

Structure and properties of silk sericin obtained from different silkworm varieties

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Abstract

Recently, many researchers have studied silk sericin because of its high water retention, good wound healing, good cyto-compatibility, and blood-glucose- and cholesterol-lowering effects. Although sericin film can be used in wound dressing and cosmetic packs, its poor mechanical properties have prevented its use in industrial fields. In the present study, sericin was obtained from different silkworm varieties, and the effect of silkworm variety on the structure and properties of sericin was examined. Except for a small difference in serine content, no significant difference in sericin was noted among the silkworm varieties. In addition, silkworm variety almost had no effect on solution viscosity, implying that it does not influence the molecular weight of sericin. Mechanical properties of sericin film were strongly affected by silkworm variety. Wonwon 126 showed the best mechanical properties, while N74 and Geumgwangju displayed the worst properties.

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Introduction

Removal of sericin from silk fibers improves the luster and feel of silk textiles, and so, sericin was considered a useless protein. However, silk sericin was recently reported to exhibit inhibition of ultraviolet B-induced apoptosis in human skin keratinocytes (Dash *et al.*, 2008), high water retention, wound-healing properties (Nagai *et al.*, 2009), cyto-compatibility (Tsubouchi *et al.*, 2005), and blood-glucose- and cholesterol-lowering effects (Limpeanchob *et al.*, 2010; Seo *et al.*, 2011). Therefore, researchers are studying sericin for various biotechnological applications, including cosmetics and wound dressing.

Sericin film can be used in various applications. However, sericin film is too brittle, showing poor mechanical properties

that restrict its application in various biotechnological fields. Recently, Jo and Um (2015) reported that formic acid casting can induce a two-fold increase in the mechanical properties of sericin film. However, the mechanical properties of sericin film need to be enhanced to a greater extent for biomedical and cosmetic applications.

Silk is produced by the silkworm (*Bombyx mori*), and many varieties of *B. mori* exist. Recently, Chung *et al.* (2015) reported that molecular weight (MW), solution viscosity, and film mechanical properties of regenerated silk fibroin (SF) were significantly different depending on the silkworm variety. They suggested the possibility of improving the mechanical properties of sericin film.

In the present study, nine different *B. mori* varieties were used

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to produce silk sericin and the effect of silkworm variety on the structure and properties of silk sericin and its film was examined.

Materials and Methods

Preparation

Silk cocoons of the same silkworm variety used in the previous study were selected (Chung *et al.*, 2015). Eight different original *B. mori* varieties were grown at the Kyungpook Sericulture and Insect Research Center, and a hybrid *B. mori* variety (Baekokjam) was grown at the Yeongdeok Taeyang Farm in South Korea. Nine silk cocoon samples were produced from the nine silkworm varieties.

Preparation of silk sericin and its film has been described in the previous study (Jo and Um, 2015). Briefly, sericin was extracted by immersing silk cocoons in water at 120°C for 30 min by using an autoclave (JSAC-60, JSR, Japan). The liquor ratio was 1:25. The extracted sericin solution was filtered using a non-woven fabric and dried at 80°C in a drying oven to obtain solid sericin.

Solid sericin was dissolved in 98% formic acid at 55°C to prepare 3% (w/w) sericin formic acid solution and then poured into a petri dish. Then, it was dried in a hood at room temperature to obtain sericin film.

Measurement and characterization

Amino acid composition of sericin powder was determined using an amino acid analyzer (L-8900; Hitachi, Japan). The sericin samples were added to 6N HCl solution, and the solution was treated at 110°C for 24 h to hydrolyze the sericin samples before the amino acid analysis. Then, 0.3% (w/w) of the sericin formic acid solution was used for the rheological measurements. The complex viscosity was measured using a rheometer (MARS III, HAKKE, Germany) with a cone and plate geometry with an angular frequency of 0.1–10 rad/s at 25 °C. The radius and angle of the cone were 60 mm and 1°, respectively. FTIR (Nicolet 380, Thermo Fisher Scientific, USA) spectra of the sericin films were obtained using the ATR method.

To evaluate the mechanical properties of sericin film, breaking strength, breaking elongation, and initial Young's modulus were obtained using the Universal Test Machine (OTT-03, Oriental TM, South Korea). The tensile tests were performed using a

3-kgf load cell at an extension rate of 10 mm/min for the sericin film. The film sample had a length of 80 mm and a width of 5 mm. The gauge length (measuring length of the sample) was 30 mm. All samples were preconditioned at 20°C and 65% (RH), and seven sericin films from each silkworm variety were tested.

Results and Discussion

Amino acid composition of sericin

Silk sericin is a protein polymer that consists of various amino acids. The amino acids have polar and nonpolar groups, depending on the type of amino acid. Thus, the character and composition of an amino acid affect the properties of sericin, such as hydrophilicity and solubility in water. The amino acid composition of sericin produced from different silkworm varieties was evaluated (Table 1).

