

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Preparation of Chitin

Squid pens and shrimp shells generally compose of three major components, which are chitin, calcium carbonate, and protein.

In this research chitin was prepared from both squid pens and shrimp shells by demineralization with hydrochloric acid solution and deproteinization with sodium hydroxide solution in order to remove calcium carbonate and protein, respectively.

Table 4.1 Yields of chitin produced from squid pens

Chitin Batch no.	Weight of squid pens (g)	Weight of chitin (g)	% Yield
1	500	188	37.6
2	500	194	38.8
3	500	195	39.0
4	500	193	38.6

Table 4.2 Yield of chitin produced from shrimp shells

Chitin Batch no.	Weight of shrimp shells (g)	Weight of chitin (g)	% Yield
1	360	206.43	57.342

4.2 Characterization of β -Chitin

4.2.1 Structural Characterization of Chitin from Squid Pen (β -chitin)

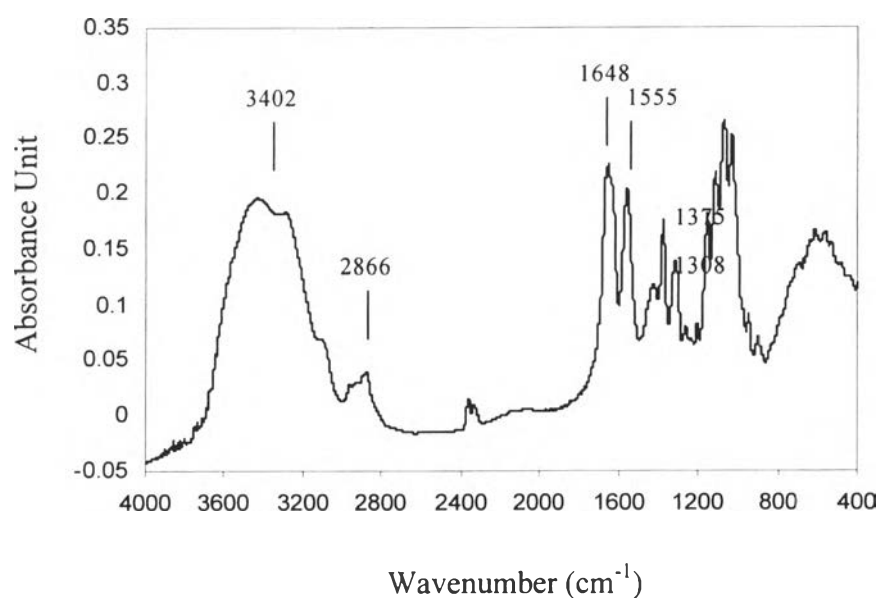


Figure 4.1 FTIR spectrum of β -chitin.

β -chitin obtained was characterized by using FTIR. The FTIR spectrum of chitin is shown in Figure 4.1 and the absorption frequencies of characteristic bands of chitin are summarized in Table 4.3.

Table 4.3 FTIR characteristic absorption bands of β -chitin

Frequencies (cm ⁻¹)	Assignment and Remarks
3402	O-H stretching
2866	C-H stretching
1648	C=O stretching of acetamind groups
1555	N-H stretching of acetamind groups
1375	CH ₃ symmetrical deformation
1308	C-N stretching of acetamind groups

4.2.2 Degree of Deacetylation

The degree of deacetylation of β -chitin was determined, based on an infrared spectroscopic measurement. The degree of deacetylation of β -chitin was 16.9%.

4.2.3 Viscosity-Average Molecular Weight

The molecular weight of β -chitin was determined by viscometric method. The molecular weight of β -chitin was derived from its intrinsic viscosity. The plot of reduced viscosity (η_{sp}/C) and inherent viscosity ($\ln(\eta_{rel})/C$) versus concentration of β -chitin solution is shown in Figure 4.2.

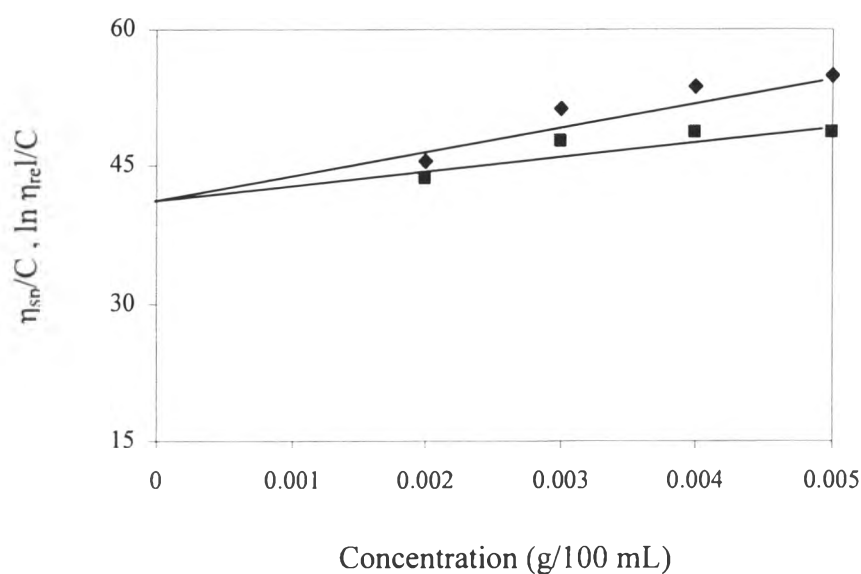


Figure 4.2 Plot of reduced viscosity and inherent viscosity versus concentration of β -chitin solution.

From the calculation, the viscosity-average molecular weight of β -chitin was 3,639,724.

