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Sericin: Structure and Properties

1.1 Type of Silk Sericin

Sericin is a natural product from the silkworm. Sericin is one of the major protein components in the cocoons of Lepidopteron insects. Sericin is a glue-like coating protein surrounded with filament protein, fibroin (Figure 1.1). In manufacturing silk, sericin is a waste product from the degumming process. The silk sericin is classified into two types based on the feeding source of the silkworms: mulberry and non-mulberry sericin. The mulberry silkworm, *Bombyx mori*, is a well-known source of commercial silk production. This worm is a completely domesticated species that feeds on mulberry leaves. *B. mori* had long been developed for an indoor cultivation for the silk industry, whereas non-mulberry silkworm or wild silkworm is the group that feeds on other leaves such as oak leaves and castor oil leaves. Most of the non-mulberry silkworms cannot be reared indoors for their whole life cycles. The well-known non-mulberry silkworms are *Antheraea*, *Samia ricini* (or *Philosamia ricini*), and *Cricula trifenestrata*. *Antheraea* is a genus of silkworm that feeds on oak leaves and produces "tasar" silk, such as *Antheraea assamensis* (producing muka silk), *Antheraea mylitta*, *Antheraea pernyi*, and *Antheraea yamamai*. *S. ricini* produces the famous "eri" silk. In the wild environment, *S. ricini* feeds on castor oil plant leaves. *C. trifenestrata* is a wild silkworm producing "cricula" silk. The diversity of silkworm sources (genus, species, and diet) may produce distinct sericin characteristics.

1.2 Localization of Silk Sericin

Sericin is located at several sites of silkworms and cocoons. In the mulberry silkworm, *B. mori*, it has been reported that sericin is present in three components including silk gland, cocoon, and floss (Gamo et al. 1977; Kikkawa 1953; Yamada 1978). For non-mulberry silkworms, sericin is also secreted in the cocoon peduncle (Dash et al. 2006). The silk gland is the site that produces sericin. In a histological study, sericin was found to be mainly synthesized in the middle and posterior of the silk gland. Sericin protein is then sent to anterior silk glands via the lumen for secretion and cocoon construction (Consortium 2008; Kikkawa 1953; Yamanouchi 1922).

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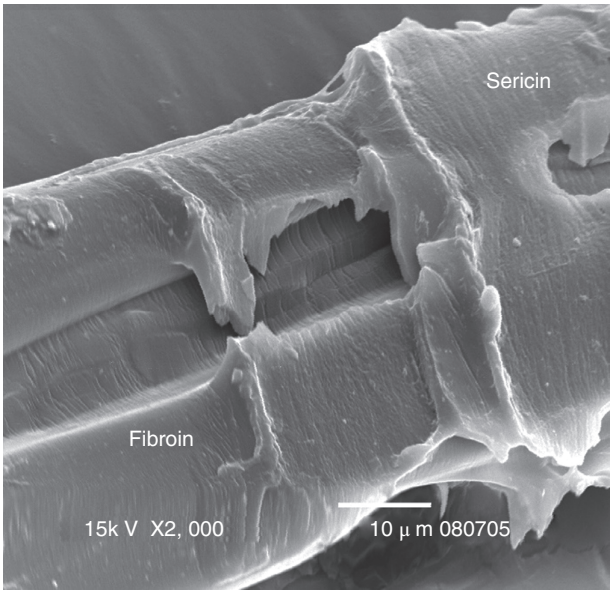


Figure 1.1 Scanning electron microscope (SEM) of a silk filament that contains fibroin and sericin.

1.3 Molecular Mass of Sericin

The molecular mass of sericin has been observed using sodium dodecyl sulfate and polyacrylamide gel electrophoresis (SDS-PAGE). The diversity pattern of sericin was investigated from various extraction sites, extraction methods, species, and strains. Figure 1.2 shows the molecular mass of sericin from different extraction methods using SDS-PAGE.

1.3.1 Middle Silk Gland Sericin

The middle silk gland (MSG, Figure 1.3) is a synthesis site for sericin in silkworm. Sericin obtained from MSG is known as native sericin. The MSG sericin measured by gel electrophoresis was found in intact bands and various protein sizes. Sericin extracted from the silk gland of mulberry silkworm was identified with three sizes of sericin including 130, 210, and 220 kDa as shown in Figure 1.4 (Sprague 1975). The study of sericin extracted from four MSG sections, including the anterior, middle to anterior, middle, and posterior sections, found five different sizes of sericin polypeptides. Two polypeptides, 177 and 134 kDa, were isolated from the anterior MSG. The middle section of the MSG had two polypeptides (309 and 145 kDa). One polypeptide (80 kDa) was found in the posterior section of the MSG (Gamo et al. 1977). Therefore, various sericin polypeptides were observed in mulberry sericin extracted from the MSG in the range between 80 and 309 kDa.

1.3.2 Mulberry Cocoon sericin

Cocoon sericin has been isolated, and the molecular mass of the protein was studied. Multiple sericin polypeptides have been extracted by several approaches including temperature, pressure, urea, acid, and alkali solution. In 1980, Tokutake reported that sericin

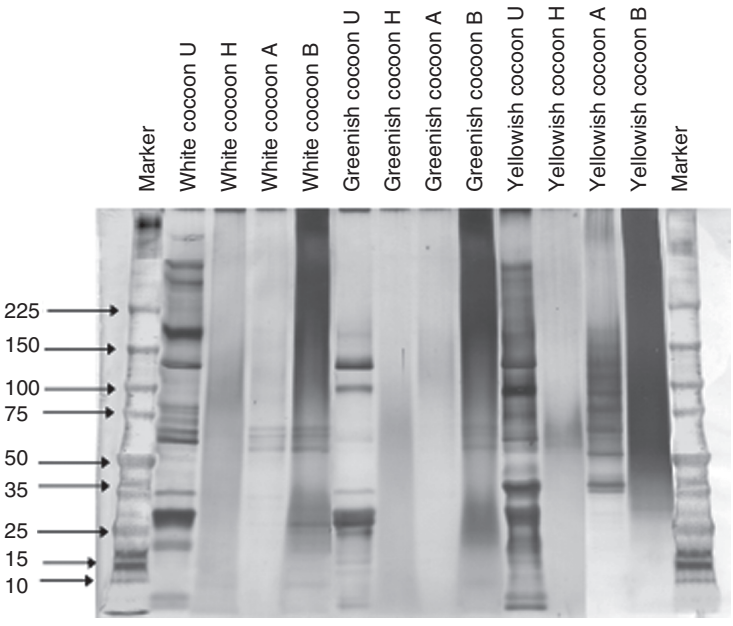
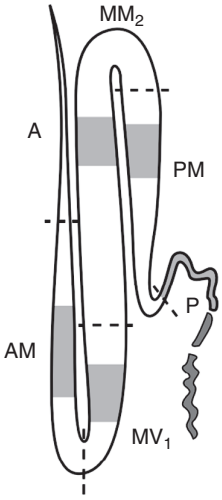


Figure 1.2 Sodium dodecyl sulfate and polyacrylamide gel electrophoresis (SDS-PAGE) of sericin extracted from white, green, and yellow cocoons using the following methods: urea solution (U), high temperature–high pressure (H), citric acid solution (A), and sodium carbonate solution (B). Different silk strains with various extraction methods show different molecular weights ranging from 10 to >225 kDa. Source: Reprinted with permission from Aramwit et al. (2010a).

Figure 1.3 Diagrammatic representation of the silk gland in the mature larvae of silkworms. Shadowed parts in the figure indicate the section used for the extraction of silk proteins, fibroin, and sericin. A, anterior gland; AM, anterior section in the middle gland; MM, middle section in the middle gland; PM, posterior section in the middle gland; P, posterior gland. Source: Reprinted with permission from Gamo et al. (1977). © 1977 Elsevier.



and fibroin protein could be separated by the precipitation of sericin in acidic conditions (pH 5.5). Later, the gel filtration approach was used to separate the precipitated sericin into five fractions (Tokutake 1980). This result suggests that sericin comprises a variety of forms. In 1982, Gamo and colleagues reported the molecular size of sericin proteins obtained by boiling with an alkali solution for extracting the protein from the cocoon. SDS-PAGE

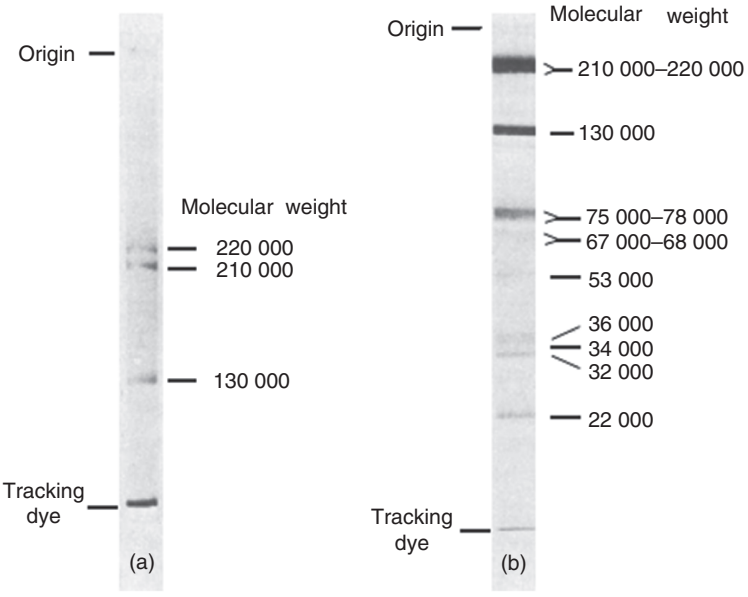


Figure 1.4 Separation of the protein components of sericin by sodium dodecyl sulfate and polyacrylamide gel electrophoresis on slabs of (a) 4% acrylamide and 0.1% bisacrylamide; (b) 8% acrylamide and 0.2% bisacrylamide. Source: Reprinted with permission from Aramwit et al. (2010a).

analysis revealed the molecular size of sericin in three different bands including the sizes >226.5, 226.5, and 218.8 kDa (Gamo 1982). In 2002, Takasu et al. compared the molecular mass between cocoon sericin and sections of the MSG. Cocoon proteins were extracted by saturated aqueous lithium thiocyanate solution. The four cocoon proteins were identified from SDS-PAGE analysis and named after the similar sizes found in subparts of the MSG. The two close bands around 250 kDa were named sericin A, as a reference to the specific size found in the anterior of the MSG. The fraction of 400 kDa was named sericin M, which was abundantly found in the middle of the MSG. The molecular size 150 kDa was named sericin P; this protein was the lowest expressed and found only in the posterior subpart of the MSG (Takasu et al. 2002). Moreover, the observation of sericin patterns was different depending on the extraction methods. In 2010, Aramwit et al. compared the extracted sericin protein pattern from four different extraction methods, including urea, autoclave (high heat and high pressure), acidic solution, and alkaline solution. Clear bands were observed with urea extraction, which found sericin in various sizes from 10 to >225 kDa. Sericin extracted by autoclave showed smear patterns ranging from 20 to 150 kDa. Acid and alkaline extraction solutions revealed band patterns mixing between clear bands and smears between 50–150 kDa and 15–75 kDa, respectively. In addition, both acid and alkaline extractions shared a clear band pattern at 50–70 kDa (Aramwit et al. 2010b). From all this information, it is evident that the extraction method affected the size and pattern of cocoon sericin proteins and is related to its biological properties.

Additionally, the different silkworm strains gave different patterns of sericin proteins. The study of *B. mori* from three strains based on the pigment color (different concentrations of

flavonoids and carotenoids in cocoons), white shell, greenish shell, and yellow shell (Figure 1.5), had variations in sericin molecular mass. Urea-extracted sericin revealed the clear bands in all strains, but different size ranges for the white shell and yellow shell strains with proteins ranging from 10 to >225 kDa, while the greenish shell strain had mass ranging from 10 to 150 kDa. The autoclave method showed smear bands ranging from 50–150, 35–100, and 35–75 kDa for the white shell, greenish shell, and yellow shell strains, respectively. For acid and alkali extraction methods, all strains also displayed different bands within the range of 35–150 and 15–75 kDa, respectively (Aramwit et al. 2010b). This suggests that not only the sericin extraction method but also the strain of silkworms affected the molecular mass structure of extracted-sericin proteins.

