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Structure and Thermal Characteristics of *Bombyx mori* Silk Fibroin Films: Effect of Different Organic Solvents

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Abstract: The aim of this study compare the effect of different organic solvents on structure and thermal characteristics of Silk Fibroin (SF) films. Silk fibroin films were prepared by casting the SF solution using evaporating technique. The SF films were treated with various organic solvents; 80% acetone, 80% ethanol, 80% ethyl acetate and 80% methanol. After treatment, they were investigated their secondary structure and thermal behavior using Fourier transform infrared (FTIR) spectroscopy and thermogravimetric analysis, respectively. Fourier transform infrared results found that the secondary structures of SF films were changed after treatment SF films with ethanol, ethyl acetate and methanol, except acetone. From the results of FTIR, TG, DTG and heat flow thermograms, methanol showed higher effect to enhance the stability of SF films compared to other. It is a promising that ethanol, ethyl acetate and methanol could be used to enhance the stability of SF films.

Key words: Silk fibroin, organic solvent, secondary structure, thermal stability

INTRODUCTION

Biomaterials have attracted much attention in various applications. Silk Fibroin (SF), a natural fibrous protein produced by *Bombyx mori* (*B. mori*) silkworm (Jin *et al*., 2002), has been as a focusing materials for nontextile applications including biomedical and biotechnological fields (Tasukada *et al*., 1995). Silk fibroin is composed of excellent biological, chemical, physical as like as mechanical properties (Altman *et al*., 2003). Silk fibroin is valuable and can be processed into different forms including film, membranes (Freddi *et al*., 1997), gel, powder and porous (Tasukada *et al*., 1995) depending on applications (Park *et al*., 2004). Among them, SF film has wildly been applied in various fields (Kweon *et al*., 2000; Acharya *et al*., 2009). In addition, SF is also exhibited unique properties including nontoxicity, biocompatibility and biodegradability (Foo and Kaplan, 2002).

Silk fibroin films often require chemical treatments in order to enhance their stability and mechanical properties (Tsukada *et al*., 1995). Among chemical agents, methanol is one of frequently used to improve that stability and mechanical properties. However, treatment of

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SF with methanol can induce high β-sheet structure ratios, resulting of brittle materials and also occur instantly in an uncontrollable crystallization (Jeong *et al.*, 2006). In the present, there are many reports about solvent which were induced the secondary structure of the SF film. However, comparison their effect is a little conformation, especially in details.

In this study, SF films were prepared and treated with different kinds of organic solvents. The goal of the study is to explore structure and thermal characteristic of SF films after treatment with those of organic solvents using Fourier transform infrared (FTIR) spectroscopy and TA instrument, respectively. The effect of each organic solvent on SF film was compared and discussed.

MATERIALS AND METHODS

This study was carried out for 3 months from April 10, 2009 to July 10, 2009. The SF solution, SF films were prepared and analyzed for thermal properties at Department of Chemistry while characterization of secondary structure was performed at the Central Instrument, Faculty of Science, Mahasarakham University, Thailand.

Materials

The *B. mori* silk cocoons were kindly supplied from Silk Innovation Center (SIC), Mahasarakham University, Thailand. The cocoons were kept in air-dried room until use. Chemical reagents in analytical grade were used.

Preparation of SF Solution

The *B. mori* cocoons were washed with distilled water to exclude those of impurity and dried in oven at 40°C. They were boiled in 0.5% (w/v) $NaHCO_3$ solution at 95°C for 30 min, rinsed with distilled water. This step was performed twice to obtain pure SF. The cocoons was dissolved using tertiary solvent system of $CaCl_2$-Ethanol-H_2O (1:2:8 by mole), with magnetic stirred at 90-95°C for 2-3 h. The SF solution was then dialyzed using dialysis bag against distilled water for 3 days to exclude salts. The obtained solution was calculated percent weight and kept for preparing SF films.

SF Films Preparation

Silk fibroin films were prepared by evaporation method. The 1% (w/w) SF solution was cast on the 5 cm diameters polystyrene plates each with 10 mL. The plates were dried at 40°C in oven for 3 days. The SF films with a thickness about 50 μm were obtained.

Organic Solvents Treatment

The SF films were immersed in different kinds of organic solvents: 80% acetone, 80% ethanol, 80% ethyl acetate and 80% methanol for 30 min. After finish in each solvent, the SF films were left in air-dried and transferred into vacuum oven to make sure the films were completely dried. They were stored in desiccators prior to investigate.

Secondary Structure Analysis

All of treated organic solvents SF films were analyzed for their secondary structure using FTIR spectrometer (Perkin Elmer-Spectrum Gx, USA) in the spectral region of ~4000-500 cm^{-1} with 4 cm^{-1} spectral resolution and 32 scans.