Interestingly, Gumgwangju (32.2%) and Imbakgalwon (31.8%) showed lower serine contents than the other varieties (34.8–36.5%). Other than that, no significant differences were observed among the varieties.

Rheological properties of the sericin solution

Rheology of silk solution has been studied because it provides indirect information on MW and affects the processability of silk solution, including wet- and electro-spinning (Kim and Um, 2014; Cho *et al.*, 2012; Ko *et al.*, 2013; Yoon *et al.*, 2013; Chung and Um, 2014; Yoo and Um, 2013).

Therefore, to obtain indirect information on MW and rheology behavior of sericin, complex viscosity was measured as a function of angular frequency (Fig. 1). Regardless of the silkworm variety, all sericin formic acid solutions showed similar viscosity values. This indicates that the MW of sericin from different silk varieties is similar.

Considering that regenerated SF showed somewhat different MW and solution viscosity according to the silkworm variety (Chung *et al.*, 2015), it is interesting to note that sericin showed no significant difference in solution viscosity among the silkworm varieties. That is, N74 and Wonwon 126 showed the highest (0.75 Pa·s) and lowest (0.20 Pa·s) solution viscosity for regenerated SF among the nine silkworm varieties. Although we could not elucidate the reason for the different effects of

Table 1. Amino acid composition of silk sericin produced from different silkworm varieties

Silkworm variety Amino acid (mol %)	Baekokjam	Geumgwangju	Hansang II	Hongbak	ImbakGalwon	N74	SK	Wonjam 125	Wonwon 126
Asp	13.4	13.1	13.4	13.3	12.8	13.2	13.5	13.2	13.8
Thr	8.6	7.9	8.2	8.2	7.7	8.5	8.6	8.2	8.8
Ser	36.5	32.2	35.1	35.5	31.8	34.8	36.5	35.9	35.5
Glu	3.8	4.2	4.0	4.0	4.5	4.0	3.7	4.1	3.9
Gly	13.8	14.8	14.1	14.2	14.6	14.6	14	13.8	13.5
Ala	4.8	5.7	4.8	4.6	5.4	4.5	4.8	5.3	4.3
Cys	1.8	2.8	2.3	2.4	3.0	2.1	1.7	2.0	2.0
Val	3.3	3.5	3.5	3.4	3.3	3.4	3.4	3.3	3.4
Met	0.2	0.4	0.2	0.2	1.4	0.3	0.2	0.3	0.2
Ile	0.6	0.6	0.7	0.6	0.6	0.5	0.6	0.6	0.6
Leu	0.9	0.9	0.9	0.8	0.9	0.8	0.9	0.9	0.9
Tyr	3.3	3.3	3.1	3.2	3.4	3.3	3.1	3.0	3.4
Phe	0.7	1.3	1.1	1.0	1.4	1.2	0.7	0.7	0.9
Lys	3.4	4.0	3.7	3.6	4.0	3.5	3.4	3.8	3.5
His	1.4	1.6	1.6	1.5	1.4	1.5	1.7	1.6	1.5
Arg	2.8	2.9	2.7	2.8	2.9	3.0	2.6	2.8	2.9
Pro	0.8	0.9	0.7	0.7	0.9	0.9	0.7	0.6	0.7

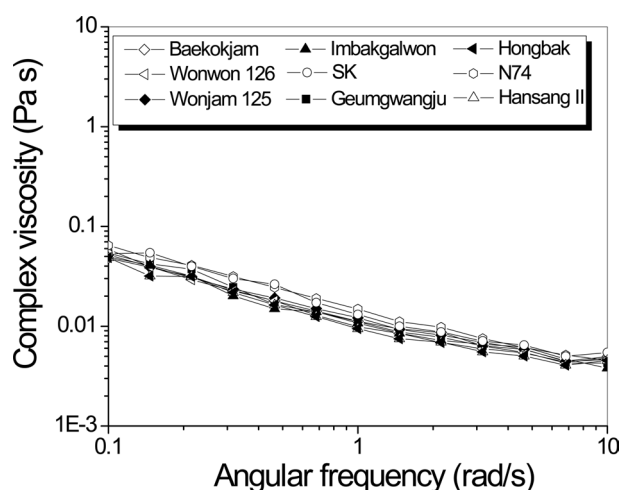


Fig. 1. Complex viscosity of 0.3% (w/w) silk sericin formic acid solution produced from different silkworm varieties.

silkworm varieties on the MW of SF and sericin in this study, we assumed that it may be related to the fabrication method for SF and sericin. That is, in case of sericin, one fabrication step (treatment of silk cocoons in an autoclave) is needed to obtain sericin. In case of SF, more preparation steps are necessary to

produce regenerated SF. The fabrication steps for regenerated SF include (1) degumming of silk, (2) dissolution of degummed silk, and (3) dialysis. Because silk cocoons have different structures depending on the silkworm variety, MW (or solution viscosity) of regenerated SF may differ after the fabrication steps. That is, SF of some silkworm varieties may decompose to a greater or lesser extent during the fabrication steps.