4.3 Characterization of α -Chitin

4.3.1 Structural Characterization of Chitin from Shrimp Shell (α -chitin)

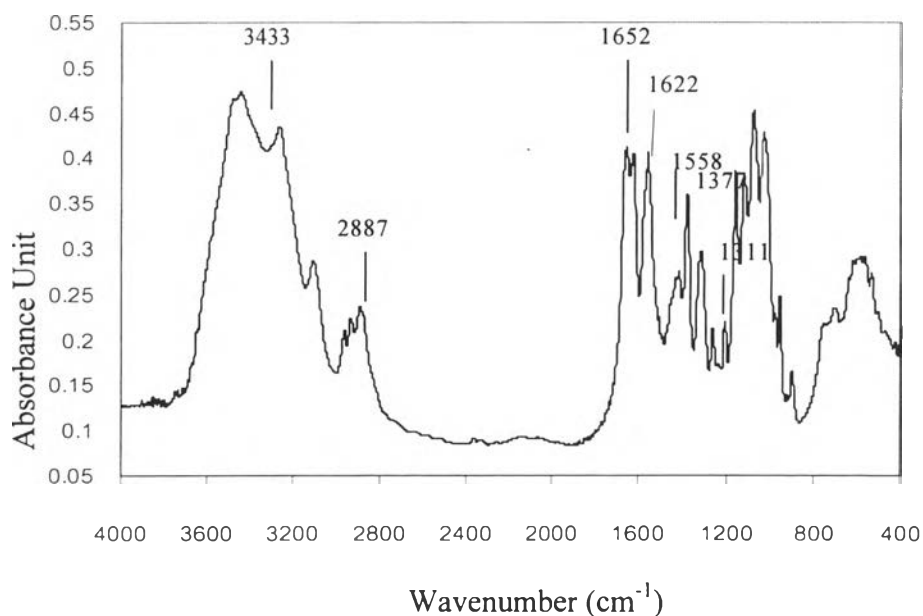


Figure 4.3 FTIR spectrum of α -chitin.

α -chitin obtained was characterized by using FTIR. The FTIR spectrum of α -chitin is shown in Figure 4.3 and the absorption frequencies of characteristic bands of chitin are summarized in Table 4.4

Table 4.4 FTIR characteristic absorption bands of α -chitin

Frequencies (cm ⁻¹)	Assignment and Remarks
3433	O-H stretching
2887	C-H stretching
1652 and 1622	C=O stretching of acetamind groups
1558	N-H stretching of acetamind groups
1377	CH ₃ symmetrical deformation
1310	C-N stretching of acetamind groups

4.3.2 Degree of Deacetylation

The degree of deacetylation of α -chitin was determined based on infrared spectroscopic measurement. The degree of deacetylation of α -chitin was 17.66%.

4.3.3 Viscosity-Average Molecular Weight

The molecular weight of α -chitin was determined by viscometric method. The molecular weight of α -chitin was derived from its intrinsic viscosity. The plot of reduced viscosity (η_{sp}/C) and inherent viscosity ($\ln(\eta_{rel})/C$) versus concentration of α -chitin solution is shown in Figure 4.4.

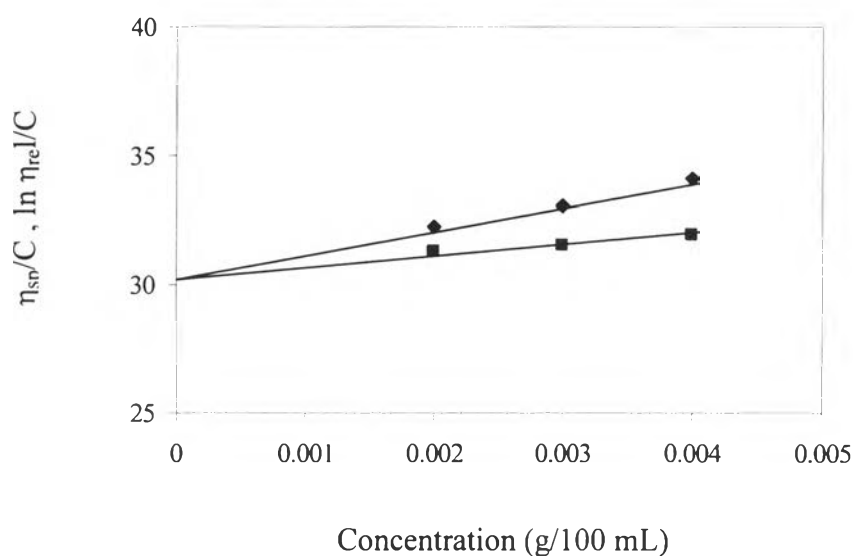


Figure 4.4 Plot of reduced viscosity and inherent viscosity versus concentration of α -chitin solution.

From the calculation, the viscosity-average molecular weight of α -chitin was 2,418,421.

4.4 Preparation of Chitosan

Chitin obtained was deacetylated by refluxing the chitin flakes in NaOH solution 50% w/w at 100°C for 2 h and repeating 3 times. Then chitosan with mainly reactive amino groups would be obtained.

Table 4.5 Yields of chitosan produced from β -chitin

Chitosan Batch no.	Weight of chitin (g)	Weight of chitosan obtained (g)	% Yield
1	50	27.80	55.6
2	50	33.0	66.0
3	50	33.0	66.0

4.5 Characterization of Chitosan

4.5.1 Structural Characterization

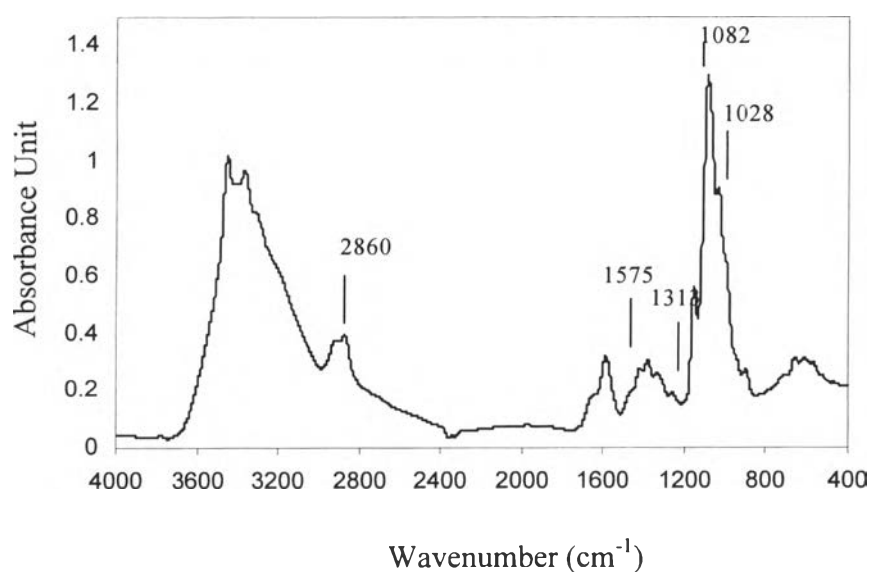


Figure 4.5 FTIR spectrum of chitosan.

Chitosan obtained was characterized by using FTIR. The FTIR spectrum of chitosan is shown in Figure 4.5 and the absorption frequencies of characteristic bands of chitosan are summarized in Table 4.6.

