

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Characterization of natural rubber and deproteinized natural rubber

4.1.1 Protein content analysis of natural rubber latex

To preliminarily investigate the deproteinization of natural rubber latex, the protein content of the deproteinized natural rubber latex was measured by the modified Lowry method. As shown in table 4.1, it showed that the protein content of the deproteinized natural rubber latex was 37.95 ug/ml while that of the raw natural rubber latex was 3675.78 ug/ml. After the enzymatic treatment (Perella et al., 2002), the proteins in the natural rubber latex were effectively removed, resulting in a protein removal as high as 98.97 %. Moreover, the % removal of soluble protein content increased with increasing protease concentration.

Table 4.1 Effect of Protease concentration on % Protein Removal

Protein content of natural rubber	Protease content	Protein content of deproteinized natural rubber	% Removal of soluble protein content
3675.78 ug/ml	0.005 g/ml	93.75 ug/ml	97.45
3675.78 ug/ml	0.015 g/ml	70.27 ug/ml	98.09
3675.78 ug/ml	0.02 g/ml	37.95 ug/ml	98.97

4.1.2 FT-IR spectra of the natural rubber and deproteinized natural rubber

The FT-IR spectrum of natural rubber and deproteinized natural rubber films were shown in figure 4.1. The results showed that characteristic peak at

1734 cm^{-1} corresponds to carbonyl group of amino acid and the peak at 1537 cm^{-1} is N-H stretching of a few proteins (Boonsong et al., 2008). These spectra of the deproteinized natural rubber film were decreased. It can be concluded that the proteins were removed or the protein content of deproteinized natural rubber was decreased.

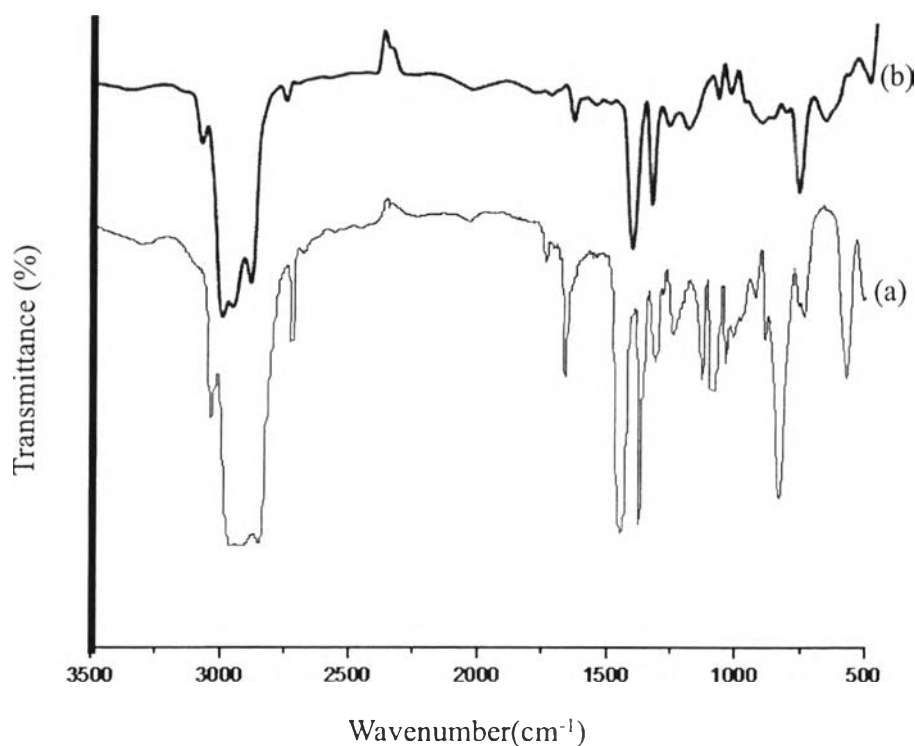


Figure 4.1 The FT-IR spectrum of (a) natural rubber and (b) deproteinized natural rubber.

4.2 Characterization of chitin whisker

4.2.1 Yield production

In general, chitin from animals occurs associated with other constituents, such as lipids, calcium carbonate, proteins and pigments, so, the shrimp shells were treated with chemicals to extract chitin. The shrimp shells were first

cleaned and treated with HCl and NaOH to remove calcium and other minerals, and proteins, respectively. Demineralization occurs according to the following reaction (Belgacem et al., 2008):



Afterwards, chitin was ground to be powder for use to crush to be the small flakes for use in chitin whisker preparation. The yields of chitin and chitin whisker production was represented in table 4.2.

Table 4.2 Yields of chitin and chitin whisker production

Material	Dry Weight (g)
Shrimp shells	100
Demineralized shrimp shells	45.00
Chitin	30.83
» Chitin whisker	20.34

4.2.2 FT-IR spectra of the chitin and chitin whisker

The FT-IR spectrum of chitin and chitin whisker were shown in figure 4.2. It can be seen that the amide I peak (–CONH–) was observed at 1656 and 1621 cm^{-1} and amide II peak at 1560 cm^{-1} , respectively (Morin et al., 2002). The spectrum demonstrates that the successive treatments were strong enough to eliminate all the proteins and obtain pure chitin because of the absence of protein peak at 1540 cm^{-1} . Moreover, the spectrum of chitin whisker exhibited sharper characteristic peaks and splitting of the amide as compared to the chitin flake.

