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EFFECT OF STARCH ON PROPERTY OF SILK FIBROIN/KERATIN BLEND FILMS

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ABSTRACT: This work was aimed to study the effect of starch on silk fibroin (SF)/keratin (K) blend films properties. The SF and K solutions were mixed with starch, homogeneously stirred and poured into polystyrene culture plates. The mixture solution was then dried in an oven at 40 °C for 3 days. The films were then investigated for their morphology, secondary structure and thermal properties by using scanning electron microscope (SEM), Fourier transform-infrared (FT-IR) spectrophotometer, Thermogravimetric analysis (TGA). The results found that each film had different patterns of surfaces depending on ratio used. The structure of almost films co-existed with random coil and α-helix structures which resulted to increase the flexibility and of film. The structure of the films changed to β-sheet after blending between SF and K according to H-bond formation and increased thermal stability of the films. This result indicated that starch helped to decrease the crystalline structure of the film which increased their flexibility.

Keywords: Biopolymer, Morphology, Secondary structure, Thermal property

1. INTRODUCTION

Silk is a natural fibrous protein produced from silkworm which had a unique characteristic. It has been proved that silk fiber composed of several excellent biological properties including high strength, biocompatibility, low inflammatory respond and biodegradability [1]. Recently, silk is increasingly interested for applications, especially biomedical and drug controlled release [2],[3]. Silk component, fibroin, has been reported as an important biomaterial which can be constructed in various forms [4]. In addition, silk fibroin has been blended with other materials to obtain novel materials such as collagen [5] PLGA and alginate [6] or hyaluronan [7]. Keratin, a kind of high cystein content fibrous protein, has been applied in various fields include tissue engineering [8]. Silk fibroin/keratin blend materials are a little informations available, especially in Thailand. However, both of structural proteins have some limitation properties for use from their fragile and low flexibility. Therefore, we had chosen starch, a polysaccharide with high polarity, for improving the properties of the silk fibroin (SF)/keratin (K) blend films. The property of the blended films such as morphology, secondary structure and thermal stability were investigated.

2. METHODS

2.1 Preparation of Silk Fibroin Solution

Silk fibroin (SF) solution was prepared by firstly boiling twice of *B. mori* cocoons in 0.5% (w/v) Na_2CO_3 solution at 90 °C for 30 min in each times, then washed with distilled water to obtain silk fibroin (SF). The pure SF was then dissolved using tertiary system of $CaCl_2$:Ethanol:H_2O (1:2:8 by mol) at 70-75 °C for 60 min. The hydrolysate SF was dialyzed against distilled water with dialysis membrane (MW cut off 10 kDa) for 3 days for excluding salt. The concentration of SF solution was adjusted to 1% (w/v) by distilled water.

2.2 Preparation of Keratin Solution

Keratin would be extracted from human hair which would be collected from hairs salon in Maha Sarakham Province, Thailand. The hairs were immersed in hexane solution for 12 h to remove some lipid components, and air-dried at room temperature for 1 day. The 1 g of dried hair would be dissolved in 20 mL of the mixture solution of 0.02 M sodium hydroxide (NaOH), 0.5 M urea, 0.26 M sodium dodecyl sulfate and distilled water at 70 °C with stirring until the hair would be absolutely dissolved. The solution would be dialyzed against distilled water for 3 days with dialysis tube (MW. cutoff = 3,500 Da) to obtain keratin solution. The keratin concentration would be diluted to 2% w/v against distilled water before use.

2.3 Silk Fibroin/Keratin Blend Films Preparation

The silk fibroin (SF)/keratin (K) blend films were prepared by mixing 1% wt. of SF solution and 1% wt. K solution at different ratios; 1:1, 1:3, and 3:1. The 21 mL of mixture was then mixed with

0.25g starch. The solution was boiled to dissolve the starch before pouring on the polystyrene plates. They were taken to an oven at 40 °C for 3 days to obtain the blend films. In addition, the native SF and K films were also prepared as comparison film property.

2.4 Characterization of Films

All films were cut and observed their morphology 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. The secondary structures of the films were analyzed using FTIR spectroscopy (Perkin Elmer-Spectrum Gx, USA) in the spectral region of 4000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans. Thermal properties were also measured using thermogravimetric analyzer (TGA), TA instruments, SDT Q600 (Luken'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°C to 1000 °C at a heating rate of 20 °C/min. The TGA was carried out in nitrogen with the flow rate of 100 mL/min. 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).