Table 4.6 FTIR characteristic absorption bands of chitosan

Frequencies (cm^{-1})	Assignment and Remarks
1575	NH deformation
1313	CN stretching
1082 and 1028	C-O stretching vibration

4.5.2 Degree of Deacetylation

The degree of deacetylation of chitosan was determined based on infrared spectroscopic measurement. From the step of preparation of chitosan, the deacetylation was performed repeatedly to achieve chitosan with higher degree of deacetylation. The degree of deacetylation of chitosan in each treatment is shown in Table 4.7

Table 4.7 Degree of deacetylation of chitosan

No. of Treatment	% Degree of deacetylation
1	90.78
2	91.31
3	94.64

4.5.3 Viscosity-Average Molecular Weight

The molecular weight of chitosan was determined by viscometric method. The molecular weight of chitosan was derived from its intrinsic viscosity.

The plot of reduced viscosity (η_{sp}/C) and inherent viscosity ($\ln(\eta_{rel})/C$) versus concentration of chitosan solution is shown in Figure 4.6.

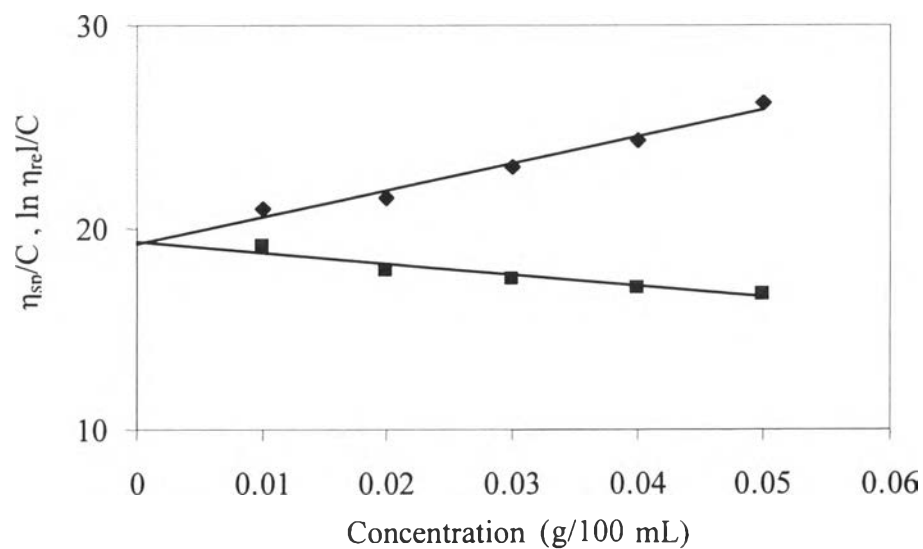


Figure 4.6 Plot of reduced viscosity and inherent viscosity versus concentration of chitosan solution.

From the calculation, the viscosity-average molecular weight of chitosan after each treatment is shown in Table 4.8.

Table 4.8 The viscosity-average molecular weight of chitosan

No. of treatment	Viscosity-average molecular weight
1	1,581,333
2	1,550,726
3	1,526,599

4.6 Characterization of Chitin Whisker

A transmission electron micrograph obtained from a diluted suspension of hydrolyzed shrimp shell chitin is shown in Figure 4.7.

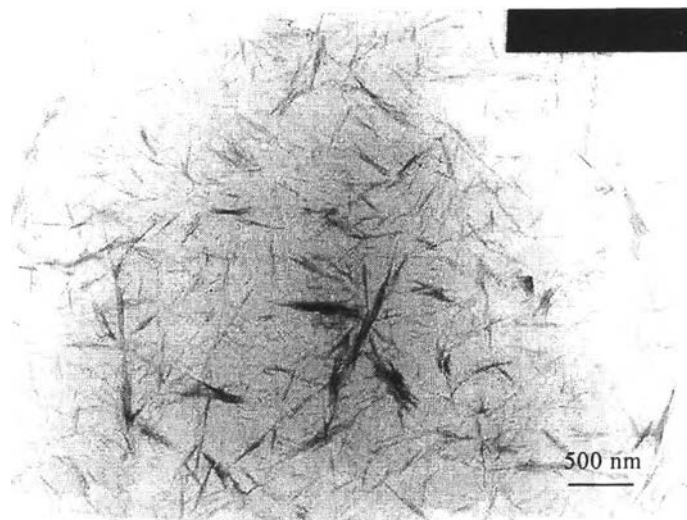


Figure 4.7 Transmission electron micrograph of a dispersion of hydrolyzed shrimp shell chitin.

After acid hydrolysis, the suspension of chitin whiskers displayed a colloidal behaviour. This is due to the presence of positive charges (NH_3^+) at the surface of the whiskers, resulting from the protonation of amino groups at acidic pH (Marchessault, 1959). The suspension contained chitin fragments consisting of both individual microcrystal and associated microcrystal. The chitin fragments consisted of slender rods with sharp points and a broad distribution in size as shown in Figure 4.8. They had a length ranging from 150 nm up to 800 nm and a width ranging from 5 to 70 nm. More than 75% of the whiskers had a length below 420 nm. The average length and width were estimated to be around 417 and 33 nm, respectively. The average aspect ratio (L/d , L being the length and d the diameter) of these whiskers was around 17. These dimensions are close to those reported for chitin whiskers obtained from squid pen which have a length from 50 to 300 nm, a width of

10 nm and an aspect ratio of 15 nm (Paillet and Dufresne, 2001), and also close to chitin whiskers obtained from crab shell which have a length from 100 to 600 nm and a width of 15 nm and an aspect ratio of 16 nm (Nair and Dufresne, 2003).

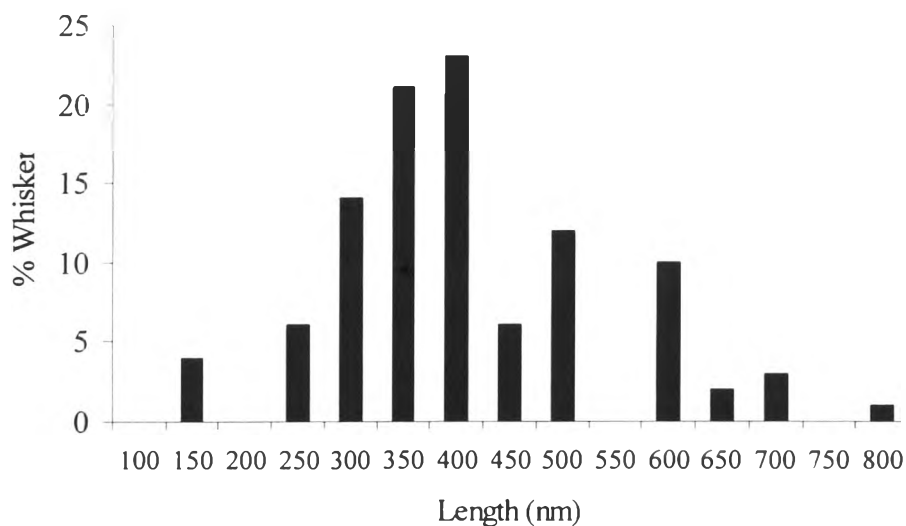


Figure 4.8 Histogram of the length distribution of chitin whiskers from shrimp shells.

