function of the equation for predicting the sericin yield of *A. atlas* (a) and *S. ricini* (b), where Y is the response (sericin yield), $X_1$ = temperature, and $X_2$ = time.

$$Y = 9{,}75 + 2{,}28X_1 + 0{,}99X_2 + 1{,}36X_1X_2 - 1{,}06X_1^2 - 0{,}82X_2^2 \quad (6)$$

Description: $X_1$= 3,10; $X_2$= 3,17

$$Y = 22{,}17 + 1{,}53X_1 + 1{,}18X_2 - 0{,}25X_1X_2 - 0{,}45X_1^2 + 0{,}87X_2^2 \quad (7)$$

Description: $X_1$= 1,82; $X_2$= -0,41

The equation function has an $R^2$ value of 0.9779 and 0.9056 for the *A. atlas* and *S. ricini* equation. These values indicate that the independent variables can affect the dependent by 97.79% and 90.56%. The value of $R^2$, which is close to 1, indicates that the resulting equation function has very good goodness of fit.

Table 3. The yield of silkworm sericin *Attacus atlas* and *Samia cynthia ricini* with *Central Composite Design trial design* (CCD)

| *Attacus atlas* | | | *Samia cynthia ricini* | | |
|---|---|---|---|---|---|
| Temperature (°C) | Time (minute) | Sericin (%) | Temperature (°C) | Time (minute) | Sericin (%) |
| 110 | 30 | 5.62 | 85 | 25 | 19.75 |
| 110 | 50 | 5.14 | 85 | 55 | 22.39 |
| 120 | 30 | 7.56 | 105 | 25 | 23.03 |
| 120 | 50 | 12.51 | 105 | 55 | 24.68 |
| 115 | 40 | 10.59 | 95 | 40 | 23.09 |
| 115 | 40 | 9.49 | 95 | 40 | 21.32 |
| 115 | 40 | 9.85 | 95 | 40 | 21.24 |
| 115 | 40 | 9.69 | 95 | 40 | 22.27 |
| 115 | 40 | 9.11 | 95 | 40 | 22.26 |
| 122.07 | 40 | 10.94 | 107.07 | 40 | 23.76 |
| 107.93 | 40 | 4.61 | 82.93 | 40 | 19.63 |
| 115 | 54.14 | 9.49 | 95 | 57.07 | 25.41 |
| 115 | 25.86 | 7.03 | 95 | 22.93 | 22.09 |

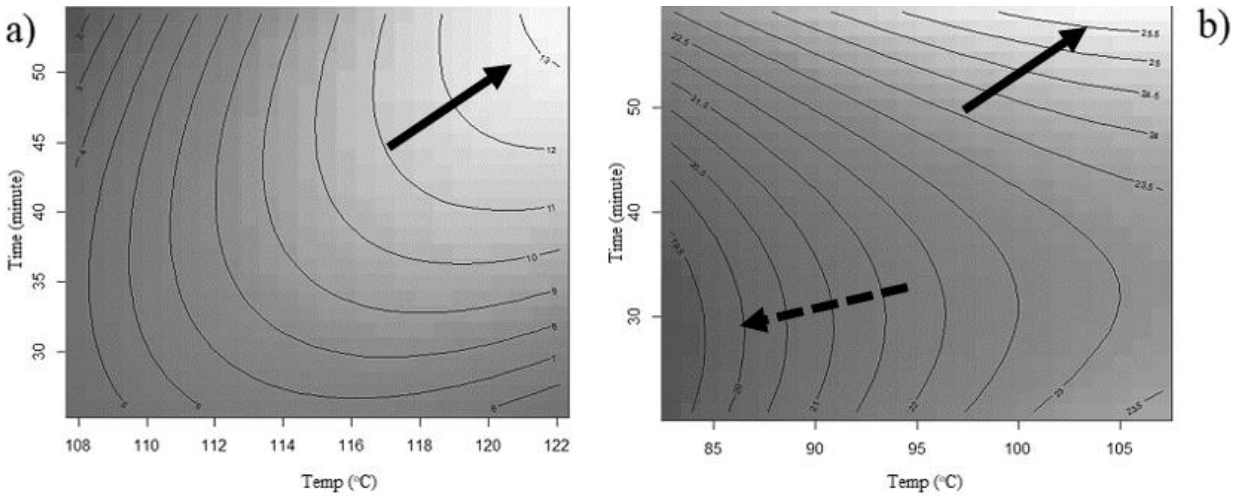


Figure 2. Contours of sericin protein yield at the optimum point, a) *Attacus atlas* (NaOH 0.08 N; 130.52 °C; 71.71 minutes), and *Samia cynthia* ricini (NaOH 0.03 N; 113.20 °C; 33.78 minutes)