1.3.3 Non-mulberry Cocoon and Peduncle Sericin

Non-mulberry cocoon sericin has been studied in *S. ricini*, *A. assamensis*, and *A. mylitta*. In 2004, Ahmad et al. observed non-mulberry sericin at 66 kDa from *S. ricini* and *A. assamensis* (Ahmad et al. 2004). In 2007, Dash et al. reported a 70 kDa sericin extracted from *A. mylitta* (Dash et al. 2007). Isolated sericin from non-mulberry cocoons' molecular mass revealed a range between 66 and 70 kDa, which is smaller than observations for mulberry sericin.

The differences in the sericin extraction methods had dissimilar protein patterns in non-mulberry cocoon sericin from *S. ricini*, *A. assamensis*, and *A. mylitta*. The autoclave technique showed smear bands in all sericin species. The high temperature and high pressure from this extraction method might degrade all types of sericin protein. For the urea extraction method, there appeared a diversity of sericin protein sizes. *S. ricini* sericin was detected at the size higher than 300 kDa and in the range of 200–250 kDa. *A. assamensis* was observed in two bands with a size >250 kDa and at approximately 90 kDa. For *A. mylitta*, three bands were revealed with sizes of 250, 200, and 70 kDa (Sahu et al. 2016). These non-mulberry sericins detected by this study are composed of a high molecular mass in between 200 and 300 kDa, which was close to the high molecular mass of mulberry sericin. A similar study was performed comparing five extraction methods: urea, autoclave, conventional, acidic solutions, and alkaline solutions in *A. assamensis* and *S. ricini*. *A. assamensis* showed smeared bands in urea, autoclave, and conventional methods, whereas acidic and alkali solutions displayed a clear band at 75 kDa. For *S. ricini*, a clear band at 75 kDa was revealed

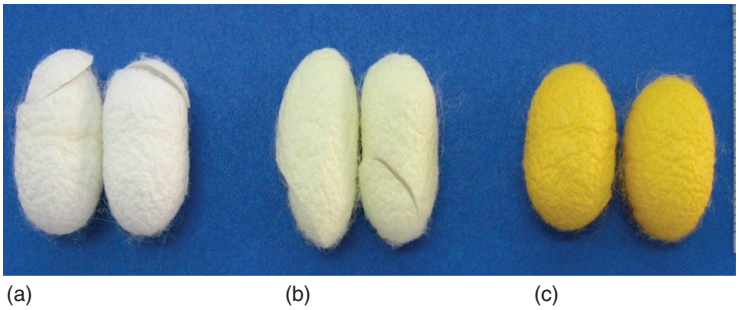


Figure 1.5 Physical appearance of silk cocoons, while shell (a), green shell (b), and yellow shell (c).

along with smeared protein in urea, conventional, and alkali solutions. Smears with no intact bands of sericin proteins were observed from the acid and autoclave extraction methods (Kumar and Mandal 2017). These data showed that cocoon sericin protein patterns were different depending on their species and extraction methods. Therefore, the cocoon sericin protein might have a diversity of structures.

The peduncle is a strong filament in a ring form for attaching the non-mulberry cocoon to the branches of a tree. Sericin extracted from the peduncle of *A. mylitta* had a single band detected at 200 kDa (Dash et al. 2006). The size of this protein is similar to that observed for the MSG sericin extraction of *A. mylitta* (Dash et al. 2009). This sericin protein might have a major role in the action of the *A. mylitta* peduncle.

1.4 Layers of Sericin

The microstructure of silk gland sericin has been observed in silkworm. Histological studies have shown that sericin can be clearly divided into three distinct parts. Sericin I is the inner layer connected to fibroin. Sericin II is the middle layer and is the most abundant type. Sericin III is the outer layer, which covers the outside and is mostly mucous (Kikkawa 1953).

Cocoon sericin could be separated into three layers based on its solubility properties from extraction methods such as temperature, pressure, urea, acid, or alkali solution. Three layers have been divided into outer, middle, and inner layers, which are connected to fibroin. The amino acid composition of each layer has been defined differently. The pattern of amino acid residues (mol%) was used to identify the type of sericin protein. Fifteen amino acids have been identified. Four residues, including serine, threonine, glycine, and aspartic acid, were present at higher levels in all three layers (Shaw and Smith 1951).

1.5 Sericin Amino Acid Components

The amino acid composition of sericin has been reported from parts of silkworms and cocoons, including the silk gland, cocoon, floss, and peduncle. The percentage of amino acid contents in the total amino acid component (mol%) could indicate the individual structure of each sericin. The application of percent amino acid components was used as a reference for the distinction between sericin and fibroin silk protein (Gamo et al. 1977).

1.5.1 Silk Gland of Mulberry Sericin

The amino acid residue composition in the silk gland of mulberry silkworm is revealed in Table 1.1. Most of the common amino acid composition (>10 mol%), which was found in all extraction methods, is serine and aspartic acid (including asparagine). In 1975, Sprague reported the amino acid content of sericin with three different molecular masses, including 220, 210, and 130 kDa. The four major amino acid residues, including aspartic acid, glutamic acid, lysine, and serine, had averages of 27, 22, 19, and 14 mol%, respectively (Sprague 1975). In 1977, Gamo et al. reported the amino acid composition of sericin

Table 1.1 Amino acid components from silk glands of mulberry silkworms.

Group of silkworm sericin	Mulberry							
Reference	Sprague (1975)			Gamo et al. (1977)				
Species (strain)	*B. mori*			*B. mori*				
Source	Silk gland			Middle silk gland				
Detail	SDS-PAGE (220 kDa)	SDS-PAGE (210 kDa)	SDS-PAGE (130 kDa)	s-1 middle	s-2 anterior	s-3 middle to anterior	s-4 posterior	s-5 anterior
Name	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%
Alanine	4.00	4.50	2.90	6.20	6.90	6.20	4.20	7.20
Arginine	—	—	—	4.00	3.90	3.00	3.60	3.50
Aspartic acid + asparagine	32.00	26.00	23.00	13.00	12.90	12.60	11.60	13.90
Cysteine	—	—	—	Trace	1.00	0.50	0.70	2.50
Glutamic acid + glutamine	20.00	28.00	17.00	5.40	5.20	10.70	6.10	10.30
Glycine	7.80	8.70	8.70	18.00	12.50	14.20	11.40	17.50
Histidine	—	—	—	1.60	1.50	0.80	1.90	3.10
Isoleucine	—	—	—	1.60	1.30	0.40	1.80	2.20
Leucine	1.70	1.50	3.60	2.10	1.50	0.60	2.80	2.70
Lysine	16.00	12.00	29.00	2.20	4.80	5.30	2.20	6.30
Methionine	—	—	—	Trace	Trace	Trace	Trace	Trace
Phenylalanine	—	—	—	1.50	0.70	0.40	3.40	1.20
Proline	—	—	—	Trace	Trace	Trace	Trace	Trace
Serine	15.00	15.00	11.00	29.10	30.20	38.10	32.50	16.40
Threonine	4.20	4.40	5.20	8.50	8.00	4.10	11.10	4.00
Tryptophan	—	—	—	—	—	—	—	—
Tyrosine	—	—	—	3.30	6.80	2.10	2.80	9.00
Valine	—	—	—	3.70	2.80	1.00	3.80	0.30

SDS-PAGE, sodium dodecyl sulfate and polyacrylamide gel electrophoresis.
*Gray highlight: top four highest contents of amino acids (mol%).

from different sections of the MSG, including the middle (fraction: s-1), anterior (fraction: s-2 and s-5), middle to anterior (fraction: s-3), and posterior (fraction: s-4) sections (Gamo et al. 1977). Serine residues had an average of 30 mol% in all sections. The exception is the anterior MSG fraction s-5, which secreted serine content of 16 mol%. This fraction (s-5) contains serine in amounts that are half that of the other fractions. The minor component amino acids of all fractions were glutamic acid (average 15 mol%) and aspartic acid (average 13%). Threonine was found as a high percentage residue in fractions s-1, s-2, and s-4, on average 9%, while fraction s-3 and s-5 had a glutamic acid-rich component, on average 10%, instead of threonine residue. This data meant that sericin at different sections of the silk gland presented unique characteristics of sericin structure.

1.5.2 Sericin from Mulberry Cocoons

The amino acid composition of cocoon sericin was studied from various extraction methods, species, and strains. Amino acid components from mulberry silk sericin were different (Table 1.2). In mulberry cocoon sericin, the highest three amino acid components commonly found were serine (average 32 mol%), glycine (average 17 mol%), and aspartic acid (average 16 mol%). The less abundant sericin amino acid is threonine (average 8 mol%). However, the amino acid composition of sericin is different based on the extraction methods. Sericin from the acid precipitation method was found to have alanine-rich residues (15 mol%) instead of threonine. Alanine richness is not commonly found in other extraction methods (Tokutake 1980). Different sericin fractions showed differences in amino acid contents, such as the fraction sericin A (13 mol%) having glutamic acid richness. Meanwhile, the other fractions, sericin M and sericin P, showed high threonine contents at an average of 11 mol% (Takasu et al. 2002). The strain of silkworm did not show differences in the ratio of the amino acid residues by the autoclave extraction method (Aramwit et al. 2009). Cocoon sericin containing high amounts of serine, glycine, and aspartic acid is common. However, the different extraction methods may affect some amino acid contents, such as glutamic acid, threonine, and alanine. In addition, strains of silkworms revealed the different amino acid components from alkali extraction methods (Table 1.2). The alkaline extractions from three different silkworm strains found different components. The fourth top amino acid composition was found to be glutamic acid rich in Chul 3/2 and Chul 4/2 strains (average 7 mol%), whereas Chul 1/1 had threonine (7 mol%) (Aramwit et al. 2010a). The major amino acid residues of cocoon sericin were similar. Only some less common amino acid components such as threonine, glutamic acid, and alanine were different among extraction methods and strains. The modification of sericin structure by chemical solution was supported by the study of the properties of sericin being improved (Teramoto et al. 2004). Therefore, the extraction method interferes with the sericin structure.

Floss is a soft filament that covers the mulberry silk cocoon of *B. mori*. Its function is to protect and hang the cocoon on a tree branch. Floss sericin has been extracted and tested for the amino acid content (Table 1.2) (Yamada 1978). Three major amino acid residues were serine (40 mol%), glycine (18 mol%), and aspartic acid (10 mol%). The serine of floss sericin revealed around twofold higher percentage content when compared to cocoons. The differences in amino acid composition in floss and cocoons may reflect their specific properties and functions.

Table 1.2 Amino acid composition of sericin obtained from the cocoons of mulberry silkworms.

Group of silkworm sericin	Mulberry silkworm sericin											
Reference	Yamada (1978)			Tokutake (1980)	Gamo (1982)	Takasu (2002)			Teramoto et al. 2004	Aramwit (2009)		
Species (strain)	*B. mori* (mandarina)			*B. mori* (shugetsu × Hosho)	*B. mori*	*B. mori*			*B. mori*	*B. mori* (chul 1/1)	*B. mori* (chul 3/2)	*B. mori* (chul 4/2)
Source	Floss	Cocoon	Cocoon	*Cocoon*	*Cocoon*	Cocoon	Cocoon	Cocoon	*Cocoon*	Cocoon	Cocoon	Cocoon
Detail	Floss sericin	Inner cocoon	Outer cocoon	Acid precipitation	S×2 (227 kDa)	Sericin A (250 kDa)	Sericin M (400 kDa)	Sericin P (150 kDa)	Sericin	Autoclave	Autoclave	Autoclave
Name	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%
Alanine	4.43	4.84	5.15	15.20	4.40	5.50	4.10	8.10	5.00	4.10	4.45	4.98
Arginine	3.30	3.03	3.41	3.02	3.20	2.90	3.40	4.00	3.20	2.87	2.95	3.09
Aspartic acid + asparagine	10.20	18.61	18.30	12.90	14.90	13.30	15.70	11.30	16.30	15.64	15.62	15.97
Cysteine	Trace	0.42	0.64	Trace	—	0.10	0.00	—	—	0.44	0.43	0.27
Glutamic acid + glutamine	4.31	4.90	4.78	4.25	11.10	12.80	3.10	3.10	4.70	4.61	4.76	4.86
Glycine	18.17	16.90	16.70	24.20	14.90	14.30	16.00	14.10	15.30	15.03	15.09	15.14
Histidine	0.68	0.86	0.99	0.98	1.00	1.00	1.30	—	1.40	1.06	1.22	1.37
Isoleucine	0.67	0.60	0.67	1.82	0.60	0.20	0.50	0.80	0.60	0.56	0.65	0.61

