Songklanakarin J. Sci. Technol.
32 (1), 17-22, Jan. - Feb. 2010

Songklanakarin Journal
of Science and Technology

http://www.sjst.psu.ac.th

Original Article

Extracted sericin from silk waste for film formation

Rungsinee Sothornvit[1]*, Rungsima Chollakup[2] and Potjanart Suwanruji[3]

[1] *Department of Food Engineering, Faculty of Engineering at Kamphaeng San,*
Center of Excellent for Agricultural and Food Engineering,
Kasetsart University, Kamphaeng San Campus, Nakhon Pathom, 73140 Thailand.

[2] *Kasetsart Agricultural and Agro-Industry Product Improvement Institute (KAPI),*

[3]*Department of Chemistry, Faculty of Science,*
Kasetsart University, Chatuchak, Bangkok, 10900 Thailand.

Received 28 September 2007; Accepted 19 February 2009

Abstract

Sericin is the second main component in cocoons, which are removed in the silk reeling process of the raw silk industry and in the silk waste degumming of the spun silk industry. The main amino acid of sericin, serine, exhibits a skin moisturing and antiwrinkle action, which is interesting to use for film formation in this study. The extraction conditions of sericin from two silk wastes, pieced cocoon and inferior knubbs were studied to find the optimum extraction conditions. Boiling water extraction was considered based on the response surface methodology (RSM) in order to identify the important factors for the sericin extraction. The two factors considered were time and temperature. Both factors were needed to be independent parameters in the predicted equation in order to improve the model fit with $R^2 = 0.84$. The components of extracted sericin were 18.24% serine, 9.83% aspatate, and 5.51% glycine with a molecular weight of 132 kDa. Film formation from extracted sericin was carried out to find the optimum conditions. Extracted sericin could not form a stand-alone film. Therefore, polysaccharide polymers, such as glucomannan, were incorporated with glycerol to form a flexible film. Sericin-based films were characterized for its properties in terms of solubility and permeability before application. It was found that sericin-based films showed a film flexibility and solubility without an increasing film water vapor permeability.

Keywords: sericin, film, RSM, permeability, solubility

1. Introduction

The use of recovered sericin from silk reeling and degumming processes in the silk industry will add more value to sericin. Moreover, the recovery of sericin will minimize the BOD and COD values in the waste water. Since sericin is composed of 80% amino acids that contain hydrophilic groups, such as serine, aspatate, and glycine, it can absorb moisture very well. Sericin is also a good antioxidant. Therefore, sericin is used in many applications, for example, in the medical field as an anticoagulation agent and as an anticancer agent. In cosmetics, sericin is used in skin and hair products. Sericin that has a molecular weight of more than 20 kDa can be used as medical biomaterials, degradable biomaterials, compound polymers, functional biomembranes and hydrogels (Zhang, 2002; Lee *et al.*, 2004; Kongdee *et al.*, 2005).

Sericin is interesting to determine its use as a biopolymer film. Biopolymer films are generally made from thin sheets of biomaterials such as proteins, carbohydrates, fats and biomass. These films can be used for food coatings to prevent water and oxygen permeation between food products and the atmosphere. In this research, the extraction conditions in boiling water of sericin from silk waste were

---

*Corresponding author.
Email address: fengrns@ku.ac.th

studied in order to predict the equations for computing the amount of extracted sericin under a defined condition using Response Surface Methodology (RSM). Then, sericin was used to study the feasibility to form film and determine its properties.

2. Material and Methods

2.1 Sericin extraction and properties

2.1.1 Sericin extraction in boiling water

Two types of silk waste, pierced cocoons (the result of breeding moths that have emerged from their cocoons to produce eggs required for the next crops) and inferior knubbs (outer portion of the cocoon layer obtained in the first process of reeling cocoons), from *Bombyx mori* (Thai Golden Silk, Multivoltine strain) were used in this study. These raw materials were analyzed moisture and sericin contents following the standard method (ISA, 1993). The sericin contents were determined based on the gravimetric method. Sericin extraction in boiling water was studied with two important factors, which were temperature in the range of 82-120°C (above 105°C using autoclave) and time in the range of 10-60 min. The experimental design chosen for this study was the central composite design (CCD) and then was performed by response surface methodology (RSM). This design was a full factorial design with all combinations of the factors at two levels (high, +1, and low, -1). The centre point (coded level 0), which was the midpoint between the high and low levels, was repeated with five points. The range and levels of the independent variables with actual and coded levels were given in Table 1. The total number of test runs needed for this design was 13.