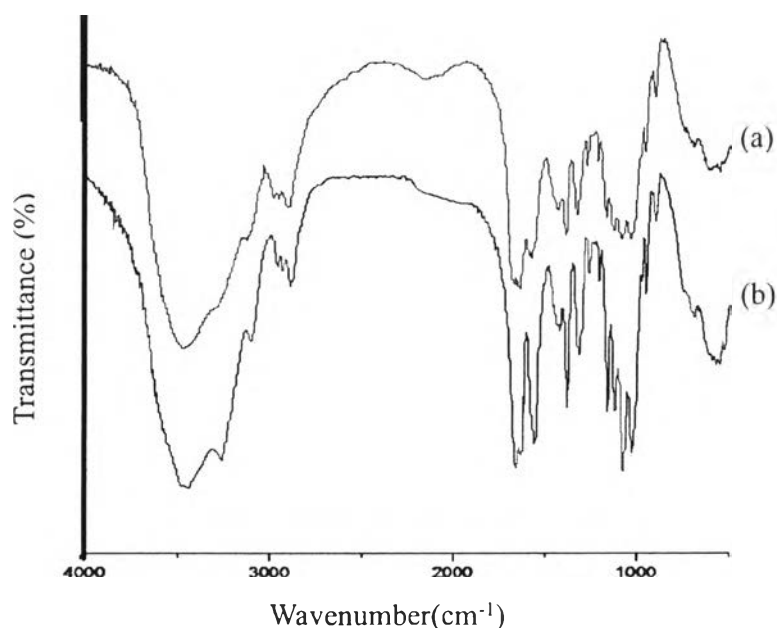


Figure 4.2 The FT-IR spectrum of (a) chitin and (b) chitin whisker.

4.2.3 The morphology of chitin whisker

The protonation of the amino groups ($-\text{NH}_3^+$) in chitin after acid hydrolysis using hydrochloric acid resulted in positive charges appearing at the surfaces of chitin whisker. Thus, the repulsion forces of these cationic charges led to the stable colloidal suspension of chitin whisker in water (Marchessault, et al., 1959). On the other hand, the chloride ions (Cl^-) from HCl 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 individual whisker has a rod-like structure and the whiskers exhibited a broad distribution in both width and length as shown in figure 4.3. The average length (L) and width (d) of the whiskers were 443 and 34 nm, respectively, and the average aspect ratio (L/d) was 13.



Figure 4.3 TEM micrograph of chitin whisker suspension.

4.3 Characterization of coconut oil/pluronic emulsion

4.3.1 Effect of Pluronic concentration and emulsion stability

The effect of Pluronic concentration and emulsion stability was shown in table 4.3. The stability of emulsion was determined by varying critical micelle concentrations (cmc) of pluronic and the ratios of pluronic to coconut oil. It revealed that the emulsion stability increased with the increasing of the ratio of pluronic to coconut oil and the concentration of pluronic. This should be due to the interaction between the pluronic molecules.

Table 4.3 Stability time of coconut oil/pluronic emulsion

Pluronic : Coconut oil (volume : volume)	Stability Time of emulsion (h.)		
	10xcmc* of pluronic	50xcmc of pluronic	100xcmc of pluronic
80:20	2	8	12
70:30	8	24	72
60:40	12	48	120
50:50	10	36	96
40:60	8	12	24
30:70	3	8	16
20:80	0.33	0.67	1

*cmc of pluronic (F-68) is 0.334 g/L.

4.3.2 The size of micelle

The size of micelle by polarized optical microscope was shown in figure 4.4. The size was determined by varying the ratios of pluronic to coconut oil. It was established that the micellar size is the result of two opposite contributions, the repulsive interactions between the head group of the ionic surfactant, which prevent the micellar increase, and the steric interactions favored by the size of the head group (Hierrezuelo et al., 2004). In case of 50/50, the micelle size was 593 ± 3.52 nm, 60/40 was 572 ± 3.19 and 70/30 was 657 ± 4.21 . It can be concluded that the emulsion stability increased with the decreasing of the size of micelle.

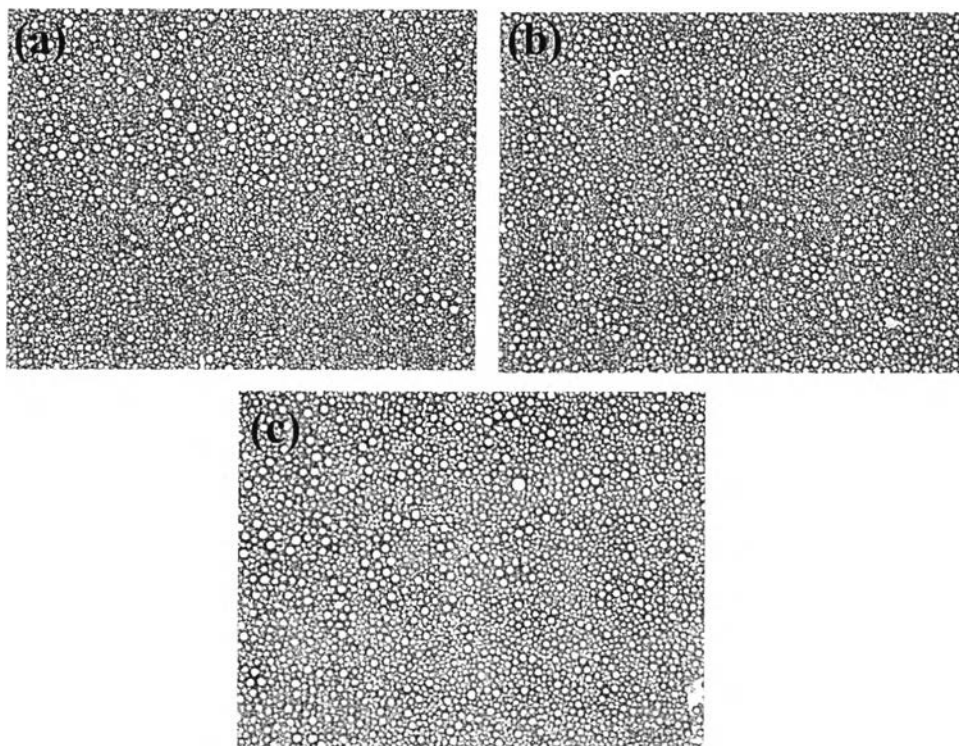


Figure 4.4 The size of micelle by polarized optical microscope with the volume ratio of pluronic to oil as (a) 50/50, (b) 60/40 and (c) 70/30.