4.7 Characterization of Chitin Whisker-Reinforced Films

The chitin whisker-reinforced films were prepared by solution casting. Transparent films with a thickness around 30-50 μm were obtained.

4.7.1 Crytallinity of the Chitin Whisker-Reinforced PVA Films

Figure 4.9 shows the WAXD pattern of chitin whisker-reinforced PVA films. Spectrum (a) shows a typical peak of PVA that appears at $2\theta = 20^\circ$. Spectrum (g) is the typical X-ray diffraction pattern of chitin whisker. Chitin whisker has two distinct crystal forms which shows strong reflection at $2\theta = 9.1^\circ$ and $2\theta = 19.2^\circ$. The X-ray diffraction patterns of chitin whisker-reinforced PVA films show that the film with low chitin whisker content had a broad peak at around $2\theta = 20^\circ$ and when chitin whisker content increased, the peak at $2\theta = 20^\circ$ became sharper and began to show reflection at around $2\theta = 9^\circ$. This suggested that the relative

crystallinity of the nanocomposite films increased upon increasing of chitin whisker contents.

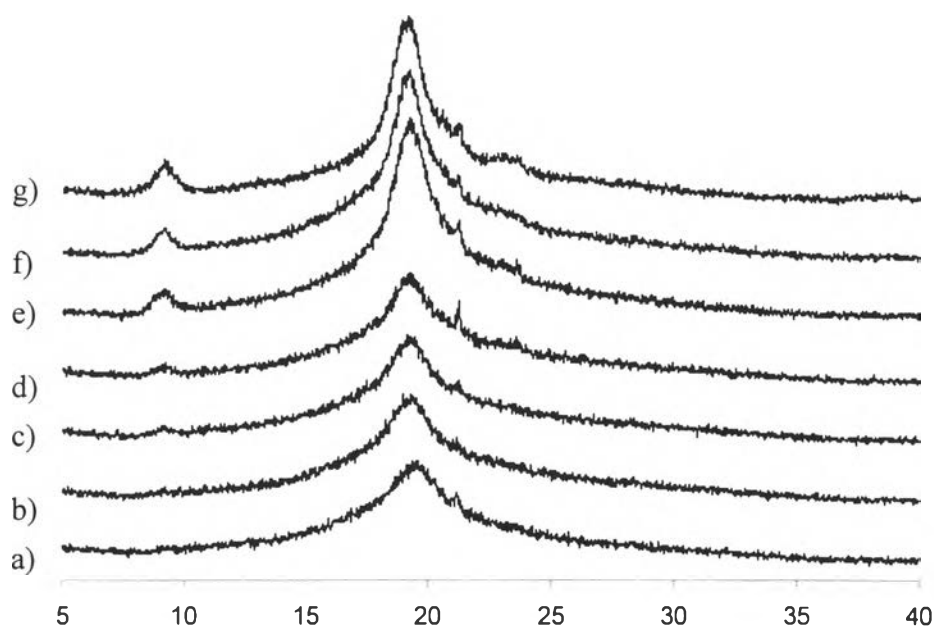


Figure 4.9 WAXD patterns of chitin whisker-reinforced PVA films containing different chitin whisker contents. (a) pure PVA, (b) 2.96 wt% whisker content, (c) 7.40 wt% whisker content, (d) 14.8 wt% whisker content, (e) 22.2 wt% whisker content, (f) 29.6 wt% whisker content and (g) chitin whisker.

4.7.2 Crystallinity of the Chitin Whisker-Reinforced Chitosan Films

Figure 4.10 exhibits WAXD patterns of chitin whisker-reinforced chitosan films. Spectrum (a) shows broad peaks of chitosan at $2\theta = 11.4^\circ$ and $2\theta = 21.4^\circ$. Spectrum (g) shows strong reflection of chitin whisker at $2\theta = 9.1^\circ$ and $2\theta = 19.2^\circ$. The films with higher content of chitin whisker showed sharper peak at around $2\theta = 20^\circ$ and began to show reflection at $2\theta = 9^\circ$. According to X-ray diffraction patterns, it indicated that the higher chitin whisker content, the more crystallinity of the nanocomposite films was observed.

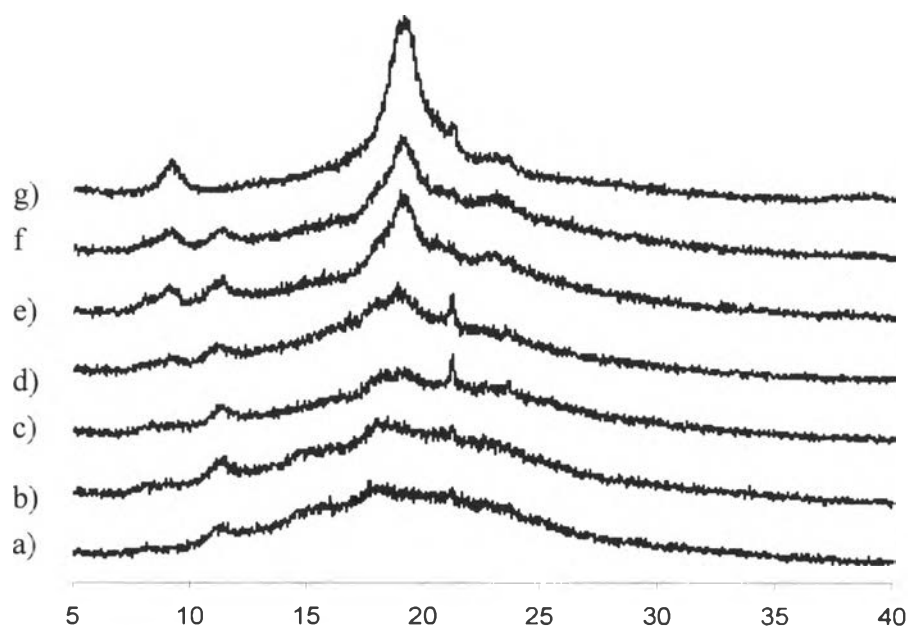


Figure 4.10 WAXD patterns of chitin whisker-reinforced chitosan films containing different chitin whisker contents. (a) pure chitosan, (b) 2.96 wt% whisker content, (c) 7.40 wt% whisker content, (d) 14.8 wt% whisker content, (e) 22.2 wt% whisker content, (f) 29.6 wt% whisker content and (g) chitin whisker.