Table 1. CCD experimental design for the independent variables of sericin extraction.

Expt. No.	Independent variables			
	Actual level		Code level	
	Temperature	Time	Temperature	Time
1	93	23	-1	-1
2	112	23	+1	-1
3	93	48	-1	+1
4	112	48	+1	+1
5	82	35	-1.414	0
6	120	35	+1.414	0
7	105	10	0	-1.414
8	105	60	0	+1.414
9	105	35	0	0
10	105	35	0	0
11	105	35	0	0
12	105	35	0	0
13	105	35	0	0

The sericin extraction was carried out by subjection silk waste of 20 grams in boiling water at a liquid ratio of 1:30 with various temperatures and times. After extraction, sericin solution was separated by hydraulic pressing at 2.5 MPa for 1 min. The silk waste was washed with hot water (100 ml) at 80°C and then pressed again for 1 min to separate the extracted solution. The collected solution was measured for volume and the % dry solid was determined by hot air oven method at 105°C in order to calculate the weight of total extracted soluble solid. Protein concentration in the sericin solution was determined using the Lowry method (Lowry *et al.*, 1951). The percentage of sericin extraction was calculated based on sericin content in the raw material at different experimental designs. RSM was applied to analyze the effect of independent variables on the response parameter (% sericin extraction) by matching the response studied (Y) with the code factors using the polynomial model associated with the experimental design as defined in Equation 1.

$$Y = A_0 + \sum A_i X_i + \sum A_{ii} X_i^2 + \sum A_{ij} X_i X_j \qquad (1)$$

where Y is the dependent variable, A_0 is the constant coefficient, A_i is the linear coefficient, A_{ii} is the quadratic coefficient, and A_{ij} is the two factors interaction coefficient.

2.1.2 Statistical analyses

Statgraphic 3.0 for Windows (Statistical Graphics Corp.) was used for regression and ANOVA analysis. Response surface graphs were obtained from the regression equation in actual levels of variables, keeping the response function on the Z axis with X and Y axes representing the independent variables.

2.1.3 Properties of extracted sericin

Sericin extracted from pierced cocoons at the optimum condition was dried using Spray Dry method at a temperature inlet of 150°C and a temperature outlet of 75°C. Amino acid contents and molecular weight (MW) were determined by using an Amino Acid Analyzer and by applying sodium dodecyl sulfate polyacrylamide gel electrophoresis (12% SDS-PAGE) and a staining with Coomassie Brilliant Blue.

2.2 Film formation and properties

2.2.1 Extracted sericin film formation

Aqueous solutions of sericin and glucomannan in the ratio of 1:1 or 2:1 with 2% w/w and addition of 0%, 15% or 30% (w/w) of glycerol (gly) plasticizer were mixed together and heated in a microwave for 2-3 min. Solutions were cooled to room temperature (RT). A 3 g of total solids was poured into 14.5-cm internal diameter, rimmed, smooth glass casting plates and dried at 60°C for 8 hrs until dried films can be released intact from plates.

2.2.2 Film solubility measurement (total soluble matter)

A piece of film sized 7.5 mm x 15.0 mm was cut and dried in an oven at 70°C for 1 hr and then weighed to obtain the initial film dry weight. The piece of film was then placed into a test tube with 10 ml water. The test tube was capped and shaken slowly on a shaker for 24 hrs at RT. The remain-ing film after immersing in the solution was dried in the oven at 70°C for 1 hr to determine the film final dry weight. The percentage of total soluble matter (% TSM) of the film was calculated from Equation 2:

$$\%TSM = \left(\frac{initial\ dry\ film\ wt - final\ dry\ film\ wt}{initial\ dry\ film\ wt} \right) \times 100 \qquad (2)$$