(Continued)

Table 1.2 (Continued)

Group of silkworm sericin	Mulberry silkworm sericin											
Reference	Yamada (1978)			Tokutake (1980)	Gamo (1982)	Takasu (2002)			Teramoto et al. 2004	Aramwit (2009)		
Species (strain)	*B. mori* (mandarina)			*B. mori* (shugetsu × Hosho)	*B. mori*	*B. mori*			*B. mori*	*B. mori* (chul 1/1)	*B. mori* (chul 3/2)	*B. mori* (chul 4/2)
Source	Floss	Cocoon	Cocoon	*Cocoon*	*Cocoon*	Cocoon	Cocoon	Cocoon	*Cocoon*	Cocoon	Cocoon	Cocoon
Detail	Floss sericin	Inner cocoon	Outer cocoon	Acid precipitation	S×2 (227 kDa)	Sericin A (250 kDa)	Sericin M (400 kDa)	Sericin P (150 kDa)	Sericin	Autoclave	Autoclave	Autoclave
Name	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%
Leucine	0.85	0.90	1.17	1.99	1.40	0.50	0.90	1.60	1.30	1.00	1.15	1.11
Lysine	1.89	2.26	2.89	2.07	6.00	5.40	1.80	1.00	2.70	2.35	2.51	2.78
Methionine	0.12	0.10	0.11	0.11	—	—	—	—	—	3.39	0.57	0.18
Phenylalanine	0.43	0.42	0.47	0.69	0.40	0.40	0.20	0.70	0.40	0.28	0.39	0.36
Proline	0.66	0.51	0.56	0.69	—	—	0.60	1.30	0.70	0.54	0.62	0.71
Serine	40.28	28.12	29.05	18.90	36.80	39.00	35.40	33.20	34.20	33.63	34.50	33.84
Threonine	6.29	11.39	8.44	5.21	4.00	3.30	9.70	12.20	8.00	8.16	8.43	8.34
Tryptophan	—	—	—	—	—	—	—	—	—	—	—	—
Tyrosine	4.09	3.28	3.39	4.10	0.10	0.70	4.00	4.60	2.90	3.45	3.64	3.47
Valine	3.46	2.67	2.91	3.34	1.20	0.70	3.20	3.90	3.30	2.88	3.04	2.92

Table 1.2 (Continued)

Reference	Aramwit et al. (2010)											
Species (strain)	*B. mori* (Chul 1/1)				*B. mori* (Chul 3/2)				*B. mori* (Chul 4/2)			
Source	Cocoon	Cocoon	Cocoon	Cocoon	Cocoon	Cocoon	Cocoon	Cocoon	Cocoon	Cocoon	Cocoon	Cocoon
Detail (extraction method)	Autoclave	Urea	Acid	Alkali	Autoclave	Urea	Acid	Alkali	Autoclave	Urea	Acid	Alkali
Name	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%	mol%
Alanine	4.1	4.33	3.72	4.21	4.45	3.8	3.57	3.96	4.98	4.63	3.56	4.4
Arginine	2.87	5.41	4.92	4.92	2.95	5.21	4.87	3.79	3.09	5.71	5.24	4.83
Aspartic acid + asparagine	15.64	18.31	15.93	19.88	15.62	17.93	16	21.58	15.97	17.69	16.61	19.92
Cysteine	0.44	0.39	0.53	0.23	0.43	0.33	0.5	0.19	0.27	0.42	0.52	0.16
Glutamic acid + glutamine	4.61	5.27	5.75	5.93	4.76	6.02	5.4	7.66	4.86	5.97	5.88	7.03
Glycine	15.03	11.23	10.49	11.01	15.09	10.75	10.38	11.16	15.14	10.96	10.69	12.58
Histidine	1.06	3.26	2.47	1.72	1.22	2.82	2.83	2.38	1.37	2.5	2.29	2.15
Isoleucine	0.56	0.96	0.87	0.75	0.65	0.95	0.9	0.87	0.61	0.74	0.66	1.03
Leucine	1	1.58	1.43	1.56	1.15	1.58	1.44	1.51	1.11	1.63	1.37	1.81
Lysine	2.35	3.14	3.48	2.89	2.51	3.55	3.03	2.71	2.78	2.5	3.16	3.08
Methionine	3.39	0.12	0.06	0.15	0.57	0.08	0.06	0.13	0.18	0.06	0.05	0.15
Phenylalanine	0.28	0.6	0.71	0.81	0.39	0.66	0.67	0.72	0.36	0.63	0.57	0.81
Proline	0.54	1.46	0.78	1.24	0.62	0.79	0.73	0.92	0.71	1.16	0.79	1.01
Serine	33.63	31.27	31.86	30.01	34.5	32.24	32.01	28.41	33.84	30.69	31.95	27.59
Threonine	8.16	8.36	8.51	6.49	8.43	8.78	8.78	6.09	8.34	9.04	8.3	5.56
Tryptophan	—	—	—	—	—	—	—	—	—	—	—	—
Tyrosine	3.45	0.36	5.56	5.24	3.64	1.24	5.81	4.92	3.47	2.67	5.59	4.9
Valine	2.88	2.96	2.95	2.94	3.04	3.28	3.03	3.03	2.92	2.98	2.76	2.99

1.5.3 Sericin from Non-mulberry Cocoons

The cocoon sericin from non-mulberry silkworm has been reported from two species, *A. mylitta* (Dash et al. 2007, 2008) and *C. trifenestrata* (Yamada and Tsubouchi 2001). The amino acid analysis revealed three major components, including serine, glycine, and threonine (Table 1.3). Interestingly, glutamic acid, which has a high content in mulberry sericin, is less observed in non-mulberry sericin. The serine component in cocoon sericin of *A. mylitta* averages 19 mol%, which is lower than that in mulberry sericin (average 32 mol%). However, *C. trifenestrata* serine showed 40 mol%, which is higher than mulberry cocoon sericin but similar to the floss of mulberry sericin. Non-mulberry cocoon sericin showed a

Table 1.3 Amino acid composition of sericin obtained from non-mulberry silkworms.

Group of silkworm sericin	**Non-mulberry**				
Reference	**Dash (2006)**	**Dash (2006)**	**Dash (2007)**	**Dash (2008)**	**Yamada and Tsubouchi (2001)**
Species (strain)	***A. mylitta***	***A. mylitta***	***A. mylitta***	***A. mylitta***	***Cricula trifenestrata***
Source	**Peduncle**	**Cocoon**	**Cocoon**	**Cocoon**	**Cocoon**
Detail	**Peduncle sericin**	**Cocoon sericin**	**Sericin (70 kDa)**	**Cocoon sericin**	**Crude sericin (400 kDa)**
Name	**mol%**	**mol%**	**mol%**	**mol%**	**mol%**
Alanine	4.80	6.01	2.95	6.01	4.90
Arginine	4.01	3.36	2.87	3.36	2.90
Aspartic acid + asparagine	—	—	—	—	2.60
Cysteine	—	—	—	—	—
Glutamic acid + glutamine	11.16	5.70	5.98	5.70	1.50
Glycine	24.40	18.11	19.20	16.11	20.80
Histidine	6.30	11.15	13.51	10.15	—
Isoleucine	2.10	1.56	1.11	1.56	0.80
Leucine	0.10	1.76	1.25	1.76	1.10
Lysine	0.90	2.95	2.20	2.95	0.70
Methionine	0.80	—	—	—	—
Phenylalanine	—	—	—	—	—
Proline	1.20	1.28	0.98	1.28	2.50
Serine	21.42	17.78	19.40	19.78	39.80
Threonine	10.20	12.22	12.32	13.22	13.10
Tryptophan	—	—	—	—	—
Tyrosine	0.70	2.38	1.94	2.38	7.10
Valine	2.40	1.29	1.01	1.29	—

very low percent molecular content of aspartic acid, but a high component of threonine (average 12 mol%). The minor amino acid component in *A. mylitta* is histidine (average 12 mol%), and it is tyrosine in *C. trifenestrata* (7 mol%). Cysteine and phenylalanine were not found in non-mulberry sericin. From the amino acid composition of non-mulberry cocoon sericin and mulberry cocoon sericin, it seems that the structure of sericin is different and may lead to different structures and biological properties.

The peduncle, which has a function in hanging *A. mylitta* cocoons on the tree, had an amino acid composition that was high in glycine (24 mol%), serine (21 mol%), glutamic acid (11 mol%), and threonine (10 mol%) (Dash et al. 2006). The amino acid content is similar to the cocoon sericin of *A. mylitta*, which has no aspartic acid residue. A major component of glutamic acid was observed, which was different from the cocoon part that had a low mol% component. The amino acid composition of the peduncle sericin was different from the cocoon sericin. Therefore, the peduncle sericin may have a special property specific to its function.

Overall, a high serine content (>10 mol%) was found in all parts of the silkworm for all species and extraction methods. The other major amino acids, glycine, threonine, aspartic acid (including asparagine), glutamic acid (including glutamine), lysine, histidine, and tyrosine, were found to vary in each part. The different amino acid components of sericin might be related to its structure, property, and function (Figures 1.6 and 1.7).

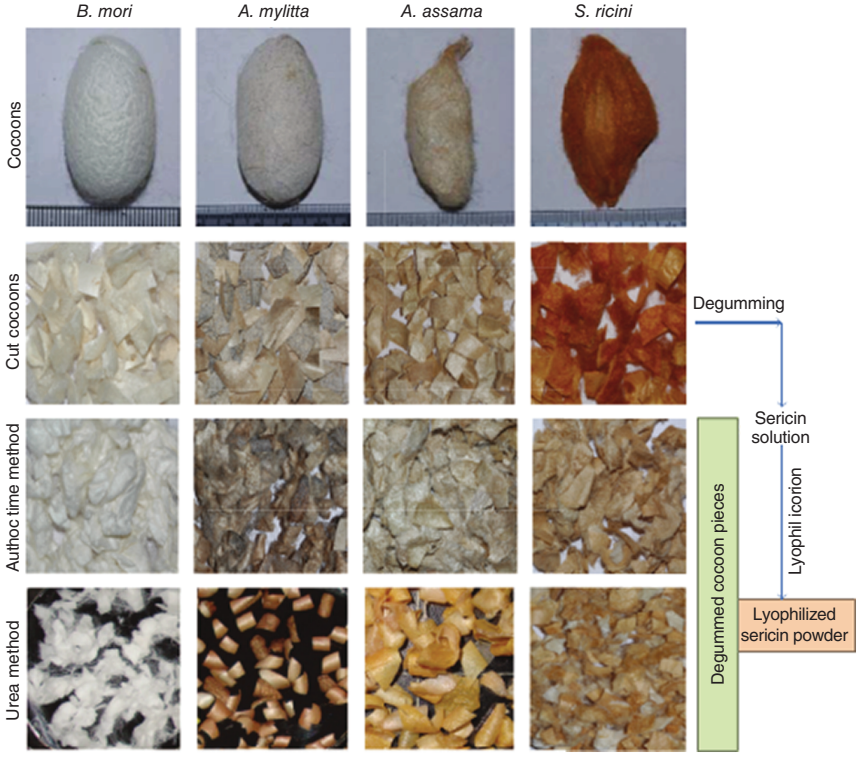


Figure 1.6 Physical appearance of mulberry and non-mulberry silk cocoons before and after degumming with different methods of sericin isolation. Source: Reprinted with permission from Sahu et al. (2016).

