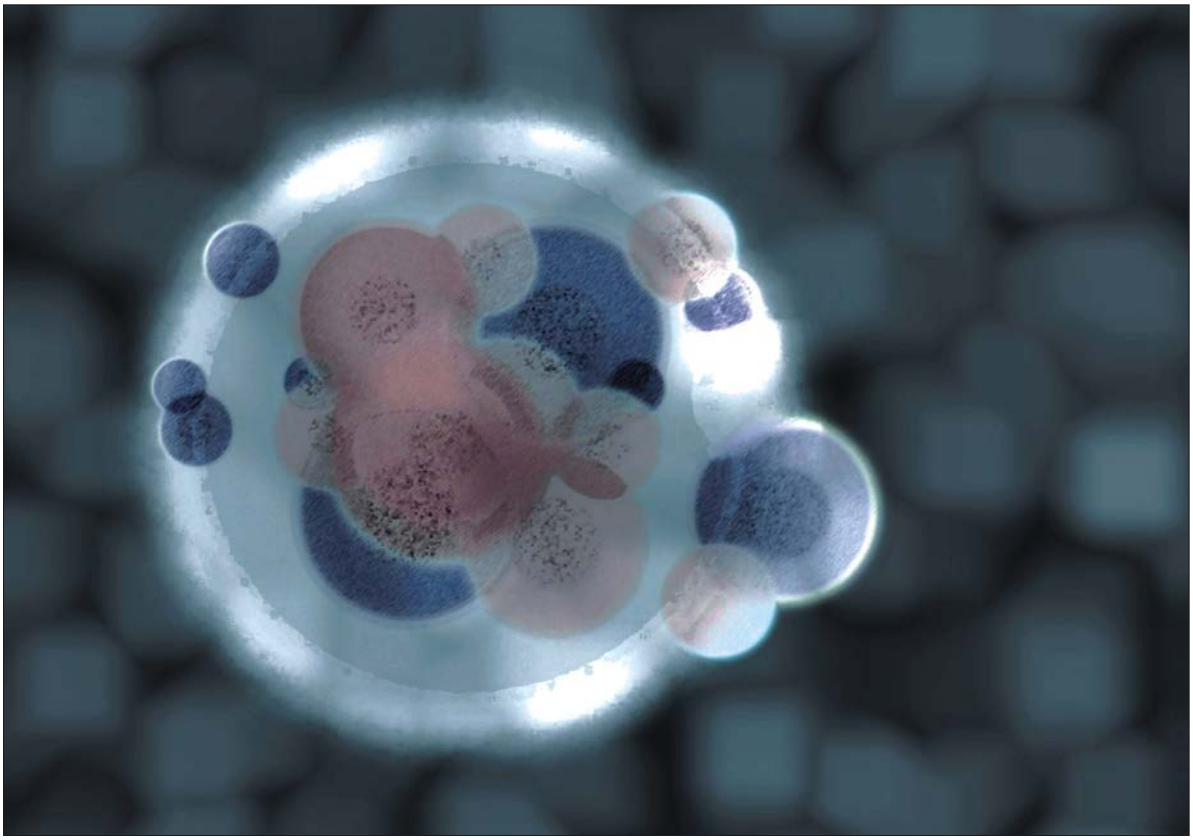

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Cross-Section Images of Eri (*Samia ricini*)-Silk Fibers and Their Secondary Structures after Treatment with Different Organic Solvents