**Optimum Variable Verification**

The prediction of sericin yield from the *A. atlas* and *S. ricini* equation yields 14.69% and 23.31% sericin. To verify the optimum point of the independent variables (temperature and time), verification is carried out in the laboratory. Laboratory verification results yielded 17.39 ± 1.24% for *A. atlas* sericin and 20.24 ± 2.30% for *S. ricini* sericin. The predicted yield of sericin was within the range of verified results in the laboratory, although the *A. atlas* and *S. ricini* sericin value was higher in the laboratory and predicted result. This is probably because the *goodness of fit* of the equation function for *A. atlas* is higher than for *S. ricini*. The sericin value was within the normal range for the yield of wild silkworms at 17%-25%. This is higher than the sericin yield of *S. ricini* by Prasong et al. (2009), which is equal to 15.7%. Therefore, the function of the equation, the optimum point of temperature and

time, and the NaOH concentration can produce sericin protein on a larger scale.

**Characteristics of Sericin**

**Sericin morphology**

Identification of sericin morphological characteristics is very important as an initial step to determine the ability of its extraction technique. Figure 3 presents the SEM results of the cocoon shells of *A. atlas* and *S. ricini* before and after extraction. Figures 3A1 and 3A2 show a reduction in sericin around the fiber. Sericin has been released from the fiber, and Figures 3B1 and 3B2 indicate a reduction around the fiber, and the results are cleaner than in 3A2. This is consistent with the yield of *S. ricini* sericin, which is greater than that of *A. atlas*. The different yields in the two types of cocoon shells are due to the total proteins in the skins. The content in the dry matter of *A. atlas* and *S. ricini* cocoon shell was 92.23% and 97.33%. Extraction with alkaline solvents can also release hydrogen bonds into smaller molecules, as seen in Figure 3B3.

**Sericin Molecular Weight**

The SDS PAGE analysis yielded a molecular weight (BW) for *A. atlas* and *S. ricini* sericin of 8.99 kDa and 7.08 kDa, and the sample used was 2 (Figure 4).

This research shows that the small BM is included in the Ser2-*small* group, both *A. atlas* and *S. ricini* sericin BM. The grouping of BM sericin is Ser1 with a BM above 250 kDa, Ser3 250 kDa, Ser2-large 225-230 kDa, and Ser2-small below 130 kDa (Takasu et al., 2010). It uses a type of alkaline solvent, NaOH, which produces a small BM of sericin. This is reinforced by Aramwit et al. (2010), who stated that different solvents in the extraction of *B. mori* sericin could yield different BMs of sericin. Furthermore, *B. mori* sericin BM with urea, acid, alkali, and high-pressure extraction produces BM 10-250 kDa, BM 50-150 kDa, BM 15-75 kDa, and 25-150 kDa. Rajput and Singh also produced the same result (2015), that extraction with different solvents will produce BM of sericin. Extraction with 1% sodium deoxycholate, enzyme, and aqueous urea yielded BM of 17.10-18.46 kDa, 3-10 kDa, and 50 kDa sericin. The BM values of sericin (*A. atlas* and *S. ricini*) were within the range of those of Aramwit et al. (2010), which used alkaline solvents in their extraction. These results are also supported by Zhang et al. (2004), where extraction with alkali produced a *B. mori* sericin MMR of less than 20 kDa. In treating strong acids or bases, the amino acids making up proteins will be released from covalent bonds, forming relatively small molecules (Lehninger, 1982).

