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Molecular conformation and crystallinity of white colored silkworm cocoons with different silkworm varieties

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Abstract

Bombyx mori silkworm has many silkworm varieties and the structure and properties of silk can be varied depending on the silkworm variety. Therefore, the choice of proper silkworm variety can be an effective way to control and improve the performance of silk materials in biomedical and cosmetic applications. As a preliminary study, in the present study, thirty nine *Bombyx mori* silkworm cocoons were prepared and their molecular conformation and crystallinity were examined. Molecular conformation and the crystallinity of silkworm cocoons were different depending on the silkworm variety. Interestingly, the crystallinity index of outside of cocoon was different from that of inside of cocoon and the difference between the crystallinities of outside and inside of cocoon was varied depending on the silkworm variety. In this study, the silkworm variety cocoons were classified into three groups (Group 1, Group 2, and Group 3) based on the difference of crystallinities between outside and inside.

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Introduction

Silk is composed of silk fibroin and sericin. Silk fibroin has a good blood compatibility (Sakabe *et al*., 1989; Um *et al*., 2002), good cyto-compatibility (Minoura *et al*., 1995) and low inflammatory reaction (Meinel *et al*., 2005). Also, sericin has high UV (Gulrajani *et al*., 2009) and oxidation resistances (Zhaorigetu *et al*., 2007) and high water retention (Padamwar *et al*., 2005), and heals wounds (Aramwit *et al*., 2009; Nagai *et al*., 2009). Owing to these useful properties as a biomaterial, the silk materials have been considered as a good biomaterial candidate in biomedical and cosmetic applications.

The silk is produced by the nature: silkworm. There are many kinds of silkworm in nature. In case of *Bombyx mori* silkworm, there were more than three hundred silkworm varieties in South Korea. In the previous studies, the molecular weight, mechanical properties, and electro-spinning performance of regenerated silk fibroin were reported to be affected by the silkworm variety (Chung *et al.,* 2015a; Park and Um, 2015), while its wet-spinning performance did not alter by the silkworm variety (Jang and Um, 2015). Solution viscosity and mechanical properties of sericin were strongly influenced by the silkworm variety (Chung *et al*., 2015b). Kim and Um (2019) found that the characteristics of raw sericin were also affected by the silkworm variety. Lee *et al*. (2017) reported that the cocoon color depended on the silkworm variety. These results of studies indicate that the structure and properties of silk materials (fibroin and sericin) are different depending on the silkworm variety.

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Although the effects of silkworm variety on the silk have been examined as above, there are much more research subject which should be explored. Also, in the previous studies, a limited number of silkworm variety silk samples (less than 10) was used. Molecular conformation and crystallinity of silk have been studied extensively because it affects chemical and physical properties of silk (Ko *et al*., 2018; Park and Um, 2018; Um *et al*., 2001; Jang *et al*., 2015; Jo *et al*., 2013; Jo and Um, 2015; Jo *et al*., 2015; Park and Um, 2016). However, the effect of silkworm variety on the molecular conformation and the crystallinity of silkworm cocoons has not been studied in detail.

Therefore, as a preliminary work, thirty nine different silkworm variety cocoons were used in the present study and the FTIR measurement was conducted to examine the effect of silkworm variety on the molecular conformation and crystallinity of silkworm cocoons.

Materials and Methods

Cocoon preparation and silkworm rearing

Thirty nine different original *Bombyx mori* silkworm varieties were grown at Kyungpook National University. Thirty nine silkworm cocoon samples were produced in total from the thirty nine silkworm varieties. All silkworm varieties larvae were reared at 25°C on fresh mulberry leaves. Pupae and cocoons were kept at 25°C.

