



# The role of glycerol and water in flexible silk sericin film



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## ABSTRACT

Silk sericin (SS) can be obtained as a byproduct during the silk fiber process, but its application has been limited due to the brittleness of the SS film. To enhance the flexibility of the SS film, glycerol (Glc) has been added as a plasticizer. The addition of Glc enhanced the elongation property of the SS film when the Glc content was 50–70 wt% of SS. Glc also induced the structural transition of SS from a random coil structure to a  $\beta$ -sheet structure. The inconsistent increase of elongation and  $\beta$ -sheet structure of the SS/Glc film were explained by the content of moisture in the SS/Glc film. The moisture content of the SS/Glc film increased proportionally when the Glc content was higher than 50 wt% of SS, which was the same Glc content range that exhibited the plasticizing effect. Therefore, the plasticizing effect on the SS film may occur not only because of Glc but also because of water. Furthermore, water also contributed to the increase in the  $\beta$ -sheet structure development. Our results suggest that the moisture content in the plasticized protein film may play an important role when the plasticizer has hygroscopic properties.

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## 1. Introduction

Presently, biopolymers draw the attention of many material researchers and industries as a substitute for petroleum-based polymers. The petroleum-based polymers and their products are not degraded via typical biodegradation pathways and will persist in the environment for many years, which can lead to various problems such as taking up too much room in landfills and long-term toxic effects [1,2]. However, biopolymers such as proteins and polysaccharides can be degraded into nontoxic materials by biological systems. To be used as alternatives to synthetic polymers, a biopolymer should have equal or better performance. To enhance the mechanical properties of biopolymers, various additives such as fillers, cross-linking agents, and plasticizers are applied. For example, to increase the stiffness, nano- or micro-sized fillers can be added [3,4]. The low wet strength problem can be solved by adding a cross-linking agent [5,6], and adding plasticizer can introduce thermoplasticity or increased ductility [7–10].

Silk sericin (SS) constitutes 25% of the total silk protein and is usually removed before textile or biomedical application of silk fibroin. According to the FAO statistics from 2004 to 2013, the average annual global production of reelable silkworm cocoon and raw silk fiber was approximately 529,000 ton and 162,000 ton, respectively [11]. Considering that the SS content in raw silk fiber is approximately 20%, approximately 32,400 ton of SS had been abandoned ever year from 2004 to 2013. Therefore, there were continuous efforts to recover SS from the waste of the degumming solution and find a proper application for the recovered SS [12–18].

Like other biopolymers, SS has to overcome inherent drawbacks to be used as a polymeric material [4,13]. One of the drawbacks is the brittleness of pure SS. To solve the problem, blending with a synthetic polymer [14,18,19] or dissolving in a solvent other than water [20,21] has been proposed. However, a simple way to overcome the brittleness of pure SS is to add a plasticizer. Plasticizers are used to improve the processing ability of the polymer or to impart flexibility to the product. According to Subramanian [22], plasticizers have average molecular weights between 300 and 600 and consist of linear or cyclic carbon chains (14–40 carbons). The small molecular size of plasticizers can occupy intermolecular spaces between polymer chains, thereby increasing the free volume between polymer chains. Furthermore, plasticizers can change the three-dimensional molecular organization of polymers, reduce the energy required for

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molecular motion and prevent the formation of secondary bonding between the chains [8,23]. Thus, these combined effects will eventually increase the mobility of the polymer chains, which results in enhancing the flexibility of the polymer matrix.

Protein-based films have suitable properties such as good mechanical and barrier properties for application as food packaging materials [8,24,25]. Proteins such as maize, peanut, wheat, and milk have been fabricated into films for such an application with the addition of a plasticizer. The most commonly used plasticizers for proteins are polyols such as glycerol, ethylene glycol, sorbitol, etc. [9,26–29]. These plasticizers have different numbers and positions of hydroxyl groups, which will affect the final properties of the plasticized protein films. Among the various plasticizers, glycerol (Glc) was most effective and widely used to enhance the flexibility or elongation of protein-based films.

Previously, Glc were also added in SS-based materials, and the elongation of SS-based materials has been increased with the amount of Glc added [4,30–32]. For example, Zhang et al. prepared a highly extensible SS film by adding Glc as a plasticizer [30]. They added up to 40 wt% of Glc, which resulted in the improved elongation of the sericin film from 0.73 to 354%. They concluded that a higher amorphous SS film was obtained due to the addition of Glc. However, the secondary structure transition of SS due to the Glc was not sufficient to support the increased amorphous structure of the plasticized SS film. In addition, a clear peak blue shift of the amide I in the FT-IR spectra was observed, indicating  $\beta$ -sheet structure development. The development of  $\beta$ -sheet structure of SS in the presence of Glc has been also observed in our previous result [4]. So far it is unclear why the elongation of SS/Glc film increases despite of the occurrence of  $\beta$ -sheet structure.

In this study, SS films were prepared using a solution-casting method in the presence of Glc, and their performance and structure were evaluated. The aim of this study is to elucidate the reason of enhanced elongation of SS/Glc film despite of the presence of increased  $\beta$ -sheet structure. Here, we focused on the moisture content of SS/Glc film, and tried to explain the role of Glc and water on the plasticizing effect of SS film.