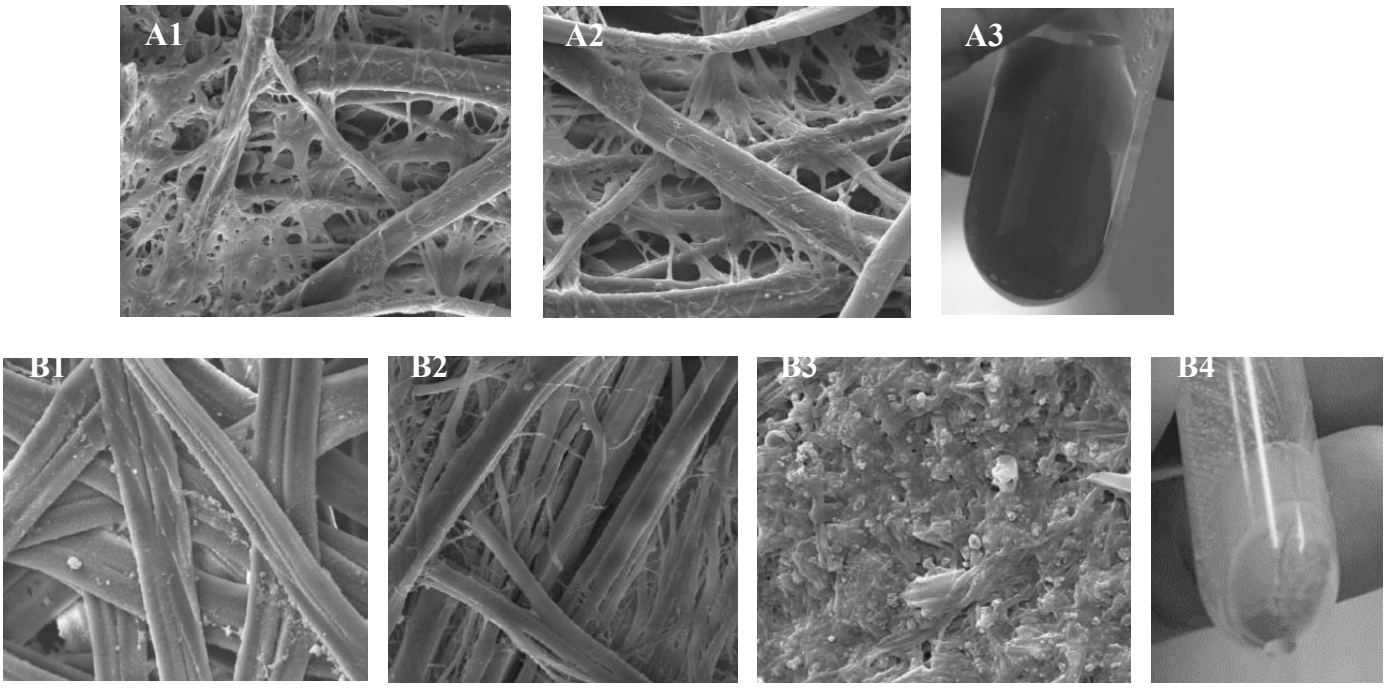


Figure 3. SEM photo, A1. *Attacus atlas* cocoon skin before extraction 1000x magnification, A2. *Attacus atlas* fibers after 1000x magnification extraction, and A3. *Sericin Attacus atlas*; while B1. the skin of *Samia cynthia ricini's* cocoon before extraction at 1000x magnification, B2. *Samia cynthia ricini* fiber after extraction with 1000x magnification, B3. *Sericin Samia cyntia ricini* 3000x magnification, and B4. *Samia cynthia ricini* sericin powder

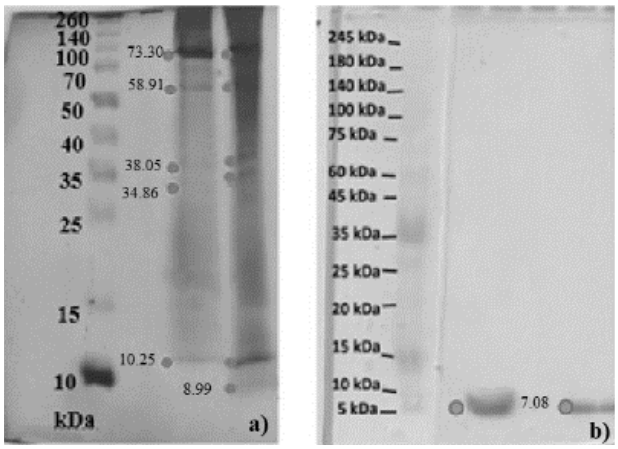


Figure 4. SDS PAGE results, a) *Attacus atlas sericin*, and b) *Samia cynthia ricini sericin* with 12.5% *separating gel* and 4% *stacking gel*

The small BM of sericin can potentially be used as a biomaterial. This is to the research results of Wu et al. (2007), where sericin with a molecular weight of 6 kDa has the ability as an antioxidant, inhibiting tyrosinase activity, and can be used as a food *supplement*. Furthermore, with low molecular weight, sericin is an antioxidant and anticancer material in cosmetics and medicine (Chang-Kee et al., 2002; Zhang, 2004).