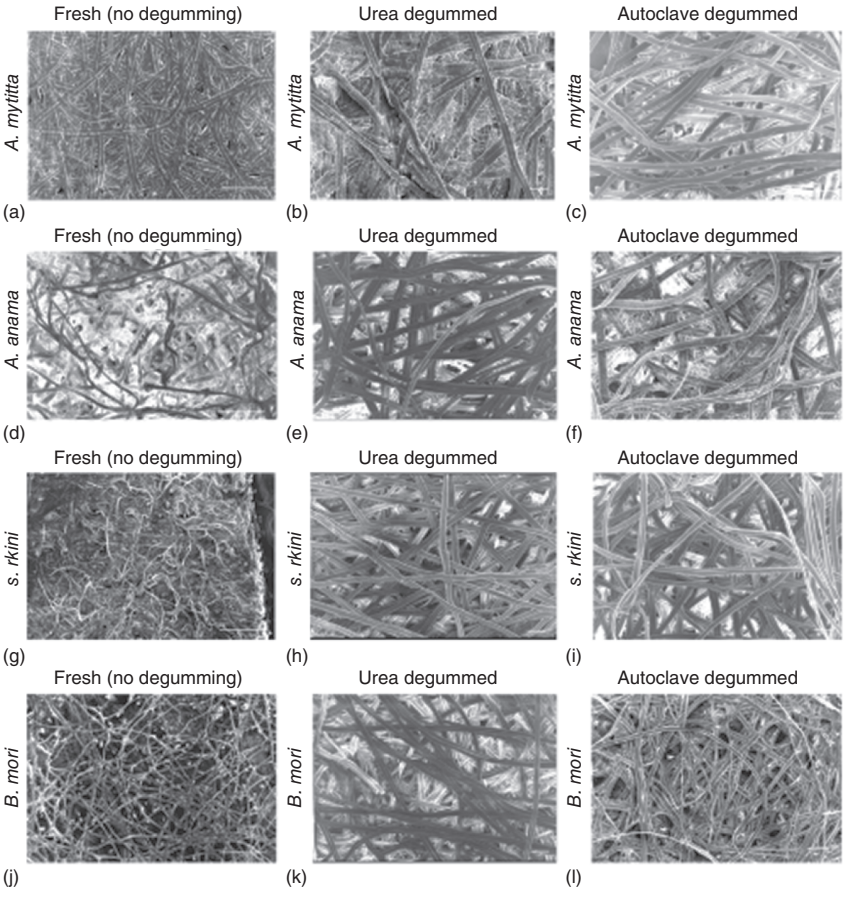


Figure 1.7 Scanning electron microscope (SEM) images of the cocoons of mulberry and non-mulberry silkworms. The cocoons are observed before (50×) and after degumming (100×) using urea and autoclave degumming methods. Scale bar represents 100 μm. Source: Reprinted with permission from Sahu et al. (2016).

1.6 Sericin Gene

Sericin proteins are translated from sericin genes, which are produced from MSG cells. Several studies have identified and characterized the sericin genes from MSG cells. The sericin genes were differently expressed in the MSG subparts (including anterior, middle, and posterior) and the stages of the silkworm larvae. Based on sericin transcripts, *B. mori* sericin genes could be separated into three types: sericin 1 (*ser1*), sericin 2 (*ser2*), and sericin 3 (*ser3*). These genes have a specific purpose for their localization as described in Chapter 11 under the *B. mori* genome (Dong et al. 2015).

The *ser1* gene was first identified by Okamoto et al., in 1982. Two sericin mRNAs extracted from the MSG were discovered at lengths of 11.0 and 9.6 kb. The mRNA complementary sequences represent similar genomic diriboxynucleic acid (DNA), which determined five exons with 114 bp internal in the repetitive region consisting of approximately 60 repeats. The repetitive region was composed of 38 amino acids which had high residues of serine

(40 mol%), aspartic acid (17 mol%), glycine (15 mol%), and threonine (10 mol%) (Okamoto et al. 1982). The composition of this region was similar to the amino acid component from the crude sericin protein extracted from the MSG. In 1986, Michaille et al. identified four variable sizes of sericin mRNAs that were compatible with 24 kb genomic DNA, including 10.5, 9.0, 4.0, and 2.8 kb (Michaille et al. 1986). The two sericin mRNAs had similar lengths between 10.5 and 9.0 kb and 11.0 and 9.6 kb, respectively, as reported by Okamoto (1982). In 1997, Garal et al. analyzed 4.0 kb of *ser1* transcripts and combined the *ser1* gene sequence from mRNA and genome sequences, which were previously reported by Okamoto and Michaille (Garel et al. 1997; Michaille et al. 1986; Okamoto et al.1982). The summarized *ser1* gene sequence revealed 23 kb with nine exons and eight introns that were elucidated (Garel et al. 1997). The four major transcripts from the *ser1* gene: 10.5, 9.0, 4.0, and 2.8 kb, were different by the absence of different exons (Michaille et al. 1986; Michaille et al. 1990). The variation of MSG sericin mRNA splicing was reported due to the production from alternative splicing of the sericin gene. The transcription of sericin was expressed in various types depending on the silkworm larval development stage (Ishikawa and Suzuki 1985). The silkworm larval stage was detected in various types of sericin transcripts in MSG cells (Couble et al. 1987). This suggests that the developmental stage induced different splicing of sericin genes in the cells. In addition, the amino acid compositions revealed in sericin M (middle of MSG) (Takasu et al. 2002) were similar to the ser1 protein (Tsubouchi et al. 2005).

The *ser2* gene, another sericin gene discovered in MSG, was first identified with two encoding matured mRNAs at lengths 5.4 and 3.1 kb by Couble et al. in 1987 (Couble et al. 1987). In 1990, Michaille et al. reported two groups of *ser2* transcripts, one of 3.1 kb and a variable-sized one between 5.0 and 6.4 kb (Michaille et al. 1990). In 2009, Kludkiewicz et al. reported two *ser2* mRNAs containing 5.7 and 3.1 kb (Kludkiewicz et al. 2009). The ser2 proteins were predicted to have 1740 and 882 amino acid residues, which were identified as 230 and 120 kDa. The entire *ser2* gene from the genomic DNA sequence database (Accession number GQ381286) is composed of 13.54 kb with 13 exons and 12 introns. The different mature mRNA sequences were observed by the deletion of the repetitive region at the exon 9a position. This evidence confirmed its generation by alternative splicing (Kludkiewicz et al. 2009). From these results, the *ser2* transcript revealed the identical sizing at the small length of 3.1 kb and the polymorphism of the long transcript gene in the range of 5.0–6.4 kb from alternative splicing at a gene exon.

The *ser3* gene was mainly obtained in the floss and outer layer of a silkworm cocoon. Takasu and colleagues identified 4.9 kb of *ser3* gene transcript. The genomic length of the *ser3* gene revealed 6.575 kb with three exons and two introns observed. Therefore, the ser3 protein consists of 1271 amino acid residues with two regions of motif sequences, 86 amino acid residues with 10 repeats, and 18 amino acid residues with 18.5 repeats. The estimated size of ser3 protein was 120 kDa (Takasu et al. 2007).

Non-mulberry *A. yamamai* had the sericin genes identified from the transcriptomic study of the last instar silk gland. Five sericin genes were discovered including *AySrn1*, *AySrn2*, *AySrn3*, *AySrn4*, and *AySrn*, with transcription size variation between 3.8 and 5.5 kb. The *AySrn* gene characters are different from *B. mori* sericins. All of the *AySrn* genes have short two exons with 22–28% of serine residues (Zurovec et al. 2016). The lower amount of exons in the sericin gene and its amino acid components are different from sericin genes in *B. mori* (3–12 exons). Therefore, the sericin proteins from different silkworm species appear to have different effects on their functions.

The *ser* genes transcribe differently depending on the silkworm larval stages. The silkworm larval (instar) is developed in five stages, and then the mature stage is started to create a cocoon. The *ser* genes were differentially expressed by the specific instar stage. The development of *B. mori* instar stage has been influenced by the sericin gene expressions. In the last silkworm stage (the fifth instar), the transcripts of *ser1* and *ser3* started increasing at day 4 of the fifth instar, whereas *ser2* transcripts were highly expressed since the third instar and decreased rapidly on day 4 of the fifth instar. This suggests that the expression of sericin genes is specific and dependent on the silkworm development stage. Moreover, sericin transcripts have investigated the expression in the fifth instar of each MSG subpart, including the anterior, middle, and posterior sections. The *ser1* transcripts were highly expressed in the middle and posterior sections of MSG cells. The *ser3* transcript was expressed at the anterior and middle MSG cells (Takasu et al. 2010, 2007). The *ser2* transcripts were detected faintly in middle MSG and highly expressed at anterior MSG cells (Takasu et al. 2010). Therefore, these three sericin genes might contain some specific purpose which are beneficial for the different developmental stages of the silkworm.

1.7 Sericin Structure

The secondary structure of sericin has been observed by Fourier transform infrared spectroscopy (FTIR). It has been reported that the silkworm species and isolation method affected to the sericin structure. Aramwit et al. reported *B. mori* cocoon sericins from four isolation methods, including autoclaving, urea, acidic, and alkaline solutions, and mainly found random coil and β-sheet structures (Figure 1.8). However, the urea extraction

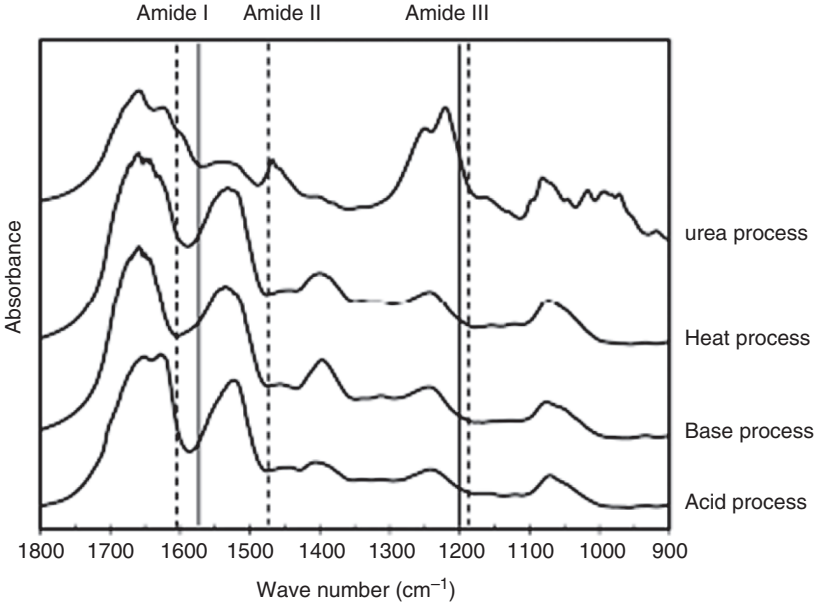


Figure 1.8 FTIR spectra of SS obtained from various extraction methods: acidic, alkaline, urea, and heat processes. Source: Reprinted with permission from Aramwit et al. (2010a). © 2010 John Wiley & Sons.

method found different peaks related to the urea solution, which suggests that urea might be integrated into the sericin structure. Therefore, the urea used in sericin extraction possibly affected the sericin protein structure and function (Aramwit et al. 2010a).

The structure of sericin from different silkworm species (*B. mori*, *A. assamensis*, and *S. ricini*) and cocoon extraction methods (urea, conventional, autoclaved, acidic, and alkaline) has been studied by Kumar et al. (Figure 1.9) (Kumar and Mandal 2017). The secondary structure of sericin protein was classified based on the percentage of α-helix, β-sheet, turns, and random coils. The sericin structures were variable among species and extraction methods. All three species had a similarly high percentage of β-sheet and random coil when obtained using the urea extraction method. The mulberry silkworms, *B. mori*, sericin extraction with an acidic solution showed a higher percentage of α-helix and turns compared to the sericin structure from the urea extraction method. Meanwhile, the conventional and alkali methods resulted in the absence of the β-sheet, whereas the autoclaved method resulted in an absence of α-helix. In contrast, non-mulberry silkworm sericins of

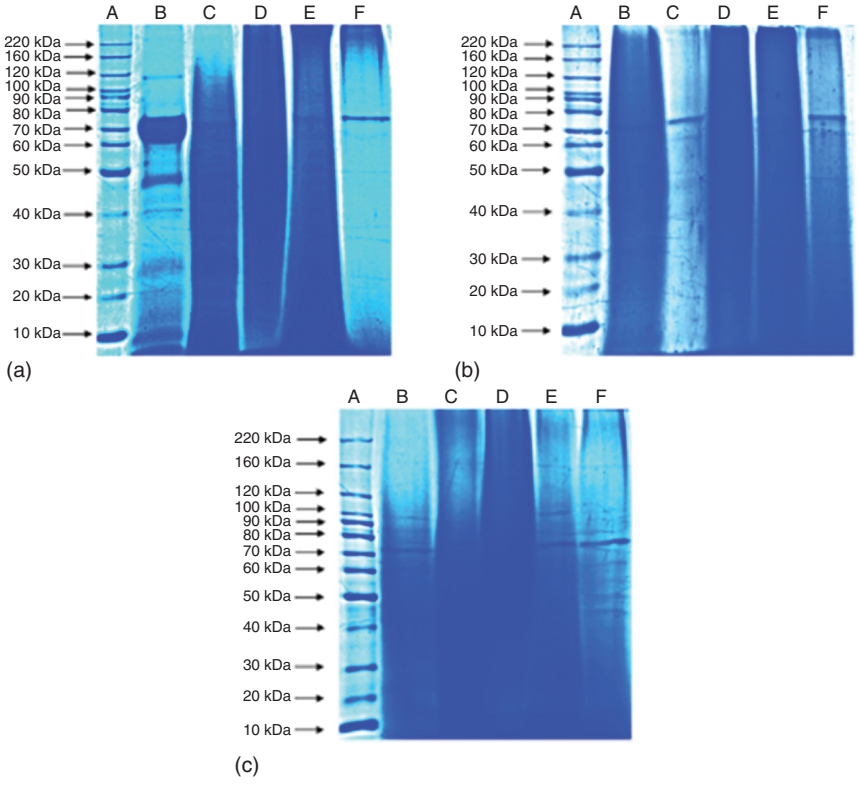


Figure 1.9 Molecular weight distribution of sericin extracted from cocoons using different extraction methods in sodium dodecyl sulfate and polyacrylamide gel electrophoresis (SDS-PAGE). 10% SDS-PAGE gel showing bands of (A) protein ladder and sericin extracted from (a) *Bombyx mori,* (b) *Antheraea assamensis,* and (c) *Philosamia ricini* using (B) urea degradation, (C) acid degradation, (D) autoclaved, (E) conventional method, and (F) alkali degradation. Source: Reprinted with permission from Kumar and Mandal (2017).