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Abstract: This study was aimed to study the surface morphology of Eri (*Samia ricini*) silk cocoons collected from different sources called China, Chaingmai, Lampoon, SIC (Silk Innovation Center) and Thai varieties. All of Eri silk fibers were prepared by hand. They were studied under scanning electron microscope (SEM) for morphological observation. The cross-section images showed China has composed of porous structures in higher ratios than Chaingmai and Lampoon, respectively. In contrast, SIC and Thai composed of nonporous structures. In addition, the effect of different organic solvents on each Eri silk fiber was also investigated. Eri silk fibers were treated with methanol, ethanol, ethyl acetate and methyl acetate in different incubation times and concentrations before examining with attenuated total reflection infrared (ATR-IR) spectroscopy. The results found that methanol showed the highest effect to change the structure of Eri fibers, compared to ethanol. Ethyl acetate and methyl acetate showed similar activity to enhance β-sheet form and lower than that of methanol and ethanol, respectively. The methanol concentrations and incubation times which suitable for China, Chaingmai, Lampoon, SIC and Thai were 40% (3 h), 40% (2 h), 60% (1 h), 60% (3 h) and 60% (3 h), respectively. From the results, SIC and Thai were considered as higher strength than those of China, Chaingmai and Lampoon. This was due to the fiber of the last group composed of porous structures in higher ratios than that of first group.

Key words: Eri silk, surface morphology, organic solvent, porous structure, secondary structures

INTRODUCTION

Silk is a fiber polymer that is spun into fibers by some Lepidoptera insects such as silkworm, spiders, scorpions, mites and flies (Altman *et al*., 2003). Each silk fiber consists of an insoluble fibroin (SF, ~72-78%) and sericin glue-like protein (~19-28%) (Gamo *et al*., 1977; Takasu *et al*., 2002). By the nature of food source, silks are generally classified into mulberry (domesticated) and non-mulberry (wild) silks (Acharya *et al*., 2009). From the past to present, silk has been used in many fields, especially silk fibroin (SF). Biomedical materials such as wound dressing, controlled drug release carriers, tissue-engineering scaffolds were applied from silk. Among the types of silk, mulberry silk has been widely studied by Tao *et al*. (2007). However, application of wild silk was also reported by Acharya *et al*. (2008, 2009) and Fang *et al*. (2009). Wild silk of *Antheraea* is the most familiar genus which was wildly studied and applied by Fang *et al*. (2009).

Eri (*Samia ricini*) is a kind of wild silk that produced and promoted to rear in Thailand, especially in the northeast area. This land usually plant of cassava which is the second host plant of the Eri silkworm. Eri cocoons are mouth-opened and high porous structure in the fiber which considered difficult reel into fibers for using. However, Eri fibers can be reeled and reported in Thailand. Mechanical properties of silk fiber are the one factor attracted to study. This property influenced with secondary structures of protein. In general, kinds of secondary structures of protein are α-helix, β-sheet, β-turn and random coil (Tao *et al*., 2007). Moreover, they can be divided as amorphous (silk random coil or I), crystal (silk II or α-helix and β-sheet) and interfacial (silk III) parts (Vepari and Kaplan, 2007). Indeed, β-sheet structure can be induced by some chemical reagents such alcohol (Jeong *et al*., 2006), salt (Vepari and Kaplan, 2007), hexane (Valluzzi *et al*., 1999) or heat (Freddi *et al*., 1997). On the other hand, differences in structure and properties of silk fiber depending on their specific source and

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function (Tsukada *et al*., 2010). Therefore, the objectives of this study are to investigate the surface morphology of Eri silk fibers collected from different sources and to study the effect of different organic solvent on secondary structures of each Eri silk fibers.

MATERIALS AND METHODS

This project was done for 3 months from August 1, 2010 to November 3, 2010. Most of the experiment was done at Department of Chemistry, Faculty of Sciences, Mahasarakham University, Thailand.

Materials

Eri cocoons: Five Eri cocoons called Chaingmai, China, Lampoon, SIC and Thai were gathered and kindly supplied by Silk Innovation Center (SIC), Mahasarakham University, Maha Sarakham, Thailand. The Eri cocoons were dried in oven at 60°C and then kept in the clean and air-dried until use.

Methods

Silk fiber preparation: Eri cocoons were boiled twice in 0.5% (by weight) of Na_2CO_3 solution at 90-95°C and thoroughly rinsed 2 times in warm distilled water. The degummed cocoons were immersed immediately to help into fibers. The reeling method was applied from hand traditional technique. The cocoons were boiled in hot water and then reeled by hand.