## 2. Experimental

### 2.1. Materials and methods

#### 2.1.1. Materials

Silk cocoons were kindly provided by the Heungjin Co. All of the other chemicals were purchased from Sigma-Aldrich (Yongin, Korea).

#### 2.1.2. SS extraction

Silk cocoons were boiled in an autoclave at 120 °C for 1 h. The liquor ratio was 1:25. After extraction, the remaining cocoons were removed from the SS solution, and the solution was filtered with a nonwoven filter paper. The final concentration of SS in the solution was 1.0 wt%.

#### 2.1.3. Preparation of SS/Glc flexible film

The SS solution was concentrated with a rotary evaporator until the concentration of SS reached 2 wt%. Glycerol was added to the SS solution as a plasticizer. Typically, to 50 ml of SS solution, different amounts of Glc were added. The SS/Glc film was prepared by casting this solution on a plastic plate having diameter of 100 mm at 50 °C in an oven for one day. The prepared flexible films were labeled according to the content of Glc relative to SS. For example, SS/Glc10 means Glc was added to 10 wt% of SS (Table 1). In addition, to compare the miscibility of Glc and sorbitol with SS, we prepared

**Table 1**

Sericin (SS) and glycerol (Glc) content of the SS/Glc flexible films and their thickness.

Sample	Sericin content (g)	Glycerol content (g)	Thickness ( $\mu\text{m}$ )
SS/Glc40	1	0.4	117 $\pm$ 7
SS/Glc50	1	0.5	120 $\pm$ 5
SS/Glc60	1	0.6	129 $\pm$ 11
SS/Glc70	1	0.7	129 $\pm$ 8
SS/Glc80	1	0.8	143 $\pm$ 8

pure SS film without plasticizer and SS/sorbitol film having sorbitol content of 50 wt% of SS.

#### 2.1.4. Mechanical characterization

The mechanical properties of SS/Glc flexible films were measured using a Universal testing machine (UTM, LRX plus, Lloyd instruments, UK). All of the samples were formed into strips of 50 mm  $\times$  50 mm, and the thickness of each film was inputted into the tester software before performing each test. The tensile test was performed according to ASTM D 638 (25.4 mm/min) at room temperature. At least 10 samples were tested for each type of flexible film.

#### 2.1.5. Morphological and structural characterizations of SS/Glc flexible films

A field emission scanning electron microscope (FE-SEM, SUPRA 55VP, Carl Zeiss, Germany) was employed to observe a cross-section of the SS/Glc films. The crystallinity of the SS/Glc flexible films was determined using a wide-angle X-ray diffraction system (WAXS, Bruker, Germany) equipped with Cu-K $\alpha$  radiation.

The secondary structure of the SS/Glc flexible films was measured using an ATR-Fourier transform infrared (FTIR, Nicolet 6700, Thermo Scientific, USA) spectrometer. The secondary structure composition of the SS/Glc film was measured as in previous study [30]. In brief, the amide I band between 1600 and 1700  $\text{cm}^{-1}$  was deconvoluted by Fourier self-deconvolution (FSD) fitting method using the Origin 8.0 software. The curve-fitting proofs were performed until  $\chi^2/\text{Dof}$  reached the minimum value with the multi-peak Gaussian fitting. The relative area of the overlapped single bands was used for estimating the proportion of secondary structures such as random coils,  $\alpha$ -helices,  $\beta$ -sheets and turns. The single bands from the flexible films were assigned to corresponding secondary structures, according to previous studies [30]: 1605–1615  $\text{cm}^{-1}$ , aggregated strands; 1616–1637  $\text{cm}^{-1}$ ,  $\beta$ -sheets; 1638–1655  $\text{cm}^{-1}$ , random coil; 1656–1662  $\text{cm}^{-1}$ ,  $\alpha$ -helices; 1663–1695  $\text{cm}^{-1}$ ,  $\beta$ -turns. ATR-FTIR results at 80 °C were obtained using a heated smart ARK accessory. After reaching 80 °C, the film was placed on the heated smart ARK for an hour before the measurement.

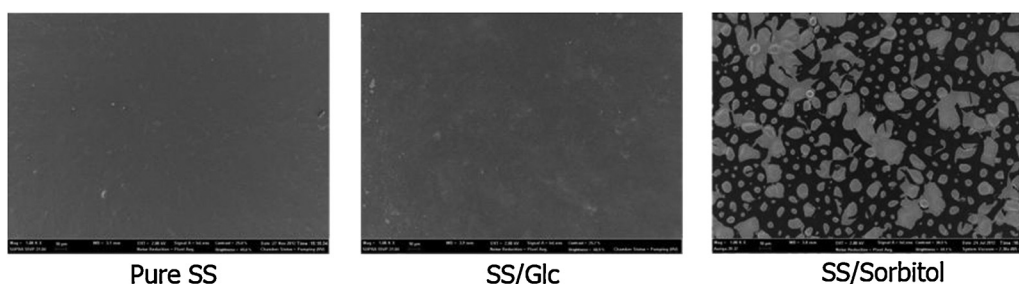
#### 2.1.6. Moisture content of SS/Glc flexible film

The moisture content of the SS/Glc flexible film was measured using a moisture analyzer (MB45, OHAUS, US). SS/Glc flexible films were tested after being standardized for 5 days at 25 °C and 35% R.H.