A. assamensis and *S. ricini* from the urea extraction method did not result in α-helix in the structure. The β-sheet structure of sericin extracted from *A. assamensis* was absent in all extraction methods. However, *S. ricini* sericin had a variety of percentages (Figure 1.10) (Kumar and Mandal 2017). The differences in the α-helix structure between mulberry and non-mulberry sericins might be from the different gene sequences of each species. This evidence may lead to a different function of each sericin protein among species. The variable structures of sericin from different extraction methods may cause the different properties and biological activities of sericin proteins.

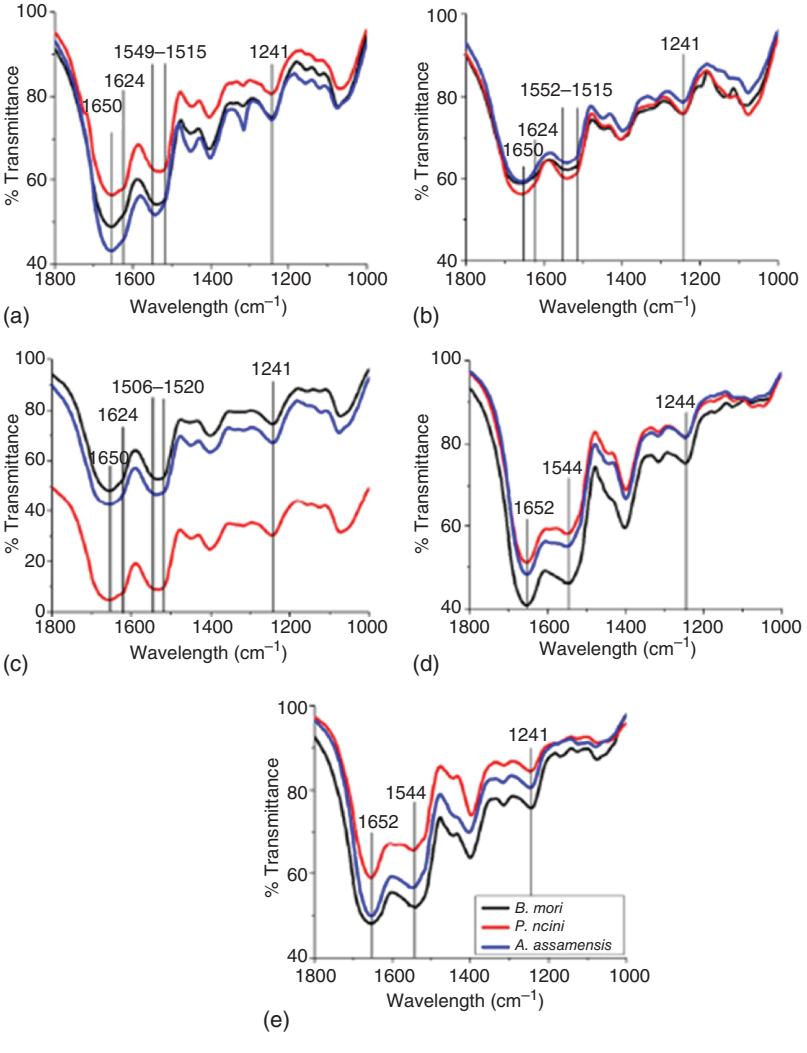


Figure 1.10 FTIR spectra of sericin extracted from the cocoons of *Bombyx mori, Antheraea assamensis,* and *Philosamia ricini* using (a) urea degradation, (b) autoclaving, (c) acid degradation, (d) conventional method, and (e) alkali degradation. Source: Reprinted with permission from Kumar and Mandal (2017). © 2017 Elsevier.

The predicted structures of three *B. mori* sericin gene sequences from *ser1*, *ser2*, and *ser3* have been observed to have a repetitive region of the sericin protein. The repetitive region of the ser1 protein is composed of 38 amino acids with approximately 60 repeats, and a 40% serine residue component was revealed in the high β-sheet content (Garel et al. 1997). The repetitive sequence of the *ser2* gene consists of 15 amino acids rich in charged residues, including lysine, aspartic acid, glutamic acid, and arginine at this region that formed the β-sheet structure of ser2 proteins (Michaille et al. 1990). The amino acid components appearing in MSG ser2 proteins were different from those found in cocoon ser1 and ser3 proteins. The ser2 proteins produced from the MSG stopped expression before the silkworm contracted the cocoon. This showed that there was very little ser2 protein left in the cocoon. Therefore, ser2 proteins were not observed in the cocoon isolate (Kludkiewicz et al. 2009; Takasu et al. 2010). The repetitive region of the *ser3* gene is composed of 86 repeating amino acids and another 8 repeating amino acids with 45% sericin residue composition. The ser3 repetitive regions were predicted to contain lower formations of the β-sheet structure than the ser1 structure (Takasu et al. 2007). The different amino acid components of each repeated sequence were formed by different structures and properties of each ser protein. This data suggests that the different *ser* genes produced the specific protein properties appropriate for each sericin layer.

1.8 Sericin Properties

1.8.1 Biophysical Properties

1.8.1.1 Water Solubility

Sericin can be dissolved in hot water and could be precipitated after exposure to cold water (Sprague 1975). The water solubility of sericin was explained by the correlation with its amino acid content. Amino acid compositions of both mulberry and non-mulberry sericin have relatively high levels of serine, glycine, and threonine. The polar hydroxyl side chain residue of serine and threonine accounting over 30% in its total amino acid profile resulted in sericin presenting strong hydrophilicity (Padamwar and Pawar 2004). The correlation between sericin structure and its water-soluble properties has been studied in mulberry (*B. mori*) and non-mulberry (*A. mylitta*, *A. assamensis*, and *S. ricini*) sericins by circular dichroism spectroscopy. The secondary structures of sericin were determined to be random coils, β-sheet, and low α-helix content. In aqueous solution, sericin rapidly changed from random coil to β-sheet. However, an FTIR study showed that the sericin powder was mostly in the random coil and α-helix conformation (Sahu et al. 2016). This evidence shows that the β-sheet conformation, which is largely seen in aqueous sericin, is the solubilized form in water. Therefore, β-sheet formation is one of the factors of sericin imparting its water-soluble properties. Additionally, temperature is also a factor that changes the sericin structure. Sericin forms an insoluble structure at high water temperatures. At low water temperatures, sericin converts from random coil to β-sheet. This property is beneficial for gel formation and can be useful for biomaterial applications (Zhu et al. 1998).

As previously discussed in the gene sequence information, the repetitive regions of the *ser* genes (*ser1*, *ser2*, and *ser3*) from *B. mori* sequences have been shown to have high

contents of β-sheet formation. The high number of repetitive regions makes it possible to increase the hydrophilic property of sericin protein. However, ser3 protein, which has a lower content of β-sheet formation than ser1 protein, was reported to be more hydrophilic than ser1 protein in a hydrophobicity prediction study (Garel et al. 1997; Takasu et al. 2007). This data suggests that the prediction method used may not be directly applicable to evaluate sericin protein properties. Therefore, to characterize sericin property, several techniques may be needed to collect the information that would be required for finding new applications.

1.8.1.2 Gelation

The gelation property of sericin has been observed under various conditions, depending on sericin solution concentration, temperature, and pH. The study of mulberry sericin from *B. mori* has revealed that gelation formed rapidly at a high concentration of sericin solution. The gelation rate was elevated at a high temperature (40 °C) and decreased at a lower temperature. Gel setting time was faster at pH 6 and became slower at a higher pH. During the gelation process, the strength of sericin increased, whereas the surface tension decreased. The secondary structure of sericin in the gelation process changed from random coil to β-sheet structure (Zhu et al. 1998; Zhu et al. 1995). This evidence suggests that sericin gelation is a thermoreversible process.

The property of sericin gelation was applied to the sericin protein as a biomaterial crosslinked with various types of polymers, such as biopolymers (polysaccharides; cellulose) (Wang et al. 2017), synthetic polymer (polyvinyl alcohol) (Aramwit et al. 2010b), or self-assembled (hydrogel) (Zhang et al. 2019). The network crosslinking between sericin and polymers was generally formed by covalent bonding at the polar functional groups of sericin amino acids (hydroxyl, carboxyl, and amino). The crosslinking could be produced by chemical and physical techniques. Chemical crosslinks were performed using agents such as glutaraldehyde (Nayak et al. 2012; Wang et al. 2017) and genipin (Aramwit et al. 2013, 2010; Wang et al. 2015). For physical crosslinks, ultraviolet (UV) light was used for photo-crosslinking bonds (Qi et al. 2018). These results demonstrated that sericin is capable of forming a gel with various polymers and several crosslinking techniques.

Gelation was also reported in non-mulberry sericin, *A. mylitta*. Similar to mulberry sericin, gel formation was observed in the crosslinking between *A. mylitta* sericin and polymers. This non-mulberry sericin was bonded with polyvinyl alcohol via a glutaraldehyde crosslinking agent (Mandal et al. 2011). Likewise, the natural polymer (cellulose) was reported to crosslink with sericin by the dual crosslinking agents, glutaraldehyde, and aluminum chloride (Nayak et al. 2014). Therefore, sericin from all sources could be efficiently used as a biomaterial application for future medicines and cosmetics.

1.8.1.3 Thermal Stability

Thermogravimetric analysis tests the mass stability of sericin according to time and temperature change. Sericins extracted from mulberry (*B. mori*) and non-mulberry (*A. mylitta*, *A. assamensis*, and *S. ricini*) silkworms have been studied for its stability as related to temperature. The non-mulberry sericins were reported to present more stability than mulberry sericin. The highest stability was for sericin from *S. ricini* (Figure 1.11) (Sahu et al. 2016). This property suggests that sericins from different species have different structures and properties.