4.3.3 The dispersion and size of micelle in the natural rubber latex

The dispersion of micelle in the natural rubber was shown in figure 4.5. These figure demonstrated that the micelle was not break down and dispersed in the matrix. The size was determined by varying the ratios of pluronic/coconut oil/natural rubber. In case of (a), the micelle size was 283 ± 7.76 nm, (b) was 228 ± 4.13 and (c) was 310 ± 6.12 . For (c), the pluronic concentration increase so the pluronic induce in agglomerate resulting in the size of micelle was increased. For (a) and (b), (a) was bigger than (b) because (a) had oil content more than (b). The oil content increased so the repulsive force between oil and oil and between oil and PPO core (hydrophobic) increased resulting in the diameter of micelle was increased.

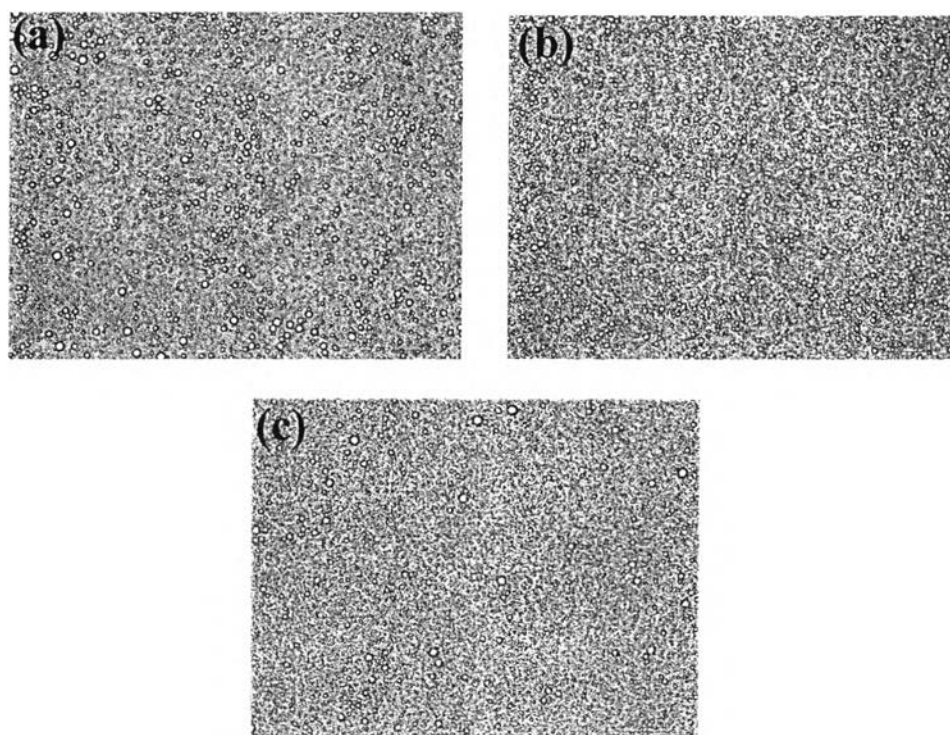


Figure 4.5 The dispersion of micelle in the natural rubber with the volume ratio of pluronic/coconut oil/natural rubber as (a) 15/15/70, (b) 18/12/70 and (c) 21/9/70.

4.4 Characterization of Natural rubber/Chitin whisker

The morphology of neat natural rubber and natural rubber with 2.5%wt 5%wt and 10%wt chitin whisker was shown in figure 4.6. The surface roughness was observed when chitin whisker was added into the films. There are no micro-scaled aggregates at 2.5 wt.% chitin whisker content. In higher chitin whisker concentration disperse well but there are some aggregation of chitin whisker and led to higher phase separation. Figure 4.7 shows representative images, staining of chitin whisker with the Amido Black 10B is a potent tool for examining the dispersion of chitin whisker within natural rubber (Watthanaphanit et al., 2009). It seem that there are no spot at 2.5 wt.% chitin whisker content so it reveal that there are no aggregates at 2.5 wt.% chitin whisker content because natural rubber latex remain protein residue. For higher chitin whisker content, it seem that there are blue spot so the blue spot refer

the chitin whisker aggregation. The chitin whisker content increased, the blue spot increased due to the separation between polar material phase of chitin whisker and non-polar material phase of natural rubber because the natural rubber is hydrophobic and chitin whisker is hydrophilic.