2.2.3 Water vapor permeability (WVP) measurement

The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh *et al.*, 1993) was used to determine WVP. Six milliliter of water was pipetted into test cups made of polymethylmethacrylate (Plexiglas) with external dimension of 8.2 cm diameter, 1.25 cm height, and 21.56 cm^3 inner volume. Films without pinholes and any defects were placed in between the cup and the ring cover of each cup coated with grease and held with 4 screws around the cup circumference. After that, the cups were placed in the constant RH cabinets (0% RH using silica gel desiccant) at 27°C. Once steady-state moisture transfer was obtained, weights were taken at 2-hrs intervals. The WVP of film was calculated from Equation 3:

$$WVP = \frac{WVTR \times thickness}{\left(p_{A1} - p_{A2} \right)} \qquad (3)$$

where WVTR is the water vapor transmission rate and p_{A1} and p_{A2} is the water vapor partial pressure inside and outside the cup, respectively.

2.2.4 Statistical analyses

A completely randomized experimental design was used to study the main factors: (1) sericin and glucomannan ratio (S:G), (2) gly content, and (3) interaction between S:G and gly. Three replications were used to determine each property. SPSS 11.0 for Windows (SPSS Inc., Chicago, IL) was utilized to calculate analysis of variance (ANOVA) at 95% confidence interval.

3. Results and Discussion

3.1 Sericin extraction and properties

3.1.1 Sericin extraction in boiling water

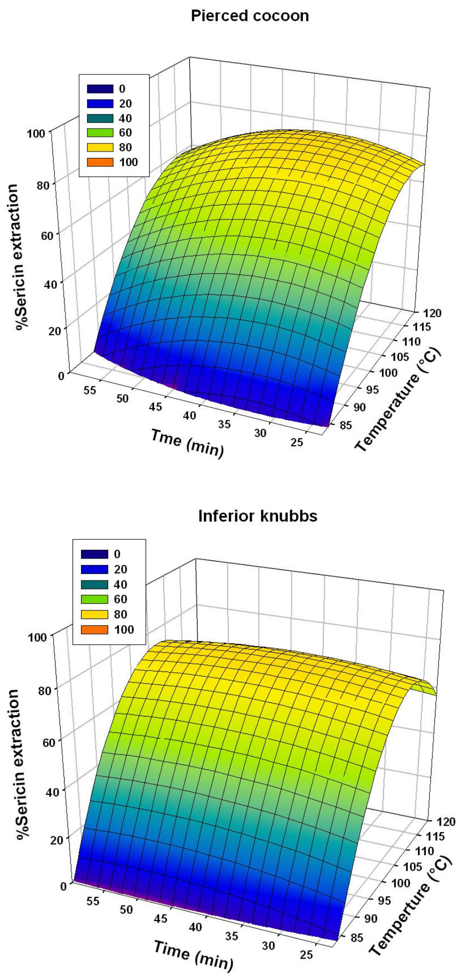

Figure 1. Response surface of %sericin extraction as a function of temperature and time.

Pieced cocoons and inferior knubbs with moisture contents of 7.91±0.07 and 8.00±0.35% had sericin contents of 26.81±1.11 and 23.15±0.68 % dry basis, respectively. After the extraction in boiling water at various temperatures and times, the effect of these two factors on the percentages of extracted sericin was evaluated and the relationship was shown in Equation 4 and 5 and the response surface graphs were shown in Figure 1 for both silk wastes. Figure 1 shows that with an increase in temperature and time for both silk wastes, %sericin extraction reaches a maximum and then decreased. At the same extraction condition, sericin extraction from inferior knubbs can give a higher yield than from pierced cocoons; although the sericin content of inferior knubbs showed a greater amount than of the pierced cocoons, because the alignment of the silk filament at the inferior knubbs was loosen than those of the pierced cocoon. Boiling water can diffuse easier to contact the surface area of interior knubbs than of the pierced cocoons for the sericin extraction process. This result can be confirmed by SEM photography of the raw material (Chollakup *et al.*, 2004). From this reason, it was found that the maximum sericin conditions of both silk waste were different, which was at 115°C, 37 min for pierced cocoons and at 108°C, 43 min for inferior knubbs, with a yield of 77% for both.