**Amino Acid in Sericin**

Analysis using HPLC Shimadzu (Kyoto, Japan) yielded the amino acid composition of the *A. atlas* and *S. ricini* sericin proteins, as shown in Table 4. The main components of sericin are serine, glycine, and threonine at 5.8%, 24.64%, and 1.45%. Meanwhile, *S. ricini* sericin consists of 8.91%, 15.34%, and 5.57% of serine, glycine, and threonine. The main amino acid sericin composition varies according to the species and type of solvent used for extraction. The percentage of serine in *B. mori* is more dominant than glycine. This differs from the non-mulberry group and the serine and glycine percentage ratio. Serine and glycine are balanced in the Antheraea sp. group, while *Cricula trifenestrata* is almost like *B. mori*. In contrast to *A. atlas* and *S. ricini*, the amino acid glycine is more dominant than serine.

The composition of the amino acids serine, glycine, and threonine results from Rajput and Singh's (2015) research. It stated that the amino acid composition of sericin in mulberry and non-mulberry silkworms differ. The percentage of non-mulberry and mulberry sericin protein in serine, glycine, and threonine is 7-16% and 16-20%, 10-20% and 8-10%, and 3-10% and 4-6%.

**FTIR Characteristics of Sericin**

The secondary structure of sericin protein was analyzed by Bruker's FTIR (USA). The analysis of sericin

Table 4. The amino acid composition of the sericin protein of the different species

| Amino acid (%) | Mulberry silkworm | | | Non-mulberry silkworm | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Bombyx mori* [1] | *Bombyx mori* [2] | *Bombyx mori* [3] | *Antheraea mylitta* [4] | *Antheraea yamamai* [5] | *Cricula trifenes-trata* [6] | *Attacus atlas* | *Samia cynthia ricini* |
| Aspartic acid | 12.99 | 19.16 | 20.18 | 0.00 | 0.00 | 0.00 | 8.70 | 8.28 |
| Glutamic acid | 4.28 | 7.34 | 6.02 | 7.14 | 10.00 | 1.62 | 10.14 | 9.66 |
| Serine | 19.03 | 27.83 | 30.46 | 23.17 | 22.35 | 42.93 | 5.80 | 8.91 |
| Histidine | 0.99 | 1.73 | 1.75 | 16.13 | 0.00 | 0.00 | 2.90 | 0.23 |
| Glycine | 24.37 | 10.91 | 11.17 | 22.93 | 22.96 | 22.44 | 24.64 | 15.34 |
| Threonine | 5.25 | 7.65 | 6.59 | 14.71 | 14.57 | 14.13 | 1.45 | 5.57 |
| Arginine | 3.04 | 5.00 | 4.99 | 3.43 | 0.00 | 3.13 | 1.45 | 5.69 |
| Alanine | 15.31 | 4.38 | 4.27 | 3.52 | 7.78 | 5.29 | 17.39 | 13.33 |
| Tyrosine | 4.13 | 4.69 | 5.32 | 2.32 | 4.32 | 7.66 | 17.39 | 5.40 |
| Methionine | 0.11 | 0.51 | 0.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.29 |
| Valin | 3.36 | 3.87 | 2.98 | 1.21 | 3.83 | 0.00 | 1.45 | 3.51 |
| Assessment | 0.69 | 1.63 | 0.82 | 0.00 | 0.00 | 0.00 | 1.45 | 5.98 |
| I-leucine | 1.83 | 1.33 | 0.76 | 1.33 | 6.54 | 0.86 | 1.45 | 3.62 |
| Leucine | 2.00 | 1.73 | 1.58 | 1.49 | 7.65 | 1.19 | 2.90 | 8.45 |
| Lysine | 2.08 | 2.14 | 2.93 | 2.63 | 0.00 | 0.76 | 2.90 | 5.75 |

[1]Tokutake (1980); [2]Wu et al. (2007); [3]Aramwit et al. (2010); [4]Dash et al. (2007); [5]Cui et al. (2009); [6]Yamada and Tsubouchi (2001).

