CHAPTER IV
RESULTS AND DISCUSSION

4.1 Morphological Properties

4.1.1 Cellulose Whisker

The TEM image of cellulose whisker, which was prepared from banana trunk or pseudo-stem, illustrates that the cellulose whisker has a needle-like structure, and the individual or aggregated fragments can be seen as shown in Figure 4.1. The width and length were estimated from the selected TEM images. The average width and length of cellulose nanofibrils were 7.3 nm and longer than 400 nm, respectively, with the aspect ratio being higher than 55, which similar to the previous reports (Dufresne, *et al.*, 1998; Cherian, 2008). Hydrolysis cellulose with sulfuric acid involves esterification reaction of hydroxyl group by sulfate ions, so that some negative charges of sulfate groups appeared at the surface of cellulose whisker resulting in the cellulose suspension exhibited stable colloidal suspension due to repulsion forces of anionic charges (Marchessault, *et al.*, 1961; Yao, 1999; Wang, *et al.*, 2007).

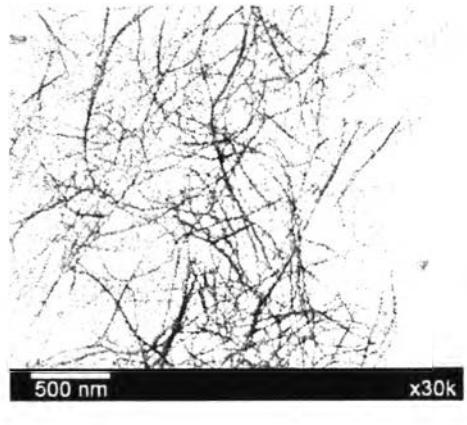

Figure 4.1 TEM image of cellulose whisker.

4.1.2 Chitin Whisker

The chitin suspension behaved colloidal suspension due to the protonation of the amino groups ($-NH_3^+$) after hydrolyzed with hydrochloric acid leading to positive charges appeared at the surfaces of chitin whisker. Hence, the stable colloidal suspension of chitin whisker was obtained because of repulsion forces of these cationic charges (Marchessault, *et al.*, 1959). In case of chitin whisker different from cellulose whisker that chloride ions (Cl^-) did not play important role on charges because chloride ions were easily eliminated by washing with distilled water for several times (Akira, *et al.*, 1999). The suspension consisted of both individual and aggregated fragments. The individual whisker, which has a rod-like structure, showed a broad distribution in both width and length as shown in Figure 4.2 Statistic evaluation of the selected TEM images suggested that the average width, length and aspect ratio of the obtained whisker were 27.1, 307.7, and 11 nm, respectively. However, the previously reported of the dimensions of chitin whisker from shrimp shells (Wattanapanit, *et al.*, 2008) gave the average width, length, and aspect ratio about 46, 343, and 7.5 nm, respectively.

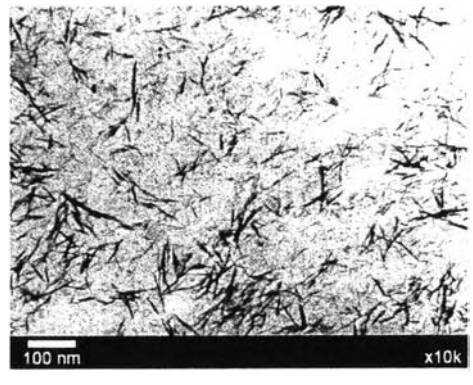

Figure 4.2 TEM image of chitin whisker.

4.1.3 Bionanocomposite Sponges

From SEM observation as shown in Figure 4.3, the top surface of neat cellulose whisker sponge, neat chitin whisker sponge, and cellulose whisker/chitin whisker sponge after lyophilization did not show open porous structure. However,

cross-section area of them exhibited porous pattern (Figure 4.4), and whiskers also could be seen with high magnitude of SEM as shown in Figure 4.5.

On the other hand, sericin containing sponges exhibited porous structure both on the top surface and throughout the sponge. It might be explained that the added of sericin might be coated on the whiskers and could reduce the floating of whiskers to the surface during freeze-drying process.