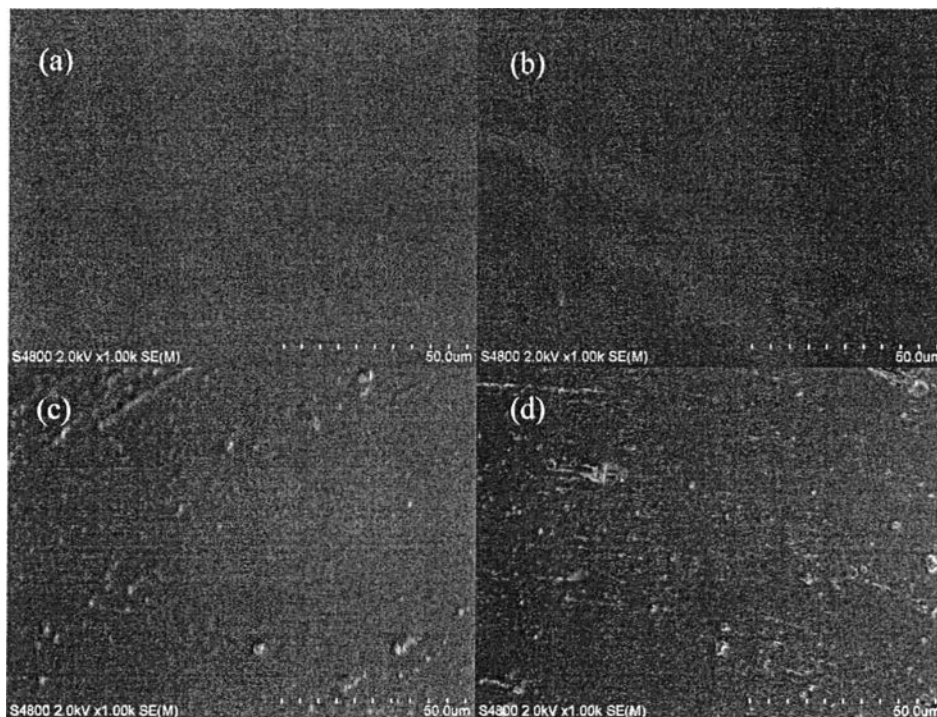


Figure 4.6 The morphology of (a) neat natural rubber, (b) natural rubber with 2.5%wt chitin whisker, (c) natural rubber with 5%wt chitin whisker and (d) natural rubber with 10%wt chitin whisker.

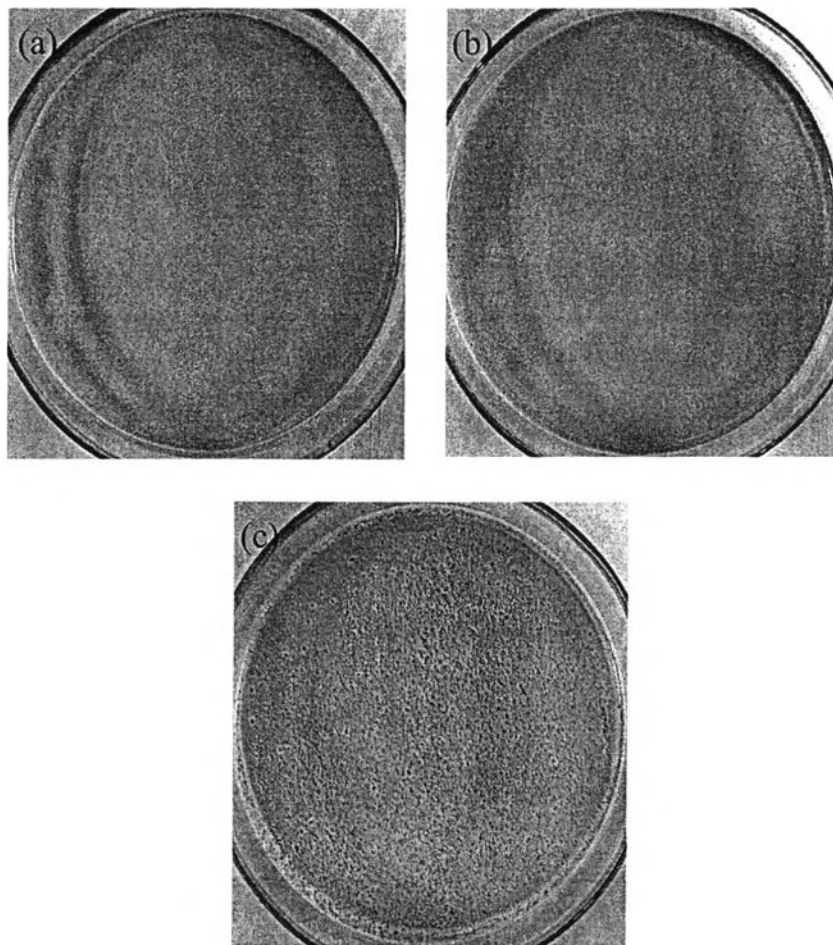


Figure 4.7 Representative images of chitin whisker in the natural rubber latex as a function of chitin whisker content (a) 2.5 wt.%, (b) 5 wt.% and (c) 10 wt.% after staining of chitin whisker with the Amido Black 10B.

4.5 Characterization of Chitin Whisker/Natural Rubber containing Pluronic and Coconut oil

4.5.1 Mechanical properties

The tensile strength of the nanocomposite films was shown in figure 4.8. It seem that the tensile strength of the nanocomposite film decreased with increasing coconut oil content and increased with the increasing of chitin whisker

content. It indicated that chitin whisker acted as reinforcement filler due to chitin whisker interfere the movement of natural rubber. On the other hand, the elongation at break of the nanocomposite films was shown in figure 4.9. It seem that the elongation at break of the nanocomposite film increased with the increasing of oil content while decreased with the increasing of whisker content. It can be concluded that coconut oil acted as plasticizer because the coconut oil have long chain on fatty acid. Then, the long chain insert between molecules cause the molecule separation and easy to move. It showed flexibility.