4.7.3 Mechanical Properties of Chitin Whisker-Reinforced PVA Films

The effects of chitin whisker content on mechanical properties of the chitin whisker-reinforced PVA nanocomposite films and chitin whisker-reinforced chitosan nanocomposite films were determined. The tensile strength and elongation at break of the chitin whisker-reinforced PVA films are shown in Figure 4.11 and Figure 4.12, respectively. In Figure 4.11, the tensile strength of pure PVA film was 55.49 MPa. Tensile strengths of the reinforced films were higher than that of the pure polymer matrix. Tensile strengths of the reinforced PVA films ranged from 63.49 to 86.39 MPa depending on the chitin whisker content. The tensile strength of the reinforced PVA film increased up to about 83.30 MPa when the chitin whisker content was 2.96 wt% and no significant increase in tensile strength was observed when chitin whisker content increased from 2.96 wt% to 29.6 wt%. These results indicated that the tensile strength of PVA films could be improved by the addition of chitin whisker into the film composition. Figure 4.12 shows the elongation at break

of chitin whisker-reinforced PVA films with different whisker contents. The elongation of whisker-reinforced PVA films remarkably decreased as compared to that of the pure PVA film. The elongation at break of pure PVA film was 14.73% whereas the values for the reinforced PVA films were in the range of 11.92% to 5.30% depending on the chitin whisker content. The higher chitin whisker content was, the more decrease in elongation at break was observed. The decreasing of the elongation at break of the reinforced PVA films is possibly due to the interaction between whisker-polymer matrix within the reinforced films which restricted the mobility of the polymer chains.

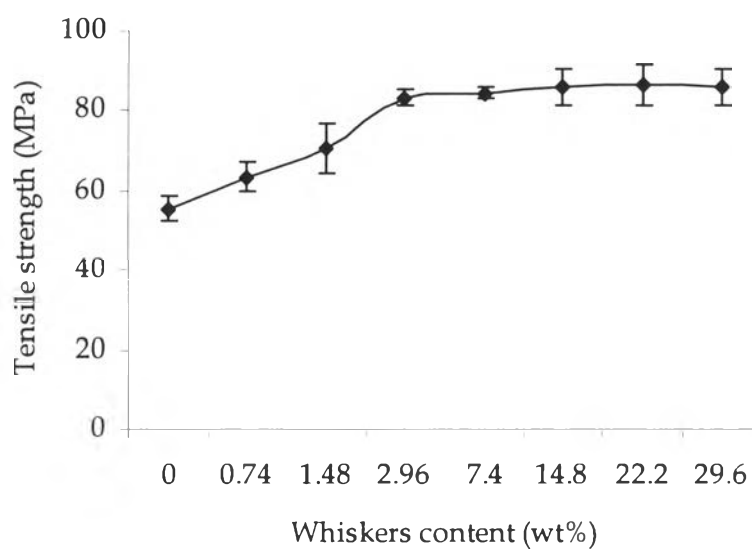


Figure 4.11 Tensile strength of chitin whisker-reinforced PVA films with different chitin whisker contents.

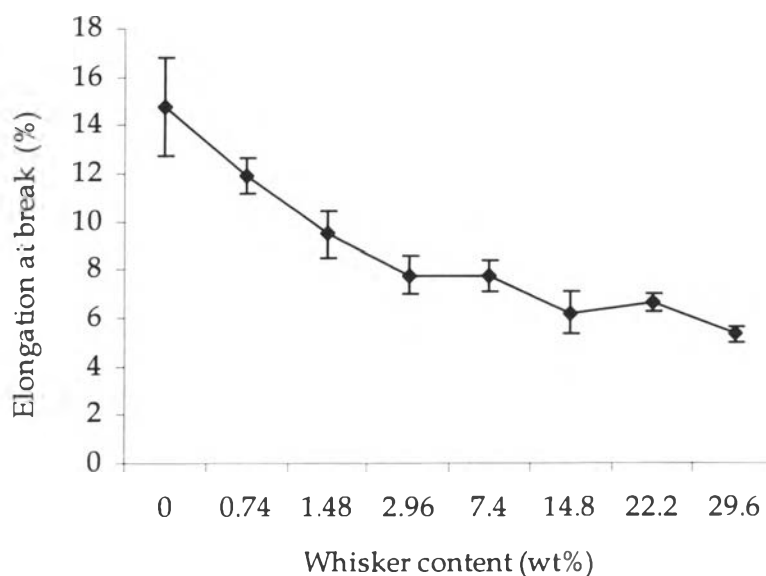


Figure 4.12 Elongation at break of chitin whisker-reinforced PVA films with different chitin whisker contents.

4.7.4 Mechanical Properties of Chitin Whisker-Reinforced Chitosan Films

The tensile strength and elongation at break of the chitin whisker-reinforced chitosan films are shown in Figure 4.13 and Figure 4.14, respectively. Figure 4.13 shows the tensile strength of chitin whisker-reinforced chitosan films with different whisker contents. The tensile strength of pure chitosan film was 64.94 MPa. Tensile strengths of the reinforced chitosan films varied between 68.81 MPa and 83.84 MPa. The increasing of tensile strength of the reinforced chitosan film increased up to 83.84 MPa when the chitin whisker content increased to 2.96 wt%. These results indicated that the tensile strength of chitin whisker-reinforced chitosan films could be improved by the addition of chitin whisker into the film composition. For the films which reinforced with 2.96 wt% to 29.6 wt% of chitin whisker content, tensile strength was no significant increase. These results suggested that film which reinforced by low chitin whisker content the whisker particle can disperse well within the film such that stress can be transfer better than an unreinforced film. For the high whisker content, the whisker may agglomerate together so it can interrupt the stress transfer in the film. Figure 4.14 shows the elongation at break of chitin whisker-reinforced chitosan films with different whisker contents. The elongation of

whisker-reinforced chitosan films slightly decreased as compared to that of pure chitosan film. The elongation at break of pure chitosan film was 11.74% whereas the values for the reinforced chitosan films were in the range of 10.84% to 6.91% depending on the chitin whisker content. The higher chitin whisker content was, the more decrease in elongation at break of the nanocomposite films was observed. The decreasing of the elongation of chitin whisker-reinforced chitosan films is possibly due to the interaction between whisker-polymer matrix within the reinforced films which restricted the mobility of the polymer chains.

