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Screening of Some Elements in Different Silk Cocoon Varieties

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Abstract: The aim of this study was to screening of some elements in different silk cocoon varieties. The varieties of silk were Thai silk (yellow color; Samrong, Nangtui, Nanglai, Huafai, Nangnoi, Nanglaung, Nangseiw, Surin4, Nongkhai012, Green and Gold colors), wild silk (Eri), Japanese and Chinese silk. The cocoons were collected and subjected for investigation of element composition by using SEM/EDX technique. The results found that the elements were arranged in following order of C>O>Ca>Si>Cl>S>Mg for inner surfaces of silk cocoons. For the outer surfaces, all elements were distributed in all Thai strain which slightly differed for each silk strain. Among them, C and O were found in high percentage in all silk cocoons both inner and outer surfaces. Japanese showed higher element than Chinese, but lower than Thai silk varieties. The result indicated that elements may be involved the silk spinning process of silk fiber to promote β-sheet structure.

Key words: Cocoon, element, silk varieties, eri, SEM/EDX, silk fibroin

INTRODUCTION

Silk is a kind of natural fiber which was produced by some insects including silkworm of Lepidopteran family; Bombycidae and Saturniidae (Dash *et al*., 2006). The silk is a protein from amino acids by peptide bond formation. In its chain, there are carbonyls and amino groups and also jointed to other side chain with amino, hydroxyl, carbonyl, guanido, imidazole and mercapto groups. These groups can be coordinated with transitional metal ions (Chen *et al*., 2005). It is well known that the properties of silk fiber properties depended on several parameters such as metal ions (Chen *et al*., 2002; Li *et al*., 2001; Hossain *et al*., 2003), viscosity and pH (Zhou *et al*., 2004).

The metal ions were reported to help in some way to refold the silk protein molecules (Knight and Vollrath, 2001). Any metal ions such as Cu^{2+} (Ochi *et al*., 2002), K^+, Ca^{2+} and Zn^{2+} (Li *et al*., 2001) on the properties of silk fiber has been reported. The results found that they could promote the random coil to α-sheet structure transition on the secondary structures. On the other hand, mechanical properties of silk are influenced by both primary structure amino acid sequence and spinning conditions such as temperature, pH, ionic strength, solvent composition and mechanical stress (Shao and Vollrath, 2002; Chen *et al*., 2001). During the fiber spinning, a combination of active dehydration, flow-induced forces and many metal ions are involved in the process (Hu *et al*., 2009). Therefore, metal composition in silk is interested to study. Many studied about metallic ions effect on silk fibroin have been reported. However, composition of metal after spinning in cocoon or fiber was rarely published. It is considered interest to investigate about the metal on them.

Therefore, this study aimed to screen the metal composition in silk cocoon both domestic (*Bombyx mori*) and wild (Eri) silks. The goal of this study is extended to screen about metal composition and different amount of them. The finding results might be led to know about metabolic relation of metal to silk fiber.

MATERIALS AND METHODS

This study was done from June 1, 2009 to December 10, 2009. Most of the experiment was carried out at Department of Chemistry and the Central Instrument Center, Faculty of Science, Mahasarakham University.

Materials: Silk cocoons for investigated were selected. The domestic silk is *Bombyx mori* (*B. mori*) which was a Japanese variety (Kinshu x Showa), Chinese variety and Thai varieties (locally called Samrong, Nangtuy, Nanglai, Huafai, Nangnoi, Nangluang, Nangsiew, Surin4,

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Nongkhai012). These cocoons were yellow color. In addition, silk cocoons with green and gold color were also collected for screening. For wild silk, Eri was chosen to compare with domestic silks. The food plant of *B. mori* was mulberry leaves and castor leaves was Eri's host plant. Both silk cocoons and food plants of silkworm were kindly supplied from Silk Innovation Center (SIC), Mahasarakham University, Thailand.

Methods

Silk cocoon preparation: All of cocoons were firstly washed with distilled water twice to exclude impurities. They were then air-dried at room temperature before taking in oven at 40°C for 24 h. The food plants of silkworm were dried in an oven at 40°C until completely dehydrated by weighing 3 times without weight changed. The cocoons were kept in a plastic bag until use.

Screening of metal compositions: Each sample was separately detected for quantitative metal determination. The samples were mounted on stubs with conductive carbon tape. All samples were analyzed for the metal compositions using Scanning Electron Microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan) and operating at 15 kV. The analyses of the data were mapped using Smiling program.