Thermal Behavior Measurement

All of SF films cut into 8-10 mg were loaded in a platinum crucible. The thermogravimetric analysis was then performed using TA instruments, SDT Q 600

(Luken's drive, New Castle, DE). The samples were non-isothermal heated from 50 to 1000°C at a heating rate of 10°C min^{-1}. The TGA was carried out in nitrogen with the flow rate of 100 mL min^{-1}. The data were recorded with TA instrument's Q series explorer software. The analyses of the data were done using TA Instrument's Universal Analysis 2000 software (version 3.3B).

RESULTS

FTIR Spectra of SF After Treatment

The data showed some different characteristics which divided into two profiles. The FTIR of SF films treated with ethyl acetate, methanol and ethanol (group 1) showed similar profiles while SF film control has similar profile of acetone (group 2). The FTIR results found the different absorptions at amide 1 (1700-1600 cm^{-1}), amide 2 (1600-1500 cm^{-1}) and amide 3 (1300-1200 cm^{-1}). Generally, the absorption bands of SF films of group 1 appeared at 1635 cm^{-1} (amide 1), 1525 cm^{-1} (amide 2) and 1240 cm^{-1} (amide 3). The absorption bands of SF films of group 1 showed intense absorption bands in higher wave number compared to group 1 at 1650 cm^{-1} (amide 1), 1540 cm^{-1} (amide 2) and 1243 cm^{-1} (amide 3) as shown in Fig. 1.

Thermal Decomposition Analysis

Thermal characteristics of SF films were presented by TA analysis. It shows that the initial weight loss at below 100°C, was due to the water evaporation (Kweon *et al.*, 2001). At temperature over 200°C, the weight loss was occurred rapidly. TG curves indicated that ethanol and methanol have same maximum decomposition at about 305°C, ethyl acetate at about 302°C and the lowest for acetone at about 298°C (Fig. 2). All of SF films did not completely decompose even at 1000°C. The results also found that SF films underwent of single thermal decomposition stage at about 300°C. However, many shoulder peaks were also observed. The detail of those decomposition peaks were clearly evidenced by differential thermogravimetric (DTG) curves. The maximum decomposition temperatures of SF films treated with acetone, ethyl acetate, ethanol and methanol were 298, 302, 305 and 305°C, respectively (Fig. 3).

Heat Flow Determination

With heat flow thermograms as shown in Fig. 4, the peak at below 100°C distributions to the moisture loss of the SF appeared in all of SF films. Those of SF films showed multiple endothermic and exothermic peaks after treatment with different solvents. The results found

