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The Effect of Temperatures and Incubation Times on Some Properties of Silk Fibroin/Chitosan Blend Films

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Abstract: The objective of this work was to study the effect of temperatures and incubation times on Silk Fibroin (SF)/Chitosan (CS) blend films properties. The films were firstly prepared by mixing the SF and CS solution in homogeneously blended, then cast on the polystyrene culture plates before taking into the oven at 40°C for 3 days. The obtained SF/CS blend films were treated at 90 and 120°C, each for 30, 60 and 90 min, respectively. All of films were characterized for their morphology and secondary structures by using SEM and FTIR, respectively. The results showed that SF/CS films have homogeneous texture without phase separation. However, they also appeared some particles dispersed and embedded on their surfaces. The surfaces of the films slightly increased smooth texture when increased both temperatures and incubation times. The results were also noted that the characteristics were varied by those different temperatures. FTIR spectra indicated that the absorption bands at amide regions (I, II and III) of the SF were similar profile. Moreover, treatment the blended films with temperatures resulted to changed the secondary structures of the films. This means the ratio of β-sheet were increased. It can be expected that water evaporation by increasing temperature resulted to make the molecules of SF and CS close up and help to form H-bonds between them.

Key words: Chitosan, film, property, silk fibroin, temperature

INTRODUCTION

Natural polymers have highly focused for biomedical application, especially biodegradable materials in last decade (Nair and Laurencin, 2007). Many advantages properties of the natural polymers such as non-toxicity, biodegradability and biocompatibility have been reported (Noi *et al*., 2009). However, some disadvantages properties such as high cost, impurity, mass production or reproducibility of the natural polymers were also questioned (Wilaiwan *et al*., 2010). Moreover, natural homopolymer demands are not sufficient for application. Therefore, blend polymers are interested and highly discovered to substitute the weakness point of individual natural polymer (Cheung *et al*., 2008; Fan *et al*., 2008).

Silk is a natural fibrous produced by some insects including silkworm (Vepari and Kaplan, 2007.). It is a strong fibrous protein. Each silk fiber composed of at least 2 main proteins that are core fibrous fibroin and glue-like sericin (Altman *et al*., 2003; Nuanchai *et al*., 2009). Generally, sericin can be dissolved in polar solution such as hot water and acid and base solutions since it comprises of highly hydrophilic and bulky amino acids. On the other hand, fibroin is known as insoluble fibrous. This was due to it does not dissolve easily in general solvents, except concentration of salt solution including LiBr (Zhang *et al*., 2007) or $CaCl_2$ (Hino *et al*., 2003). From the past until now, silk fibroin (SF) has been used and extended studied in various applications, especially for tissue engineering and drug delivery system (Prasong, 2011). Moreover, it can be performed in various forms, depending on applications. Among them, SF films have been studied and applied. However, films prepared from SF were hardly used according to its very brittle and easy fragile (Srisuwan *et al*., 2008; Nuanchai *et al*., 2010; Lu *et al*., 2010). To improve this point, SF blended with polar materials such as chitosan (He *et al*., 2010), gelatin (Prasong, 2010), collagen (Zhou *et al*., 2010) or sugar (Baimark *et al*., 2009; Acharya *et al*., 2008) have been studied.

Chitosan (CS), a derivative of chitin, is a heteropolysaccharide polymer. It composes of acetyl group which is glucosamine and N-acetyglucosamine units linked by 1,4-glycosidic bonds. The CS showed many excellent properties including biocompatibility (Jones *et al*., 2009), biodegradability (Yang *et al*., 2009), flexibility (Wang and Li, 2007) and anti-infection (Chung *et al*., 2003). In addition, it is a high polar polymer and could be interacted well with other substances (Griffon *et al*., 2006). In this research, the SF/CS blend films were prepared by evaporation method. Effects of temperatures and incubation times on the blended films properties were investigated.

MATERIALS AND METHODS

This study was done for 5 months from April 1, 2011 to September 1, 2011. The experiment was performed at Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand.

Materials: Silk cocoons, *B. mori* locally called Nang Noi, were kindly supplied from Silk Innovation Center (SIC), Mahasarakham University, Thailand. Chitosan (90% deacetylation and molecular weight of 15 kDa) was purchased from Seafresh Chitosan Lab Co., Ltd., Thailand. All using chemicals were analytical grade obtained from commercially.