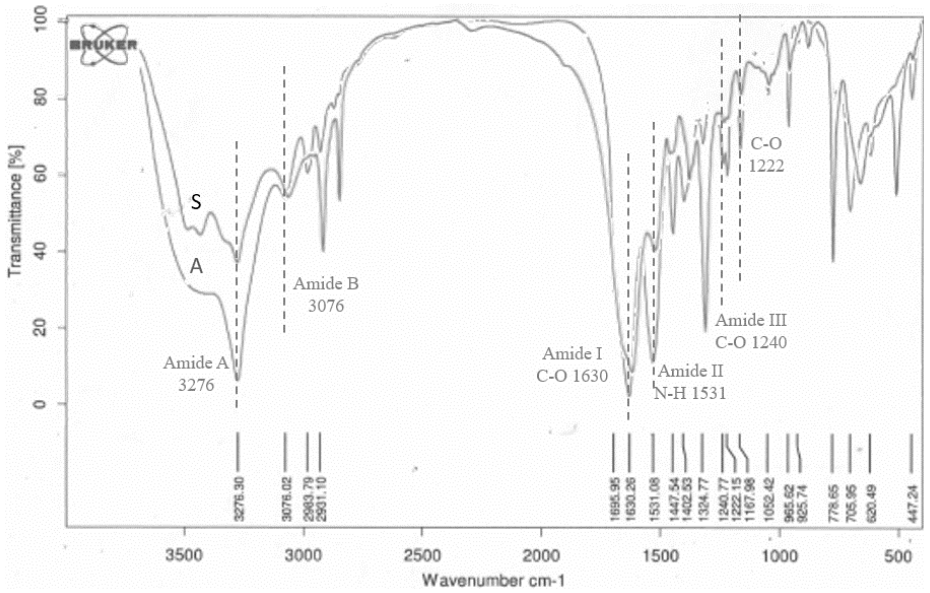


Figure 5. FTIR sericin *Attacus atlas* (A) and *Samia cynthia ricini* (S)

*A. atlas* and *S. ricini* yielded Amide I C-O, Amide II N-H, Amide III C-O, Amide A, and Amide B at a wavelength of 1,630 $cm^{-1}$, 1,531 $cm^{-1}$, 1,240 $cm^{-1}$, 3,276 $cm^{-1}$ and 3,076 $cm^{-1}$ as shown in Figure 5. This is consistent with the secondary structure of sericin, namely Amida I at a wavelength of 1643–1644 $cm^{-1}$ as C=O stretching vibration, Amida II at 1,538-1,540 $cm^{-1}$ as N-H bending, and C-N stretching vibration, Amida III at 1,240 $cm^{-1}$ as a C-N stretching couple with N-H bending vibrations, Amida A at 3,265 $cm^{-1}$ and Amida B at 3,062 $cm^{-1}$ as hydrogen bonds (Ahn et al., 2001; Krisha et al., 2006). Sericin with alkaline extraction is at a wavelength spectrum of 1,652 $cm^{-1}$, 1,544 $cm^{-1}$, and 1,241 $cm^{-1}$ as Amide I, II, and III (Kumar and Mandal, 2017). Based on these results, the extraction of sericin did not change the secondary structure of the protein.

## CONCLUSION

RSM optimization produced *Attacus atlas* and *Samia cynthia ricini* sericin extraction formulation at NaOH concentrations of 0.08 N and 0.10 N, temperature 130.52 °C and 113.20 °C, time 71.71 and 33.78 minutes, and produced a yield of 17.39 ± 1.24% and 20.24 ± 2.30%. The higher sericin yield value in *S. ricini* is consistent with the SEM results, where the fiber is cleaner than *A. atlas* after extraction treatment. The sericin had *Ser2small* type BM characteristics in *A. atlas* and *S. ricini* sericin at 8.99 kDa and 7.08 kDa. The amino acids produced by the two species are also of the same type, where the serine percentage is smaller than glycine. The resulting sericin extraction formulation also did not change the secondary structure of the protein in *A. atlas* and *S. ricini*. Therefore, this formulation can maximize the yield and maintain the chemical characteristics.

## ACKNOWLEDGMENT

The authors are grateful to the IPB University for providing funding through the Young Lecturer Research scheme (5540/IT3.L1/PT.01.03/M/T/2021) to carry out this research.

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