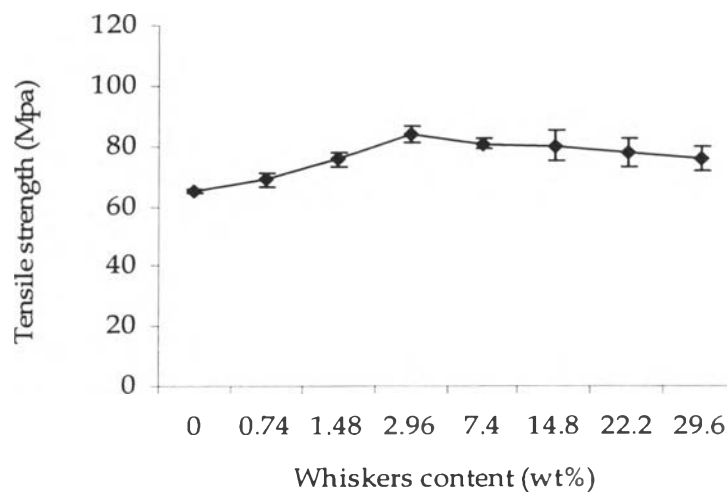


Figure 4.13 Tensile strength of chitin whisker-reinforced chitosan films with different chitin whisker contents.

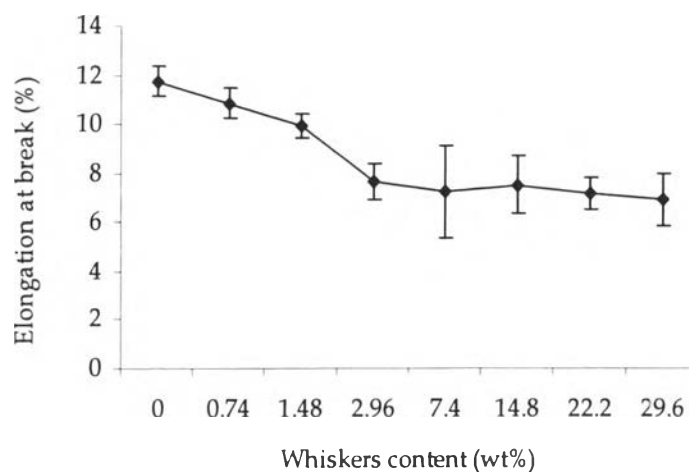


Figure 4.14 Elongation at break of chitin whisker-reinforced chitosan films with different chitin whisker contents.

These results correspond to the study of Nair and Dufresne (2003) who prepared crab shell chitin whisker-reinforced natural rubber nanocomposites and indicated that the improvement in mechanical properties of the reinforced natural rubber nanocomposites could originate from the possible interaction between whisker-polymer matrix.

4.7.5 Thermal Properties of Chitin Whisker-Reinforced PVA Films

Thermal degradation behavior of the nanocomposite films was examined by thermogravimetric analysis (TGA).

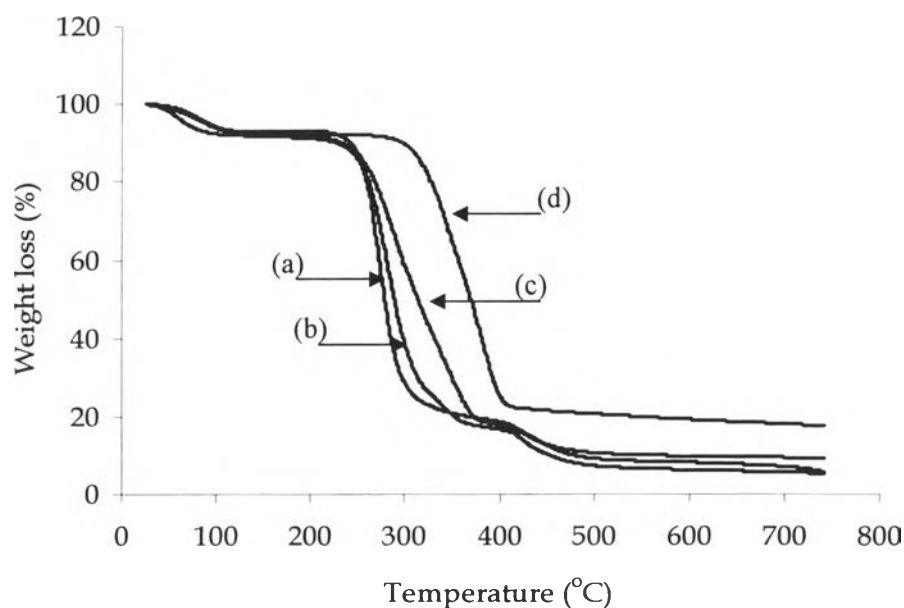


Figure 4.15 TGA thermograms of reinforced PVA films. (a) pure PVA, (b) 14.8 wt% chitin whisker content, (c) 29.6 wt% chitin whisker content and (d) chitin whisker.

Figure 4.15 shows the TGA curves of chitin whisker-reinforced PVA films containing different chitin whisker contents. According to the TGA curves, pure PVA film was found to degrade at 274°C while chitin showed degradation temperature at 347°C. The degradation temperatures of PVA films reinforced with 14.8 wt% and 29.6 wt% chitin whisker contents showed a difference at the mid-point of the slope. Moreover, weight losses of the reinforced films were lower than that of

pure PVA film and were in the range between weight losses obtained for pure PVA and chitin whisker when compared at the same temperature. Furthermore, the increasing of chitin whisker content resulted in the decreasing of the weight losses of chitin whisker-reinforced PVA films.

4.7.6 Thermal Properties of Chitin Whisker-Reinforced Chitosan Films

Figure 4.16 shows the TGA curves of the reinforced chitosan films. The degradation temperatures of pure chitosan and chitin whisker were 290°C and 347°C, respectively. For chitosan films reinforced with 14.8 wt% and 29.6 wt% chitin whisker content, there were no significant change in degradation temperature compared to that of pure chitosan film and the percent weight losses were slightly different.