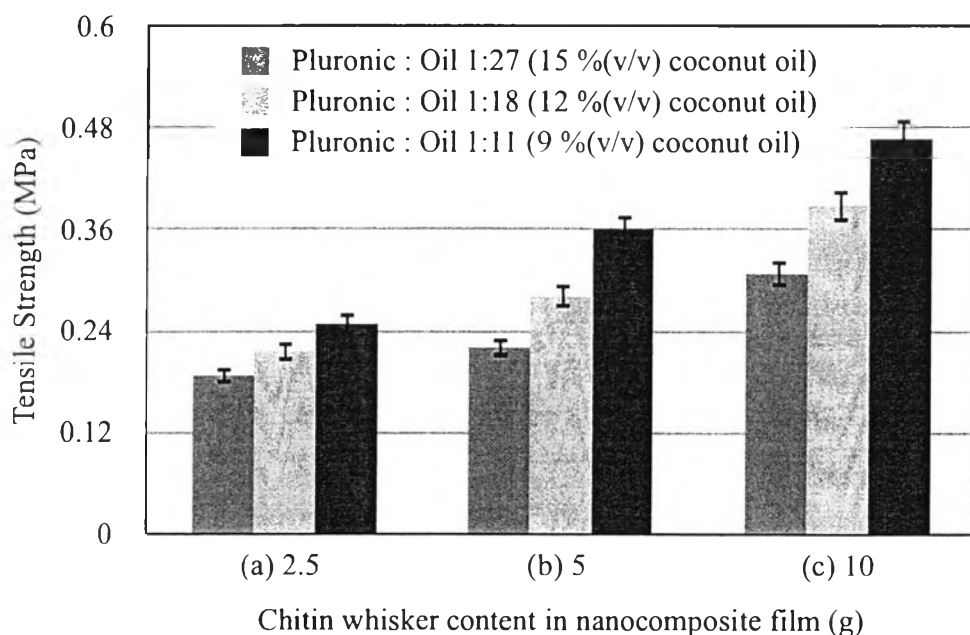


Figure 4.8 Tensile strength of nanocomposite films as a function of chitin whisker content (a) 2.5 wt.%, (b) 5 wt.% and (c) 10 wt.%.

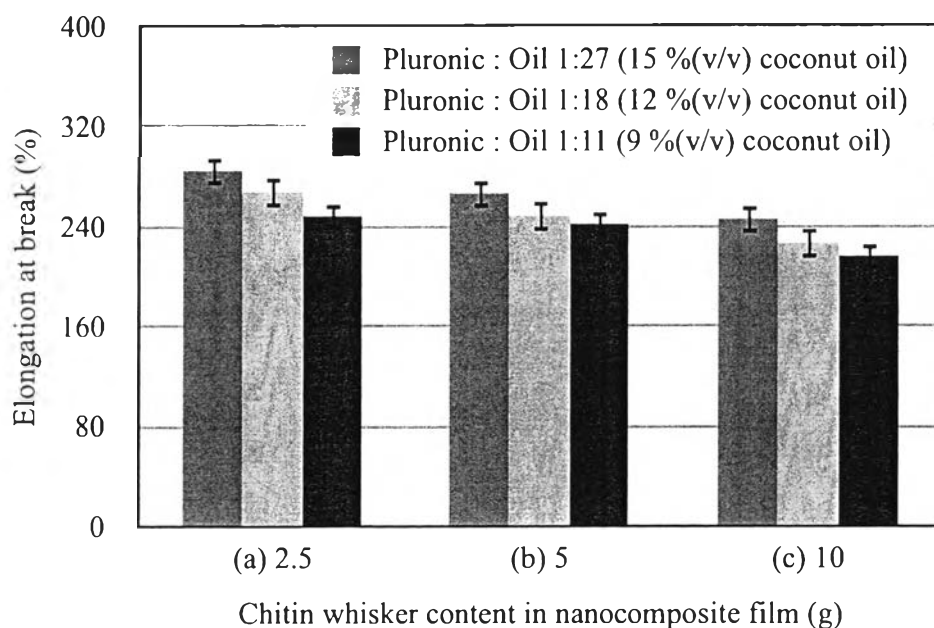


Figure 4.9 The percentage of elongation at break of of nanocomposite films as a function of chitin whisker content (a) 2.5 wt.%, (b) 5 wt.% and (c) 10 wt.%.

4.5.2 The degree of swelling and weight loss of the nanocomposite films

The degree of swelling and weight loss of the nanocomposite films after submersion in buffer pH 5.5 at 37 °C for 6, 12 and 24 hr were shown in figure 4.10 and figure 4.11, respectively. When the coconut oil released, the film occur void. Then, the buffer solution penetrate to film so the sample was swollen. The bigger micelle size, it obtain the bigger void. From the figure 4.10, it showed that the degree of swelling increased with decreasing chitin whisker content. From these results, it is assumed that the formation of a hydrogen-bonded network of whiskers within the matrix is a reason for the lower degree of swelling. From the figure 4.11, it showed that the percentage of weight loss increased with decreasing chitin whisker content. Therefore, it can be confirmed that chitin whiskers can improve the shape stability of these materials and chitin whisker also obstruct the oil releasing.