Morphology observation: Eri silk fibers of all sources were cut and observed under the scanning electron microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan). The samples were sputter coated with gold by double side of carbon for enhancing surface conductivity.

Solvents and incubation times: The organic solvents used in this study are methanol, ethanol, ethyl acetate and methyl acetate. The incubation times were assigned for 1, 2 and 3 h while the concentrations of organic solvents were adjusted at 40, 60 and 80% (v/v). All of Eri silk fibers were immersed in each organic solvents and concentration at designation time points. The fibers were left in air-dried before further determination.

Secondary structure determination: All Eri silk fibers were determined their secondary structures using attenuated total reflection infrared (ATR-IR) spectroscopy (Perkin Elmer-Spectrum Gx, USA) in the spectral region of ~2000-500 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans.

RESULTS AND DISCUSSION

The morphology of silk fibers might be differed when produced by different varieties of silkworms. From SEM micrographs, they can be divided into 2 groups; porous and non-porous structures. The first group composed of China (Fig. a1), Chaingmai (Fig. b1), and Lampoon (Fig. c1) while the second one composed of SIC (Fig. d1) and Thai (Fig. e1), respectively. SIC have most identical 2 filaments in each fiber without separation part of cross-section as like as Thai. However, Thai slightly differed from SIC in fiber shape. China composed of the highest porous content than those of porous Eri group. Considering from cross-section images, porous in each variety slightly differed. Lampoon has dense texture than China and both are significantly differed comparison to Chaingmai. The last Eri showed variety of pore sizes which indicated as non-homogeneous texture. As shown in Fig. 2, the absorption peaks of amide I showed at 1685 and 1643 cm^{-1}, amide II at 1543 cm^{-1}, respectively. Figure 3 showed absorption peaks after incubation of China and Chaingmai (Fig. 4) silk fibers with 40% methanol. After 1 and 2 h, the absorption peaks of China showed at 1642 cm^{-1} (amide I) and 1542 cm^{-1} (amide II). Difference peaks occurred when incubated the silk fiber for 3 h, especially amide II (1559, 1522 cm^{-1}). As shown in Fig. 4, the absorption peaks of Chaingmai silk fiber showed amide I region at 1653 cm^{-1}, amide II (1575 cm^{-1}) in 40% methanol. Increase of incubation time, the absorption peaks were changed into lower wave number for 2 h at 1647, 1533 cm^{-1} (amide I, II) and 3h at 1638, 1541 cm^{-1} (amide I, II). 60% methanol showed suitable concentration for enhancing the β-sheet structure of Lampoon (Fig. 5), SIC (Fig. 6) and Thai (Fig. 7). ATR-IR spectra indicated that incubation time did not affect on the transitional changes of absorption peaks. All of silk fibers showed absorption peaks at ~1637 and 1541 cm^{-1} for amide I and amide II, respectively. However, Thai have an amide II region with gradually decreased of wave number when increased the incubation time.

The cross-section of silk fibers differed slightly in their filament structures. Generally, wild silk have a porous structure and contain fine tubules (Akai and Nagashima, 1997). Comparison to previous report, the present result showed slightly different since all of silk cocoons are Eri. There have also been reported about some different points between domesticated (*B. mori*) and wild silk (Saturniidae family) (Mahendran *et al*., 2006a, b). The main different point is tripeptide sequences of Arg(R)-Gly(G)-Asp(D) which is a recognition site for cell adhesion (Pierschbacher and Ruoslahti, 1984; Ruoslahti and Pierschbacher, 1987). In addition, it is result