## 3. Results and discussion

### 3.1. Morphology and mechanical properties of the SS/Glc flexible films

Among the various requirements of a plasticizer, the plasticizer should be compatible with polymer to achieve the original goal of plasticization. Although Glc is the most widely used plasticizer for protein films, we first checked the miscibility of Glc with SS prior to initiating more detailed studies with Glc. Fig. 1 shows the



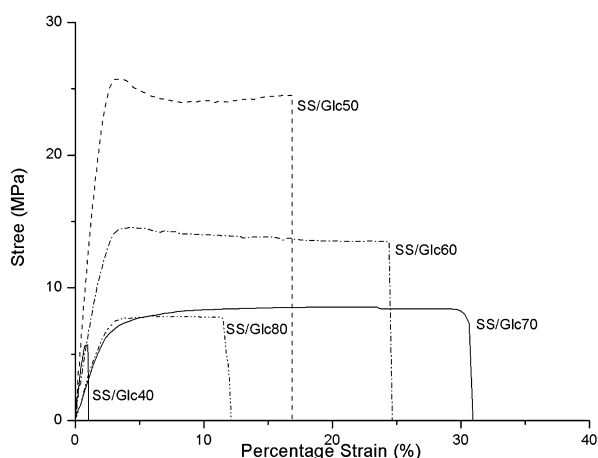
**Fig. 1.** FE-SEM images of pure SS, SS/Glc and SS/sorbitol films. The Glc and sorbitol content was 50 wt% of SS.



**Fig. 2.** The flexibility of the SS/Glc film when Glc was added at a level of 70 wt% of SS.

surface image of the pure SS, SS/Glc50 and SS/sorbitol film. While the addition of Glc to the SS film created a smooth surface topology, sorbitol exhibited a clear phase separation with SS even though it had been used successfully for other proteins [9,28,29]. In addition, the SS film prepared with sorbitol was still brittle, indicating no plasticization effect on the SS film due to immiscibility with the SS. Fig. 2 shows the flexibility of the SS/Glc70 film, and the bending performance was greatly enhanced by the addition of Glc.

Fig. 3 shows the representative S-S curve, and Fig. 4 shows the ultimate strength, Young's modulus and elongation at breaking of the SS/Glc film according to the Glc content from 50 to 80 wt% of SS. The pure SS film and SS/Glc films with Glc content less than 30 wt% of SS were too brittle and SS/Glc films with Glc content higher than 90 wt% of SS were too weak to test with a mechanical tester. However, when Glc was added to 50–80 wt% of SS, the