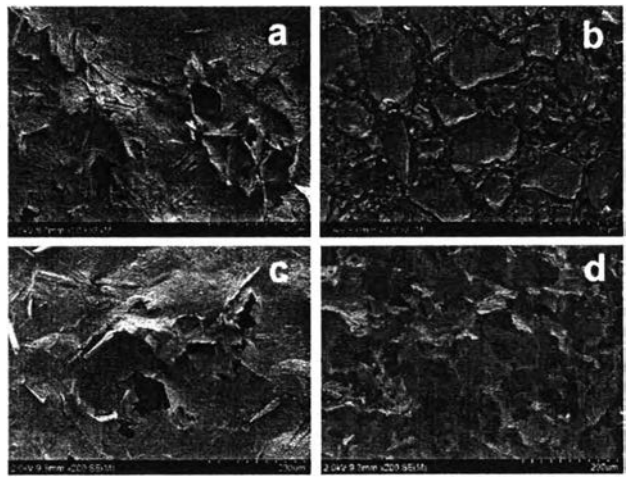

Figure 4.3 SEM images of top surface of (a) cellulose whisker, (b) chitin whisker, and the bionanocomposite sponges having ratios of cellulose whisker : chitin whisker : sericin of (c) 50:50:0 and (d) 50:50:50.

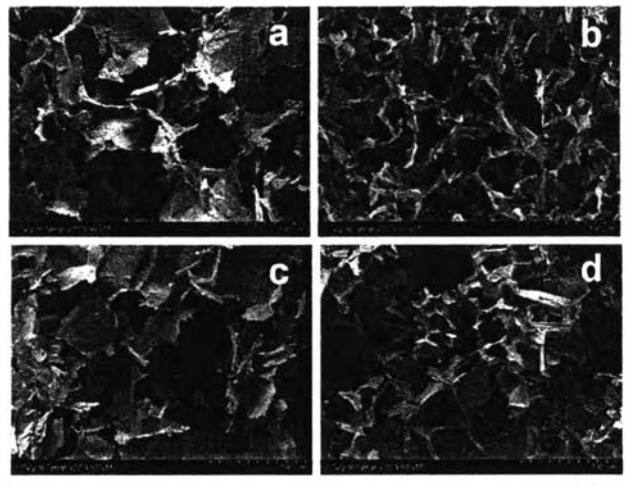

Figure 4.4 SEM images of cross-sectional surface of (a) cellulose whisker, (b) chitin whisker, and the bionanocomposite sponges having ratios of cellulose whisker : chitin whisker : sericin of (c) 50:50:0 and (d) 50:50:50.

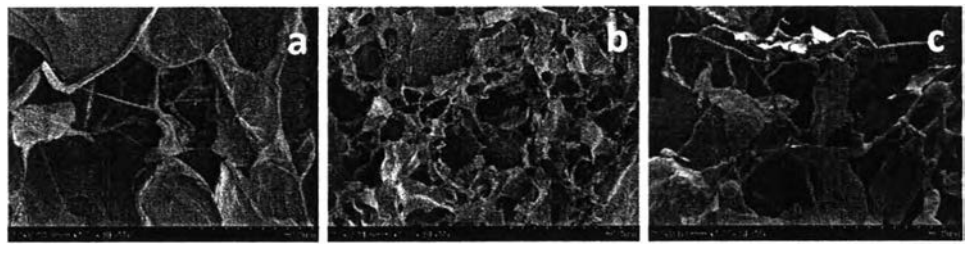

Figure 4.5 SEM images of the cross-section of (a) cellulose whisker, (b) chitin whisker, and (c) 75:25:50 cellulose whisker/chitin whisker/sericin sponges.

In case of bionanocomposite sponges containing sericin, ranging from 25, 50, 100wt% of cellulose whisker/chitin whisker composite, both top surface and cross-sectional area exhibited porous structure. Nevertheless, different sericin content showed no significant difference in terms of granularity and porosity was observed with increasing sericin content and fixed the ratio of cellulose whisker:chitin whisker was 50:50, as shown in Figure 4.6.

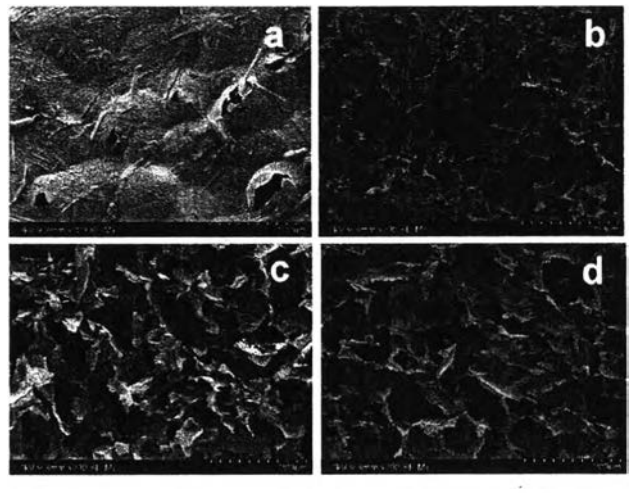

Figure 4.6 SEM images of cellulose whisker/chitin whisker/sericin sponges with the ratio (a) 50:50:0, (b) 50:50:25, (c) 50:50:50, and (d) 50:50:100 cellulose whisker:chitin whisker:sericin sponges.