Preparation of Silk Fibroin (SF) solution: Silk fibroin solution was prepared by firstly boiling twice of *B. mori* locally called Nang Noi 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 calculated using evaporation method. Finally, the SF concentration was adjusted to 1% (w/v) by distilled water.

Preparation of Chitosan (CS) solution: Chitosan (CS) solution of 1% (w/v) was prepared by weighing 1 g of CS powder and then added 100 mL of 2% acetic acid before mixing in the flask. They were then stirred using magnetic bar at room temperature until completely dissolved.

Preparation of SF/CS blend films: Each 10 mL of 1% (w/v) of SF and CS solutions were mixed homogeneously. They were then cast on the polystyrene plates before drying in an oven at 40°C for 3 days to obtain the blended films. In addition, native SF and CS films were also prepared for comparison.

Temperature treatments: All of films were treated with different temperatures at 90 and 120°C and each for 30, 60 and 90 min, respectively. The treated films were kept for further characterization.

Characterization of treated films: Morphology of treated film surfaces and cross-section were examined by Scanning Electron Microscopy (SEM) using a JEOL JSM-6460LV SEM. The films cross-section was obtained by cutting with paper scissors. The samples were coated with gold for enhancing conductivity before observation.

Secondary structures and interactions of the treated films were investigated by FTIR spectroscopy using a Perkin-Elmer Spectrum GX FTIR spectrometer with air as the reference. The resolution of 4 cm^{-1} and 32 scans were chosen in this study.

RESULTS

Morphology of native CS films: The morphology of native CS films indicated particles like-beads covered surface areas of the films in all treated temperatures which resulted to rough surfaces. With cross-sections, separations of their texture were appeared. However, the films were co-adhesive throughout the film matrices, as shown in Fig. 1 and 2. Considering for each point of temperatures, the smooth of film surfaces have gradually increased when treated at higher 60°C for 60 min.

Morphology of native SF films: Native SF films have smoother surfaces than other films. The changes of their morphology occurred when treated at 90°C and gradually increased as the temperature increase. The edge of native SF films showed wave-like shape and fragile. The texture and surfaces of the native SF films fused together at 90°C and returned to smooth again when treated at 120°C as shown in Fig. 3 and 4.

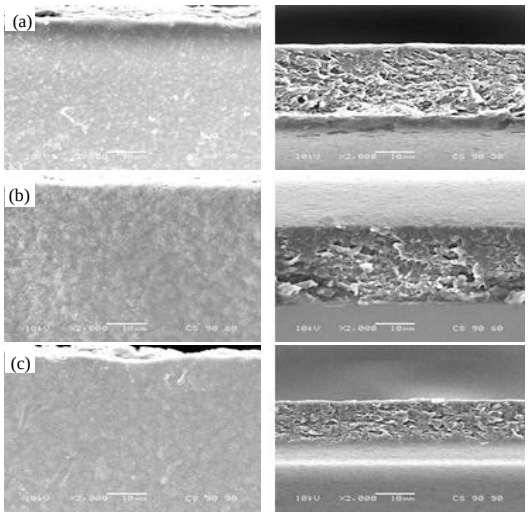

Fig. 1(a-c): SEM micrographs of native chitosan (CS) films treated at 90°C for different incubation times; (a) 30, (b) 60 and (c) 90 min. Notes surfaces (left column) and cross-section (right column) were presented

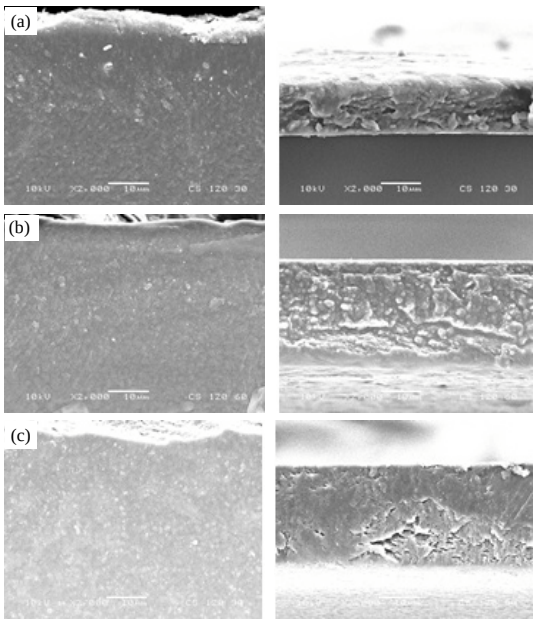

Fig. 2(a-c): SEM micrographs of native CS films treated at 120°C for different incubation times; (a) 30 (b) 60 and (c) 90 min. Notes surfaces (left column) and cross-section (right column) were presented