Measurement and characterization

The color and external feature of silkworm cocoons were photographed using a digital camera (PC1310, Canon, China). To examine the molecular conformation of outside and inside of silkworm cocoons, FTIR spectroscopy (Nicolet 380, Thermo Fisher Scientific, USA) was performed in the attenuated total reflection (ATR) method. The scan range was 4000 cm^{-1} to 650 cm^{-1} and the scan number and the resolution were 32 and 8 cm^{-1}, respectively. Seven samples were measured for each silkworm variety cocoon. The crystallinity index was calculated as the intensity ratio of the 1260 and 1235 cm^{-1} of the FTIR spectrum using the following equation (Chung and Um, 2014; Kim and Um, 2014):

$$\text{Crystallinity index (\%)} = \frac{A_{1260cm^{-1}}}{A_{1235cm^{-1}}} \times 100$$

$A_{1235cm^{-1}}$: Absorbance at 1235 cm^{-1}
$A_{1260cm^{-1}}$: Absorbance at 1260 cm^{-1}

Results and Discussion

Table 1 showed the external shape of silkworm cocoons with different silkworm varieties. As can be seen in the table, the size and shape of silkworm cocoons were different depending on the silkworm variety. This might be due to that the size and shape of silkworm were various depending on the silkworm variety.

FTIR spectroscopy has been extensively used in the examination of micro-structure of silk materials because it provides the information for the molecular conformation of silk (Lee *et al*., 2018). Fig. 1 displayed FTIR spectra of outside of silkworm cocoon with different silkworm varieties. All silkworm cocoons showed IR peaks at 1515 cm^{-1} in amide II band attributed to β-sheet crystallite indicating all silkworm cocoon samples have β-sheet crystallite (Lee *et al*., 2018). It is interesting to note that the silkworm cocoons showed IR peaks at different positions in amide I band depending on the silkworm variety. That is, R501 and 35 showed a IR peak at 1645 cm^{-1} and a shoulder at 1620 cm^{-1} in amide I band attributed to random coil conformation and β-sheet crystallite, respectively. This

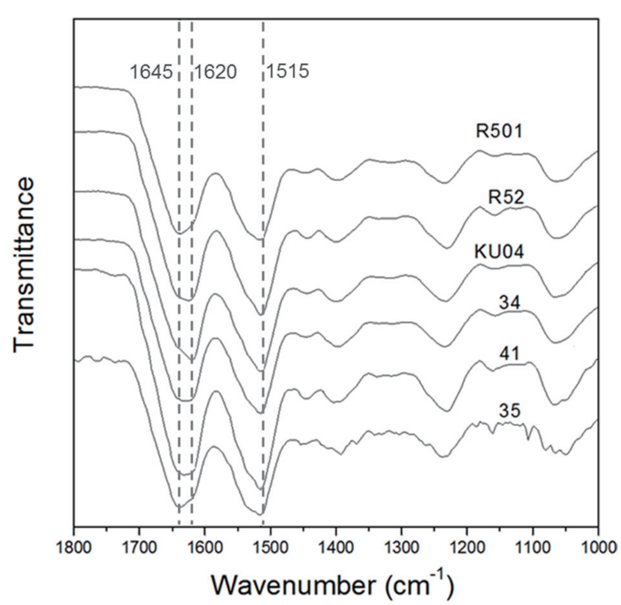
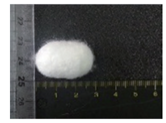
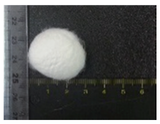
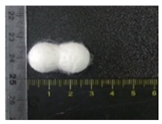
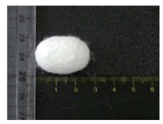
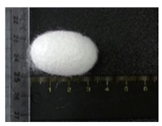
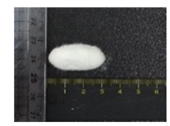
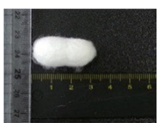
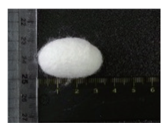
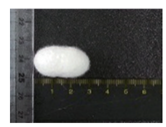


Fig. 1. Representative FTIR spectra of outside of silkworm cocoon with different silkworm varieties.