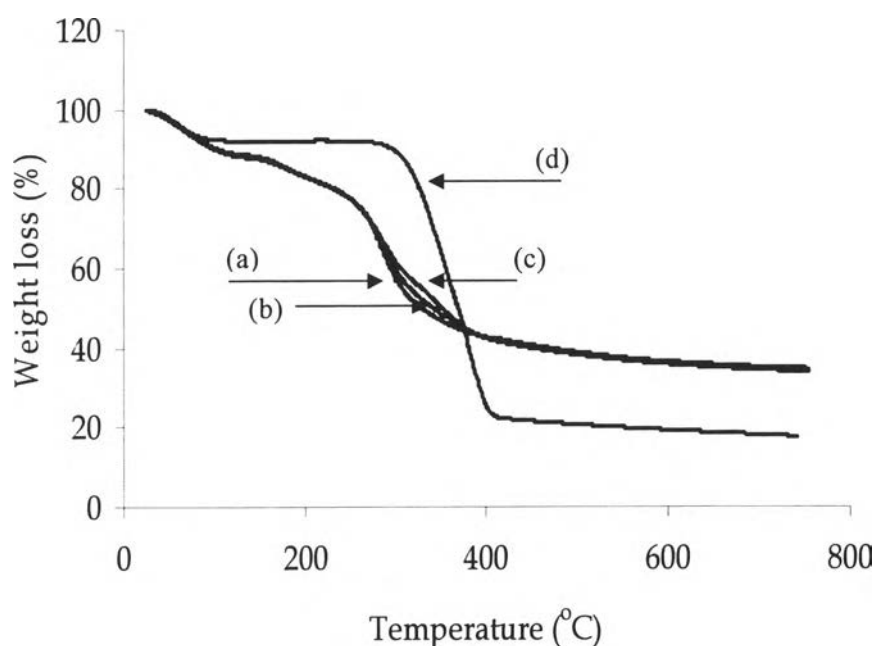


Figure 4.16 TGA thermograms of reinforced chitosan films. (a) pure chitosan, (b) 14.8 wt% chitin whisker content, (c) 29.6 wt% chitin whisker content and (d) chitin whisker.

It may be concluded that degradation temperatures of the nanocomposite films were not significantly affected by the presence of chitin whisker in the film and it seemed to be independent of chitin whisker content.

4.7.7 Weight Loss of the Films without Heat Treatment

The weight losses of the chitin whisker-reinforced films without heat treatment after immersing in water for 24 h were determined. The percent weight loss of the reinforced PVA films is shown in Table 4.9. Weight loss of pure PVA film in water was 90.82%. When chitin whisker contents in the PVA nanocomposite films increased, the weight losses of the reinforced PVA films decreased. The lowest weight loss of 60.28% was obtained for the film with 29.6 wt% chitin whisker content. It may be concluded that the addition of chitin whisker into the PVA film could reduce the weight loss of the PVA nanocomposite film after immersion in water.

Table 4.9 Percent weight loss in water of PVA and chitin whisker-reinforced PVA films with and without heat treatment

Whisker content (wt%)	Weight Loss (%)		
	Without heat treatment	Autoclaved 5 min	Autoclaved 10 min
0	90.82±3.08	36.78±0.94	13.58±1.74
0.74	86.90±6.90	28.66±3.18	12.02±0.24
1.48	84.25±8.77	18.57±1.50	10.19±1.84
2.96	82.25±5.39	19.28±1.20	9.53±0.65
7.40	65.46±8.97	18.00±0.22	8.92±0.95
14.8	60.37±1.47	18.70±1.86	7.80±1.51
22.2	60.30±5.60	17.76±2.57	7.03±2.24
29.6	60.28±1.81	17.05±2.43	7.22±0.61

For determination of weight loss, the film has to retain its shape more than 24 h. Chitosan film and chitin whisker-reinforced chitosan films cannot retain their shape in water so weight losses of the films in water could not be determined. However, pure chitosan film and chitosan nanocomposite films with 0.74 wt% to 7.40 wt% chitin whisker content could retain their shape in the water for 4 h. For chitosan nanocomposite films with 14.8 wt% chitin whisker content, the film could

retain their shape in water for 6 h. For chitosan films reinforced with 22.2 wt% and 29.6 wt% chitin whisker, the films could retain their shape in water for 18 h. It may be concluded that the addition of chitin whisker to prepare chitosan nanocomposite films could help the nanocomposite films to retain shape their in water. The reason for this is possibly due to the formation of chitin whisker-polymer matrix interaction.

4.7.8 Heat Treatment

In some applications, especially in biomedical field, PVA and chitosan films may be in contact with aqueous solution that can cause them to dissolve or not able to retain their shape before the desired time. It has been reported that crosslinking in polymer could be promoted by heat treatment (Vazquez-Torres *et al.*, 1993; Ogawa, K., 1991; Lim *et al.*, 1999; Toffey *et al.*, 1996; Muramatsu *et al.*, 2003). In order to reduce weight loss of chitin whisker-reinforced PVA and chitosan films in water, heat treatment was used. The nanocomposite films were treated by saturated steam in an autoclave at 110°C for 5 and 10 min. For PVA and chitin whisker-reinforced PVA films, the change in color was not observed after heat treatment. The films remained transparent. Table 4.9 shows the weight loss of PVA and chitin whisker-reinforced PVA films after heat treatment for 5 and 10 min. For the films treated in an autoclave for 5 min, the weight loss of pure PVA film was 36.78% and weight loss of PVA nanocomposite films decreased as chitin whisker content in nanocomposite films increased. The weight loss of PVA nanocomposite film could reduce to 17.05% which was about 53.6% reduction as compared to pure PVA film for the PVA nanocomposite film with 29.6 wt% chitin whisker content. For PVA nanocomposite films after heating in an autoclave for 10 min, the weight loss of pure PVA film was 13.58% and weight losses of PVA nanocomposite films decreased from 12.02% to 7.22% as chitin whisker contents increased from 0.74% to 29.6%. Comparison of the weight losses before and after heat treatment, it was found that the weight loss of the films remarkably decreased by heat treatment in the autoclave and the reduction of weight loss increased with increasing of chitin whisker content and heating time in the autoclave.

Table 4.9 Percent weight loss in water of PVA and chitin whisker-reinforced PVA films with and without heat treatment

Whisker content (wt%)	Weight Loss (%)		
	Without heat treatment	Autoclaved 5 min	Autoclaved 10 min
0	90.82±3.08	36.78±0.94	13.58±1.74
0.74	86.90±6.90	28.66±3.18	12.02±0.24
1.48	84.25±8.77	18.57±1.50	10.19±1.84
2.96	82.25±5.39	19.28±1.20	9.53±0.65
7.40	65.46±8.97	18.00±0.22	8.92±0.95
14.8	60.37±1.47	18.70±1.86	7.80±1.51
22.2	60.30±5.60	17.76±2.57	7.03±2.24
29.6	60.28±1.81	17.05±2.43	7.22±0.61

After heat treatment in the autoclave for 5 and 10 min, weight losses in water of chitosan film and chitosan nanocomposite films were determined. For chitosan and chitin whisker-reinforced chitosan films, the changes in color of the films were observed. The color of the films changed from transparent before heat treatment to pale yellow after heating the films in the autoclave for 5 min and to yellowish brown after heating the films in the autoclave for 10 min. Table 4.10 shows the weight losses in water of chitosan and chitin whisker-reinforced chitosan films with heat treatment. In case of heat treatment for 5 min, the weight losses decreased from 15.37% for pure chitosan film to 10.64% for the nanocomposite film with 29.6 wt% whisker content. At heat treatment for 10 min, the weight losses decreased from 11.27% for pure chitosan film to 9.62% for the nanocomposite film with at 29.6 wt% chitin whisker content. The percent reduction of weight loss of chitosan nanocomposite films compared to pure chitosan film after heat treatment for 5 and 10 min were about 33% and 17%, respectively. It can be concluded that, when chitin whisker content increased, the weight loss decreased and the increasing of the heating time could reduce the weight loss of the films.