4.2 Characterizations

4.2.1 Fourier Transform Infrared Spectroscopy (FT-IR)

After boiling silk cocoons under high temperature and high pressure by using autoclave, the obtained sericin had light orange-yellowish which FTIR spectra is shown in Figure 4.7. The broad peak at 3300 cm^{-1} was corresponding to the NH stretching band. Sericin exhibited absorption bands at 1657, 1529, and 637, which are characteristics of amide I, amide II, and amide V, respectively (Cho, *et al.*, 2003).

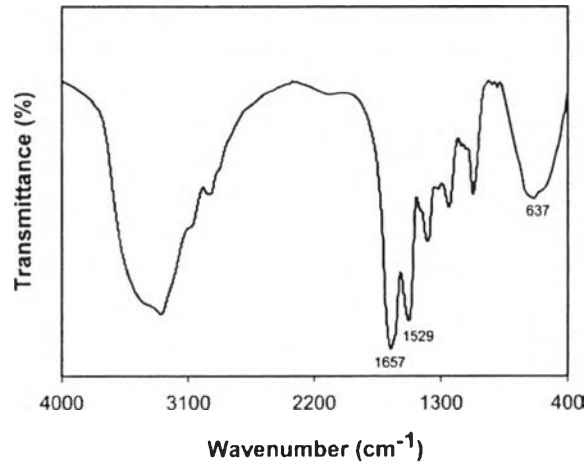

Figure 4.7 FTIR spectrum of sericin.

While the cellulose whisker showed the peaks in the area of 3300-3400cm^{-1} due to OH stretching vibration of –OH groups, which is related to their main composition in cellulose as shown in Figure 4.8. The absorption peak at 2922 cm^{-1} is the characteristic of aliphatic saturated C-H stretching vibration in cellulose and hemicelluloses.

Remarkable observation on the difference between cellulose and cellulose whisker is an intensity of peak at 1640 cm^{-1}. This peak corresponds to C=C vibration and is an indicative of lignin (Wang, *et al.*, 2009). Thus, the peak intensity at 1640 cm^{-1} of the cellulose whisker was lower than that of cellulose because the lignin was further removed out after acid hydrolysis of cellulose. The 1440 cm^{-1} and 1350 cm^{-1} peaks in the raw cellulose represent C–H deformation vibration of lignin (Sain & Panthapulakkal, 2006; Sun, *et al.*, 2000), and O-C stretching of hemicelluloses and lignin (Cherian, *et al.*, 2008), respectively. The 1250 cm^{-1} peak is indicative of lignin (Paul, *et al.*, 2008). These peaks decrease in case of cellulose whisker due to partial removal of hemicelluloses and lignin.

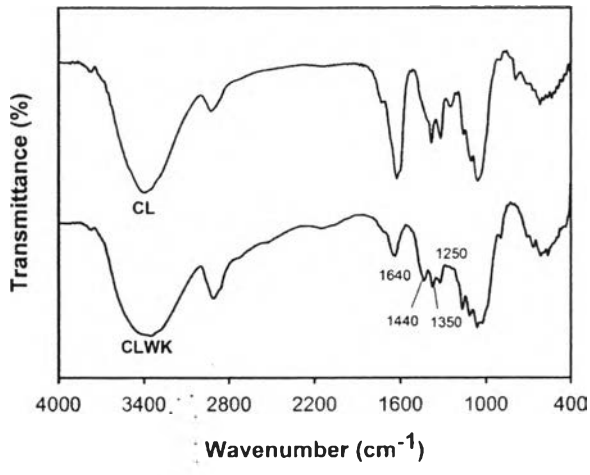

Figure 4.8 FTIR spectra of banana trunk (CL) and cellulose whisker (CLWK).

FTIR spectrum of chitin whisker (Figure 4.9) was similar to that of the anhydrous chitin where three strong absorption peaks in the carbonyl region at 1676 cm^{-1} (amide II), 1646 cm^{-1} (amide II), and 1569 cm^{-1} (amide I) were observed (Muzzrelli, 2005).