Table 1. Pictures of white colored silkworm cocoon with different silkworm varieties used in this study.

Silkworm variety	34	8032	KU04	52	19	07	S39
							
	KL70	M01	124	KA46	RET32	KOK	9F
							
	KB32	108	35	41	JB31	33	40
							
	R501	03	XE28	S12	S19	S25	S10
							
	R52	S11	113	09	125	30	M44
							

indicates that random coil and β-sheet conformations co-exist in the silkworm cocoons and that random coil conformation more exists in the silk than β-sheet crystallite. On the other hand, KU04 and R52 showed a IR peak at 1620 cm^{-1} and a shoulder at 1645 cm^{-1} implying that β-sheet crystallite more exists than random coil conformation in the silks. In case of 34 and 41, they showed similar IR absorption intensity at 1620 cm^{-1} and 1645 cm^{-1} indicating both conformations almost co-exist equally.

This result implies that the molecular conformation and degree of crystallinity of silkworm cocoon might be different depending on the silkworm variety. Therefore, to examine the effect of silkworm variety on the crystallinity of silkworm cocoons quantitatively, the crystallinity index of silkworm cocoons was calculated. It was reported that the morphology of outside and inside of silkworm cocoon are different (Chung *et al.*, 2015a). We can examine the crystallinity of both (outside and inside) surfaces of silkworm cocoon using ATR-IR technique because ATR-IR technique provides information of surface of sample. Therefore, we conducted IR measurements on outside and inside of silkworm cocoons and the results were shown in Fig. 2~4.

Fig. 2 showed the crystallinity index of outside of silkworm cocoon with different silkworm varieties. As can be seen in the figure, the crystallinity of outside of silkworm cocoon ranged from 51% to 62% depending on the silkworm variety. This result reconfirms that the molecular conformation of silkworm cocoon is different depending on the silkworm variety as shown in Fig. 1.

Fig. 3 exhibited the crystallinity index of inside of silkworm cocoon with different silkworm varieties. The crystallinity of silkworm cocoon ranged from 57% to 62% depending on the silkworm variety. Interestingly, the silkworm variety silk (sample 35) with the lowest crystallinity (51.6%) for outside of cocoon showed a crystallinity index of 58.8% for the inside of cocoon. Although sample 3 showed the lowest crystallinity index (57.4%) for inside of cocoon, it displayed medium level crystallinity (59.8%) for outside. These results imply that the crystallinity indexes of outside and inside of silkworm cocoon are different