RESULTS

Different of cocoon colors and silk strain were considered and selected for study. The color characteristics of the silk cocoon used in this work is shown in Table 1. Thai silk strain is almost yellow, except Surin4, Green and Gold. Japanese and Eri are white while Chinese is pink pale color. The elemental compositions was analyzed by EDX instrument as shown in Table 2 and 3 for inner and outer surfaces cocoons, respectively. The results revealed that the emission of X-rays character spectrum of carbon (C), oxygen (O), calcium (Ca), magnesium (Mg), sulphur (S), silicon (Si), calcium (Ca), molybdenum (Mo), sodium (Na) and aluminum (Al). For inner surfaces, it was found that C showed the highest percentage of all elements about 51.58 (in gold cocoon)-58.32 (Nangsiew). The other elements were arranged in following order of C>O>Ca>Si>Cl>S>Mg (Table 2). Among the silk variety, green cocoons composed all of elements; the next is gold cocoon and then Nangsiew. C and O were found in high percentage in all silk cocoons. Chinese and Japanese varieties showed very similar both percentages and kind of elements with slightly differ from Thai silk variety. Eri (wild) cocoon showed high level of 2 metals which were Ca and Mg and also with Si. In Thai silk varieties, element components were slightly differed in percentages and element types. All of them found Ca with highest percentages of 0.54 for Nangluang. The Surin4 showed similar in element compositions compared to Nangsiew, green and gold cocoons. Mg found in Eri,

Table 1: Different colors of cocoon shell varieties

Silk varieties	Color of cocoon shells
Eri	White
Chinese	Pink pale
Japanese	White
Samrong	Strong yellow
Nangtui	Strong yellow
Nanglai	Yellow
Huafai	Strong yellow
Nangnoi	Strong yellow
Nangluang	Yellow
Nangsiew	Strong yellow
Surin4	White
Nongkhai012	White pale
Green	White with green
Gold	Brown with gold

Table 2: Types and contents of some elements founded in the inner surfaces of cocoon

	Types of element and contents (weight %)									
Silk varieties	C	O	Ca	Mg	S	Si	Cl	Mo	Na	Al
Eri	52.06	47.16	0.14	0.28	nd	0.37	nd	nd	nd	nd
Chinese	56.51	42.84	0.15	nd	0.41	nd	0.10	nd	nd	nd
Japanese	56.50	42.52	0.24	0.09	nd	0.09	nd	0.56	nd	nd
Samrong	58.08	41.43	0.32	nd	nd	nd	0.17	nd	nd	nd
Nangtui	57.10	42.11	0.25	nd	0.20	0.11	0.22	nd	nd	nd
Nanglai	55.54	43.91	0.28	nd	nd	nd	0.15	nd	nd	nd
Huafai	54.02	45.49	0.30	nd	nd	nd	0.20	nd	nd	nd
Nangnoi	55.50	44.07	0.12	nd	nd	0.12	0.19	nd	nd	nd
Nangluang	56.87	42.15	0.54	nd	nd	0.07	nd	0.37	nd	nd
Nangsiew	58.32	40.89	0.34	0.10	0.19	0.16	nd	nd	nd	nd
Surin4	56.06	42.36	0.36	0.28	0.19	0.47	nd	0.29	nd	nd
Nongkhai 012	55.25	44.11	0.24	nd	nd	0.21	0.19	nd	nd	nd
Green	54.35	43.27	0.49	0.13	0.11	0.80	0.13	0.33	0.27	0.12
Gold	51.58	45.46	0.18	1.32	0.10	1.26	0.12	nd	nd	nd

nd: Not detected

Table 3: Types and contents of some elements founded in the outer surfaces of cocoon

	Types of element and contents (weight %)									
Silk varieties	C	O	Ca	Mg	S	Si	Cl	Mo	Na	Al
Eri	55.01	44.88	nd	nd	0.03	0.06	nd	nd	nd	nd
Chinese	49.68	48.08	nd	nd	nd	0.28	nd	1.47	nd	0.06
Japanese	54.92	43.22	0.33	0.71	0.23	0.35	0.25	nd	nd	nd
Samrong	57.37	41.47	0.47	nd	0.09	0.37	nd	nd	nd	0.22
Nangtui	57.07	41.26	0.41	0.31	0.38	0.17	0.13	nd	0.27	nd
Nanglai	53.95	44.48	0.49	0.08	nd	0.26	nd	0.50	0.24	nd
Huafai	53.95	44.69	0.37	0.14	0.26	0.18	0.15	nd	0.26	nd
Nangnoi	53.69	45.26	0.46	0.30	0.17	nd	nd	nd	nd	0.11
Nangluang	56.16	42.30	0.77	0.29	0.35	0.13	nd	nd	nd	nd
Nangsiew	59.48	39.36	0.38	0.22	0.15	0.26	0.15	nd	nd	nd
Surin4	54.16	44.53	0.50	0.17	0.08	0.31	0.09	nd	nd	0.14
Nongkhai 012	45.03	44.19	1.12	0.59	nd	6.65	nd	nd	2.43	nd
Green	56.63	41.60	0.51	nd	nd	0.26	0.35	0.65	nd	nd
Gold	54.51	44.00	nd	0.64	nd	0.71	0.12	nd	nd	nd