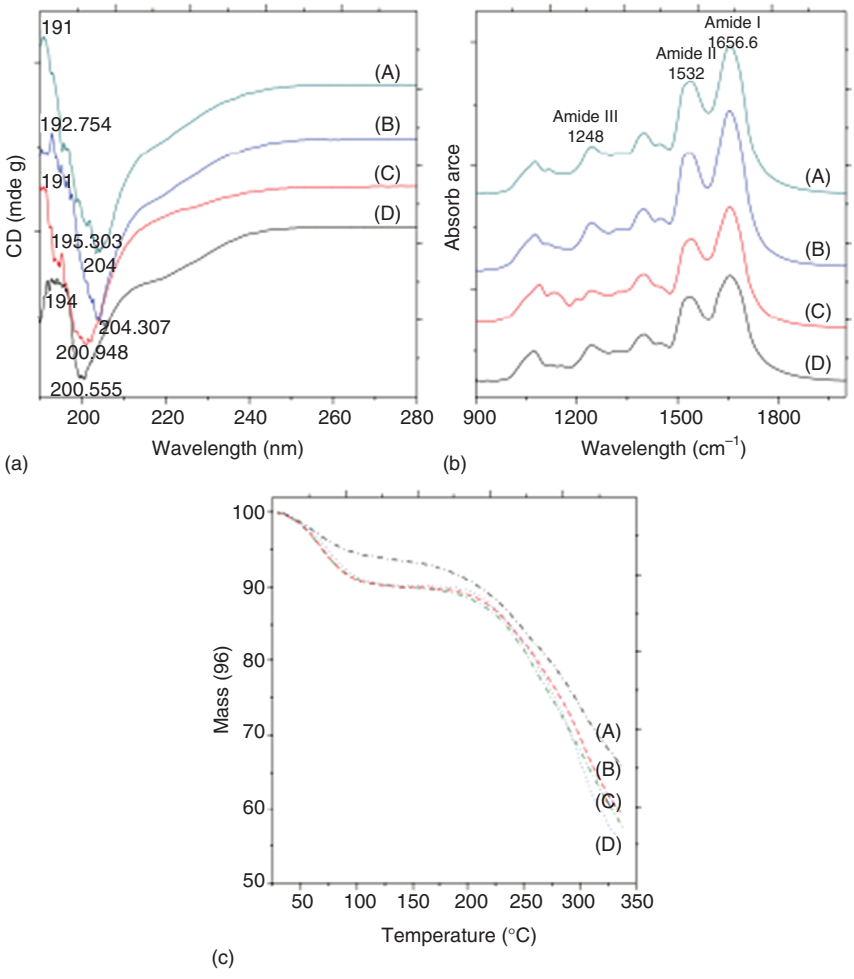


Figure 1.11 (a) Circular dichroism (CD) spectra of 0.1% w/v sericin solution from cocoons of different species: (A) *A. mylitta*, (B) *A. assamensis*, (C) *S. ricini*, (D) *Bombyx mori*; (b) FTIR spectrum of sericin powders from the various species: (A) *A. mylitta*, (B) *A. assamensis*, (C) *S. ricini*, (D) *B. mori*; (c) thermogravimetric analysis (TGA) curves of lyophilized sericin powders of (A) *A. mylitta*, (B) *A. assma*, (C) *S. ricini*, (D) *B. mori*. Source: Reprinted with permission from Sahu et al. (2016). Licensed under CC BY 4.0.

1.8.1.4 Ultraviolet (UV) protection

Sericin has shown the ability to protect cells from UV radiation. The study of the photoprotective properties of *B.mori* reported that sericin was effective in reducing skin oxidative stress (Zhaorigetu et al. 2003), inhibiting UVB-induced apoptosis (Dash et al. 2008), and absorbing UVC radiation (Kiro et al. 2017). Non-mulberry sericin from *A. assamensis* and *S. ricini* was reported to increase cell viability against both UVA and UVB more than *B. mori* sericin (Kumar et al. 2018). The *A. assamensis* sericin enhanced collagen production from both UVA and UVB radiation, while the *B. mori* sericin enhanced protection only from UVA (Kumar and Mandal 2019). This information seems to indicate that non-mulberry sericins have photoprotective properties that are better than those of mulberry sericin.

1.8.1.5 Adhesion Properties and Electrostatic Interaction

The adhesion property is important for silkworm in its developmental stages, especially during the cocoon stage. The adhesion property of sericin is beneficial in cementing cocoon scaffolding and attaching the cocoon to the tree branch by floss or peduncle. The study of crude ser2 proteins extracted from the anterior MSG showed that the tensile strength needed to detach the adhesive from wooden surfaces was about $120 \pm 30\,N/cm^2$. The adherence strength was higher than starch glue ($42 \pm 20\,N/cm^2$) but less than bone glue ($502 \pm 132\,N/cm^2$). The adhesion property was facilitated by the high contents of charged amino acid in ser2 proteins, which provide the electrostatic interactions between the wooden surface and ser2 proteins (Kludkiewicz et al. 2009).

1.8.2 Biochemical Activity

Sericin has had several biochemical activities beneficial for medical applications as discussed below.

1.8.2.1 Anti-tyrosinase Activity

Tyrosinase is an enzyme that plays a major role in melanin synthesis, which plays a key role in cell protection from UV damage (Cichorek et al. 2013), and it is also a key enzyme in melanogenesis on the skin (Schallreuter et al. 2008). Sericin anti-tyrosinase activity was investigated in various studies by *in vitro* assay using mushroom tyrosinase. The mulberry silkworm, *B. mori*, was subjected to isolate sericin protein for anti-tyrosinase activity assay. The sericin collected by the heat isolation method had a 50% inhibitory concentration (IC50) of tyrosine activity observed at a concentration of 10 mg/ml (Kato et al. 1998; Wu et al. 2008). Sericin from urea extraction revealed the highest anti-tyrosinase activity among other methods (including autoclave, acidic, and alkaline extraction methods) (Aramwit et al. 2010a). The sericin isolated by the autoclave method (high heat and high pressure) revealed an IC_{50} of 1–7 mg/ml depending on the silkworm strain in the study (Aramwit et al. 2010a; Manosroi et al. 2010). Sericin from the acidic extraction method showed some activity against tyrosinase (Aramwit et al. 2010a). Two reports revealed no inhibitory activity from sericin extracted by the alkaline method (Aramwit et al. 2010a; Kumar and Mandal 2019). In contrast, there is a report that revealed that the sericin from the alkaline extraction method had IC_{50} values of 3–19 mg/ml from various silkworm strains (Manosroi et al. 2010). This suggests that different factors of sericin extraction methods and silkworm strains affect sericin protein in tyrosinase inhibitory activity. The alkali extraction method might interfere with the *B. mori* sericin anti-tyrosinase activity. However, a recent study of sericin isolated from the cocoon of non-mulberry silkworms reported that the sericin isolated by an alkali extraction method from *A. assamensis* and *S. ricini* had anti-tyrosinase activity with IC_{50} values of 6 and 10 mg/ml, respectively (Kumar and Mandal 2019). This information shows that the sericin proteins are different in their biological properties with each species. The evidence of this is supported by a report of *B. mori* sericin extracted from several strains with different silkworm diets. The results showed that the diet influenced the sericin property related to anti-tyrosinase activity (Chlapanidas et al. 2013). Additionally, the *B. mori* sericin extracted from different colors of the cocoon (flavonoids and carotenoids) showed that the color of the cocoon is associated

with an increase of the inhibitory effect of tyrosinase. The elimination of cocoon color from sericin extraction reduced the anti-tyrosinase activity (Aramwit et al. 2010a). There are many factors of sericin anti-tyrosinase activity including genes, species, extraction methods, and cocoon colors.

1.8.2.2 Anti-elastase Activity

Elastase is a proteolytic enzyme that functions in the degradation of the elastin and consequently in the skin losing elasticity. The expression of elastase protein can be induced by UV radiation (Suganuma et al. 2010). Sericin isolated from various strains of *B. mori* cocoons first had its anti-elastase activity discovered by Chlapanidas et al. in 2013 (Figures 1.12 and 1.13) (Chlapanidas et al. 2013). Non-mulberry, *Antheraea* spp. (tasar), sericin revealed anti-elastase activity. Interestingly, tasar sericin extracted from the waste products of the silk industry also retained anti-elastase activity (Jena et al. 2018). This anti-elastase property of sericin is beneficial in sun protection. Therefore, sericin was proposed to be used for application in cosmetic products.

1.8.2.3 Antioxidant Activity

Sericin isolated from the cocoon of *B. mori* has shown antioxidant properties when measured using 1,1-diphenyl-2-picrylhydrazyl, chemiluminescence, oxygen radical absorbance capacity, electron spin resonance, and other techniques. The sericin extracted from high pigment cocoon strains (yellow–green cocoon) revealed higher anti-oxidative activity than low pigment cocoons (white cocoon) (Takechi et al. 2014). Previous information has shown that the sericin protein extracts have different sizes for the cocoons of various color strains (Aramwit et al. 2010b). The chemical is accumulated in the sericin layer (Ma et al. 2016). Moreover, chemicals such as flavonoids have been reported to have antioxidant properties (Heim et al. 2002).

An antioxidant assay using a skin fibroblast cell line (AH927) by pre-incubated sericin before hydrogen peroxide-stimulated oxidative stress showed antioxidant properties in sericins extracted from the cocoons of both mulberry (*B. mori*) and non-mulberry

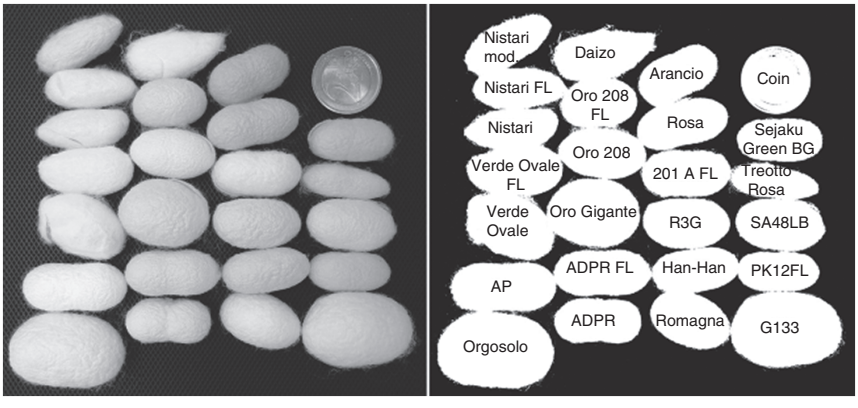


Figure 1.12 Image of 24 *Bombyx mori* cocoon strains fed with artificial and/or mulberry leaf diets. Source: Reprinted with permission from Chlapanidas et al. (2013). © 2013 Elsevier.

Sample	Mean K_{CL}	St. dev. K_{CL}	Mean V_{max}	St. dev. V_{max}
201 A	13.109	4.326	1.275	0.11
ADPR	16.252	8.796	1.201	0.206
ADPR (FL)	13.032	3.655	1.162	0.107
AP	17.313	7.891	1.229	0.225
Arancio	20.903	6.211	1.527	0.249
Daizo	25.21	9.282	1.376	0.244
G133	14.011	4.397	1.179	0.113
Han-Han	15.159	4.237	1.314	0.108
Nistari (FL)	24.522	14.403	1.463	0.318
Orgosolo	19.149	7.174	1.005	0.13
Oro 208	20.455	6.23	1.307	0.188
Oro gigante	27.084	18.583	1.514	0.386
PK12 (FL)	38.495	32.269	1.681	0.627
R3G	20.382	11.089	1.417	0.277
Romagna	43.763	25.109	1.536	0.527
SA48LB	11.542	3.478	1.17	0.091
Sejaku green BG	23.411	12.909	1.404	0.228
Treotto rosa (FL)	47.178	47.702	1.765	0.862
Verde ovale	11.992	3.525	1.148	0.088
Verde ovale (FL)	19.431	11.688	1.261	0.248

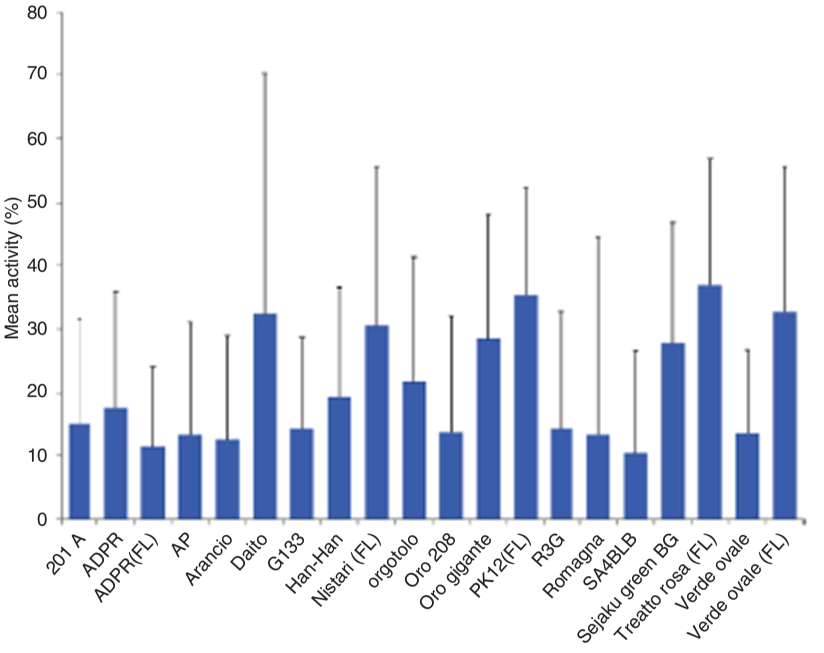


Figure 1.13 Anti-elastase activity of sericin samples as a function of the strain and diet. Source: Reprinted with permission from Chlapanidas et al. (2013). © 2013 Elsevier.

silkworms (*A. mylitta*) (Dash et al. 2008). The sericin extraction method also affected the antioxidant activity. In *B. mori* sericin, the highest antioxidant activity was for sericin isolated by the autoclaving method and the lowest activity was for sericin isolated by the acidic method (Kumar and Mandal 2017). Unlike mulberry sericin, *A. assamensis* and *S. ricini* showed that the conventional method resulted in the highest observed antioxidant activity (Kumar and Mandal 2017). In addition, the sericin obtained from waste products from the *Antheraea* spp. (tasar) silk industry also retained its antioxidant activity (Jena et al. 2018). Not only the *in vitro* assay but also the *in vivo* experiment with rats orally treated with sericin showed antioxidant activity in the rat brain homogenate (Banagozar Mohammadi et al. 2019). These results might be beneficial for sericin applications in pharmaceuticals in the future. Moreover, the advantageous sericin anti-oxidative stress property was used for the cryoprotection of several cell types, such as human hepatocytes (Miyamoto et al. 2010), adipose tissue-derived stem cells (Miyamoto et al. 2012), islet cells (Ohnishi et al. 2012), bovine embryonic cells (Isobe et al. 2013), and buffalo spermatozoa (Kumar et al. 2015).