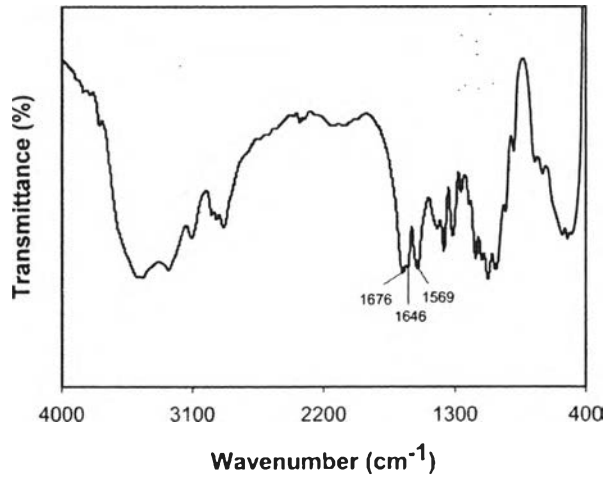

Figure 4.9 FTIR spectrum of chitin whisker.

Since all components of the composites have characteristic absorption bands in a near region, no significant changes in the peak location from the pure components were seen, as shown in Figure 4.10. However area of –OH broad peak

around 3300 cm^{-1} decreased in case of cross-linked cellulose whisker, due to some –OH groups were used in cross-linking reaction. Like cellulose whisker, peak of –NH stretching of cross-linked chitin whisker sponge also reduced. Although, no significant changing peak, in case of sericin-containing bionanocomposite showed distinct peak at 1660 cm^{-1} which is indicative of –C=N– vibration (Nyquist, *et al.*, 1978). Because amine groups in sericin can react with glutaraldehyde to obtain –C=N– bond.

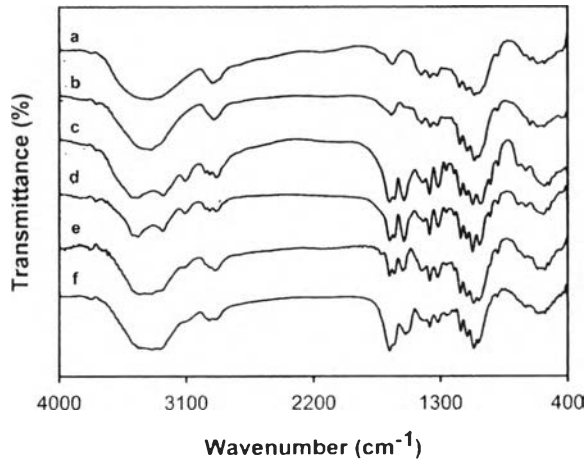

Figure 4.10 FTIR spectra of (a) cellulose whisker, (b) cross-linked cellulose whisker, (c) chitin whisker, (d) cross-linked chitin whisker, (e) 50:50:50 cellulose whisker:chitin whisker:sericin, and (f) 50:50:50 cross-linked cellulose whisker:chitin whisker:sericin bionanocomposite sponges.

4.2.2 X-Ray Diffraction Analysis (XRD)

The characteristic diffractions of raw banana trunk and banana cellulose whisker are shown in Figure 4.11. Raw banana trunk showed the diffraction peak 2θ at 22.5°, 29° and 41°, while cellulose whisker showed the diffraction peak 2θ at 16°, 22.5° and 27.5°. The patterns indicated that the celluloses obtained from raw banana presented a typical form of cellulose I since there was the main peak at 22.5°, which indicating the 020 plane (Li, *et al.*, 2009; Klemm, *et al.*, 2005; Ouajai and Shanks, 2005). Whereas, cellulose whisker showed the diffraction peak at 22.5° for cellulose I form became sharper, due to increase of crystallinity. Moreover, the crystalline transformation to cellulose II could be seen after acid hydrolysis, which

was presented at 2θ = 15° (110 plane). The sharply peaks which appeared at 27°, 28°, and 42° might indicated the mineral of cellulose from environment. So, acid hydrolysis exhibited more efficient removal of hemicelluloses and lignin, which are existed in the amorphous regions. This increase of crystallinity after acid treatment has been reported by several authors (Cherian, *et al.*, 2008; Azizi, *et al.*, 2004; Cherian, 2010; Chen, *et al.*, 2011).

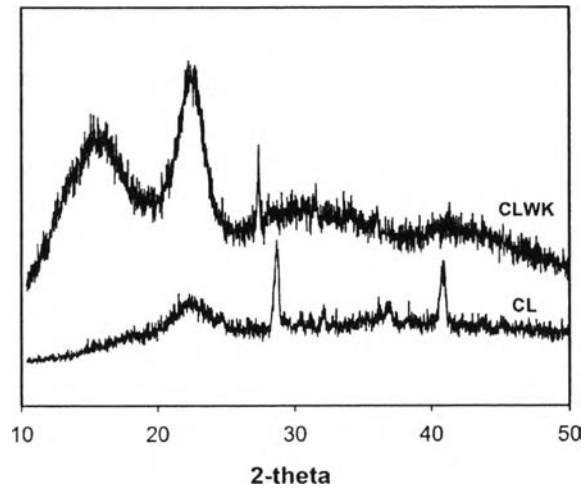

Figure 4.11 X-ray diffraction spectra of dried banana trunk (CL) and cellulose whisker (CLWK).