3. RESULTS AND DISCUSSION

3.1 Morphology of Films

Native SF film had slight rough surfaces with homogeneous texture observed by cross-section (Fig. 1a) as like as the native K film (Fig. 1b). The surfaces of the blended films had rougher surfaces than native films and appeared particles like-beads covered film surfaces and textures (1:3 ratios, Fig. 1e). It was also found that the surfaces and texture of the blend films were depended the ratios of SF/K used. The compatible film texture and surfaces of the blend ratio at 1:1 (Fig. 1d) was found than other ratios (Fig. 1c and 1e).

The morphology of the film is affected by the chemical composition of each material [9]. SF composes of main similar monomer which affected to smooth surface and homogeneous texture. Keratin composes of many kinds of protein sizes which resulted to rough surface of the film. In case of the blend films, unequal ratios appeared the particle of starch granule in the film. This suggested that the starch molecule might be more contents than the suitable ratio need to interact with SF and K molecule.

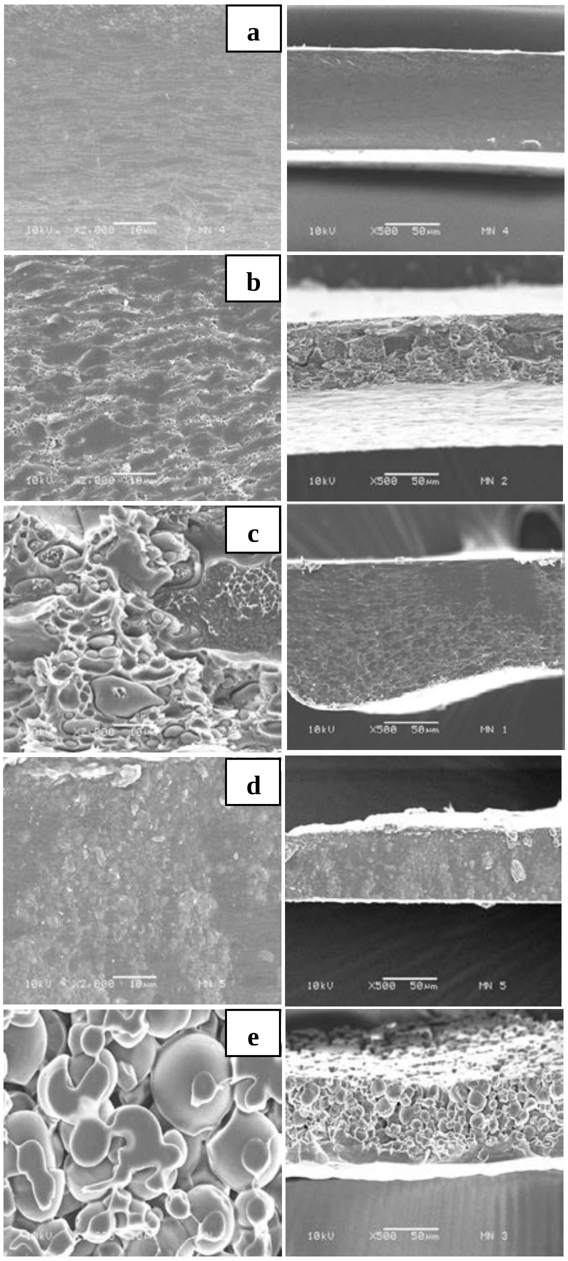

Fig. 1 SEM micrographs of various films; native silk fibroin (d), keratin (b) and silk fibroin/keratin blend in different ratios of 3:1 (c), 1:1 (d) and 1:3 (e). Left presented as surface area and right as cross-section. (All bars = 50µm)

3.2 Secondary Structure of Films

Figure 2 shows the FTIR spectra of the various types of films. In general, the films were varied of absorption peaks. The native SF film showed the absorption peaks 1660, 1548 and 1249 cm^{-1} whereas the native keratin film had the absorption peaks at 1652, 1547 and 1242. The absorption peaks of the blend films showed different patterns according the blend ratios. At the ratio of SF/K as 1:3, the absorption peaks found at 1659, 1525 and 1249 cm^{-}

^{1}. The 1:1 ratio indicated the absorption peaks at 1649, 1525 and 1312cm^{-1}. At the ratio of SF/K as 3:1, the absorption peaks found at 1691, 1526 and 1228 cm^{-1}.

The results indicated that SF/K blend with starch had increased in the strength of films. This was observed from the absorption peaks at amide II (1525 cm^{-1}) which are dominantly appeared after mixing with starch. This peak is designated as β-sheet structure [10]. Moreover, the absorption peaks about 100-900 cm^{-1} were also appeared. These peaks are responding of saccharide structure [11].