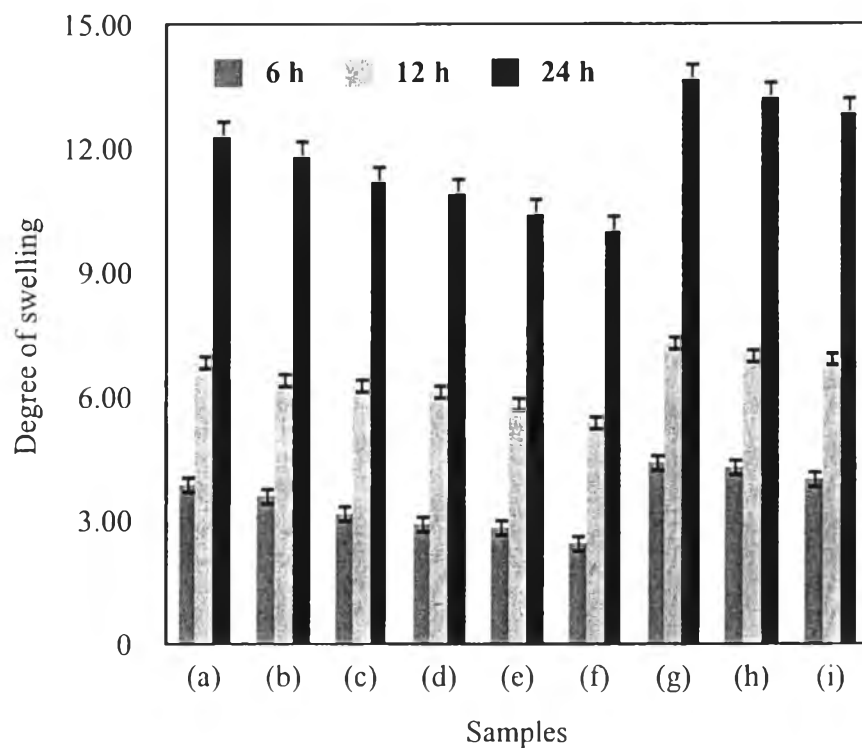


Figure 4.10 Degree of swelling of nanocomposite films with the weight ratio of pluronic/coconut oil/natural rubber/chitin whisker as (a) 0.5/13.6/21/2.5, (b) 0.5/13.6/21/5, (c) 0.5/13.6/21/10, (d) 0.6/10.9/21/2.5, (e) 0.6/10.9/21/5, (f) 0.6/10.9/21/10, (g) 0.7/8.2/21/2.5, (h) 0.7/8.2/21/5 and (i) 0.7/8.2/21/10 after submersion in buffer pH 5.5 for 6, 12 and 24 hr at room temperature.

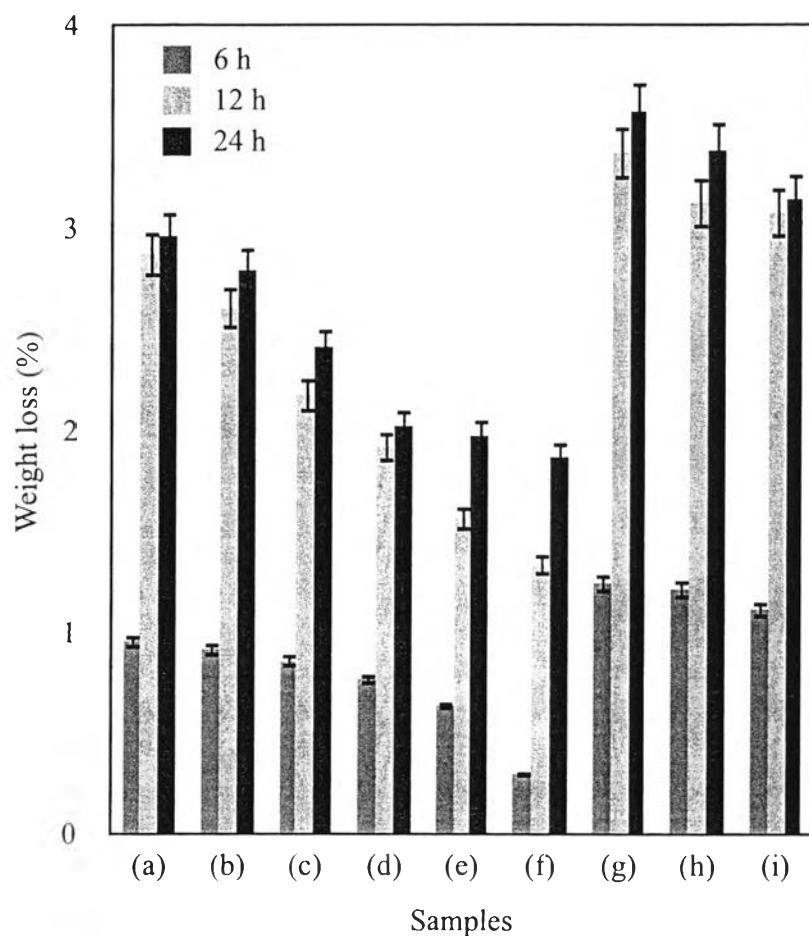
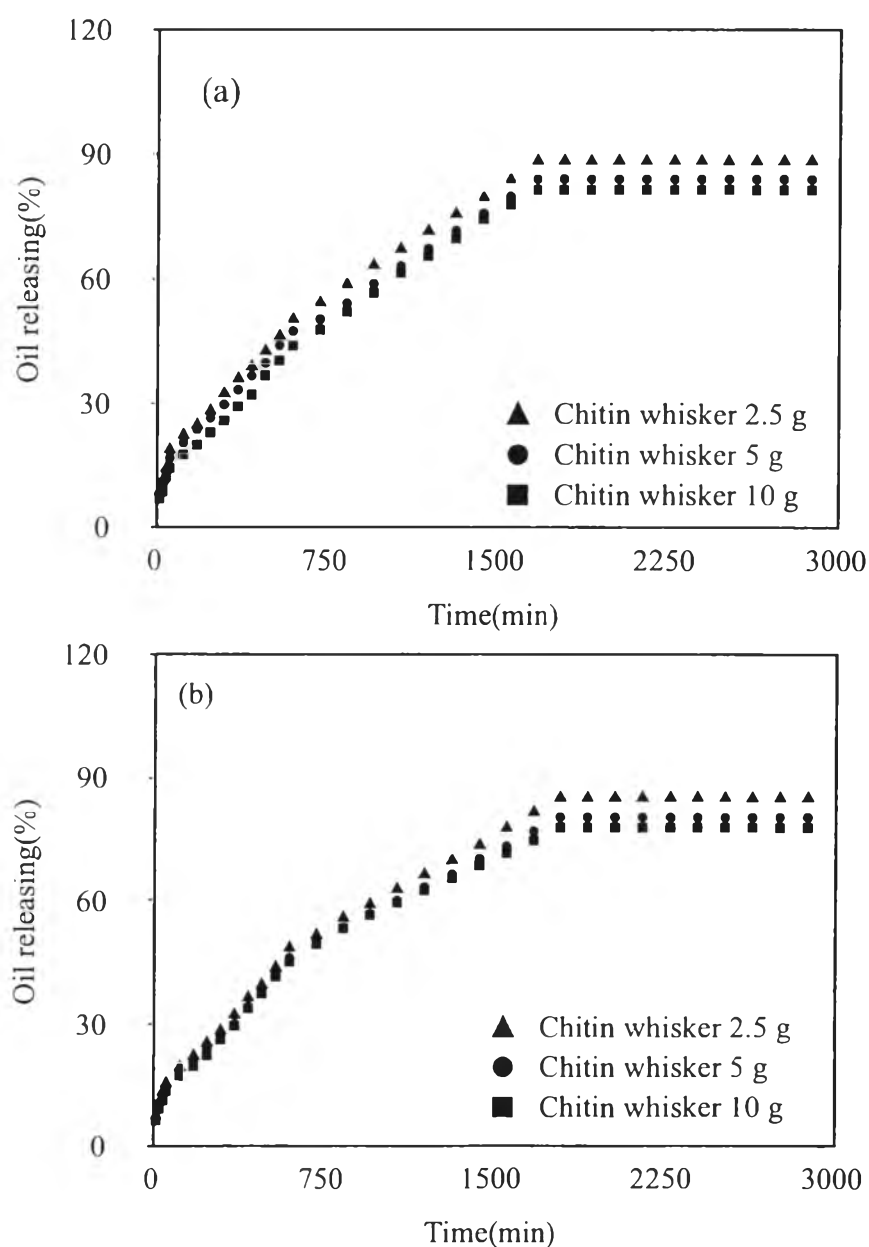


