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Preparation and Characterization of Eri (*Philosamia ricini*) Silk Fibroin Powder

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Abstract: Eri (*Philosamia ricini*) cocoons were dissolved with 9 M $Ca(NO_3)_2$ and then dialyzed against distilled water for 3 days. The Silk Fibroin (SF) solution was used as substrate to prepare SF powder by using freeze-drying method. The secondary structure and thermal behavior of SF powder were determined by FT-IR and TGA analyzer, respectively. The SF powder was arranged in micrometer sizes. FT-IR spectra indicated that the SF powder composed of α-helix and β-sheet structures and differed from silk cocoon. Thermal properties were studied by thermogravimetric technique. Difference in thermal properties between Eri silk cocoon and SF powder was found. It is a promising that conformational structure and thermal properties were influenced by changing of silk forms.

Key words: Silk fibroin, silk powder, secondary structure, thermal decomposition

INTRODUCTION

Natural polymers have been wildly used in biotechnological and biomedical applications. These were due to their unique properties including nontoxicity, biocompatibility and biodegradability. Unfortunately, natural homopolymer is inadequate to meet the diversity of demands for biomaterials by itself (Kweon *et al*., 2001). Silk is a kind of natural polymer which produced by the family Bombycidae (domestic silk; *Bombyx mori*) and Saturniidae (wild silk; *Antheraea pernyi*, *Philosamia ricini*, etc.) of the order Lepidoptera (Dash *et al*., 2007). Silk is one of the most valuable materials for applications such as the textile and biomedical devices (Taddei *et al*., 2006). Generally, silk fiber consists of two main proteins; fibroin and sericin. Silk Fibroin (SF) is a typical fibrous protein and shows excellent both physical and chemical properties. SF has been used in various fields such as cosmetics, food additives and medical materials (Min *et al*., 2004). In addition, SF can be prepared and used in various forms including gel, powder, fiber or membrane, depending on application (Park *et al*., 2004).

The efforts at adjusting or preparing the biomaterial forms to their required functions are the aims in last decades. The silk powder is a kind of material form and now produced commercially as an additive in cosmetics and functional foods. Moreover, potential application of silk powder including surface coating, fibre treatment, fillers in films, ink, wound care, enzyme immobilization, composite scaffold for cell growth and drug delivery have also been reported by Rajkhowa *et al*. (2009). Making available novel versatile biomaterials addressing a broad range of biomedical needs is a crucial technological challenge (Taddei *et al*., 2006). Recently, much attention has been studied on *B. mori* either in producing process or application forms (Park *et al*., 2004). Contrast few studies have been carried out on the wild silk SF, especially Eri (*P. ricini*) silk.

Silk Fibroin (SF) powder can be prepared either by a solution route or by a mechanical method. With the solution route, SF is firstly dissolved in a concentrated salts followed by removal of the salts and then produced regenerated SF powder (Yoshimizu and Asakura, 1990; Jin and Young, 2001). On the other hand, the mechanical method avoids lengthy, costly and environmentally sensitive production process (Rajkhowa *et al*., 2009). It has been reported that dissolution of the SF fiber of wild silk is hardly work. In addition, dissolution of SF is often required for non textiles applications (Kweon *et al*., 2000).

Therefore, the aims of this study are to prepare and characterize SF powder of Eri (*P. ricini*) silk. The conformation structure and thermal properties of the silk were investigated by using Fourier Transform Infrared (FT-IR) and TA-Instrument TG SDT Q600 thermogravimetric analyzer, respectively.

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MATERIALS AND METHODS

This study was constructed for 5 months from October 1, 2008 to March 5, 2009. The preparation of SF powder and characterization were performed at the Central Instrument, Faculty of Science, Mahasarakham University.

Materials: The Eri (*P. ricini*) silk cocoons were kindly supplied from Silk Innovation Center (SIC) Mahasarakham University, Thailand. The cocoons were degummed twice using 0.5% Na_2CO_3 (w/v) and thoroughly rinsed 2 times in warm distilled water. They were then air-dried at room temperature.

Dissolution of silk fibroin: The Eri SF was dissolved with 9 M Ca $(NO_3)_2$ solution followed by Tao *et al*. (2007). Briefly, dried Eri SF was mixed with dissolving solution of 1 g SF to 10 mL of 9 M $Ca(NO_3)_2$ solution. The solution was firstly warmed to about 90°C on the hot plate, then gradually added SF into the solution and magnetic stirred at 100-105°C until SF completely dissolved (approximately 45 min). The SF hydrolysate was filtrated and then dialyzed in cellulose tube against distilled water for 3 days at room temperature.