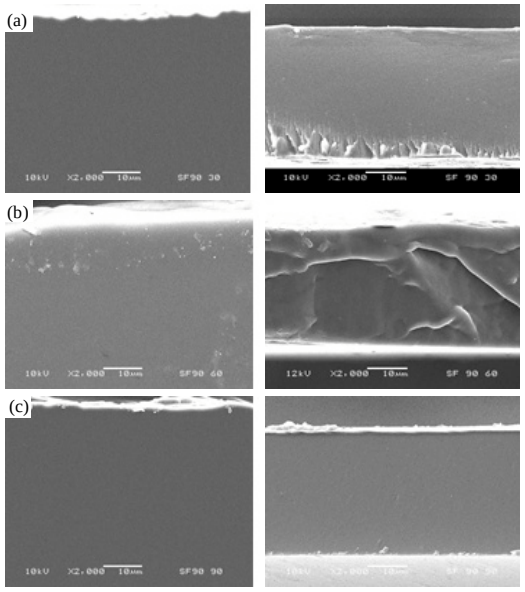

Fig. 3(a-c): SEM micrographs of native silk fibroin (SF) films treated at 90°C for different incubation times; (a) 30, (b) 60 and (c) 90 min. Notes surfaces (left column) and cross-section (right column) were presented

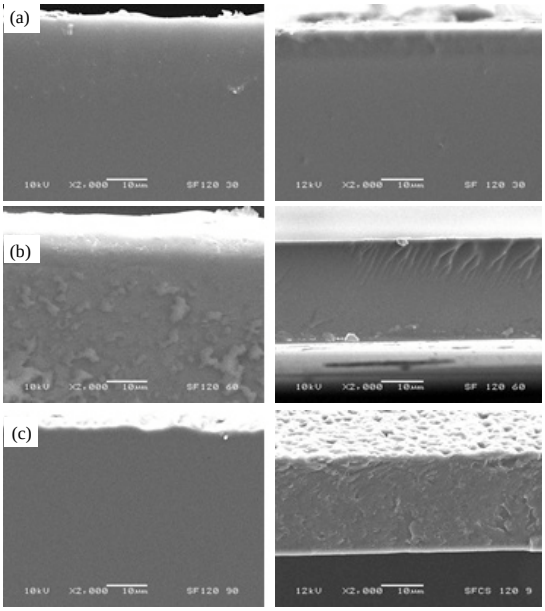

Fig. 4(a-c): SEM micrographs of native SF films treated at 120°C for different incubation times; (a) 30, (b) 60 and (c) 90 min. Notes surfaces (left column) and cross-section (right column) were presented

Morphology of SF/CS blend films: The morphology of SF/CS blend films showed in Fig. 5 and 6. In general, the surfaces of the blended films have rougher than native films since they have particles like-beads covered throughout the film textures. It was also found that the particles shapes were varied in different temperatures and incubation times. The particles changed with different shapes such as sponge or fused together to form bigger areas. The cross-section of the SF/CS blend films showed smoother textures compared to the native CS films. In addition, the texture of films were adhered together to form dense surfaces.

FTIR spectra of native CS films: FTIR spectra of native CS films showed similar regions of absorption peaks at ~3000-3500, 1500-1600 and 1000-1100 cm^{-1} after treatment at 90°C. These peaks slightly changed, especially at 1500-1600 cm^{-1} when treated at 120°C. The CS films showed absorption peaks at 1540 cm^{-1} when incubated at 90°C for 30 min, then changed to higher wave number at 1572 cm^{-1} after treatment for 60 and 90 min (Fig. 7). The changes of this peak did not clearly observe when treated at 120°C in all incubation time. However, the absorption peaks at about 1100-1200 cm^{-1} were varied depending on the