For pierced cocoons,

$$Y = -948.66+17.27X_1+1.726X_2-0.0763X_1^2+0.0078X_1X_2 -0.0352X_2^2 \quad (4)$$

$R^2 = 0.84$, p-value 0.0097

Maximum condition at 115°C, 37 min, %Sericin extraction = 77.14%

For inferior knubbs,

$$Z = -1344.24+25.73X_1+1.56X_2-0.1181X_1^2-0.0060X_1X_2 -0.0106X_2^2 \quad (5)$$

$R^2 = 0.83$, p-value = 0.0120

Maximum condition at 108°C, 43 min; %Sericin extraction = 77.10%

As Y = %sericin extraction,

X_1 = Temperature (°C), X_2 = Time (min)

3.1.2 Properties of extracted sericin

SDS gel electrophoresis was used to determine the molecular weight of sericin from pierced cocoons. It was found that the extracted sericin had a molecular weight distribution above 132 kDa compared with a standard marker as shown in Figure 2. This result was different from sericin extracted from *Bombyx mori* cocoons with saturated aqueous salt solution, which had molecular weights of 150, 250, and 400 kDa (Takasu *et al.*, 2002). It was implied that the extracted sericin was partly degraded by boiling water at high temperatures. The amino acid compositions of sericin were given for 18 kinds as shown in Table 2. The main amino acids in the sericin extracted from the pierced cocoons by boiling water were serine (32.74%), aspartic acid (17.64%), and glycine (9.89%). This composition was similar to those sericin extracted from the Bivoltine strain of different sources; 1) raw material with serine 31.99%, aspartic acid 15.74%, and glycine 14.20% (Vaithamsat and Kitpreechavanich, 2008), 2) degumming solution with serine 27.30%, aspartic acid 18.80%, and glycine 10.70% (Wu *et al.*, 2007), and 3) hot water-soluble sericin with serine 28.00%, aspartic acid 17.97%, and glycine 16.29% (Zhang *et al.*, 2004). The extracted sericin was used to make biopolymer since it had a high molecular weight (more than 20 kDa) and can absorb moisture well. These properties can be used for film formation.

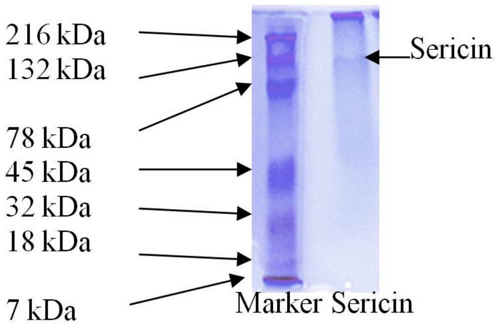

Figure 2. SDS-PAGE of extracted sericin.

Table 2. Amino acid composition of sericin from pierced cocoons compared with a reference sericin.

Amino acid	Percent of gram amino acid in 100 g protein			
	Sericin from this study[a]	Sericin from raw material[b]	Sericin from degumming solution[c]	Hot water-soluble sericin[d]
Aspartic acid	17.64	15.74	18.80	17.97
Serine	32.74	31.99	27.30	28.00
Glutamic	7.31	6.28	7.20	6.25
Glycine	9.89	14.20	10.70	16.29
Histidine	1.81	1.49	1.70	1.32
Arginine	6.16	4.29	4.90	3.52
Threonine	5.51	7.73	7.50	7.78
Alanine	3.86	4.85	4.30	5.20
Proline	0.59	0.71	1.20	ND
Cystine	ND	0.20	0.30	0.69
Tyrosine	4.63	3.01	4.60	2.87
Valine	3.14	3.30	3.80	3.77
Methionine	0.11	ND	0.50	0.79
Lysine	3.05	4.17	2.10	1.21
Isoleucine	1.04	0.72	1.30	0.64
Leucine	1.44	0.96	1.70	1.21
Phenylalanine	1.08	0.37	1.60	0.64

[a] Silk waste (pierced cocoon) of B. mori Multivoltine strain

[b] Raw material sericin of B. mori Bivoltine strain (Vaithamsat and Kitpreechavanich, 2008)

[c] Degumming solution of B. mori Bivoltine strain (Wu *et al.*, 2007)