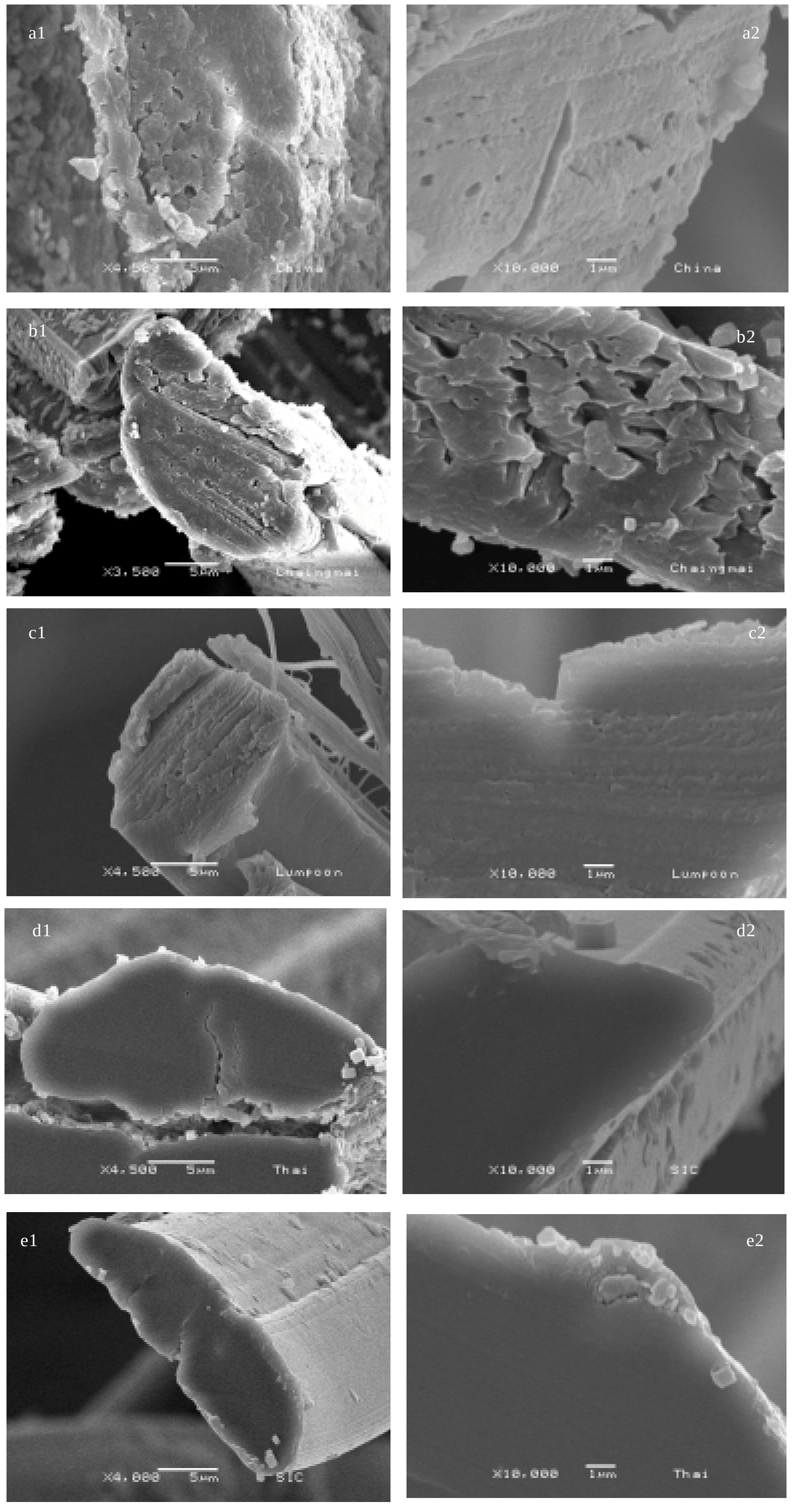

Fig. 1: Cross-section images of Eri silk fibers gathered from different sources; (a) (China), (b) (Chaingmai), (c) (Lampoon), (d) (SIC) and (e) (Thai). Low magnification presented in left and high magnification presented in right