Figure 4.11 Percentage of weight loss of nanocomposite films with the weight ratio of pluronic/coconut oil/natural rubber/chitin whisker as (a) 0.5/13.6/21/2.5, (b) 0.5/13.6/21/5, (c) 0.5/13.6/21/10, (d) 0.6/10.9/21/2.5, (e) 0.6/10.9/21/5, (f) 0.6/10.9/21/10, (g) 0.7/8.2/21/2.5, (h) 0.7/8.2/21/5 and (i) 0.7/8.2/21/10 after submersion in buffer pH 5.5 for 6, 12 and 24 hr at room temperature.

4.6 Oil releasing behavior

The release characteristic of coconut oil from the nanocomposite films is shown in figure 4.12. It was found that the release of coconut oil from the nanocomposite films decreased with the increasing of the chitin whisker content. It can be concluded that the presence of chitin whisker obstruct the coconut oil

releasing. From the figure 4.13, it showed the comparison in the different ratio of pluronic to oil in the equal chitin whisker content. The oil releasing of 70:30 is more than 50:50 and 50:50 is more than 60:40. Moreover, the percentage of oil releasing corresponded to the stability time and micelle size of emulsion. The smallest micelle can prolong the releasing due to the least repulsive force of head chain of micelle resulting in the most stability so the most prolong releasing.



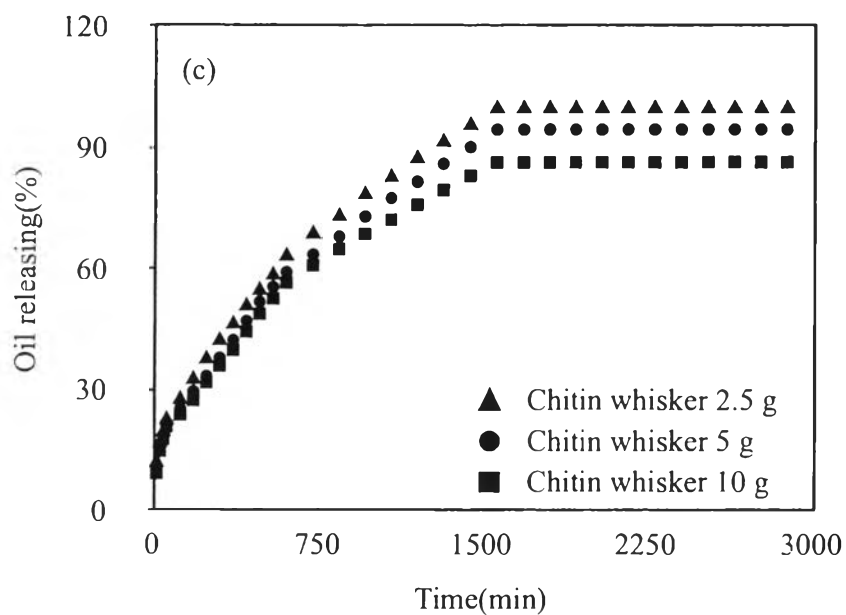
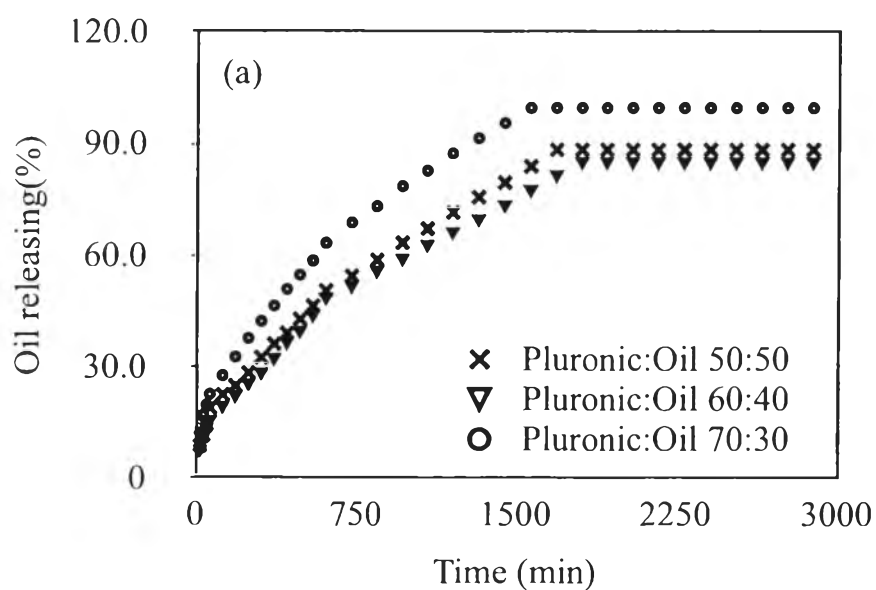