SF powder preparation: There are many reports about SF powder preparation such as spray drying (Rajkhowa *et al*., 2009). However, lyophilization or freeze-drying method is a simple and popular method (Yao *et al*., 2004). Fifty milliliters of SF solution was freeze-dried about 2 days. The obtained SF powder was then grinned by using mortar and pestle to obtain fine powder. The SF powder was returned to dehydrate in vacuum oven before storing in desiccators. They were then observed their appearances and also measured particle sizes by sieving method.

Investigation of SF powder: The samples were prepared using KBr disc in the ratio of 9:1 (KBr : sample) and then analyzed with FT-IR (Perkin Elmer-Spectrum Gx, USA) in the spectral range of 4000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans. A TA-Instrument TG SDT Q600 thermogravimetric analyzer was used to determine the thermal behavior. The analysis condition was 50-1000°C for heating sample at 20°C min^{-1} rates under nitrogen atmosphere. Difference of silk structure between Eri and *B. mori* was compared.

RESULTS

The Eri SF powder could be prepared. Generally, the powder appeared light white color like cocoon and luster.

Table 1: Absorption bands of FT-IR spectra of the SF in different forms

	Amide (cm^{-1})			
SF forms	I	II	III	IV
Eri fiber	1655, 1702	1559	1235	694
Eri powder	1643	1542	1235	694
B. mori powder	1655/1725	1559	1235	704

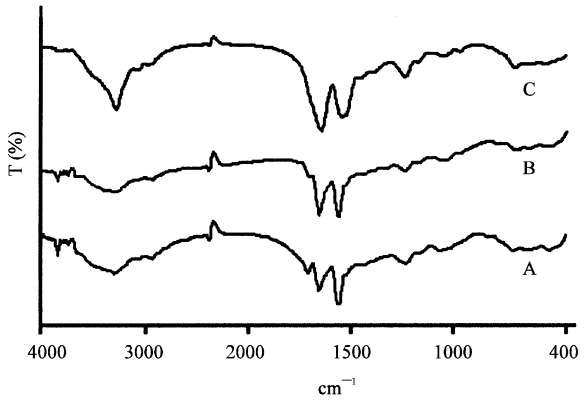

Fig. 1: FTIR spectra of the different forms of SF, (A) *B. mori* SF powder, (B) Eri SF fiber and (C) Eri SF powder

SF powder was arranged in the micrometer of their sizes under 200 micrometers pore sieved.

FT-IR spectra: Table 1 showed the absorption bands of silk cocoon and SF powder. The absorption bands of Eri silk cocoon appear at 1655 cm^{-1} with shoulder peak at 1702 cm^{-1} (amide I), 1559 cm^{-1} (amide II), 1235 cm^{-1} (amide III) and 695 cm^{-1} (amide IV). Contrast FT-IR spectrum of the SF powder showed intense absorption bands at 1643 cm^{-1} (amide I), 1542 cm^{-1} (amide II), 1235 cm^{-1} (amide III) and 695 cm^{-1} (amide IV). The FT-IR result of *B. mori* powder appeared at similar absorption bands of Eri SF powder, except at amide IV (Fig. 1).

Thermogravimetry: At low temperature, Eri silk has decomposed weight higher than that of *B. mori* silk. However, both SF fiber and powder of *B. mori* silk started decomposition at lower temperature than Eri silk about 300°C which completely decomposed at 360°C (Fig. 2). The Eri silk underwent of three thermal decomposition stages which are 200 to 300°C, 300 to 350°C and complete decomposition at approximately 350 to 400°C. The detail of those decomposition peaks were clearly evidenced by differential thermogravimetric (DTG) curves (Fig. 3). The maximum decomposition temperatures of Eri SF fiber were 235, 350 and 400°C, while Eri SF powder was 235, 350 and 390°C.

