Silk Fibroin/Starch Blend Films: Preparation and Characterization

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Abstract: This study was aimed to prepare silk fibroin (SF) of Thai silk (Bombyx mori) and cassava starch blend films by solvent evaporation method. The SF solution was firstly prepared from the cocoons by dissolving pure silk fibroin with tereary solvent system of CaCl₂: Ethanol: H₂O (1:2.8 by mol). Cassava starch solution could be prepared by boiling at high temperature. The mixture of the SF and cassava starch solution was then blended and cast on the polystyrene plates. The obtained films were investigated on their morphology, secondary structures and thermal properties by using SEM, FTIR and thermogravimetry, respectively. The results found that SF blended with starch could be formed the higher ratio of β-sheet. With cross-section images, the blend films showed homogeneously texture. In addition, the blend film gradually increased its thermal properties when the starch blended ratio was increased. In conclusion, the SF/starch blend films could be adjusted their properties by varying the blend ratio.

Key words: Blend films, morphology, silk fibroin, starch, thermal properties

INTRODUCTION

Silk is a natural protein, produced by some insects including silkworm. Each silk fiber generally composed of 2 main proteins, sericin and fibroin (Altmann et al., 2003). Silk fibroin (SF) was obtained by degumming sericin with salt. It is a fibrous protein and has been used in various fields for long history (Nogueira et al., 2010). In general, SF is classified into domestic (B. mori) and wild type. The SF macromolecules have been studied as biomaterial for textile and different textile applications (Min et al., 2004, Rajkhowa et al., 2009). This was due to its excellent properties including biodegradability, biocompatibility and low inflammatory reaction (Foo and Kaplan, 2002). On the other hand, SF is an inexpensive material and friendly environment. Moreover, SF can be performed in different types depending on applications (Park et al., 2004).

Film is one kind of SF which wildly prepared and used. However, some disadvantages of the SF films such as very brittle and fragile were also occurred. This problem can be improved by blending SF solution with other polymers (Fan et al., 2008). Polar or hydrophilic molecules such as chitosan (Baimark et al., 2009), gelatin (Wacharin et al., 2009) as well as polyester polymers (Cheung et al., 2008) have been used for improving the SF properties.

Starch, a non-toxic and one kind of polar molecule was chosen to blend with SF solution. It is a biodegradable, biocompatible, edible and inexpensive natural polymer. With previous works, starch has been widely applied (Elfrstrand et al., 2009, Fang et al., 2008; Loksuan, 2007). Indeed, hydrophilic starch is incompatible with hydrophobic polymer and the blend tends to phase separation (Du et al., 1997). However, the starch blend with hydrophobic might be prepared to obtain water insoluble. Recently, many researchers have considered preparing and characterize starch blend films (Das et al., 2010; Parvin et al., 2010). Previous reports have also been blended the starch with other biodegradable polymers into improve their physical properties (Godbole et al., 2003).

MATERIALS AND METHODS

This study was carried out for 3 months from May to August 2010. All of the experiments were carried out at Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand.

Materials: Bombyx mori (locally called Nang-Lai) silk cocoons were kindly supplied by Silk Innovation Center (SIC), Mahasarakham University, Thailand. Cassava starch (96% starch, amylose content 16.8%) was purchased from Bangkok Inter Food Co., Ltd., Thailand. All used chemicals were analytical grade obtained commercially.
Methods

Preparation of silk fibroin and starch solution: The B. mori silk cocoons of Thai variety (locally called Nang-Lai) were firstly excluded sericin by boiling twice in 0.5% Na2CO3 solution at 95°C for 30 min. They were rinsed with distilled water and then air-dried at room temperature. Pure SF fibers were dissolved in the mixture of CaCl2-Ethanol-H2O (1:2:8) with stirring until absolutely dissolved. The SF solution was then dialyzed against distilled water for 3 days. After filtration, then the SF concentration was adjusted to 1% w/v with distilled water. The 1% w/v starch solution was prepared by dispersing starch powder in hot water at 80°C. It was then stirred until gelatinization.

Preparation of SF/starch blend films: SF and starch solutions were firstly mixed with different ratios of 4/0, 3/1, 2/2, 1/3 and 0/4 (w/w) using magnetic stirrer for 1 h before casting in polystyrene plates and dried. The plates were then left in oven at 40°C for 3 days to obtain SF/starch blend films.

Characterization of SF/starch blend films: Secondary structure and conformational change of the blend films were measured by using Fourier Transform Infrared (FTIR) spectroscopy (Perkin-Elmer Spectrum GX, USA) with air as the reference. This work, resolution of 4 cm⁻¹ and 32 scans were chosen.

Morphology of the SF/starch blend films were observed under scanning electron microscope (SEM, JEOL JSM-6460LV, Japan). The films were firstly cut into small pieces and then mounted on the stub with double side carbon tapes. The samples were then sputter coated with gold to enhance surface conductivity.