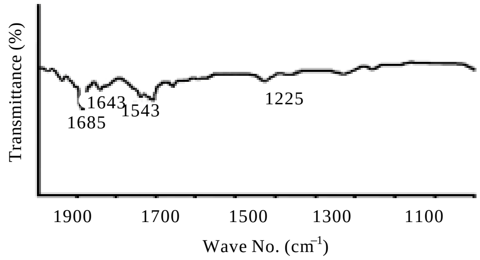

Fig. 2: ATR-IR spectrum of Eri silk fibroin (control)

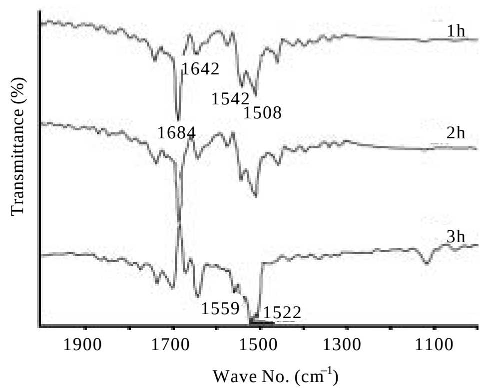

Fig. 3: Effect of incubation times on secondary structure of Eri (China) silk fiber treated with 40% methanol

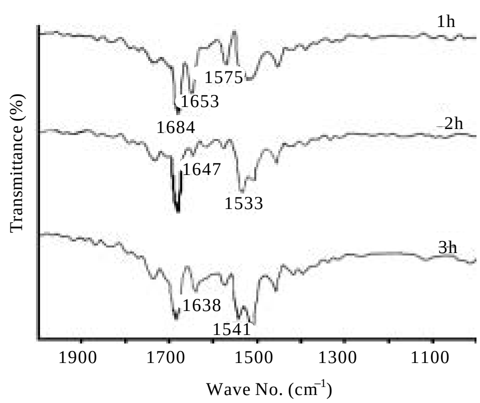

Fig. 4: Effect of incubation times on secondary structure of Eri (Chaingmai) silk fiber treated with 40% methanol

to the rough surface of the silk fiber as well. This tripeptide is not found in silk fibroin fiber of *B. mori* (Acharya *et al*., 2009). On the other hand, the amino acid composition of Eri silk fibroin differed from mulberry silk, especially alamine content. Silk fibroin of the Eri or other wild silks composed of alanine residues in higher content

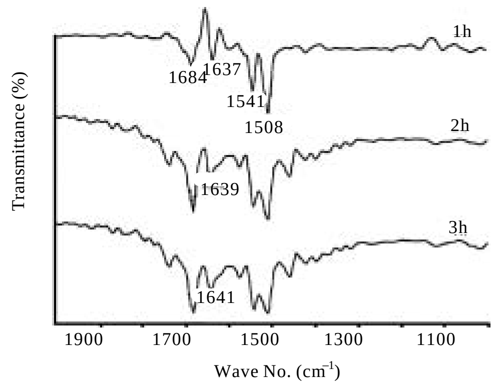

Fig. 5: Effect of incubation times on secondary structure of Eri (Lampoon) silk fiber treated with 60% methanol

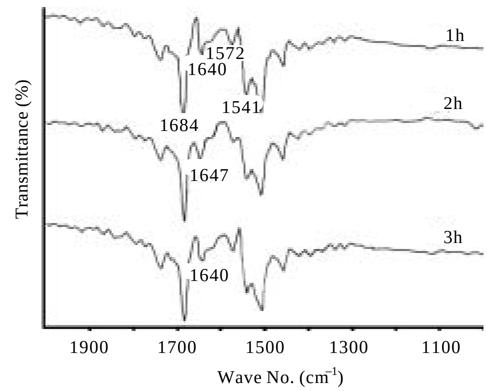

Fig. 6: Effect of incubation times on secondary structure of Eri (SIC) silk fiber treated with 60% methanol