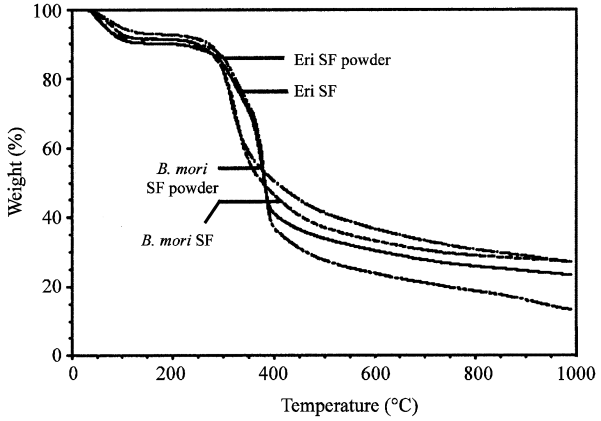

Fig. 2: TG curves of SF in different forms

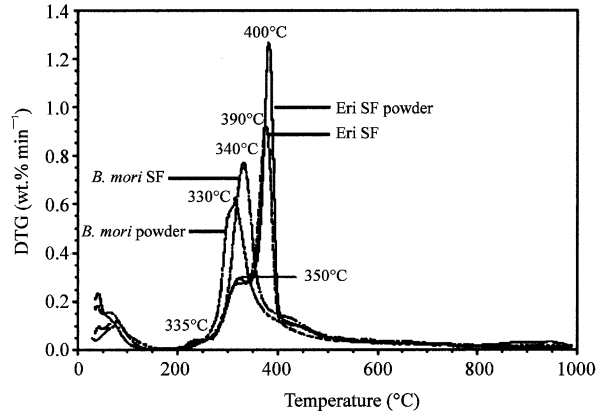

Fig. 3: DTG curves of the SF in different forms

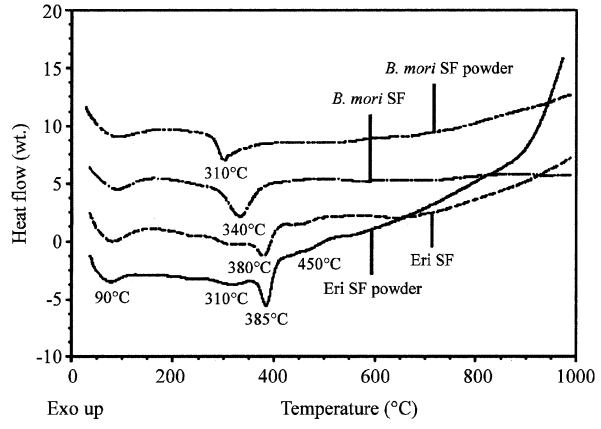

Fig. 4: DSC thermograms of the SF in different forms

Differential scanning calorimetry: With DSC thermograms, the peak at below 100°C distributions to the dehydration of the SF appeared in all of samples. The Eri silk showed multiple endothermic peaks at approximately 310, 380 and 450°C. Contrast the *B. mori* silk occurred strong single step at 310 and 340°C for SF powder and SF fiber, respectively (Fig. 4).

DISCUSSION

Eri SF solution could be achieved by dissolving with 9 M $Ca(NO_3)_2$. The SF solution was then used to construct SF powder and subject to investigate properties. In general, silk protein composed of many amino acid residues. They were linked with peptide bonds. The secondary structures of silk protein are indicated by the amide groups of the amino acid components (Kweon *et al*., 2000; Hino *et al*., 2003). The amide I (1700-1600 cm^{-1}), amide II (1600-1500 cm^{-1}), amide III (1300-1200 cm^{-1}) and amide IV (700-600 cm^{-1}) bands were measured. From FT-IR results, the lower wave number bands of amide I and amide II of the Eri SF powder were assigned to β-sheet structures (Kweon *et al*., 2001). The result indicating that the main structures of Eri SF powder were co-existed of α-helix and β-sheet structures with predominantly β-sheet form (Tao *et al*., 2007). It is suggested that the step of SF powder preparation could be induced structural transition of the amino acid components of the SF. The result may suggest that the dehydration of moisture from the silk could be affected on the silk structure. However, FT-IR result of the Eri and B. mori SF powder were differed. Thermal properties of domesticated silk, *B. mori* took place in a single step; contrast from wild silk, *P. ricini* which underwent at least two steps (Kweon *et al*., 2000). This might be expected that structure and amino acid between *B. mori* and Eri SF were differed. In addition, decomposition temperature of Eri silk is similar to other wild silk, especially *A. pernyi*. However, they are different in details such as the endothermic peaks at about 310 and 380°C of Eri silk which were differed from *A. pernyi* (Kweon *et al*., 2000). The differences of some properties between wild silk should be affected by the unique strain and genetic information. These mean that amino acid compositions and bond formation between them were main factors on the silk characteristics. It was also observed that the maximum decomposition temperatures of Eri SF powder and SF fiber were higher than that of *B. mori* SF powder or SF fiber. The result indicated that different characteristics of the silk were influenced by the components, form or silk strains.

CONCLUSION

The Eri SF powder could be achieved by the present study. The obtained SF powder was light white color,

luster and arranged in micrometer sizes. With FT-IR spectra, the SF powder co-existed of α-helix and β-sheet structures and differed from the Eri SF fiber and *B. mori* SF powder. Those of TG, DTG and DSC indicated that the thermal behavior could be affected by silk form and strain.

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