[d] Hot water-soluble sericin of B. mori Bivoltine strain (Zhang *et al.*, 2004)

3.2 Film formation and properties

3.2.1 Extracted sericin film formation

Extracted sericin could not form a stand-alone film by itself, although its molecular weight is high. Therefore, other biopolymers, such as glucomannan, were selected to be incorporate with sericin to provide the structure of the film. We also hypothesized that sericin might act as a plasticizer similar to other polyols, such as glycerol. Thus, we designed an experiment to determine the effect of both sericin itself and glycerol content on the film properties. Sericin films were yellow and transparent. The addition of a plasticizer produced more flexible films, while a reduction of sericin provided more transparent films. The average thickness of the sericin films was 0.14±0.02 mm.

3.2.2 Sericin and glucomannan ratio (S:G) effects

TSM: Film solubility is an important functional property. For example, films for water soluble packages must be readily soluble, but films on high moisture foods must be insoluble. The S:G had a significant effect on the percentage of total soluble matter (%TSM) ($p \leq 0.05$) as shown in Figure 3. The less amount of sericin (S:G=1:1) maintained the film integrity in water better than S:G at 2:1.

WVP: WVP of sericin films from S:G=1:1 was different from that of S:G=2:1 ($p \leq 0.05$) as shown in Figure 4. An increasing sericin content showed a slightly lower WVP of films without adding glycerol. The lower amount of glucomannan added facilitated the film formation, while the sericin itself provided a flexible film and easy peeling films from the casting plates without plasticizer needed. Increasing glycerol and sericin contents provided a higher film WVP. We hypothesized that sericin might be acting as a plasticizer. These results are consistent with the results of TSM, which showed an increase in TSM at a higher amount of sericin.

3.2.3 Glycerol content effects

TSM: The plasticizer amount produced a significant effect on %TSM ($p \leq 0.05$) as shown in Figure 3. Generally, plasticizer reduces intermolecular forces along protein chains and increases the polymer free volume. It was found that an increase in plasticizer content produced a linear increase in water-soluble matter content in fish myofibrillar protein films (Cuq *et al.*, 1997). It can be assumed that the addition of a plasticizer provided a higher TSM.

WVP: The plasticizer content created a significant difference in the film WVP. As expected, an increasing gly content presented a significant increase in the sericin film WVP (Figure 4) and in most edible films, such as whey protein films (Sothornvit and Krochta, 2000), and also in polysaccharide films (Schultz *et al.*, 1949). As mentioned above, plasticizer increases the polymer free volume; therefore, there is greater space for water and other molecules to migrate. At S:G = 2:1, there was a significant higher effect on WVP at 15% gly. It might be the effect of gly over the effect of S:G. However, the effect of gly on WVP was lower at S:G=1:1.

4. Conclusion

In this study, the sericin extraction conditions in boiling water from silk wastes are optimized and predicted by an equation with $R^2 = 0.84$, which is derived based on the RSM. The two factors considered time and temperature are resulted in an increasing sericin extraction with an increase of both factors at the optimum condition. The composition of the extracted sericin shows mainly serine with 18.24% with MW 132 kDa. Extracted sericin is prepared for film formation with other biomaterials such as glucomannan. Sericin-glucomannan films made good films with greater solubility.

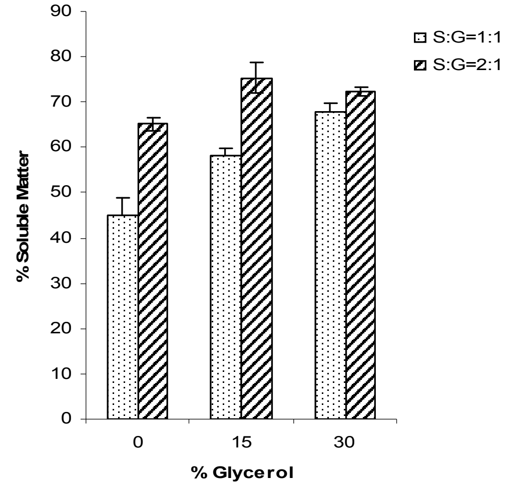

Figure 3. Effect of the sericin to glucomannan ratio (S:G) and glycerol content on %TSM of the films.