1.8.2.4 Anti-lipid Peroxidation Activity

Sericin protein was first investigated for anti-lipid peroxidation activity in 1998 by Kato et al. In *B. mori* sericin, cocoon sericin extracted by heating showed inhibitory activity of lipid peroxidation by thiobarbituric acid reactive substances (TBARS) and conjugated diene assays during an *in vitro* test with rat brain homogenization (Kato et al. 1998). A similar observation of anti-lipid peroxidation has been observed from rats with oral treatment with sericin. The rat brain homogenization showed that sericin reduced the activity of lipid peroxidation by TBARS assay (Banagozar Mohammadi et al. 2019). Non-mulberry sericins from *A. assamensis* and *S. ricini* have also been reported to have activity against lipid peroxidation. The activity against lipid peroxidation has been found to be 75–90% depending on the sericin concentration. However, the extraction methods, including autoclaving and alkali, did not significantly affect the anti-lipid peroxidation activity (Kumar and Mandal 2017). Not only the sericin from cocoon has the anti-lipid peroxidation activity, but also the sericins obtained from waste products from the *Antheraea* spp. (tasar) silk industry also retain their anti-lipid peroxidation activity (Jena et al. 2018). The anti-lipid peroxidation activity by sericin could be found in both mulberry and non-mulberry sericin. The activity is maintained in several sources of sericin, such as cocoon and waste products from the silk industry. However, the extraction method affected its activity.

1.8.3 Biological Activity

1.8.3.1 Anti-inflammatory Activity

The anti-inflammatory activity by *B. mori* sericin has been reported in several studies. In an experiment of sericin treatment in rat wounds, it was revealed that sericin initially induced the activity of pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β). However, after long-term treatment for seven days, the inflammation did not progress. In this study, sericin induced inflammation at the starting point of treatment but it did not accelerate the progression of wound inflammation (Aramwit et al. 2009). An *in vivo* acute inflammation model of carrageenan-induced paw edema showed that sericin at a high concentration (0.080 mg/ml) significantly inhibited the inflammation

induced by carrageenan (Figure 1.14) (Aramwit et al. 2013). The histopathological changes of the rat tissues indicated that there was less cellular infiltration in the dermal layer of indomethacin-treated (positive control) and sericin-treated rats, while water-treated tissues showed a massive cellular infiltration in the dermal layer (Figure 1.15) (Aramwit et al. 2013).

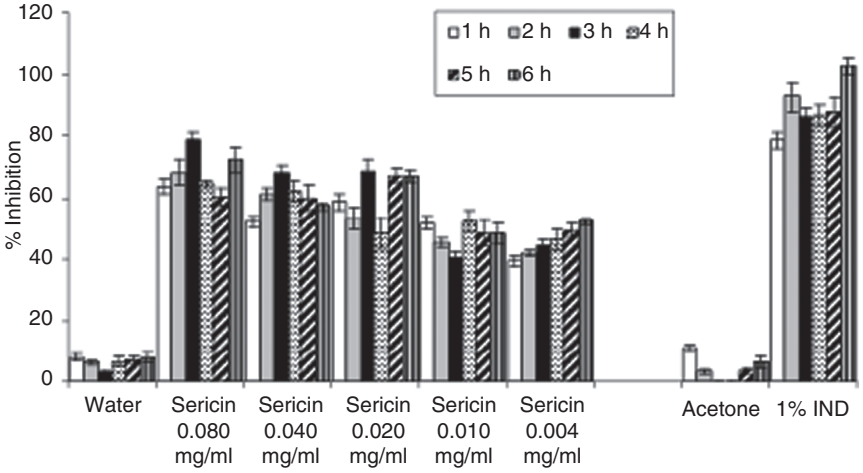


Figure 1.14 Percentage of edema inhibition, induced by carrageenan injection, from sericin at different concentrations at various time points. Source: Reprinted with permission from Aramwit et al. (2013). © 2013 SAGE Publications.

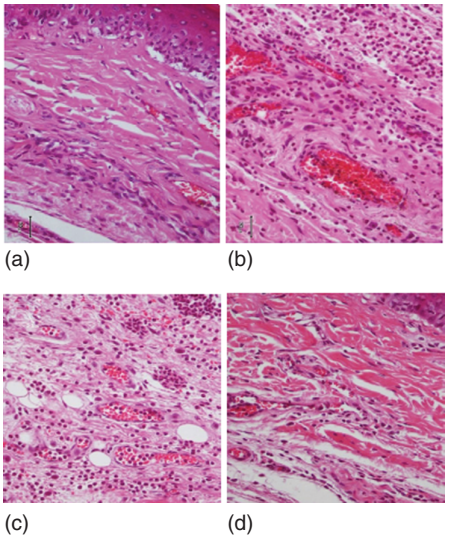


Figure 1.15 Histological changes in rat tissue (×64) (a) normal histological structure of the epidermal and dermal layers with no obvious cellular infiltration, (b) water-treated tissues followed by carrageenan injection with massive cellular infiltration in the dermal layer, (c) indomethacin-treated tissues followed by carrageenan injection show less cellular infiltration in the dermal layer compared with the water-treated tissues, (d) sericin-treated (0.080 mg/ml) tissues followed by carrageenan injection shows almost intact dermis with little cellular infiltration. Source: Reprinted with permission from Aramwit et al. (2013).

Moreover, sericin was reported to be effective for suppressing the inflammatory mediators, cyclooxygenase-2 enzyme and nitric oxide production (Aramwit et al. 2013). Additionally, the effect of sericin increased anti-inflammatory cytokine expression, including IL-4, IL-10, and transforming growth factor-β, and reduced the production of the allergic chemokine ligands 8 (CCL8) and CCL18 (Aramwit et al. 2018). This information suggests that the effect of sericin interferes with several mechanisms related to reducing inflammation. The effect of sericin on the inflammation of the neurological systems in animal models showed that sericin decreased cytokine expression, including the nuclear factor kappa-light-chain-enhancer of activated B cells, TNF-α, and IL-1β proteins in the brains of the mouse model (Banagozar Mohammadi et al. 2019). This anti-inflammatory activity of sericin could be used for several applications such as wound healing (Aramwit et al. 2013), nanomicelles for tumor treatment (Deng et al. 2019), or nanoparticles for drug delivery (Suktham et al. 2018; Yalcin et al. 2019).

1.8.3.2 Anti-tumor Activity

Sericin has been found to be active against several anti-tumor cells. The *B. mori* sericin suppressed several carcinogeneses such as colon and skin tumors. The inhibitory activity varies depending on the type of cancer cells. In colon tumors, sericin was reported to have the ability to reduce colon tumors by reducing colonic 8-hydroxydeoxyguanosine (oxidative stress in colon cancer) and 4-hydroxynonenal (inhibits cell proliferation). Furthermore, sericin was induced by nitric oxide synthase protein to kill tumor cells (Zhaorigetu et al. 2001). In skin cancer, sericin has a strong anti-tyrosinase activity, which is a key enzyme in

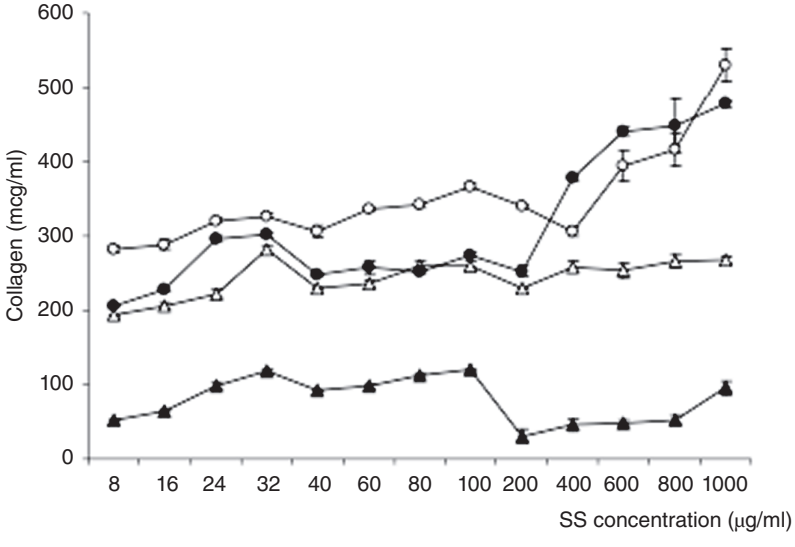


Figure 1.16 Collagen type I production in fibroblast cell line L929 when various sericin concentrations were added into the culture medium for 24 h to make the final concentration of sericin in each well 8–1000 μg/ml. Δ is acid-degraded sericin, o is alkali-degraded sericin, • is heat-degraded sericin, and ▲ is urea-extracted sericin. Source: Reprinted with permission from Aramwit et al. (2010b). Licensed under CC BY 3.0.

melanogenesis (Aramwit et al. 2018; Kumar and Mandal 2019). Sericin reduced skin tumors from UV radiation by antioxidant activity to reduce oxidative stress, decrease cyclooxygenase-2, and lower cell proliferation on the skin (Zhaorigetu et al. 2003). In addition, sericin downregulated the expression of a melanogenesis regulatory gene, microphthalmia-associated transcription factor, in melanocytes (Aramwit et al. 2018). By this information, it is shown that sericin is effective against tumor cells by attacking several melanogenesis involvement proteins.

Non-mulberry sericins from *A. assamensis sericin* and *S. ricini* have also shown anti-tumor activity via anti-tyrosinase secretion. Interestingly, their activity is more effective than mulberry sericin (Kumar and Mandal 2019). In *in vitro* anti-tumor testing, non-mulberry sericin actively destroyed human squamous carcinoma (A431) and human tongue carcinoma (SAS) cells. It has been reported that sericin from both mulberry and non-mulberry extractions killed the cancer cells by upregulating the tumor suppressor gene, *p53* (Kumar and Mandal 2019).

1.8.3.3 Inducing Collagen Production

Sericin has been reported to induce fibroblast cell proliferation and collagen production. It has been reported that sericin induces fibroblast cell proliferation (Aramwit et al. 2009).