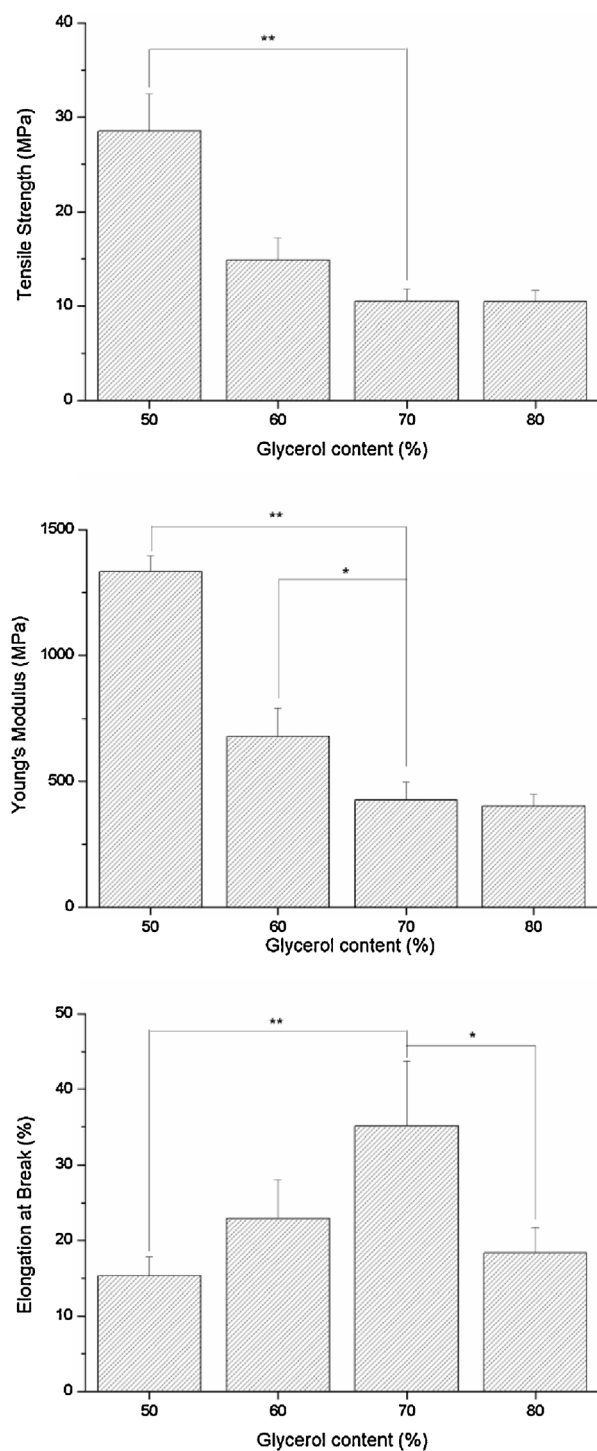


**Fig. 3.** Representative stress–strain curves of a flexible SS film containing varying amounts of glycerol.

films became stable and flexible, and we were able to measure the mechanical properties. However, the elongation of SS/Glc40 is still low, indicating a low plasticizing effect at this level of Glc concentration. In a previous study, the plasticizing effect of Glc was observed even at a Glc content at 10 wt% of SS, but the thickness of the film was only 80  $\mu\text{m}$  [30]. Since the average thickness of the SS film in this study was 0.11–0.14 mm, the difference in the Glc content required to exhibit the plasticizing effect is due to the different thicknesses of the SS film from both studies. The elongation at breaking of the SS film was increased up to  $35.1 \pm 8.61\%$  when the Glc content was 70 wt% of SS. The elongation of the SS/Glc film was quite small compared to the previous results of Zhang et al. [30], but it could be also explained by the differences in the film thickness. The Young's modulus and ultimate tensile strength of the SS/Glc film decreased with the increase in Glc content, which could typically be observed when the plasticizer was added. In the case of the SS/Glc70 film, the Young's modulus and tensile strength were  $425.22 \pm 72.24$  MPa and  $10.50 \pm 1.31$  MPa, respectively. However, when the Glc content reached 80 wt% of SS, the elongation at breaking decreased to  $18.31 \pm 3.40\%$  even though there were little differences in the Young's modulus and the tensile strength compared to the SS/Glc70 film. This sudden decrease in the elongation could be explained by the low content of SS in the SS/Glc80 film.

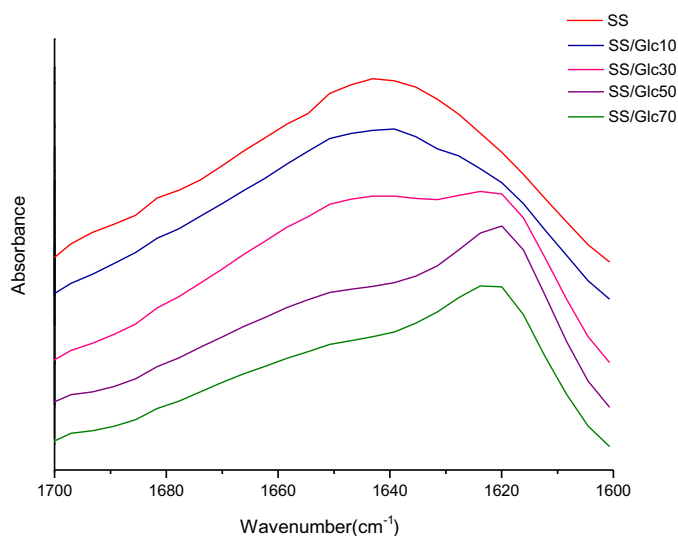
### 3.2. Secondary structure and crystallinity of the SS/Glc flexible films

Because plasticizers disturb the intra- and intermolecular interaction of polymer chains, the secondary structure of the SS was investigated according to the Glc content. The transition of the secondary structure of the SS/Glc film was measured using an ATR-FTIR spectrometer. We examined the absorbance between 1700 and 1600  $\text{cm}^{-1}$ , which results from the C=O stretching vibration of the amide group in the protein (Fig. 5). This region represents the amide I structure of SS, which provides useful information about the secondary structure of the SS film, and the absorption peak of Glc did not affect this region. The maximum peak in the amide I band of the pure SS film was observed at 1643  $\text{cm}^{-1}$ , which is usually assigned as a random coil structure. When the Glc content in the SS films is increased by more than 30%, the absorption peak at 1623  $\text{cm}^{-1}$  is developed, and finally, the maximum amide I peak shifts to 1623  $\text{cm}^{-1}$ , which corresponds to the  $\beta$ -sheet structure. For more detailed study of the transition of secondary structure according to the Glc content, a quantitative analysis on the amide I band was performed using the FSD fitting method. Table 2 shows the change in the secondary structure of the SS with an increase in the Glc content from 10 to 90 wt% of SS. The pure SS film had a predominantly random coil structure that was approximately 36.5%. When the Glc was added, the content of the random coil,  $\beta$ -turn and  $\alpha$ -helix structure decreased, while the content of the aggregated structure remained unchanged. As expected from Fig. 5, the content of the  $\beta$ -sheet structure was significantly increased



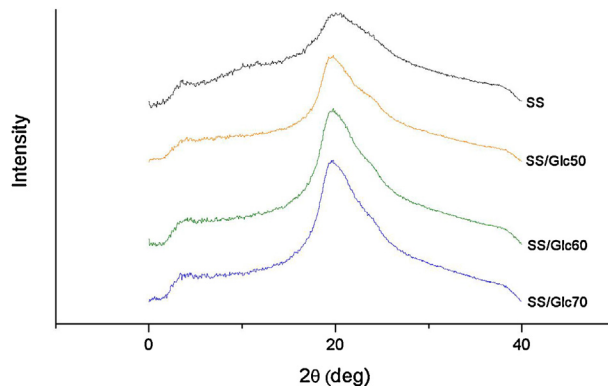
**Fig. 4.** Mechanical properties of the SS/Glc flexible films with different Glc content relatively to SS. (a) Tensile strength, (b) Young's modulus and (c) elongation at break. \* and \*\* indicate significant differences of  $p < 0.05$  and  $p < 0.01$ , respectively, as determined using Student's *t*-test.