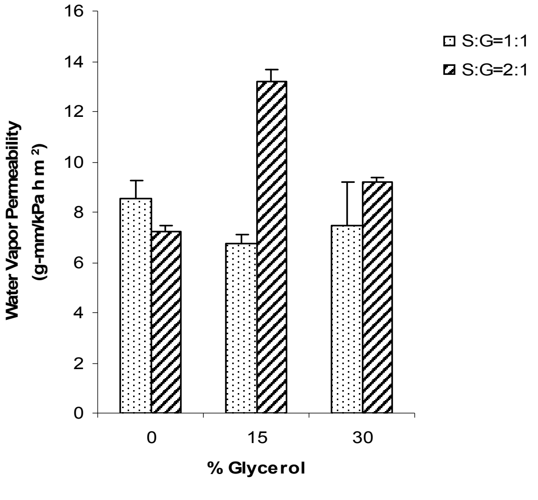

Figure 4. Effect of the sericin to glucomannan ratio (S:G) and glycerol content on WVP of the films.

Sericin films can provide a flexibility of the film without increasing the film WVP. Thus, use of sericin as a plasticizer incorporated with other biopolymers can reduce the amount of plasticizer required to impart the desired film flexibility and thus minimize the permeability of the films.

Acknowledgements

This work was supported by the Kasetsart University Research Development Institute (KURDI). The authors wish to thank Shinano Kenshi (Thailand) Co., Ltd., for supporting the raw materials, the Faculty of Ago-Industry, Faculty of Science, and KAPI, Kasetsart University, for the degumming process and instrument analyses.

References

Chollakup, R., Sinoimeri, A. and Dréan, J-Y. 2004. Characteristics of Thai Hybrid Silk Fibres from different portions of the cocoon layer wastes: Feasibility in blending with cotton fibre. Journal of Insect Biotechnology Sericology 73, 39-45.

Cuq, B., Gontard, N., Cuq, J. L. and Guilbert, S. 1997. Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. Journal of Agricultural and Food Chemistry. 45, 622-626.

International Silk Association (ISA). 1993. Spun silk: Quality limits, testing procedures and recommendations regarding quality, Group of Section IV, Zellweger Uster AG, Switzerland, pp. 5-8.

Kongdee, A., Bechtold, T. and Teufel, L. 2005. Modification of cellulose fiber with silk sericin. Journal of Applied Polymer Science. 96, 1421-1428.

Lee S.R, Miyazaki K., Hisada K. and Hori T. 2004. Application of silk sericin to finishing of synthetic fabrics. Sen-I Gakkaishi. 60, 9-15.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 93, 265-275.

McHugh, T.H., Avena-Bustillas, R. and Krochta, J.M. 1993. Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. Journal of Food Science. 58, 899-903.

Schultz, T.H., Miers, J.C., Owens, H.S. and Maclay, W.D. 1949. Permeability of pectinate films to water vapor. Journal of Physical Colloid Chemistry. 53, 1320-1330.

Sothornvit, R. and Krochta, J.M. 2000. Water vapor permeability and solubility of films from hydrolyzed whey protein. Journal of Food Science. 65, 700-703.

Takasu, Y., Yamada, H. and Tsubouchi, K. 2002. Isolation of three main sericin components from the cocoon of the silkworm, *Bombyx mori*. Bioscience Biotechnology Biochemistry. 66, 2715-2718.

Vaithamsat, P. and Kitpreechavanich, V. 2008. Sericin separation from silk degumming wastewater. Separation and Purification Technology. 59, 129-133.

Wu, J-H., Wang, Z. and Xu, S-Y. 2007. Preparation and characterization of sericin powder extracted from silk industry wastewater. Food Chemistry 103, 1255-1262.

Zhang, Y-Q 2002. Applications of natural silk protein sericin in biomaterials. Biotechnology Advance. 20, 91-100.

Zhang, Y.-Q., Tao, M.-L., Shen, W.-D., Zhou, Y.-Z., Ding, Y., Ma, Y. and Zhou, W.-L. 2004. Immobilization of L-asparaginase on the microparticles of the natural silk sericin protein and its characters. Biomaterials. 25, 3751-3759.