nd: Not detected

Japanese, Thai (Nangsiew and Surin4) green and gold cocoons. It is a surprising that Si did not found in Chinese, Samrong, Nanglai and Huafai, but all of them composed of Cl. In addition, Mo was found in Nangluang, Surin4, green and Japanese cocoons. Moreover, Na and Al metals were found only in green cocoon. For outer surfaces of silk cocoons, all element investigated were distributed in all Thai silk varieties as well as the Japanese. Green and gold colors were next content from Thai silk and the lowest was found in Eri. However, Na and Al were found in many strains of Thai silk compared to inner surfaces. Among the elements, C and O were the highest (Table 3).

DISCUSSION

Silk fiber is a kind of fibrous protein which remarkable record as second strongest fiber in the world. There are many reports about the factors affected on the mechanical strength of the silk fiber such as food and environmental including silk varieties (Shao and Vollrath, 2002). On the other hand, silk processing is also a major factor on the silk fiber strength (Zhou *et al*., 2003). The silk protein composed of eighteen kinds of amino acids. The amino acids composed of many functional groups at the end and side chain. Those of them can be interacted with transitional metal ions (Chen *et al*., 2005; Taddei *et al*., 2003). A number of studies showed that metallic ions and pH may be involved in natural silk processing in silkworm and spider (Chen *et al*., 2002; Ochi *et al*., 2002).

In the recent, there has been interest in exploiting the metal binding capacity of natural protein fiber (Goto and Suyama, 2000) as well as about incorporating metal ions into fibrous polymer (Arai *et al*., 2001; Park *et al*., 1998). The previous reports showed that many metal such as Cu, Ag, Co, Na, Ca, or Mg are found in the silk fibers (Taddei *et al*., 2003; Zhou *et al*., 2003; Ruan and Zhou, 2008). Copper (Cu) is known to induce the formation of secondary structure (β-sheet) of protein (Lim *et al*., 1999). In addition, there are many reports about calcium (Ca) and magnesium (Mg) which were involved in natural spinning process of silkworm. Ruan *et al*. (2008) reported that magnesium is an important element for silk fiber formation especially the folding of fibroin. Therefore, metal presents in the silk may affect the β-sheet structure. Ji *et al*. (2009) reported that ferric ions can induce a conformation change from helix to β-sheet form in silk fibroin as well as sodium ion (Na^+) (Ruan and Zhou, 2008). On the other hand, thermal stability of silk protein structure was acted by calcium (Hu *et al*., 2009). Moreover, the metal content in different secretory parts of silk solution pathway has been reported (Zhou *et al*., 2003, 2005; Ruan *et al*., 2008).

In this study, contents and types of elements were differed from earlier study. This is due the different of silk culture, food plant and location of sericulture since it has been reported that these parameters were affected the silk fiber characteristics. In addition, different quantities are depending on both strain and part of cocoon study. It can conclude that many elements were observed even the final process of silk fiber production. This is strong evidence that metals are concerned in the silk spinning process including the transition of the secondary structure of silk protein. It is very interesting to study in expand of the knowledge about the mechanism of those metal involved the process as well as the action of them. Furthermore, this work gave to know that metal elements found in each silk variety were significantly different. It is suggested that they may be involved the secondary structure of the silk fiber which affected to the silk fiber strength. This was due to Thai silks, wild silk (Eri), Japanese silk or Chinese silk have different in mechanical properties as like as their characteristics. However, the data should be investigated in higher information into used as supporting the description.

CONCLUSION

The SEM/EDX results revealed the character spectrum of the elements were arranged in following order of C>O>Ca>Si>Cl>S>Mg for inner surfaces of silk cocoons. For the outer surfaces, all elements were distributed in all Thai strain. Among them, C and O were found in high percentage in all silk cocoons both inner and outer surfaces. Japanese showed higher element than Chinese, but lower than Thai silk varieties. It is a promising that elements may be involved the secondary structure of silk fiber to change the helix into β-sheet form. However, the information about the action of that element has not been clear nowadays.

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