from 23.46 to 42.51% when the Glc content reached 70 wt% of SS. The results indicate that Glc induces a  $\beta$ -sheet structure transition of SS from the random coil structure, which is in agreement with previous results [4,30]. Glc has been reported to induce protein compaction, which allows the protein molecule to be more closely packed [33–35]. Therefore, a similar effect might occur here as well. In the case of SS/Glc70 film, the random coil,  $\beta$ -turn and  $\alpha$ -helix contents decreased from 36.5 to 27.4%, 21.8 to 16.9% and 15.8



**Fig. 5.** ATR-FTIR spectra of the SS/Glc flexible films with different Glc content relatively to SS.

to 10.4%, respectively, compared to the SS film. The XRD analysis was performed to check the crystallinity of the SS and the SS/Glc films (Fig. 6). The peak intensity at  $2\theta = 19.74^\circ$  increased with the increase in the Glc content, indicating increased crystallinity or regularity in the SS/Glc film. We cannot directly relate the increase in the  $\beta$ -sheet structure to the increase in crystallinity, but at least the irregular random coil structure has certainly been decreased, consistent with the ATR-FTIR results. In general, the effect of the plasticizer contributed to occupation of the intermolecular spaces between polymer chains, and one of the consequences will be the disruption of the secondary forces between polymer chains, such as hydrogen bonding. Proteins have many functional groups capable of hydrogen bonding. Except for the random coil structure, all secondary structures of a protein occur due to the formation of complex hydrogen bonds. Alpha-helix and  $\beta$ -turn structures are formed through intramolecular hydrogen bonding, and  $\beta$ -sheet structures are formed through intermolecular hydrogen bonding. Therefore, if the plasticizer successfully disrupts the hydrogen network of the protein, the secondary structure will be altered significantly. Generally, the increase in the elongation of protein films by addition of plasticizers will be the result of increased  $\alpha$ -helix,  $\beta$ -turn or random coil structures, which are more stretchable than the extended  $\beta$ -sheet structure [27,36,37]. However, in this study, the SS/Glc film increased the  $\beta$ -sheet structure in the presence of Glc. In such a case, the elongation of the protein films is expected to decrease,



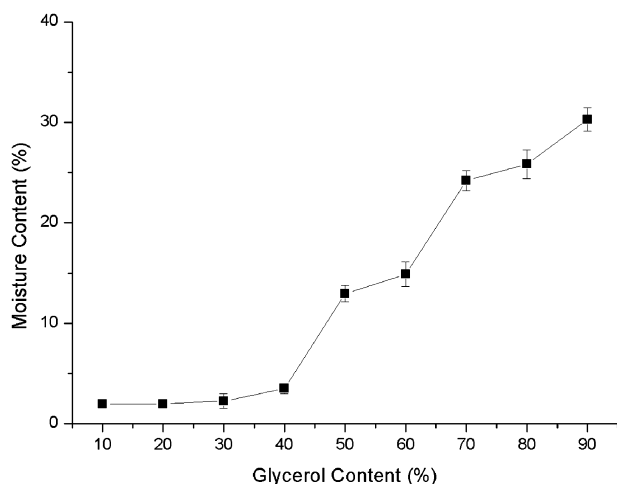
**Fig. 6.** X-ray diffractograms of flexible SS films with different Glc content relatively to SS.



**Table 2**

The relative proportion (%) of the secondary structures calculated from the areas of the deconvoluted FTIR spectra of the SS and the SS/Glc flexible films with different contents of glycerol.

	Aggregated Strands	$\beta$ -Sheet	Random Coil	$\alpha$ -Helices	Turns
SS	2.93 $\pm$ 1.00	23.46 $\pm$ 0.43	36.5 $\pm$ 0.97	15.79 $\pm$ 0.92	21.79 $\pm$ 0.59
SS/Glc10	2.96 $\pm$ 0.08	31.42 $\pm$ 1.81	32.69 $\pm$ 0.86	12.86 $\pm$ 0.53	20.08 $\pm$ 0.50
SS/Glc30	3.30 $\pm$ 0.31	38.74 $\pm$ 0.68	28.91 $\pm$ 0.22	11.21 $\pm$ 0.09	17.84 $\pm$ 0.19
SS/Glc50	2.75 $\pm$ 0.21	41.87 $\pm$ 0.22	27.74 $\pm$ 0.08	10.61 $\pm$ 0.06	17.03 $\pm$ 0.27
SS/Glc70	2.56 $\pm$ 0.50	42.51 $\pm$ 0.14	27.48 $\pm$ 0.50	10.43 $\pm$ 0.36	17.01 $\pm$ 0.44
SS/Glc90	2.73 $\pm$ 0.26	42.23 $\pm$ 0.06	27.46 $\pm$ 0.38	10.64 $\pm$ 0.30	16.94 $\pm$ 0.16

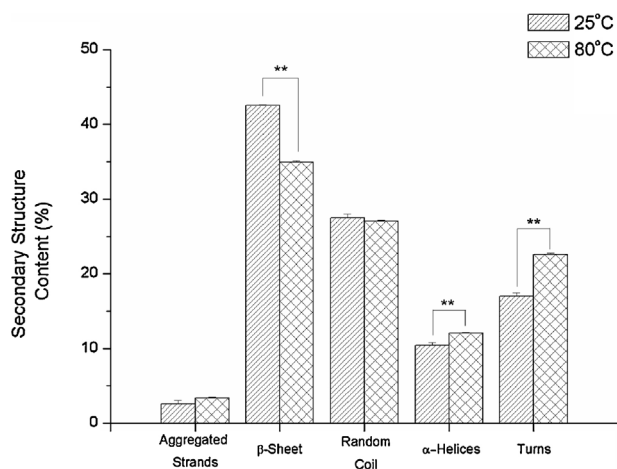


**Fig. 7.** Effect of Glc contents relatively to SS on the moisture content of flexible SS films.

but our measurement resulted in the opposite result. Therefore, the plasticizing effect of Glc in the SS film should be explained differently.