Figure 4.12 The releasing profiles of coconut oil from natural rubber/chitin whisker nanocomposite films with the weight ratio of pluronic/coconut oil as (a) 0.5/13.6, (b) 0.6/10.9 and (c) 0.7/8.2.



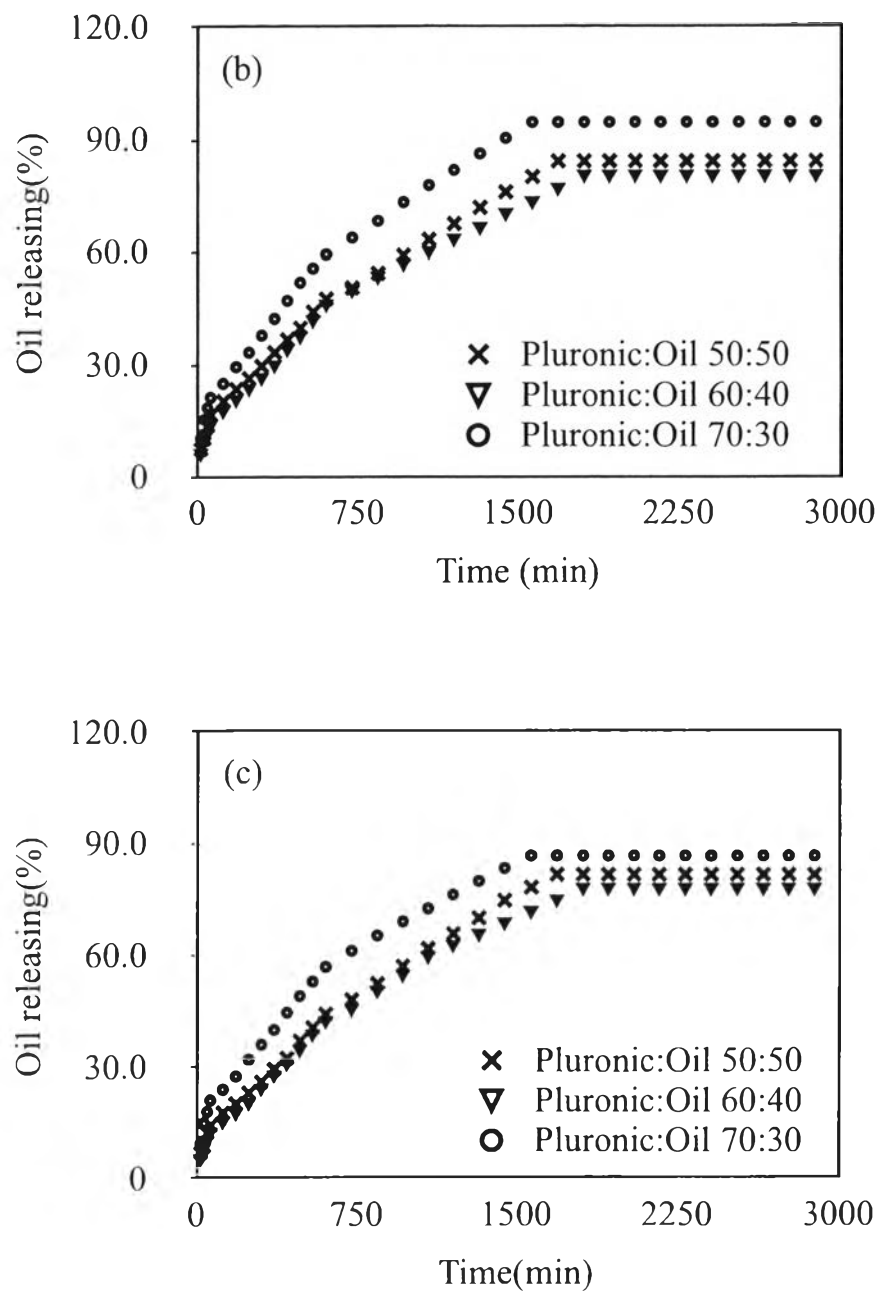


Figure 4.13 The releasing profiles of coconut oil from natural rubber/chitin whisker nanocomposite films as a function of chitin whisker content (a) 2.5 wt.%, (b) 5 wt.% and (c) 10 wt.%.