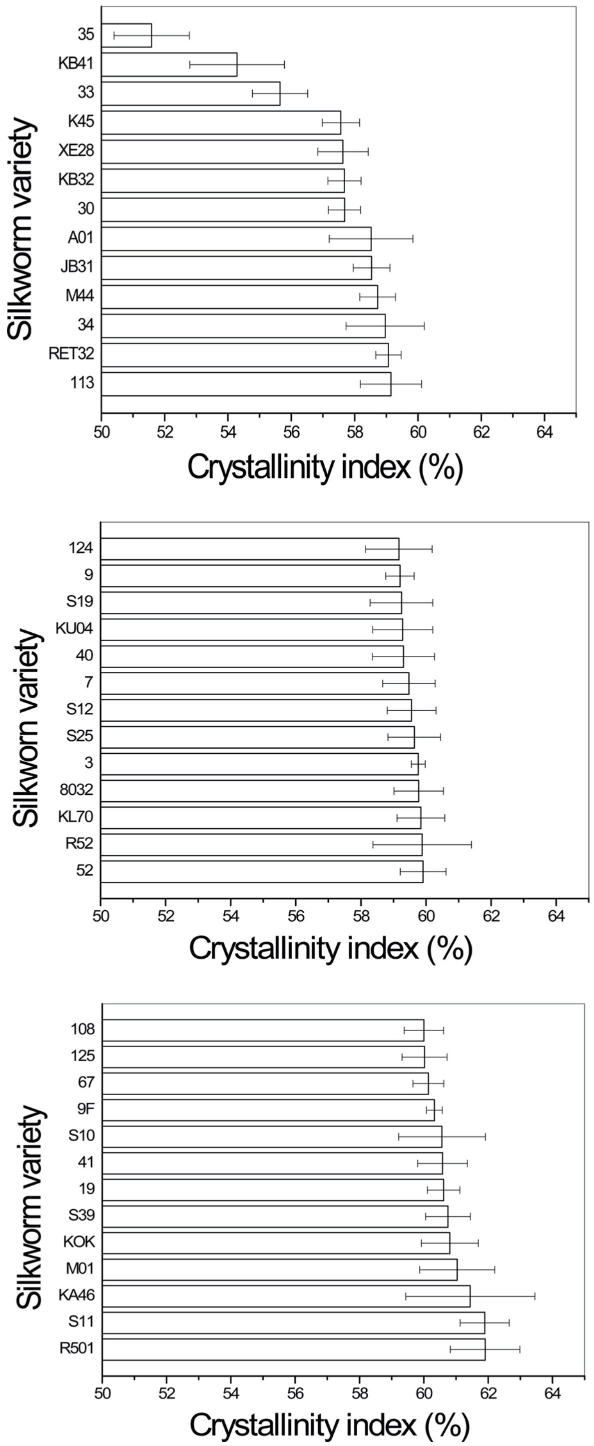


Fig. 2. Crystallinity index of outside of silkworm cocoon with different silkworm varieties.