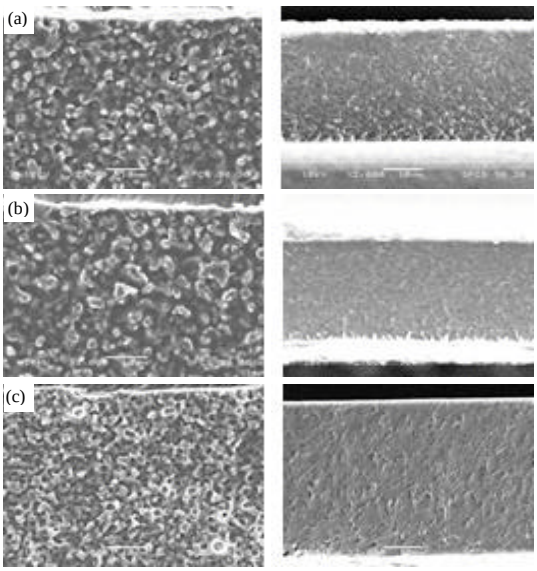

Fig. 5(a-c): SEM micrographs of silk fibroin (SF)/chitosan (CS) blend films treated at 90°C for different incubation times; (a) 30, (b) 60 and (c) 90 min. Notes surfaces (left column) and cross-section (right column) were presented

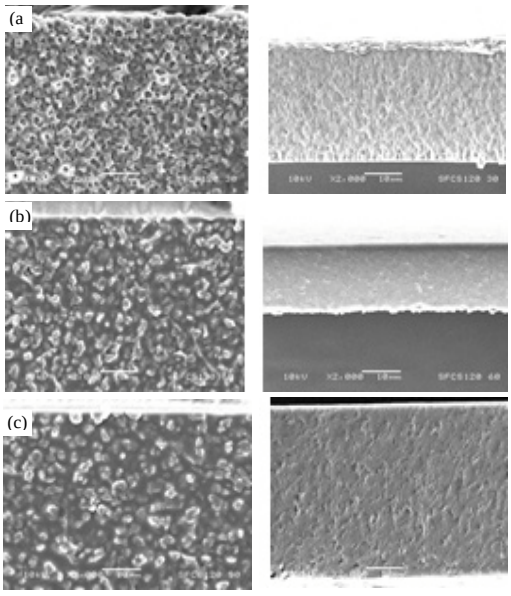

Fig. 6(a-c): SEM micrographs of SF/CS blend films treated at 120°C for different incubation times; (a) 30, (b) 60 and (c) 90 min. Notes surfaces (left column) and cross-section (right column) were presented

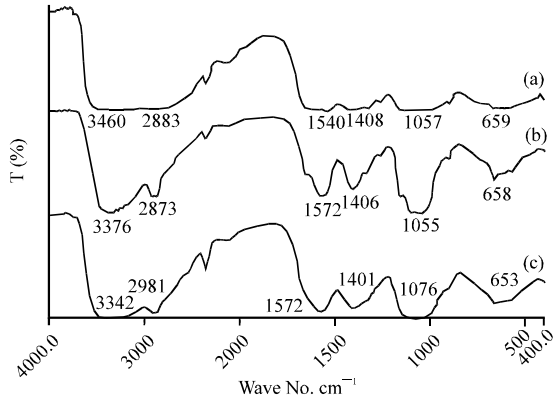

Fig. 7: FTIR spectra of native CS films treated at 90°C for different incubation times; (a) 30, (b) 60 and (c) 90 min

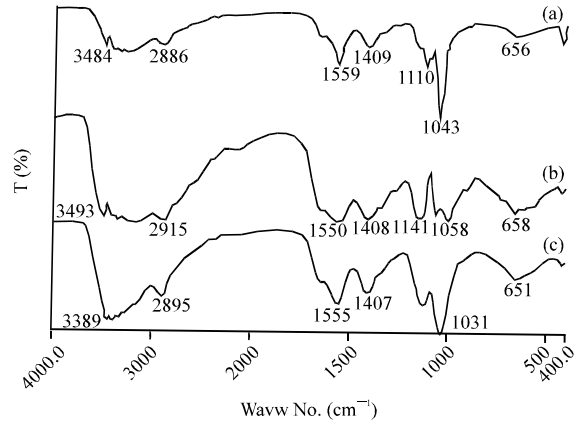

Fig. 8: FTIR spectra of native CS films treated at 120°C for different incubation times; (a) 30, (b) 60 and (c) 90 min

incubation times. The absorption peaks of the native CS films were found at 1110, 1141 and 1120 cm^{-1} when treated for 30, 60 and 90 min, respectively (Fig. 8).