### 3.3. Moisture content in SS/Glc film

An SS film containing Glc has enhanced flexibility despite an increased  $\beta$ -sheet structure and decreased random coil structure. Here, we focused on the moisture content of the SS/Glc film because the SS/Glc80 film became hygroscopic during storage of the sample. The secondary structure of SS has been reported to be greatly influenced by the presence of water [38] or ethanol [13,20,39,40]. Therefore, the presence of water might affect the secondary



**Fig. 8.** The secondary structure component distribution of flexible SS films at different temperatures. The Glc content was 70 wt% of SS. \*\* indicates significant differences of  $p < 0.01$ , respectively, as determined using Student's *t*-test.

structure of SS. First, we determined the moisture content of the SS/Glc films relative to the content of Glc in the film (Fig. 7). With up to Glc content of 40 wt% of SS, the moisture content of the SS/Glc films was less than 5%, but a sudden increase in the moisture content was observed when the Glc content was 50 wt% of SS in the film. When the Glc content exceeds 50 wt% of SS, the moisture content also increased proportionally. Because Glc is highly hygroscopic, this increase is not surprising, but in the previous section, we mentioned that the plasticizing effect of Glc occurred when the content of Glc was higher than 50 wt% of SS. Therefore, the plasticizing effect did not occur only because of Glc but also because of water. Water itself is also a strong plasticizer for most natural polymers, but it is not used as a result of its volatile properties. In our case, the water molecules were captured by the Glc, preventing evaporation during the casting process. Our results suggest that the plasticizing effect of Glc is accompanied by water rather than by Glc alone. In addition, water has a strong effect on the secondary structure of the SS. Teramoto et al. reported that the hydrated SS was stabilized by the formation of a  $\beta$ -sheet-rich structure [39]. They suggest that the  $\beta$ -sheet structure of the SS was formed by intermolecular hydrogen bonding of Ser and Thr side chains in the presence of water. Water molecules hydrate the loop structure of the SS and may reinforce the intermolecular hydrogen bonding in the hydrophobic region. However, in the dry state, the conformation of the SS maintained a disordered structure. The same result was also found in this study. Fig. 8 shows the secondary structure of the SS in the SS/Glc70 films measured by ATR-FTIR spectrometry at a different temperature. We tried to eliminate water from the SS/Glc70 film by elevating the temperature to 80 °C. As a result, the content of the  $\beta$ -sheet structure was decreased but that of the  $\alpha$ -helix and  $\beta$ -turn structure was increased at 80 °C, indicating that the elimination of water changed the secondary structure of the SS from a  $\beta$ -sheet to an  $\alpha$ -helix and  $\beta$ -turn structure. This change is in agreement with previous reports that the  $\beta$ -sheet structure of the SS is stable only in the presence of water molecules [38,40]. Upon losing water, the SS molecules became unstable and attempted to reconstruct themselves into stable form by intramolecular hydrogen bonding, resulting in the  $\alpha$ -helix and  $\beta$ -turn structure.

The roles of Glc and water can be summarized as follows. At low concentrations of Glc (up to 40 wt% of SS), Glc alone is effective for the structural transition of the SS. The original random coil structure of SS is transformed into a  $\beta$ -sheet structure in the presence of Glc (Fig. 5). The moisture content is still low up to this concentration because of the limited amount of Glc (Fig. 7), and no plasticizing effect is observed (Fig. 4). However, when the content of Glc exceeds 50 wt% of SS, the  $\beta$ -sheet structure is fully developed (Table 2 and Fig. 5), but the plasticizing effect occurs due to the water in the film (Figs. 4 and 7). In addition, water may also contribute to an increase in the  $\beta$ -sheet structure development (Fig. 8). Fig. 9 shows an illustration of the SS, Glc and water when the Glc content is below or above 50 wt% of SS. Below 50 wt% of SS, Glc induces compaction of the SS, leading to  $\beta$ -sheet formation, but the plasticizing effect is not yet observed at this concentration. However, when the content of Glc exceeds 50 wt% of SS, water penetrates between the SS molecules, especially in the random coil region. This