Molecular conformation and mechanical properties of sericin film

Molecular conformation of silk proteins has been examined because it affects the properties of silk proteins (Um *et al.*, 2001; Jo *et al.*, 2013; Lee *et al.*, 2013). Therefore, sericin film was evaluated using FTIR to examine the effect of silkworm variety on the molecular conformation of sericin film (Fig. 2). Regardless of the silkworm variety, all sericin films showed IR absorption peaks at 1612 and 1515 cm^{-1} attributable to β -sheet conformation (Jo *et al.*, 2013; Jo and Um, 2015). Jo and Um (2015) reported that sericin film is crystallized to a greater extent

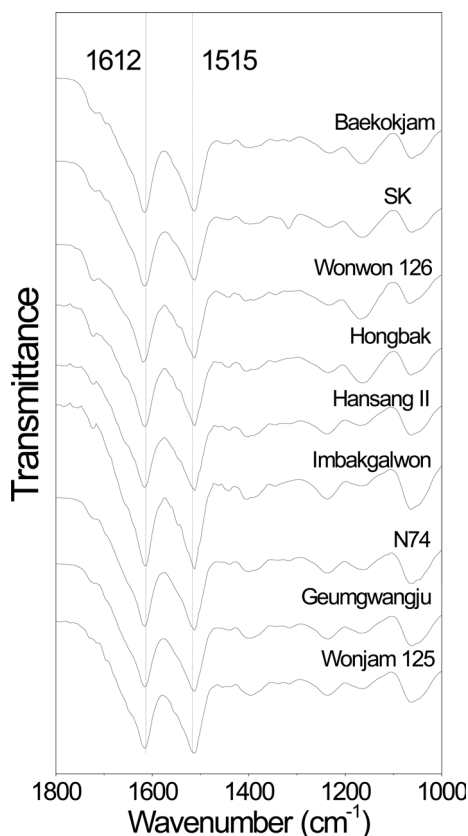


Fig. 2. FTIR spectra of silk sericin film produced from different silkworm varieties.

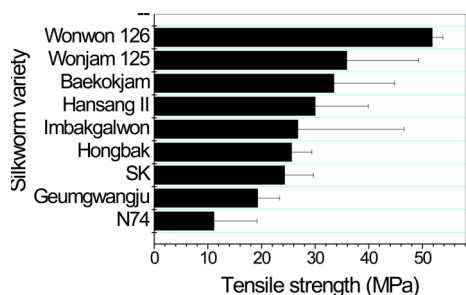


Fig. 3. Tensile strength of silk sericin film produced from different silkworm varieties.

in formic acid casting than water casting because formic acid accelerates β -sheet crystallization of sericin. Therefore, the IR results shown in Fig. 2 are consistent with the results obtained by Jo and Um (2015). No change in IR peaks among the silkworm variety samples indicates that the silkworm variety does not affect β -sheet crystallization of silk sericin film during formic acid casting.

Poor mechanical properties of sericin film have prevented the use of sericin film in industrial fields. Therefore, breaking strength, elongation, and initial Young's modulus of sericin

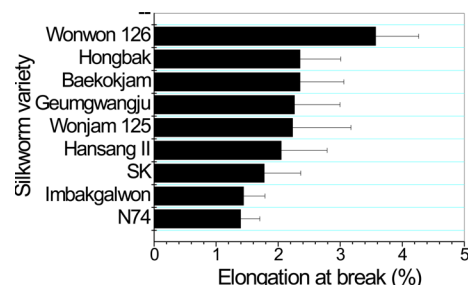


Fig. 4. Elongation of silk sericin film produced from different silkworm varieties.

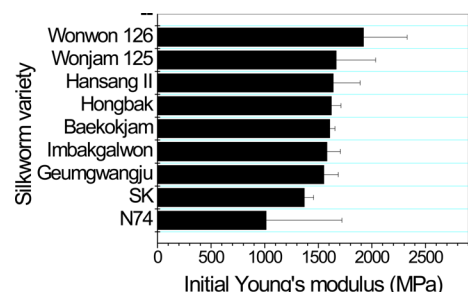


Fig. 5. Initial Young's modulus of silk sericin film produced from different silkworm varieties.

film obtained from different silkworm varieties were measured to examine the effect of silkworm variety on the mechanical properties of sericin film.