Thermal properties were measured using Thermogravimetric Analyzer (TGA), TA instruments, SDT Q600 (Laken's drive, New Castle, DE). The films weight of 8-10 mg were prepared and loaded in a platinum crucible. The samples were non-isothermal heated from 50 to 1000°C at a heating rate of 20°C min⁻¹. The TGA was carried out in nitrogen with the flow rate of 100 mL min⁻¹. The TG and heat flow 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

Secondary structure analysis: Chemical structures of the films were identified from FTIR spectra as shown in Fig. 1. The absorption bands of SF were specifically showed by amide I, II and III bands. The FTIR spectrum of SF film Trend a in Fig. 1 showed absorption bands at 1654 cm⁻¹ (amide I), 1545 cm⁻¹ (amide II) and 1242 cm⁻¹ (amide III). The native starch film (Trend e) showed absorption broad bands at 3385 and highly sharp at 2929 cm⁻¹. In addition, those of bands at about 1400 and 1000-1155 cm⁻¹ were also occurred. As shown by FTIR spectra, the intensities of starch characteristic bands at 2929, 1415 and 1000-1155 cm⁻¹ increased when the starch blended ratio was increased (Trend b-d in Fig. 1). On the other hand, the amide I and II bands of the SF film slightly shifted to lower wave number after blending with starch.

Morphology observation: With SEM images, both native SF and starch films (Fig. 2a, e) have smooth surfaces while the blend films slight rough on their surfaces (Fig. 2b-d). With cross section images, immiscibility of the SF/starch blend films occurred comparison to the native films. This immiscibility was slightly increased when increased of the starch ratio.

Thermal properties: Thermal properties of native SF, starch and SF/starch blend films were determined from Thermogravimetric (TG) thermograms as shown in Fig. 3. At low temperature, starch rapidly degraded than other films while SF was the lowest degradation. However, SF films was rapidly degraded over 200°C. All of films showed a single stage of decomposition and did not completely decompose at temperature of 1,000°C. At the last temperature, SF film had remaining weight in higher percent than others. The thermal properties of the samples clearly detailed from differential TG (DTG) thermograms as shown in Fig. 4. The temperature of maximum decomposition rate (Tm,ma) of the SF film was the lowest Tm,ma at 308°C while starch film was the highest Tm,ma at 328°C. The SF/starch blend films showed that the SF/starch at 1/3 blend ratio had Tm,ma in higher than other at 326°C.
Fig. 2: SEM micrographs of (a) SF, (b) SF/starch (3/1), (c) SF/starch (2/2), (d) SF/starch (1/3) and (e) starch films. All bars = 10 μm.

Fig. 3: TG thermograms of (a) SF, (b) 3/1 blended, (c) 2/2 blended, (d) 1/3 blended and (e) starch microparticles.

Fig. 4: DTG thermograms of (a) SF, (b) 3/1 blended, (c) 2/2 blended, (d) 1/3 blended and (e) starch films.
DISCUSSION

SF indicated various excellent properties, especially mechanically robust. It was found that the amino compositions and their structures are main factor on this property. Generally, the secondary structures of protein are indicated by the amide groups of the silk composition (Kweon et al., 2000; Hino et al., 2003). The FTIR spectra showed absorption bands of SF film at 1654 cm⁻¹ (amide I), 1545 (amide II) and 1242 cm⁻¹ (amide III), assigned to the random coil conformation (Kweon et al., 2001). The starch film showed absorption broad bands at 3385 (O-H stretching) and highly sharp at 2929 cm⁻¹ (C-H stretching), at about 1400 cm⁻¹ (C-H bending vibration) and at 1000-1155 cm⁻¹ attributed to saccharide structures, which the main structural of starch (Mundargi et al., 2008; Roy et al., 2009). The FTIR spectra of the blend films appeared those of characteristics bands of both SF and starch. The results also showed that the amide I and II bands of SF film slightly shifted to lower wave number after blending with starch. This result shows that the formation of β-sheet structures in the blend films were occurred by adding starch. In contrast, the amide III of SF shifted to higher wave number when blended with starch. This was also confirmed that the SF films changed structure from random coil to β-sheet form since the β-sheet region at amide III was appeared at 1270 cm⁻¹ (Um et al., 2001). The blend films slight rough on their surfaces comparison to the native films. It is suggested that SF and starch composed of large or various molecules which affected on the interaction together. All of films types showed homogeneous without separation throughout the surface area, indicated a high level of miscibility of the blend (Yang et al., 2000). With cross section images, immiscibility of the SF/starch blend films occurred comparison to the native films. The immiscibility was slightly increased when the ratio of starch was increased. This point was expected to the high interaction between the SF and starch by hydrogen bond result to the brittle or separate phase of the films. This suggested that the uniformly films with smooth surfaces could be obtained by adjusting appropriate ratios of starch. Thermal properties analysis showed that starch rapidly degraded than other films while SF was the lowest degradation at low temperature. However, SF films was rapidly degraded over 200°C, indicated to a depolymerization of amino acids chain of SF (Periche-Covas et al., 1993). The temperature of maximum decomposition rate (T_d max) of the films arranged with SF<SF/starch blend<starch, respectively. These results supported the FTIR results concerning the intermolecular interactions between SF and starch.

CONCLUSIONS

The SF/starch blend films with different blended ratios were successfully prepared by the simple solvent evaporation method. With SEM, all the films remained homogeneous throughout film surfaces, but the blend films have rougher surface area than the native films. In addition, the blend films indicated immiscibility surfaces with cross-section images. The intermolecular interactions between SF and starch molecules were evidenced by FTIR and TG analysis. For secondary structures determination, SF changed their structures from random coil to β-sheet structures when added starch and gradually increased follow by increasing starch content. These results affected the increasing of SF thermal properties. In conclusion, SF properties can be changed by the ratio of starch content.

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REFERENCES