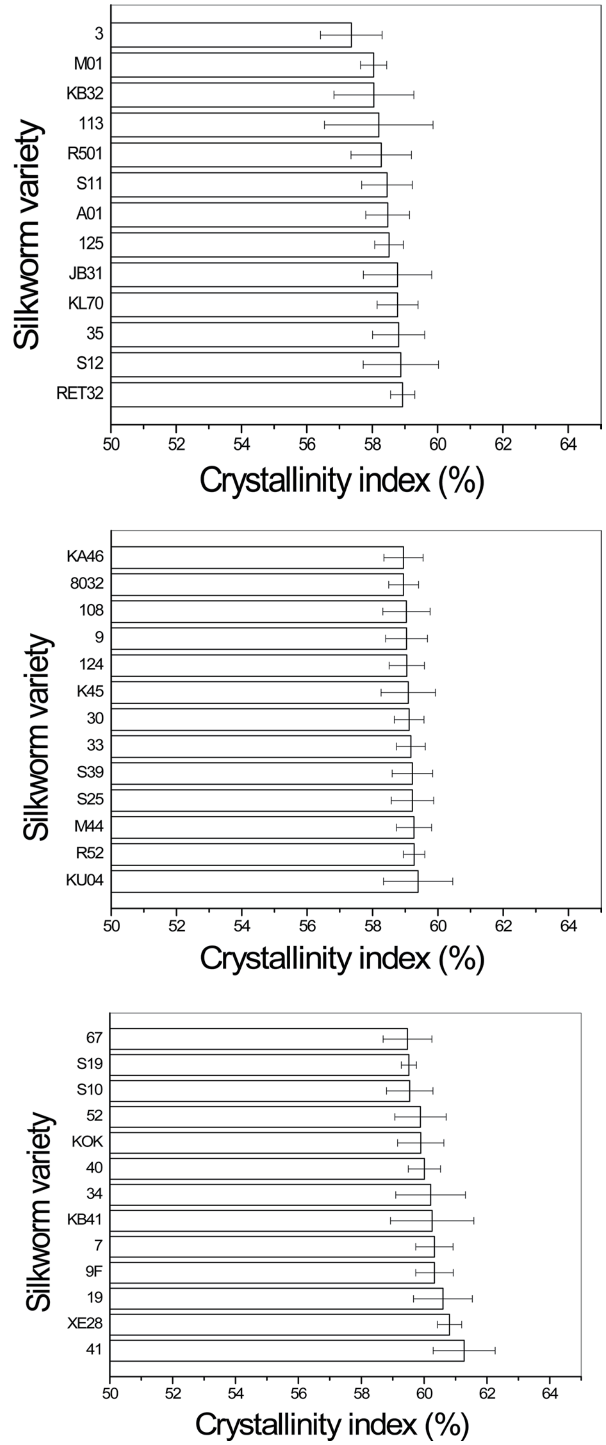


Fig. 3. Crystallinity index of inside of silkworm cocoon with different silkworm varieties.

depending on the silkworm variety.

Therefore, we plotted the crystallinity indexes of outside and inside of silkworm cocoon with different silkworm varieties and the result was shown in Fig. 4. As can be seen in the figure, the crystallinity indexes of outside and inside of silkworm cocoons were varied depending on the silkworm variety. We classified thirty nine silkworm variety silkworm cocoons into three groups by the difference between the crystallinity indexes of outside

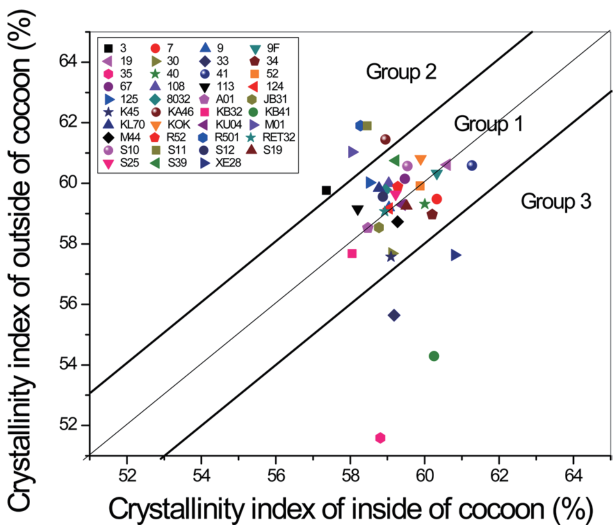


Fig. 4. Plot of crystallinity indexes of outside and inside of silkworm cocoon with different silkworm varieties.

and inside of silkworm cocoon. That is, Group 1 indicates the difference between crystallinity indexes of outside and inside is less than 2%. Group 2 indicates that the crystallinity index of outside is higher than that of inside by more than 2%. Group 3 indicates that the crystallinity index of outside is lower than that of inside by more than 2%. Most silkworm variety silkworm cocoons belong to Group 1. On the other hand, R501, M01, S11, KA46, and 3 belong to Group 2 and XE28, 35, M44, and KB41 belong to Group 3.

This result indicates the crystallinity index of silkworm cocoon and the difference of crystallinity index between inside and outside of cocoon are strongly affected by the silkworm variety. Considering the crystallinity of silk strongly affects the physical properties of silk including mechanical properties and moisture regain, it is very interesting to note that the crystallinity of silkworm cocoon varies depending on the silkworm variety, although the exact reason for this should be elucidated in the future study.

Conclusions

In this study, the molecular conformation and crystallinity of thirty nine silkworm variety silkworm cocoons were examined using FTIR spectroscopy. From the results of the measurement, it was found that the molecular conformation and crystallinity of silkworm cocoons were strongly affected by the silkworm variety. Although the more detail study should be conducted in the future to have a better understanding for the different crystallinity of silkworm cocoons depending on the silkworm variety, considering the crystallinity of silk strongly affects the physical properties of silk such as moisture regain and mechanical properties, these results can be effectively utilized in the application of silk. That is, silk can be used diversely in biomedical and cosmetic applications and various performances of silk are necessary for the successful use in these applications. Considering the various silkworm variety cocoons may provide various properties and performances in these applications, it is thought that the finding of this study will be helpful to apply the silk in the biomedical and cosmetic applications.

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