FTIR spectra of native SF films: The FTIR spectra of native SF films showed dominant peaks at amide regions (I, II and III). In general, the absorption peaks of the films were varied. The absorption peaks at about 1200-1300 cm^{-1} found stable at all temperatures. At 1500-1600 cm^{-1}, the native SF films shifted into both higher and lower wave numbers at different temperatures and incubation times as well as in the region of 1600-1700 cm^{-1}. The native SF films changed the absorption peaks of amide II region when treated at 90°C with different wave number. The absorption peaks at 1528, 1550 and 1547 cm^{-1} were occurred after incubation

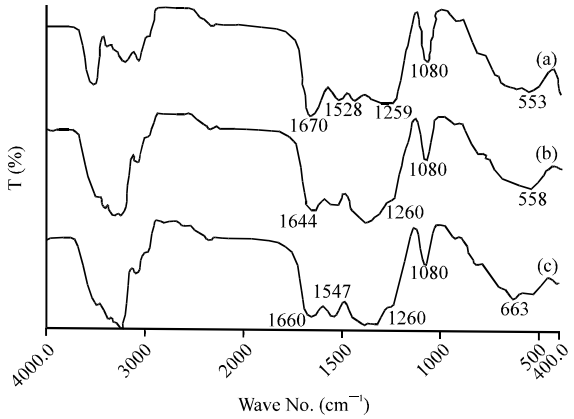

Fig. 9: FTIR spectra of native SF films treated at 90°C for different incubation times; (a) 30, (b) 60 and (c) 90 min

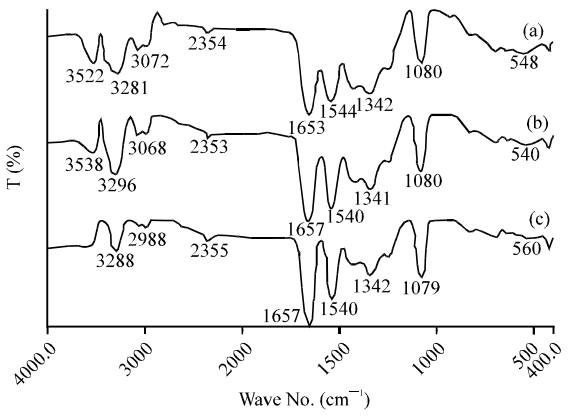

Fig. 11: FTIR spectra of SF/CS blend films treated at 90°C for different incubation times; (a) 30, (b) 60 and (c) 90 min

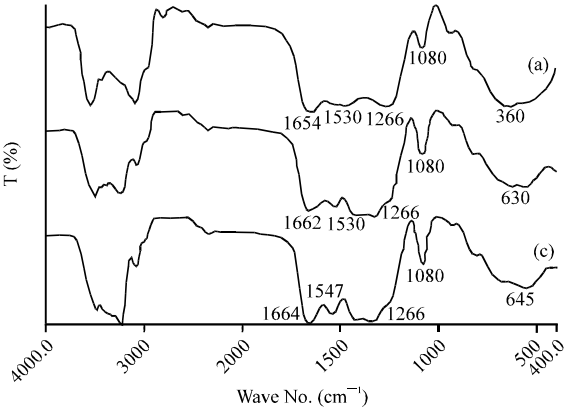

Fig. 10: FTIR spectra of native SF films treated at 120°C for different incubation times; (a) 30, (b) 60 and (c) 90 min