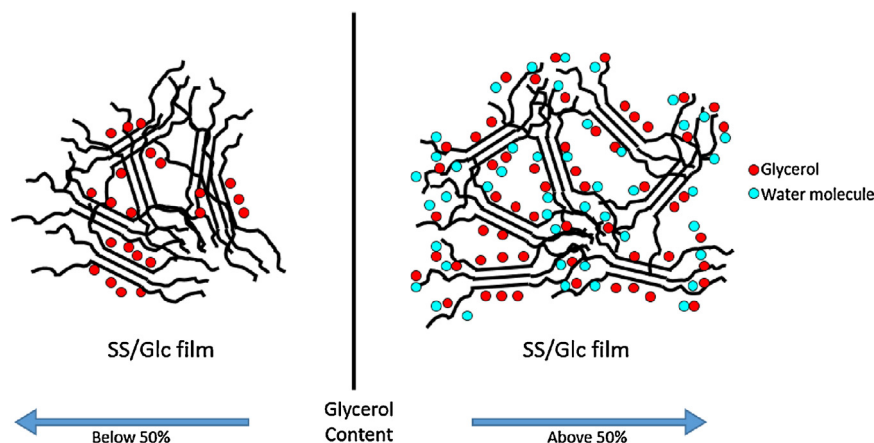


Fig. 9. Schematic model of the SS, the Glc and the water when the Glc concentration is below or above 50 wt% of SS.

penetration will increase the free volume between the SS chains, thereby inducing the plasticizing effect.

#### 4. Conclusion

In this study, we observed the enhancement of the elongation properties of the SS film by the addition of Glc as the plasticizer. The SS/Glc film exhibited increased elongation at the break when increasing the amount of Glc relative to the amount of SS. As the amount of Glc in the film increased, the  $\beta$ -sheet structure increased gradually. However, the moisture content also increased. We conclude that the Glc and the water molecule have a synergistic effect on the improved flexibility of the SS film. To date, Glc has been used as a common plasticizer for various types of protein films, but the moisture content in the plasticized protein film was not investigated. However, our results suggest that the plasticizing effect of Glc might also be affected by the increased moisture content. Therefore, the moisture content in the Glc plasticized film would be worth evaluating to understand the plasticizing effect of Glc in protein films.

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#### References

- [1] T. Mukherjee, N. Kao, PLA based biopolymer reinforced with natural fibre: a review, *J. Polym. Environ.* 19 (2011) 714–725.
- [2] T.A. Hottle, M.M. Bilec, A.E. Landis, Sustainability assessments of bio-based polymers, *Polym. Degrad. Stabil.* 98 (2013) 1898–1907.
- [3] D.F. Xie, V.P. Martino, P. Sangwan, C. Way, G.A. Cash, E. Pollet, K.M. Dean, P.J. Halley, L. Avérous, Elaboration and properties of plasticised chitosan-based exfoliated nano-biocomposites, *Polymer* 54 (2013) 3654–3662.
- [4] H. Yun, M.K. Kim, H.W. Kwak, J.Y. Lee, M.H. Kim, E.H. Kim, K.H. Lee, Preparation and characterization of silk sericin/glycerol/graphene oxide nanocomposite film, *Fiber Polym.* 14 (12) (2013) 2111–2116.
- [5] Y.C. Wei, S.M. Hudson, J.M. Mayer, D.L. Kaplan, The cross-linking of chitosan fibers, *J. Polym. Sci. Pol. Chem.* 30 (1992) 2187–2193.
- [6] Y. Wang, X. Mo, X.S. Sun, D. Wang, Soy protein adhesion enhanced by glutaraldehyde crosslink, *J. Appl. Polym. Sci.* 104 (2007) 130–136.
- [7] M.G.A. Vieira, M.A. da Silva, L.O. dos Santos, M.M. Beppu, Natural-based plasticizers and biopolymer films: a review, *Eur. Polym. J.* 47 (2011) 254–263.
- [8] N.E. Suyatma, L. Tighzert, A. Copinet, Effects of hydrophilic plasticizers on mechanical, thermal, and surface properties of chitosan films, *J. Agric. Food Chem.* 53 (2005) 3950–3957.
- [9] T.H. McHugh, M. Krochta, Sorbitol-vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation, *J. Agric. Food Chem.* 42 (4) (1994) 841–845.
- [10] R.A. Talja, H. Helén, Y.H. Roos, K. Jouppila, Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films, *Carbohydr. Polym.* 67 (2007) 288–295.
- [11] Food and Agriculture Organization of the United Nations. <http://faostat3.fao.org/>.
- [12] Y. Yang, S.M. Lee, H.S. Lee, K.H. Lee, Recovery of silk sericin from soap-alkaline degumming solution, *Int. J. Indust. Entomol.* 27 (1) (2013) 203–208.
- [13] H. Oh, J.Y. Lee, M.K. Kim, I.C. Um, K.H. Lee, Refining hot-water extracted silk sericin by ethanol-induced precipitation, *Int. J. Biol. Macromol.* 28 (2011) 32–37.
- [14] H. Oh, J.Y. Lee, A. Kim, K.H. Lee, B.S. Shin, Preparation of PVA/silk sericin blend fiber using LiCl/DMSO solvent, *Text. Sci. Eng.* 45 (1) (2008) 1–5.
- [15] H. Oh, M.K. Kim, K.H. Lee, Preparation of sericin microparticles by electrohydrodynamic spraying and their application in drug delivery, *Macromol. Res.* 19 (3) (2011) 266–272.
- [16] Y.Q. Zhang, Y. Ma, Y.Y. Xia, W.D. Shen, J.P. Mao, R.Y. Xue, Silk sericin–insulin bioconjugates: synthesis, characterization and biological activity, *J. Control. Release* 115 (2006) 307–315.
- [17] B.B. Mandal, A.S. Priya, S.C. Kundu, Novel silk sericin/gelatin 3-D scaffolds and 2-D films: fabrication and characterization for potential tissue engineering applications, *Acta Biomater.* 5 (2009) 3007–3020.
- [18] P. Aramwit, T. Siritientong, S. Kanokpanont, T. Srichana, Formulation and characterization of silk sericin–PVA scaffold crosslinked with genipin, *Int. J. Biol. Macromol.* 47 (2010) 668–675.
- [19] B. Kundu, S.C. Kundu, Silk sericin/polyacrylamide in situ forming hydrogels for dermal reconstruction, *Biomaterials* 33 (2012) 7456–7467.
- [20] H. Oh, J.Y. Lee, C.S. Ki, J.W. Kim, Y.H. Park, K.H. Lee, Preparation of silk sericin beads using LiCl/DMSO solvent and their potential as a drug carrier for oral administration, *Fiber. Polym.* 8 (5) (2007) 470–476.
- [21] M.M.R. Khan, M. Tsukada, X. Zhang, H. Morikawa, Preparation and characterization of electrospun nanofibers based on silk sericin powders, *J. Mater. Sci.* 48 (2013) 3731–3736.
- [22] M.N. Subramanian, *Plastics Additives and Testing*, first ed., Scrivener Publishing LLC, MA, 2013.
- [23] M. Wihodo, C.I. Moraru, Physical and chemical methods used to enhance the structure and mechanical properties of protein films: a review, *J. Food Eng.* 114 (2013) 292–302.
- [24] J.W. Rhim, K.W. Ng Perry, Natural biopolymer-based nanocomposite films for packaging applications, *Crit. Rev. Food Sci. Nutr.* 47 (4) (2007) 411–433.
- [25] N. Reddy, Y. Yang, Thermoplastic films from plant proteins, *J. Appl. Polym. Sci.* 130 (2) (2013) 729–738.
- [26] S. Kokoszka, F. Debeaufort, A. Hambleton, A. Lenart, A. Voilley, Protein and glycerol contents affect physico-chemical properties of soy protein isolate-based edible films, *Innov. Food Sci. Emerg. Technol.* 11 (2010) 503–510.
- [27] S. Lu, X. Wang, Q. Lu, X. Zhang, J.A. Kluge, N. Uppal, F. Omenetto, D.L. Kaplan, Insoluble and flexible silk films containing glycerol, *Biomacromolecules* 11 (2010) 143–150.
- [28] P.J.A. Sorbal, F.C. Menegalli, M.D. Huninger, M.A. Roques, Mechanical, water vapor barrier and thermal properties of gelatin based edible films, *Food Hydrocolloids* 15 (2001) 423–432.
- [29] N. Cao, X. Yang, Y. Fu, Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films, *Food Hydrocolloids* 23 (2009) 729–735.
- [30] H. Zhang, L. Deng, M. Yang, S. Min, L. Yang, L. Zhu, Enhancing effect of glycerol on the tensile properties of *Bombyx mori* cocoon sericin films, *Int. J. Mol. Sci.* 12 (2011) 3170–3181.
- [31] R. Purwar, S. Sharma, P. Sahoo, C.M. Srivastava, Flexible sericin/polyvinyl alcohol/clay blend films, *Fiber Polym.* 16 (4) (2015) 761–768.