In case of the obtained chitin whisker, it showed the main diffraction peak 2θ at 19° which corresponding to 110 plane. Moreover, it showed small diffraction peaks at 23° and 38°. Compared previous researches, diffraction peaks were occur in the same 2θ as shown in Figure 4.12 (Sajomsang and Gonil, 2010; Fan, *et al.*, 2008).

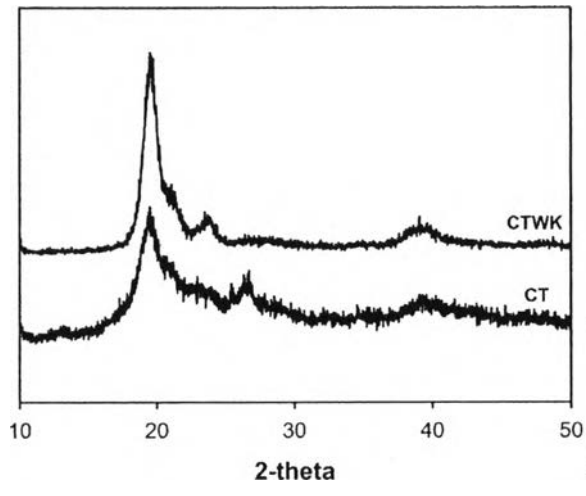

Figure 4.12 X-ray diffraction spectra of chitin powder (CT) and chitin whisker (CTWK).

4.2.3 Thermal Degradation Analysis (TGA)

The TGA and DTA data of raw banana trunk and banana cellulose whisker are shown in Figure 4.13 Both have initial weight loss started at around 50 °C which weight loss around 8%, due to evaporation of water upon heating. In addition, both of them showed only one main step around 250°C - 300°C because of the decomposition of cellulose (de Morais Teixeira, *et al.*, 2010). Due to the low decomposition temperature of hemicelluloses, lignin, and pectin (Moran, *et al.*, 2008), the curve of the original raw banana trunk showed an earlier weight loss that started at around 150°C, which reached the dominant peak at 300°C on the DTG curve accounting for the pyrolysis of cellulose.

On the other hand, the cellulose whisker showed more gradual thermal transitions, it might cause by higher crystallinity of cellulose whisker due to partial removal of hemicelluloses and lignin from the fibers resulting in harder to decompose. Furthermore, the sulfate groups of the cellulose whisker via sulfuric acid hydrolysis were acting as the flame retardants (Roman & Winter, 2004). The charred residue of cellulose whisker was lower than that of the raw banana trunk, due to cellulose whisker had lower content of lignin. Lignin is a complex phenolic polymer, which aromatic substances are not easy to decompose resulting in charred residue.

Thus, acid hydrolysis could remove the other substances to obtained pure cellulose whisker.

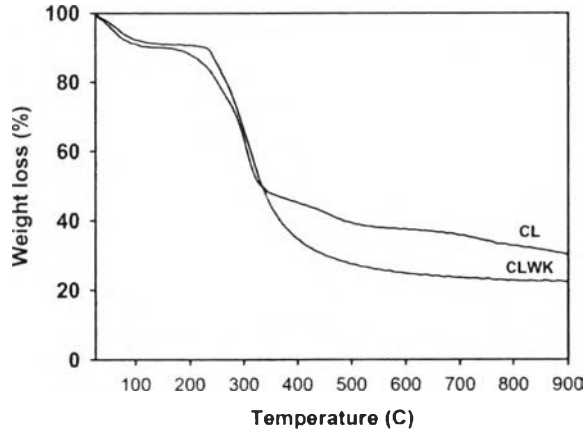

Figure 4.13 Thermal degradation profile of banana trunk (CL) and cellulose whisker (CLWK).