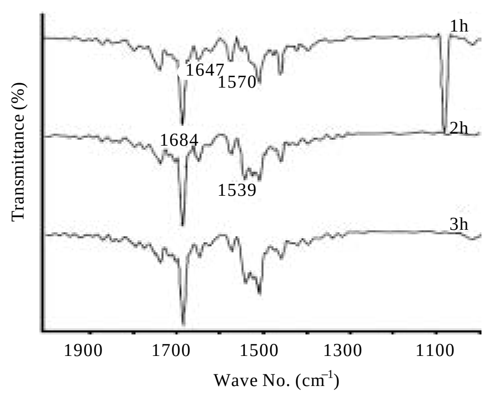

Fig. 7: Effect of incubation times on secondary structure of Eri (Thai) silk fiber treated with 60% methanol

than that of *B. mori*. The SEM micrographs showed that surfaces of Eri fiber relative to the porous structure. This was due to Eri with high porous content showed rougher surfaces than non-porous fibers. Moreover, the climate of local area of cocoon gathering might be affected on the porous as well as surfaces of the Eri silk fiber even those Eri were collected in Thailand. However, China, Chaingmai and Lampoon were the North of Thailand which has low temperature than SIC, and Thai (reared in the North-East of Thailand). Therefore, temperature may be an important factor on the properties and morphology of Eri silk fibers.

Secondary structures investigation: The secondary structures of the Eri silk fibroin treated with different organic solvents were analyzed using ATR-IR. Comparing with the control (Fig. 2), Eri fibers treated with methanol shows the most affect on the secondary structures of the fibers than other solvents. Therefore, methanol was chosen as solvent for study the suitable condition. The results found that suitable concentration and incubation times of the Eri silk fibers in methanol for China, Chaingmai, Lampoon, SIC and Thai were 40% (3 h) (Fig. 3), 40% (2 h) (Fig. 4), 60% (1 h) (Fig. 5), 60% (3 h) (Fig. 6) and 60% (3 h) (Fig. 7), respectively. The conformational structure is an important part and significantly affected on protein (Lee *et al*., 2003). From the previous reports, IR spectrum indicates typical absorption bands sensitive to the molecular conformation of protein, especially SF (Kweon *et al*., 2000; Tasukada *et al*., 1995). The structures of the silk fibroin protein are indicated by the amide I (1700-1600 cm^{-1}), amide II (1600-1500 cm^{-1}) and amide III (1300-1200 cm^{-1}) regions (Hino *et al*., 2003; Kweon *et al*., 2000). The present study found that alcohol has strongly affected on the transition of protein structure. It is suggested that alcohol can be induced the strength of protein structure of Eri fiber by increasing secondary structure (β-sheet) more than others. From ATR-IR spectra, methanol showed high effect on the transition of the SF structure. The results indicated that the amide I and amide II bands transited into lower wave number compared to other solvents. This result might be suggested that molecules composed in silk fibroin conformation were closed to form interaction. It is also thought that the strength of the Eri SF after submersion in methanol was enhanced by inducing structural transition from random coil to β-sheet structure (She *et al*., 2008). This result was similar to those of previously reported by Jeong *et al*. (2006), Kweon *et al*. (2000), Lee *et al*. (2003) and Tao *et al*. (2007). However, suitable condition for inducing the transitional change of the secondary structures of the Eri silk fiber differed depending on such Eri source.

CONCLUSION

Cross-section images showed that China composed of higher content of porous structures than that of Chaingmai and Lampoon, respectively. Surprising, SIC and Thai Eri are non-porous fibers. The porous as well as rough surfaces of the fiber might be concerned alanine residues composition. On the other hand, temperature of local area may be also influenced on the porous or surface of Eri fibers. ATR-IR spectra showed methanol was strongly affected of the transitional changes of the Eri SF compared to other solvents. The results suggested that the intermolecular interactions between amino acids molecules were formed according to water dehydration after treatments. The results also found that suitable condition for inducing secondary structures was varied by the Eri source.

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