- [32] R. Sothornvit, R. Chollakup, Properties of sericin–glucomannan composite films, *Int. J. Food Sci. Technol.* 44 (2009) 1395–1400.
- [33] S.N. Timasheff, The control of protein stability and association by weak interactions with water: how do solvents affect these processes? *Annu. Rev. Biophys. Biomol. Struct.* 22 (1993) 67–97.
- [34] P.R. Davis-Searles, A.S. Morar, A.J. Saunders, D.A. Erie, G.J. Pielak, Sugar-induced molten-globule model, *Biochemistry* 37 (1998) 17048–17053.
- [35] V. Vagenende, M.G.S. Yap, B.L. Trout, Mechanisms of protein stabilization and prevention of protein aggregation by glycerol, *Biochemistry* 48 (2009) 11084–11096.
- [36] C. Gao, M. Stading, N. Wellner, M.L. Parker, T.R. Noel, E.N. Mills, P.S. Belton, Plasticization of a protein-based film by glycerol: a spectroscopic, mechanical, and thermal study, *J. Agric. Food Chem.* 54 (2006) 4611–4616.
- [37] T. Gillgren, S.A. Barker, P.S. Belton, D.M.R. Georget, M. Stading, Plasticization of zein: a thermomechanical, FTIR, and dielectric study, *Biomacromolecules* 10 (2009) 1135–1139.
- [38] H. Teramoto, A. Kakazu, K. Yamauchi, T. Asakura, Role of hydroxyl side chains in *Bombyx mori* silk sericin in stabilizing its solid structure, *Macromolecules* 40 (2007) 1562–1569.
- [39] H. Teramoto, K. Nakajima, C. Takabayashi, Preparation of elastic silk sericin hydrogel, *Biosci. Biotechnol. Biochem.* 69 (4) (2005) 845–847.
- [40] H. Teramoto, M. Miyazawa, Molecular orientation behavior of silk sericin film as revealed by ATR infrared spectroscopy, *Biomacromolecules* 6 (2005) 2049–2057.