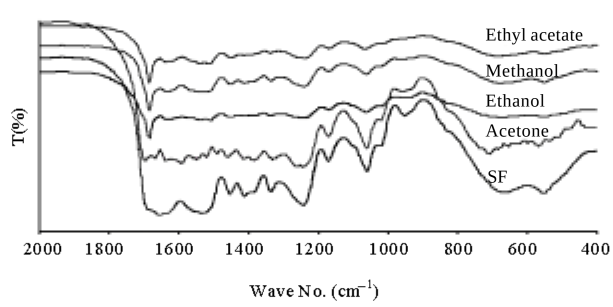

Fig. 1: FT-IR spectra of SF films treated with different solvents

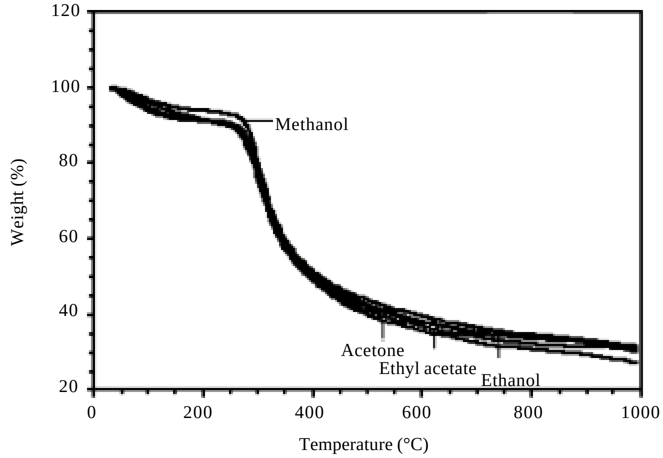

Fig. 2: TG curves of SF films treated with different solvents

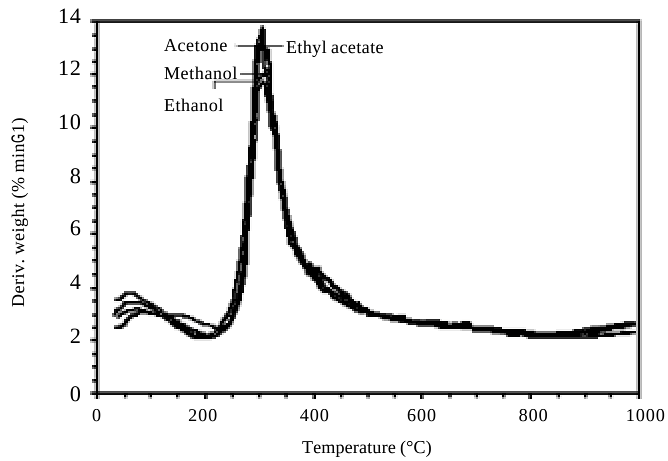

Fig. 3: DTG curves of SF films treated with different solvents

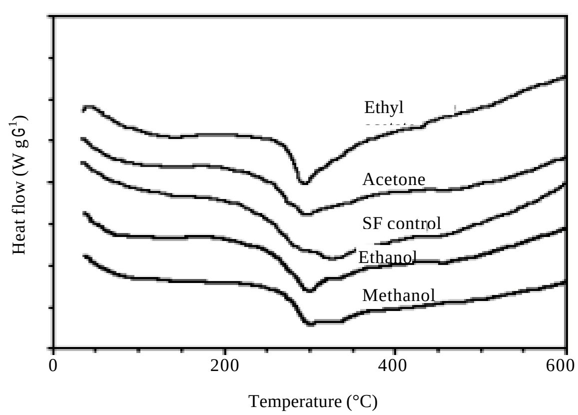

Fig. 4: Heat flow thermograms of SF films treated with different solvents

Table 1: Exo/endo transition of the SF films

List	Endo (°C)	Exo (°C)
SF	292.59, 316.26, 420.52	242.79, 309.15
SF-treated methanol	299.67. 331.66, 456.07	222.65, 322.18
SF-treated ethanol	299.67, 326.92, 456.21	252.27, 313.89
SF-treated ethyl acetate	292.82, 326.92, 448.96	226.21, 305.59
SF-treated acetate	273.60, 297.30, 318.65	233.32, 303.89

the endothermic peaks at least 3 points and exothermic peaks 2 points in all of samples. The SF-treated methanol and ethanol showed similar endothermic peaks with the highest at 456°C. However, SF-treated methanol showed higher exothermic peaks than SF-treated ethanol at 322°C. SF-treated ethyl acetate showed endothermic peaks at 448°C which was higher than native SF film, but the exothermic peaks were lower. In addition, SF-treated acetone showed the lowest both exothermic and endothermic peaks compared to others and native SF. Table 1 shows exo and endo transition of the SF films.

DISCUSSION

Secondary Structure

The effect of organic solvent on the secondary structure and thermal behavior of SF films were studied and compared. The FTIR results found that secondary structure and thermal properties of SF films were affected by organic solvent in term of enhancing structural interaction. This is an important point to change the structural of SF films (She *et al*., 2008). The secondary structures of protein are indicated by the amide groups of the silk composition (Kweon *et al*., 2000; Hino *et al*., 2003). In general, SF film is existed of random coil, α-helix and β-sheet structures. After treatment SF films with organic solvents, shift of wave length about absorption bands in each film were observed except acetone did not different. The shift of absorption bands from high to low represents β-sheet structure was formed (Tao *et al*., 2007). On the designate time, ethyl acetate, ethanol and methanol showed similar effect on the SF films which were differed from acetone. The result suggested that structural of SF could be changed by treatment with those of such solvents.

Thermal Behavior

Thermal properties of domesticated silk, *B. mori* took place in a single step; contrast from those of wild silk since it took place in several steps (Kweon *et al*., 2000). Furthermore, TG and DTG results slightly showed that SF films treated with different organic solvents have different thermal decompositions. The results found that SF films treat with those of ethanol, ethyl acetate and methanol showed higher thermal stability than that SF film treated with acetone. This point suggested that the SF film was the lowest affected from acetone or might be said that acetone did not affect on the secondary structure of SF film. To clarify the effect of solvents, endo/exo thermic peaks were described. The results found that those of SF films treated solvents occurred higher thermal stability decomposition compared to SF film, except acetone only. The results from this work related to previously reported about the effect of solvent especially alcohol on the structural changes of the SF films (Kweon *et al*., 2001; Tasukada *et al*., 1995). This illustrated that the solvents could be enhanced the molecular changed to construct higher ratio of β-sheet structure by intermolecular bonding which were increased the mechanical strength of the SF films. However, the β-sheet structure could be formed by those of the fiber reinforcement and matrix, fiber content, fiber length, fiber orientation, processing method and condition (Sofia *et al*., 2001).

CONCLUSION

The effect of different solvents on secondary structure and thermal properties of SF films were reported in this study. Before treatment, SF films composed of random coil, α-helix and β-sheet structures. The secondary structures were changed after treatment SF films with ethanol, ethyl acetate and methanol, except acetone. In addition, methanol showed higher effect to enhance the stability of SF films. This conclusion was from the results of FTIR, TG, DTG and heat flow thermograms. It is a promising that ethanol, ethyl acetate and methanol could be used to enhance the stability of SF films.

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