In case of breaking strength (Fig. 3), Wonwon 126 showed the highest strength among the samples. Geumgwangju and N74 showed the lowest values among the sericin films. For elongation, a similar trend was observed (Fig. 4). In case of initial Young's modulus, Wonwon 126 and N74 showed the highest and lowest values, respectively, among the samples. Wonwon 126 showed the best mechanical properties for sericin film, whereas Geumgwangju and N74 displayed the worst properties. These results indicate that silkworm variety significantly affects the mechanical properties of sericin film. For example, Wonwon 126 showed a five-fold higher breaking strength than N74.

Although the mechanism underlying the different mechanical properties of sericin film on the basis of silkworm variety could not be elucidated, it is interesting that Wonwon 126 showed better mechanical properties than Baekokjam (standard).

In this study, the effect of silkworm variety on the structure and properties of sericin was examined. Although the amino acid composition and solution viscosity of sericin were very similar among the silkworm varieties, the mechanical properties of sericin film were remarkably different depending on the silkworm variety. Considering that the poor mechanical

properties of sericin film has restricted its application in biotechnological fields, this finding shows that an optimum silkworm variety can be a good solution for improving the mechanical properties of sericin film. Because silkworm variety was found to affect the properties of sericin, a more detailed and related study on silkworm varieties should be performed in the future to identify better silk protein materials for various applications, including biomedical and cosmetic applications.

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References

- Cho HJ, Yoo HJ, Kim JW, Park YH, Bae DG, Um IC (2012) Effect of molecular weight and storage time on the wet- and electro-spinning of regenerated silk fibroin. *Polym Degrad Stab* 97, 1060-1066.
- Chung DE, Um IC (2014) Effect of molecular weight and concentration on crystallinity and post drawing of wet spun silk fibroin fiber. *Fiber Polym* 15, 153-160.
- Chung DE, Kim HH, Kim MK, Lee KH, Park YH, Um IC (2015) Effects of different *Bombyx mori* silkworm varieties on the structural characteristics and properties of silk. *Int J Biol Macromol* 79, 943-951
- Dash R, Mandal M, Ghosh SK, Kundu SC (2008) Silk sericin protein of tropical tasar silkworm inhibits UVB-induced apoptosis in human skin keratinocytes. *Mol Cell Biochem* 311, 111-119.
- Jo YN, Bae DG, Um IC (2013) The effect of extraction conditions and film side on the molecular conformation of silk sericin film. *Int J Indust Entomol* 26, 113-118.
- Jo YN, Um IC (2015) Effects of solvent on the solution properties, structural characteristics, and properties of silk sericin. *Int J Biol Macromol* 78, 287-295.
- Kim HJ, Um IC (2014) Relationship between the rheology and electro-spinning performance of regenerated silk fibroin prepared using different degumming methods. *Korea-Australia Rheol J* 26, 119-125.
- Ko JS, Yoon K, Ki CS, Kim HJ, Bae DG, Lee KH, Park YH, Um IC (2013) Effect of degumming condition on the solution properties and electrospinnability of regenerated silk solution. *Int J Biol Macromol* 55, 161-168.
- Lee JH, Bae CH, Park BD, Um IC (2013) The preparation of cellulose nanofibril / regenerated silk fibroin composite fiber. *Int J Indust Entomol* 26, 81-88.
- Limpeanchob N, Trisat K, Duangjai A, Tiyafoonchai W, Pongcharoen S, Sutheraawattananda M (2010) Sericin reduces serum cholesterol in rats and cholesterol uptake into caco-2 cells. *J Agric Food Chem* 58, 12519-12522.
- Nagai N, Murao T, Ito Y, Okamoto N, Sasaki M (2009) Enhancing effects of sericin on corneal wound healing in Otsuka long-evans Tokushima fatty rats as a model of human type 2 Diabetes. *Biol Pharm Bull* 32, 1594-1599.
- Seo CW, Um IC, Rico CW, Kang MY (2011) Antioxidative and hypoglycemic effects of silk fibroin/sericin mixtures in high fat-fed mice. *Int J Indust Entomol* 23, 115-122.
- Tsubouchi K, Igarashi Y, Takasu Y, Yamada H (2005) Sericin enhances attachment of cultured human skin fibroblasts. *Biosci Biotechnol Biochem* 69, 403-405.
- Um IC, Kweon HY, Park YH, Hudson S (2001) Structural characteristics and properties of the regenerated silk fibroin prepared from formic acid. *Int J Biol Macromol* 29, 91-97.
- Yoo YJ, Um IC (2013) Effect of extraction time on the rheological properties of sericin solutions and gels. *Int J Indust Entomol* 25, 180-184.
- Yoon K, Lee HN, Ki CS, Fang D, Hsiao BS, Chu B, Um IC (2013) Effect of degumming conditions on electro-spinning rate of regenerated silk. *Int J Biol Macromol* 61, 50-57.