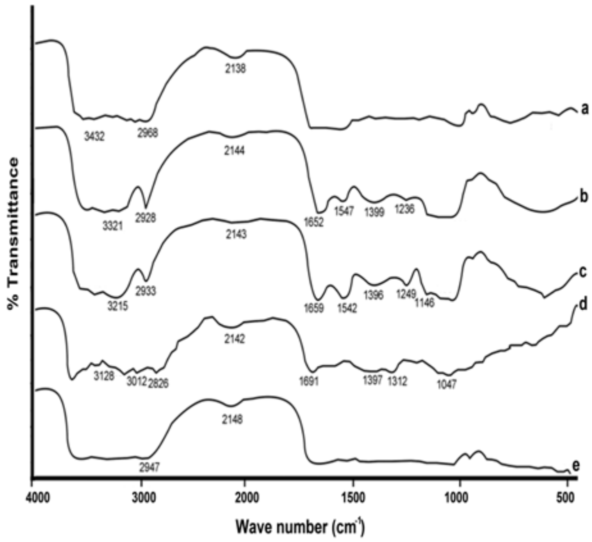

Fig. 2 FTIR spectra of various films; native silk fibroin (a), native keratin (e) SF/K blend in the ratios of 1:3 (b), 1:1 (c) and 3:1 (d).

3.3 Thermal stability

The thermal stability of the films was illustrated by TG curves (Fig. 3). Generally, the films started of decomposed at about 100 °C which was the water evaporation temperature. The main decomposition temperature of all films were higher than 300 °C which clearly indicated by DTG curves (Fig. 4). This peaks called the maximum temperature of decomposition ($T_{d,max}$) and summarized in Table 1. The native SF had $T_{d,max}$ at 310 °C while native K had $T_{d,max}$ at 309 °C. Among the blend ratios, SF/K at 1:3 had the highest $T_{d,max}$ at 315 °C, while the ratios at 3:1 and 1:1 had similar $T_{d,max}$ at 311 °C.

The increasing of $T_{d,max}$ should be resulted from the interaction via H-bonds between hydroxyl groups of starch and amino or carboxylic groups of protein [12].

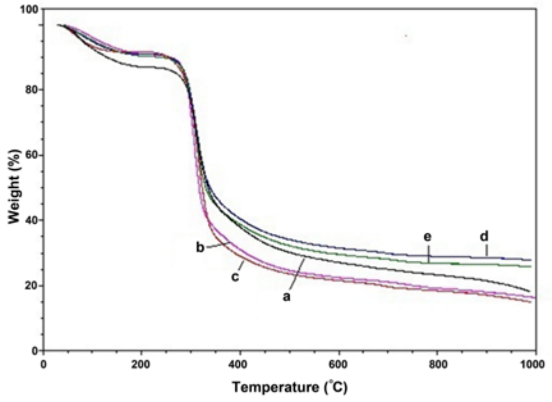

Fig. 3 TG curves of various films; native silk fibroin (a), native keratin (b), SF/K blend in the ratio of 1:3 (c), 3:1 (d) and 1:1 (e).

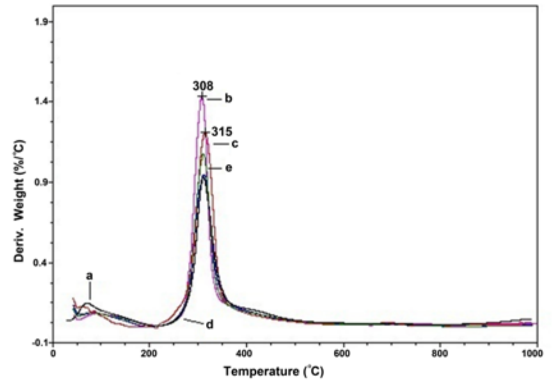

Fig. 4 DTG of various films; native silk fibroin (a), native keratin (b), SF/K blend in the ratio of 1:3 (c), 3:1 (d) and 1:1 (e).

Table 1 The $T_{d,max}$ of several films.

Films types	$T_{d,max}$ (°C)
Silk Fibroin (SF)	310
Keratin (K)	308
SF:K (1:3)	315
SF:K (3:1)	311
SF:K (1:1)	311

4. CONCLUSION

Starch is helped to increase the flexibility of the SF/K blend films since it reflected to change the secondary structure of the films from β-sheet into random coil structure. The surfaces and texture of the SF/K blend films were varied by the blending ratios with composed of particles on the texture of films and increase rough surfaces. The blend film at 3:1 ratio had the highest stability after mixing with starch. The different properties of the SF/K blend films should be led to prepare the novel materials for medical and pharmaceutical applications.

5. ACKNOWLEDGEMENTS

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