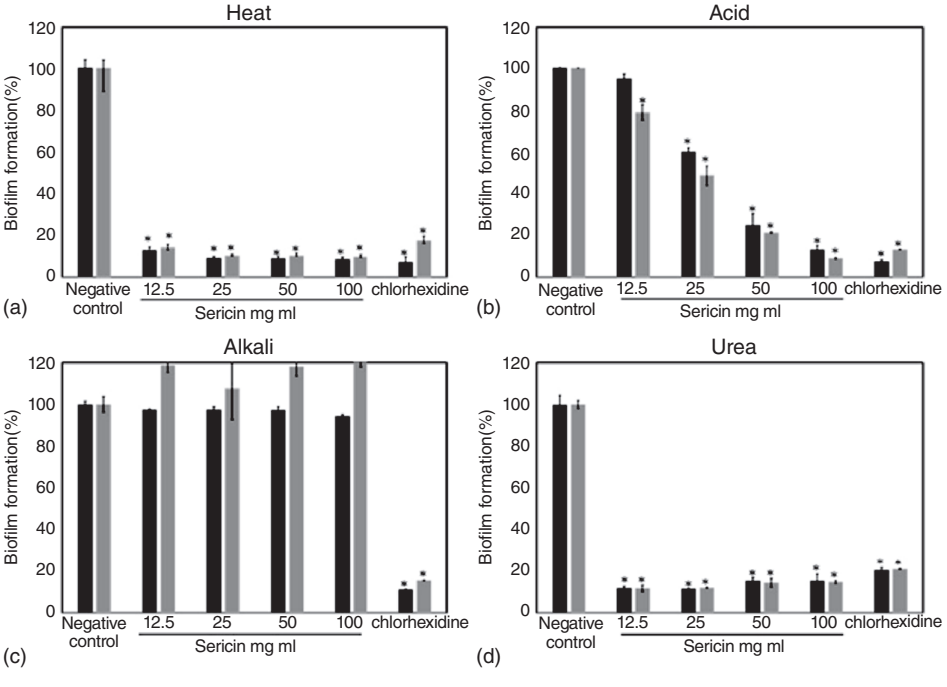


Figure 1.17 Biofilm formation percentage of *Streptococcus mutans* strains (ATCC25175 (black bar) and UA159 (grey bar)) when grown in the presence of (a) heat-extracted sericin, (b) acid-extracted sericin, (c) alkali-extracted sericin, and (d) urea-extracted sericin (12.5, 25, 50, and 100 mg/ml), brain–heart infusion medium in the absence of sericin (negative control), and 1.2 mg/ml chlorhexidine (positive control) at 37 °C for 24 h. Source: Reprinted with permission from Aramwit et al. (2020). © 2020 MA Healthcare Ltd.)

Sericin isolated from four extraction methods (heat, urea, acid, and alkali) induced collagen production depending on its concentration (Figure 1.16). However, the heat extraction method had the highest activation of collagen synthesis (Aramwit et al. 2010b). The effectiveness of collagen production is related to the amino acid composition of sericin. The various strains of silkworms revealed different amino acid compositions. The high contents of methionine and cysteine residues in sericin protein are the promoting factor for collagen production (Aramwit et al. 2009). On the other hand, non-mulberry sericins from *A. assamensis* and *S. ricini* reportedly had a protective effect from collagen degradation induced by UV radiation (Kumar and Mandal 2019). Because of this property, sericin was used for biomaterial and cosmetic applications, especially skin treatment (Akturk et al. 2011; Aramwit et al. 2015; Bakhsheshi-Rad et al. 2020; Tyeb et al. 2020).

1.8.3.4 Antibacterial Activity

Sericin has been tested for its antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Mulberry, (*B. Mori*), sericin

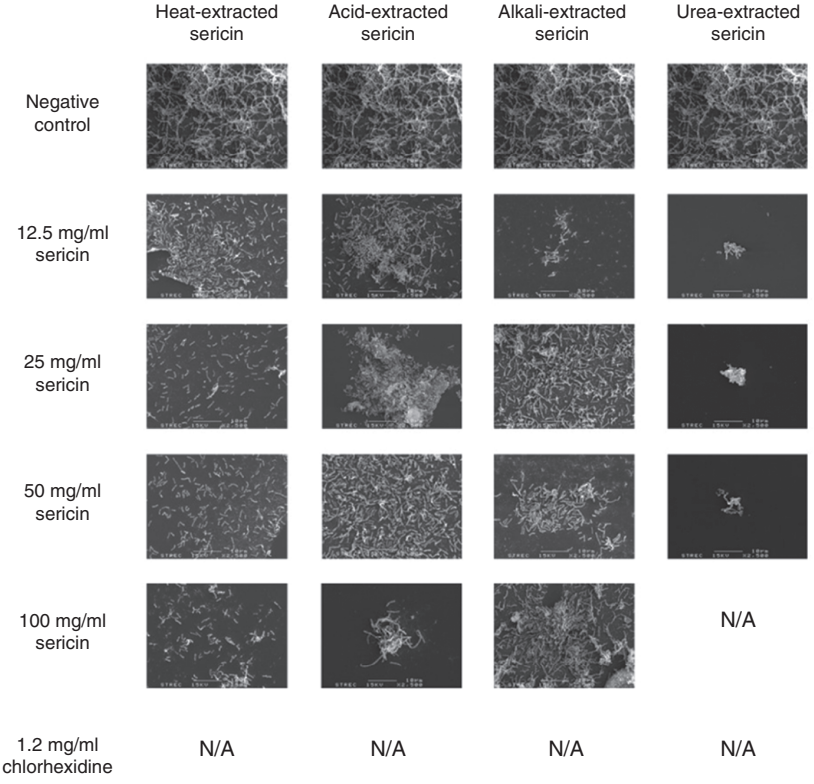


Figure 1.18 SEM images of ATCC25175 of *Streptococcus mutans* strains when grown in the presence of heat-extracted sericin, acid-extracted sericin, alkali-extracted sericin, and urea-extracted sericin (12.5, 25, 50, and 100 mg/ml), brain–heart infusion medium (negative control), and 1.2 mg/ml. Source: Reprinted with permission from Aramwit et al. (2020).

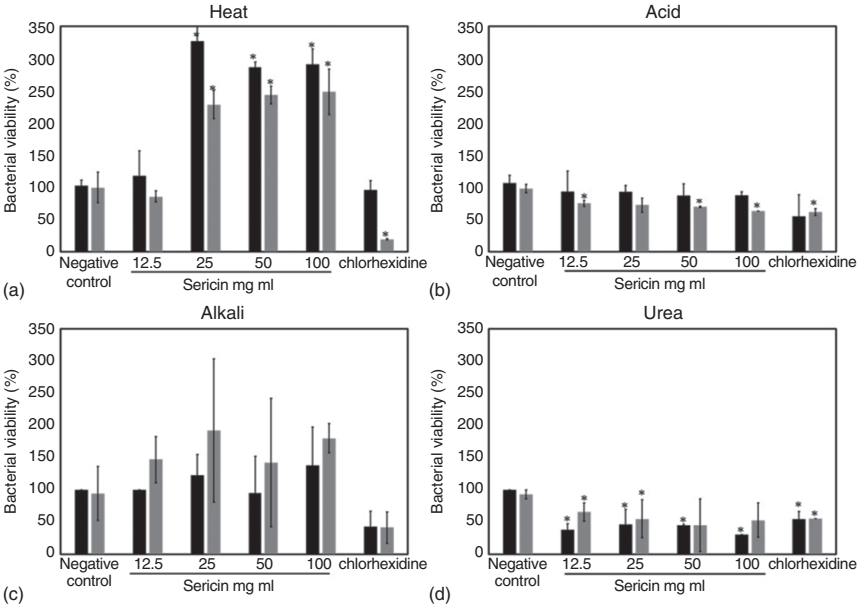


Figure 1.19 Viability percentage of *Streptococcus mutans* strains (ATCC25175 [black bar] and UA159 [grey bar]) in the biofilms after treatment with (a) heat-extracted sericin, (b) acid-extracted sericin, (c) alkaline-extracted sericin, and (d) urea-extracted sericin (12.5, 25, 50, and 100 mg/ml), brain–heart infusion medium in the absence of sericin (negative control), and 1.2 mg/ml chlorhexidine (positive control) at 37 °C for 24 h. Source: Reprinted with permission from Aramwit et al. (2020). © 2020 MA Healthcare Ltd.

caused *E. coli* cell membrane damage (Xue et al. 2016). The purity and extraction method of sericin affected its antibacterial properties. The commercially available pure sericin is active against *S. aureus* similar to antibiotic (penicillin/streptomycin) while having very low activity against *P. aeruginosa* and *S. aureus*. Cocoon sericin from autoclaving preparation slightly affected *S. aureus* but did not affect both *E. coli* and *P. aeruginosa* (Rocha et al. 2017). Antibacterial activity may not be the main property in both mulberry and non-mulberry sericin, as sericin has many other bioactivity properties for medical applications. Therefore, sericin is useful in combination with other antibacterial bioactive molecules for enhancing its activity and advancing biomaterial properties. The biomaterials developed from sericin obtained from both mulberry (*B. mori*) and non-mulberry (*A. mylitta* and *S. ricini*) sericins have been combined with other biopolymers such as chitosan nanofiber or film (Sapru et al. 2017; Shah et al. 2019; Zhao et al. 2014), or chemical agents such as silver nanoparticles (He et al. 2017; Muhammad Tahir et al. 2020; Chaisabai et al. 2018), zinc oxide nanoparticles, and anti-biofilm titanium (Ghensi et al. 2019) to further enhance the antibacterial and other biological properties.

Besides antibacterial activity, sericin has been found to inhibit biofilm formation (Aramwit et al. 2020). However, the extraction method affects this activity. It was found that urea-extracted sericin showed the highest potential anti-biofilm activity for

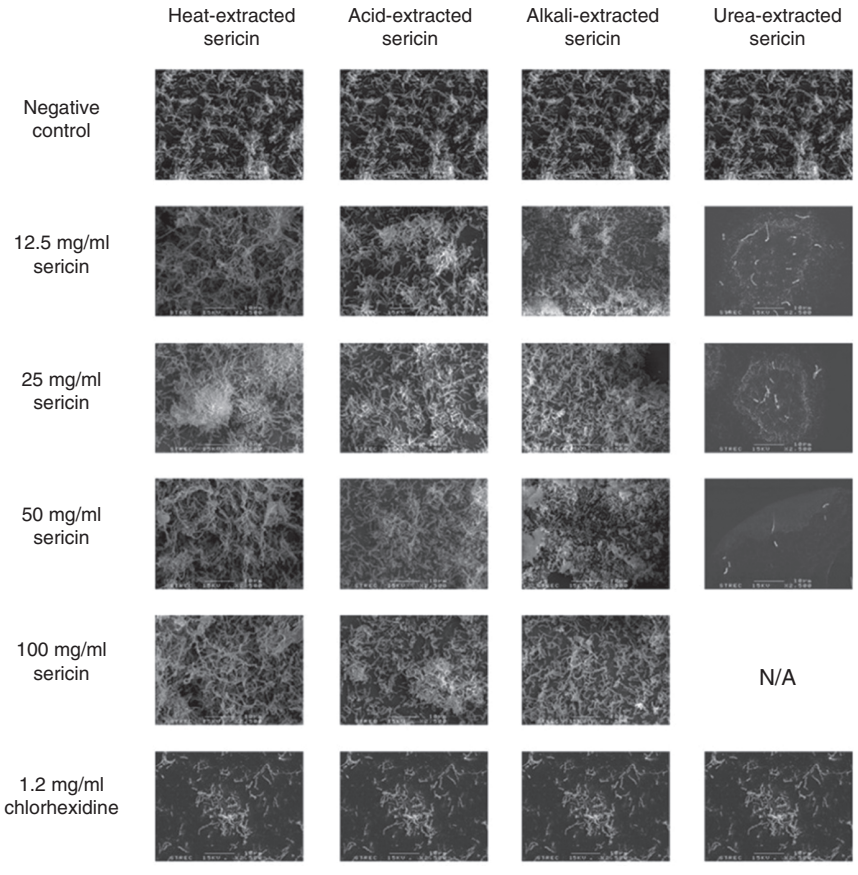


Figure 1.20 Scanning electron microscope (SEM) images of ATCC25175 of *Streptococcus mutans* strains in the biofilms after treatment with heat-extracted sericin, acid-extracted sericin, alkaline-extracted sericin, and urea-extracted sericin (12.5, 25, 50, and 100 mg/ml), brain–heart infusion medium (negative control), and 1.2 mg/ml chlorhexidine (positive control) at 37 °C for 24 h. Source: Reprinted with permission from Aramwit et al. (2020).

Streptococcus mutans in terms of both inhibition and disruption effects, compared with sericins extracted by heat, acids, or alkaline solutions (Figures 1.17–1.20). The heat-extracted and acid-extracted sericins were found to reduce the biofilm formation dose-dependently, while the alkaline-extracted sericin did not show either an inhibition or a disruption effect on the bacterial biofilm. The urea-extracted sericin also killed the bacteria residing within the biofilm, possibly due to its modified structure, which may destabilize the bacterial cell wall, leading to membrane disintegration and, finally, cell death.

From these data, it can be inferred that the sericin structure is quite complicated and varies from the nature of the silk strain and extraction methods, among other factors, which results in various biological properties. Due to these variations, the type of silkworm and processing method are the key factors for sericin selection.

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