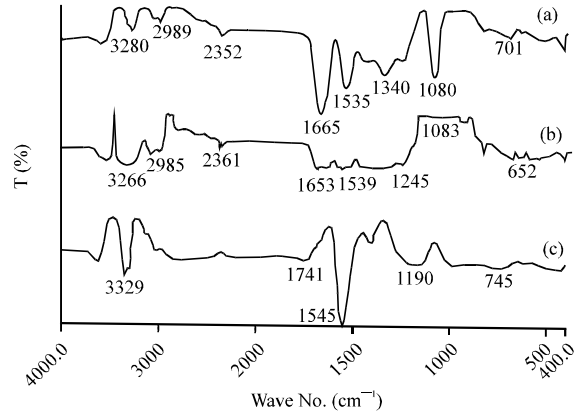

Fig. 12: FTIR spectra of SF/CS blend films treated at 120°C for different incubation times; (a) 30, (b) 60 and (c) 90 min

for 30, 60 and 90 min, respectively. Moreover, the fluctuation of the absorption peaks at amide I region (1700-1600 cm^{-1}) was also observed (Fig. 9). The similar trend was also found when treated the native SF films at 120°C. This was due to the amide II region shifted from low (1530 cm^{-1}) to high (1547 cm^{-1}) of wave number after incubation for 90 min. In addition, the absorption peaks of amide I region were also shifted from 1654 to 1664 cm^{-1} at this temperature (Fig. 10).

FTIR spectra of SF/CS blend films: The results indicated that the SF/CS blend films have similar of absorption peaks even the incubation times were differed. Increasing of temperature affected on the changes of absorption peaks as well as the incubation times. The blend films showed the absorption peaks in each native polymer. In addition, some new absorption peaks were found in the blended films which differed from the native films. The SF/CS blend films showed stable absorption peaks at amide II (1544 cm^{-1}) and amide III (1342 cm^{-1}) after treatment at 90°C for all incubation time (Fig. 11). Surprisingly, the SF/CS blend films showed irregular of absorption peaks at all regions when treated at 120°C. The different peaks were varied followed the incubation times. The absorption peaks of amide I (1665 cm^{-1}) was gradually shifted into lower (1653 cm^{-1}) wave number after treatment for 60 min, then shifted into higher (1741 cm^{-1}) wave number when treated for 90 min. In contrast, the absorption peak of amide II (1535 cm^{-1}) was slightly increased to 1539 and 1545 cm^{-1} after incubation for 60 and 90 min, respectively (Fig. 12).

DISCUSSION

In recent, Silk fibroin (SF) has been used gradually for biomedical applications (Zhou *et al*., 2010; Nogueira *et al*., 2010), cosmetics and food additives (Park *et al*., 2004). This was due to SF composed of excellent properties. In addition, it can be prepared in various forms depending on applications (Acharya *et al*., 2008). SF properties were affected by external factors such as moisture, acid-base, solvent, air and temperatures (Shao and Vollrath, 2002). Moreover, the strength of SF was adjusted by pulling, heat as well as solvent immersion (Kweon *et al*., 2000). This was due to the dehydration of water inside the SF which enhanced the crystalline formation (Nogueira *et al*., 2010).

SEM images indicated that the native SF films has smoother with homogeneous throughout the films texture as comparison to the native CS film. However, SF/CS blend films showed the highest rough surfaces without phase separation. The obtained result was similar trend with previous report (Prasong, 2011). The rough may be from the differences nature of SF and CS as well as the condition used. The film surfaces were affected by increasing of temperatures and incubation times. The native CS and SF/CS blend films have more smooth of their surfaces but native SF has surfaces like wave shape. It might be explained that increasing of temperature enhanced the solubility of materials and decreased of some particles on the film surfaces (Abbasi and Morsali, 2011).

The structure of SF was examined by FTIR. The results found that absorption peaks of the SF films treated with different temperatures and incubation times were varied. In general, native SF film composed of α-helix and random coil structures. However, the β-structures were gradually occurred when increased of temperature. The native CS films showed strong absorption peaks at 1560 cm^{-1} (N-H stretching), 1410 cm^{-1} (C-O stretching), 1335 cm^{-1} (C-N stretching), 1000 and 900 cm^{-1} (saccharide structure) (She *et al*., 2008). It is surprised that some peaks were disappeared when increased temperature. This may be suggested that the composition of CS molecules were degraded. The β-structures were dominantly found in the SF/CS blend films, especially at 120°C. This result suggested that SF and CS molecules were interacted via H-bonds by increasing of water evaporation (Prasong, 2011).

CONCLUSION

SF/CS blend films could be prepared using simple evaporation technique. The obtained blend films were homogeneous without phase separation. SEM micrographs showed that the SF/CS blend films have more rough surfaces than the native films. In conclusion, surfaces and secondary structures of the SF/CS blend films were affected by exposure to high temperature and incubation times in such temperature treated.

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