4.3 Water Absorption Properties

After immersed the bionanocomposite sponges in distilled water, the bionanocomposite sponges exhibited good water absorption ability at least 10 times compared to their dry state due to their porous structures and their hydrophilicity of the bionanocomposite sponges, as shown in Figure 4.14. The result revealed that the water absorption decreased with the content of chitin whisker except in case of no sericin sponges, pure cellulose whisker exhibited highest water absorption and significantly dropped when adding chitin whisker but when the content of chitin whisker increased, the water absorption increased by contrasted with sericin-containing sponges. This might be adding chitin whisker interrupted a network structure of cellulose whisker, but higher content of chitin whisker exhibited more ordered arrangement due to aggregation of whisker, leading to better the water absorption however it worse than pure cellulose whisker.

On the other hand in case of sericin- containing sponges showed higher water absorption due to its hydrophilicity of sericin and higher amount of sericin also leaded to higher the water absorption. But in case of high content of chitin whisker,

the water absorption dropped in case of 100wt% of sericin. It might caused by chitin whisker and sericin exhibited stronger interaction bond because of both their hydrogen bonding interaction between protein and chitin (Lu, *et al.*, 2004), and their contrast charges on surface (Nishida, *et al.*, 2011) which chitin whisker and sericin had positive and negative charges in media, respectively. This caused the water absorption dropped.

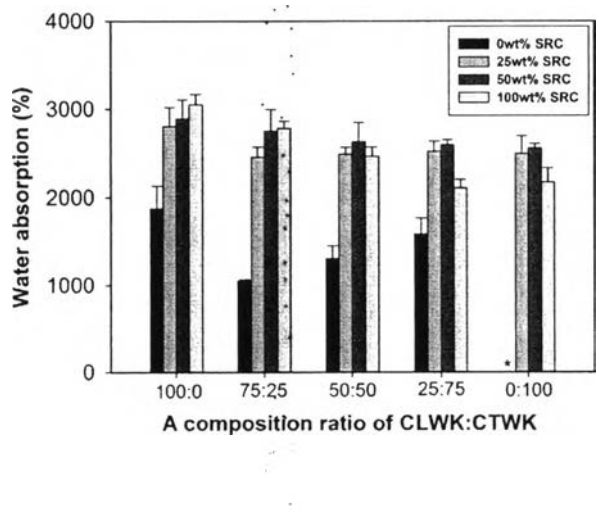

Figure 4.14 The water absorption of bionanocomposite sponges.

4.4 Sericin Releasing

Sericin is water-soluble protein which has many beneficial properties for wound healing, so that it is very useful if it releases into human body. This part investigated effect of cellulose whisker:chitin whisker (CLWK:CTWK) ratio, the effect of sericin (SRC), and the effect of lysozyme — which is an enzyme in human body that can degrade chitin — on the releasing of sericin.

4.4.1 The Effect of Cellulose Whisker and Chitin Whisker on Sericin Releasing

In order to evaluate an effect of the blend composition between cellulose whisker and chitin whisker on release behavior of the sericin from the bionanocomposite sponges, the sponges with the weight ratios of cellulose whisker to chitin whisker of 100:0, 75:25, 50:50, 25:75, and 0:100 were selected (sericin

content was fixed at 25 wt% and 100 wt%). In addition, the release behavior of sericin between the tests using media with and without lysozyme was compared. It was found that the bionanocomposite sponge at a composition of 75:25:25 exhibited the highest sericin release amount. The next compositions were 100:0:25, 50:50:25, 25:75:25, and 0:100:25, as shown in Figure 4.15. In the presence of lysozyme, release amount of sericin increased with the increase in the chitin whisker content. Sericin released from compositions 75:25:25 and 0:100:25 increased from composition 100:0:25 about 5% and 15%, respectively. This should be a result of the enzymatic degradability of chitin in the presence of lysozyme.

In case of fixed 100 wt% sericin showed same trend as 25 wt% sericin as shown in Figure 4.16. However sericin released of 25:75:100 did not increase up to 15% as showed in case of 25:75:25 in the presence of lysozyme. It might be high amount of sericin coat all around whisker surface, especially chitin whisker, leading to no surface area to interact with enzyme. Compared with low sericin content, it did not coat all surface area of whisker, so lysozyme could react with chitin resulting in enzymatic degradation of chitin and sericin could be released.