Table 4.10 Percent weight loss in water of chitosan and chitin whisker-reinforced chitosan films with and without heat treatment

Whisker content (wt%)	Weight Loss (%)		
	Without heat treatment ^a	Autoclaved 5 min	Autoclaved 10 min
0	N/A	15.37±0.45	11.27±2.35
0.74	N/A	13.41±1.09	10.27±0.71
1.48	N/A	12.31±0.13	10.06±0.11
2.96	N/A	12.10±2.40	10.04±0.21
7.40	N/A	12.24±2.99	9.30±0.55
14.8	N/A	12.62±2.66	9.58±0.93
22.2	N/A	10.06±1.62	9.01±0.31
29.6	N/A	10.64±0.57	9.62±2.49

Percent weight loss was determined from 3 samples.

^a Weight loss of the films without heat treatment could not be determined because the films completely dissolved before 24 h.

The decreasing of weight loss after heating in an autoclave could imply that heat might promote crosslinking formation between chitin whisker and polymer matrix in the nanocomposite films. Similar results have been reported by Vázquez-Torres *et al.* (1993), who studied thermally induced hydrogels of poly (vinyl alcohol)/poly(acrylic acid) blends and found that the degree of crosslinking was higher when longer heating time was used.

4.7.9 Swelling Behavior of Chitin Whisker-Reinforced Films

According to the better shape retaining after heat treatment in the autoclave of chitin whisker-reinforced PVA and chitosan nanocomposite films, the degree of swelling in water of the films was determined. Figure 4.17 shows degree of swelling in water of the PVA nanocomposite films after autoclaved at 110°C for 5 min. The swelling of the films was remarkably increased within the first 5 min and

then became rather constant within 60 min. It was found that the degree of swelling of PVA film reinforced with 29.6 wt% chitin whiskers was lower than that of PVA film reinforced with 7.40 wt% chitin whiskers. And the degrees of swelling of the chitin whisker-reinforced PVA films were lower than that of pure PVA film. This may be implied that there was crosslinking in the films that reduced swelling ability of the films.

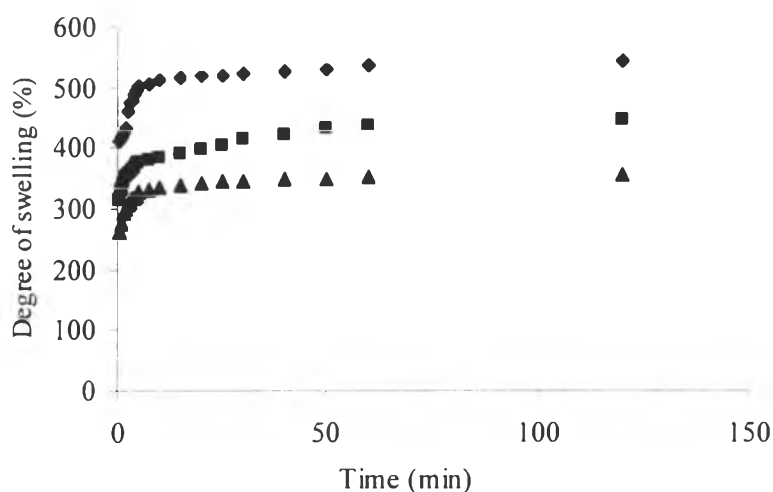


Figure 4.17 Degree of swelling of PVA and chitin whisker-reinforced PVA films after autoclaved for 5 min as a function of immersion time. (♦) pure PVA, (■) 7.4 wt% chitin whisker content and (▲) 29.6 wt% chitin whisker contents.

In Figure 4.18 the degree of swelling in water of chitin whisker-reinforced chitosan films after autoclaved at 110°C for 5 min is shown. The swelling of the films was remarkably increased within 3 min and then became rather constant within 60 min. The degree of swelling of chitin whisker-reinforced chitosan films reduced with the increasing of the chitin whisker content in the reinforced films.

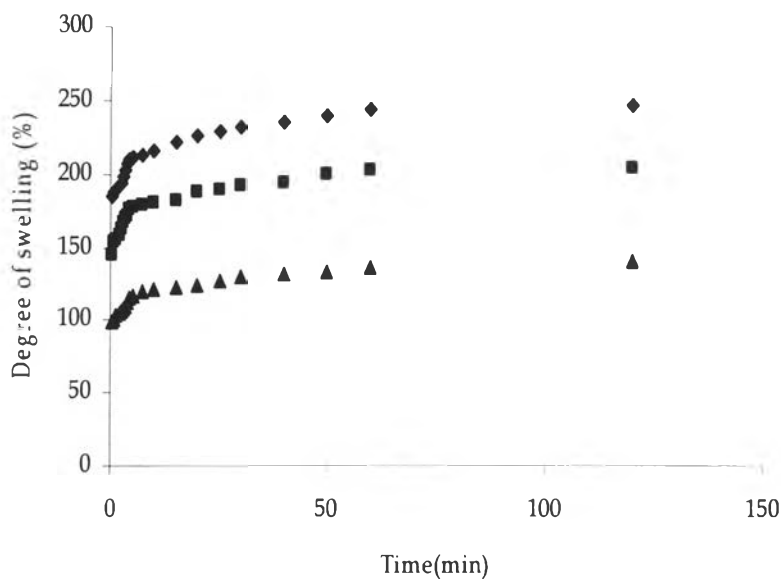


Figure 4.18 Degree of swelling of chitosan and chitin whisker-reinforced chitosan films after autoclaved for 5 min as a function of immersion time. (◆) pure PVA, (■) 7.4 wt% chitin whisker content and (▲) 29.6 wt% chitin whisker content.

Similar results were reported by Nair and Dufresne (2003a), who investigated processing and swelling behavior of crab shell chitin whisker-reinforced natural rubber. They reported that the swelling of the material was strongly reduced in the presence of chitin whisker in natural rubber matrix.