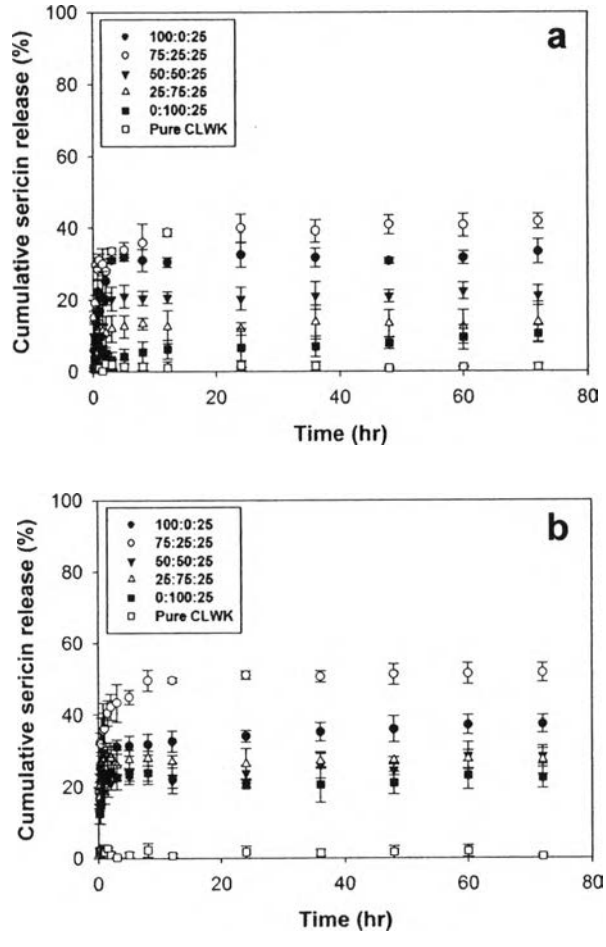

Figure 4.15 Sericin releasing profile of bionanocomposite sponges containing 25wt% sericin (a) without, and (b) with 0.1%w/v lysozyme.

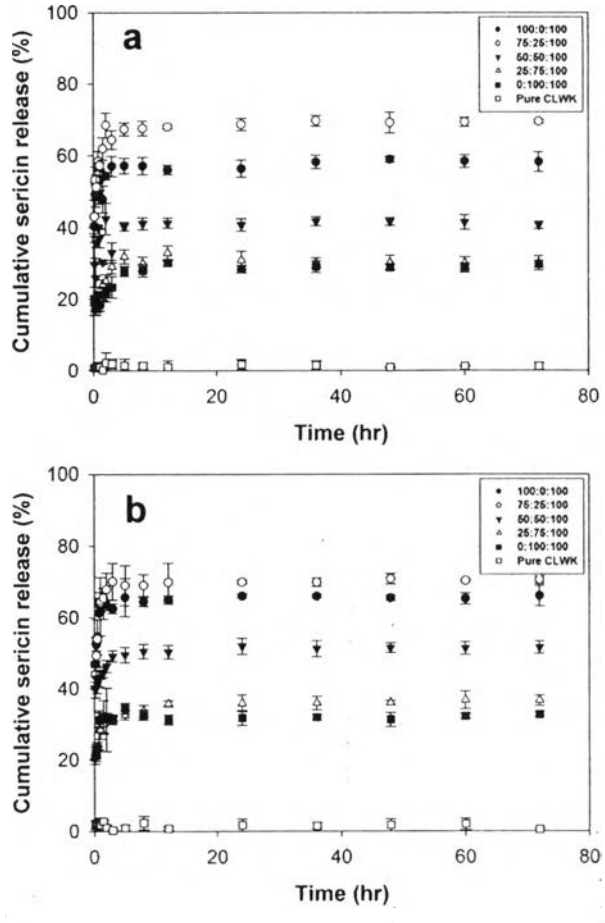

Figure 4.16 Sericin releasing profile of bionanocomposite sponges containing 100wt% sericin (a) without, and (b) with 0.1%w/v lysozyme.

4.4.2 The Effect of Amount of Sericin on Sericin Releasing

To investigated the effect of sericin; 25wt%, 50wt%, and 100wt% of whiskers (the ratio of cellulose whisker:chitin whisker was fixed at 75:25). The releasing profile revealed higher amount of sericin resulting in higher sericin released as shown in Figure 4.17. Nevertheless, lysozyme also did not significantly effect on high content of sericin due to no surface area to interact as previously mentioned.

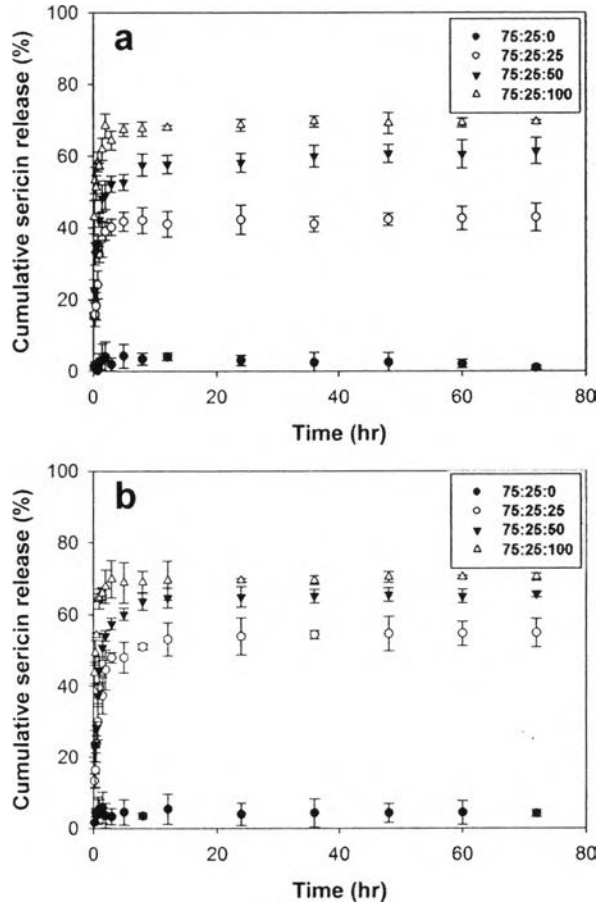

Figure 4.17 Sericin releasing profile of 75:25 cellulose whisker:chitin whisker bionanocomposite sponges containing various sericin content (a) without, and (b) with 0.1%w/v lysozyme.

In conclusion of sericin releasing, highest released in the ratio 75:25 cellulose whisker:chitin whisker and lowest in the ratio 0:100 cellulose whisker:chitin whisker both fixed amount of sericin at 25 wt% and 100 wt%. It might be sericin interacted with chitin whisker better than cellulose whisker due to chitin whisker and sericin has similar amide linkage. Thus the hydrogen bond between chitin whisker and sericin is stronger than cellulose whisker – sericin hydrogen bond, resulting in interaction bonds are difficult to break and sericin is hard to release through external environment.

In the presence of NaCl, it did not effect on 75:25:100 much more than 25:75:100 due to 75:25:100 had high content of cellulose which had weak hydrogen bond resulting in sericin easy to release even no ion in system. On the other hand, high content of chitin sponges significantly sericin released in the presence of NaCl. Because stronger hydrogen bond between chitin whisker and sericin was disturbed by sodium ion leading to sericin easier to release in the presence of NaCl, as shown in Figure 4.18.

In addition, lysozyme could effect on sericin releasing with low amount of sericin because interaction surface area is important to degrade chitin.

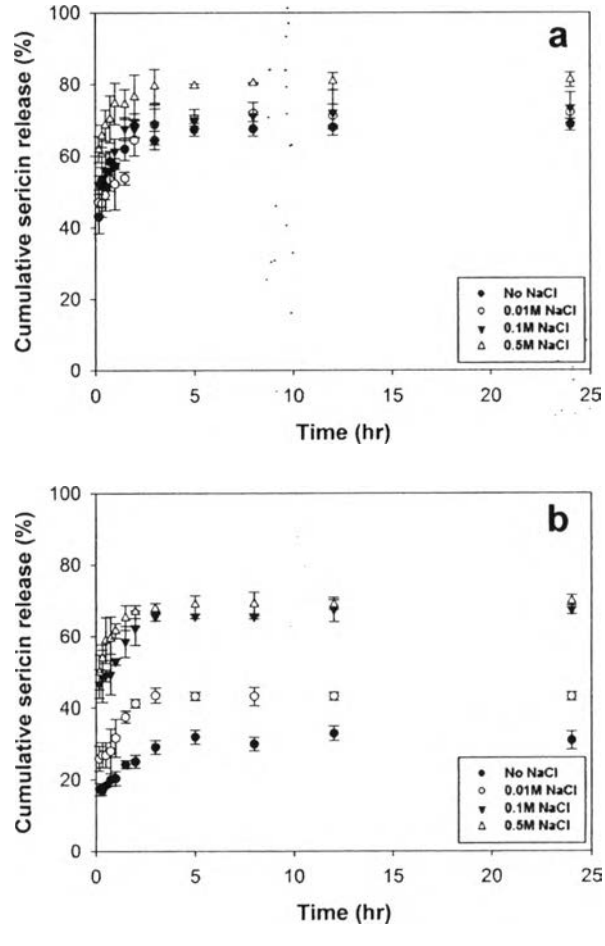

Figure 4.18 Sericin releasing profile of (a) 75:25:100, and (b) 25:75:100 cellulose whisker:chitin whisker:sericin bionanocomposite sponges in various concentrations of NaCl in tris-HCl buffer.