CHAPTER VI

RESULT AND DISCUSSION

4.1 Characterization of Chitin from Shrimp Shell

4.1.1 Morphological Analysis

To observing on the surfaceof alpha chitin, which was prepared from shrimp shell, the SEM image illustrates that the chitin powder exhibited uneven size such as large and small particular structure, and the individual or aggregated fragments can be seen as shown in Figure 4.1(A) and then more magnifying that chitin look like fibers respectively in Figure 4.1 (B).

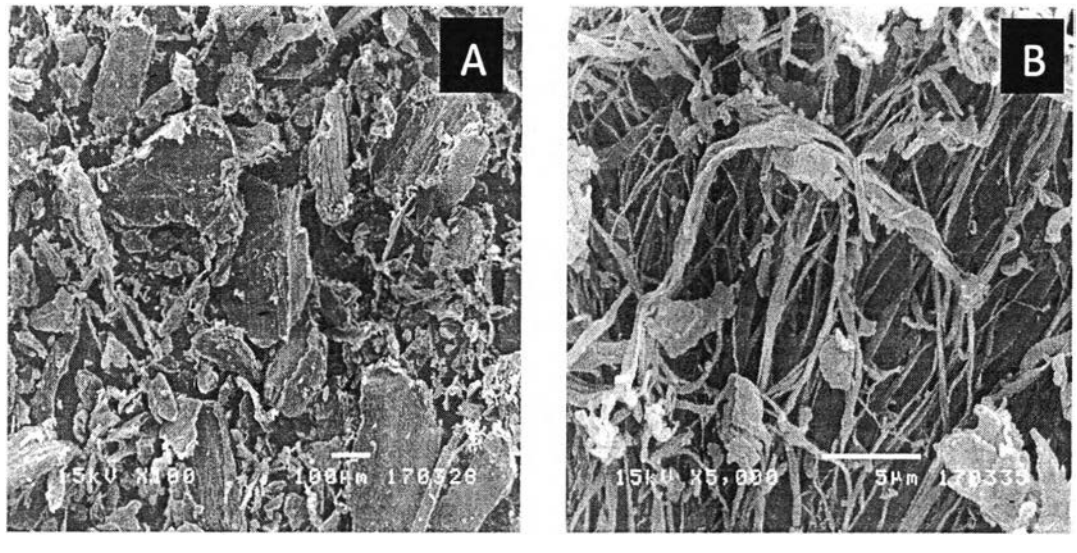


Figure 4.1 SEM image of alpha chitin obtained from shrimp shell (A) magnifying (x100) and (B) is magnifying (x5,000).

4.1.2 Chemical Analysis of Chitin

The chemical structures of the alpha chitin and the resulting were investigated with the use of the FTIR spectroscopy. As shown in Figure 4.2, the FTIR spectra of chitin original show a broad band of the OH-stretching in a wavenumber range of 3450 cm^{-1} to 3000 cm^{-1}. An characteristic peak at 2,878 cm^{-1} which can be assigned to the C-Hstretching bonds, the FTIR peaks at 1623 cm^{-1} which correspond to the amide I peak (-CONH-) and amide peak II at 1,550 cm^{-1} correspond to out of phase N-H stretching and C-N stretching. Degree of-deacetylation (Sannan, 1977) can determine in equation for low degree of deacetylation (DD). The degree of deactylation from chitin in this batch is 33.14 %

$$DD = 101\text{-}35.71(Abs_{1550}/Abs_{2878})$$

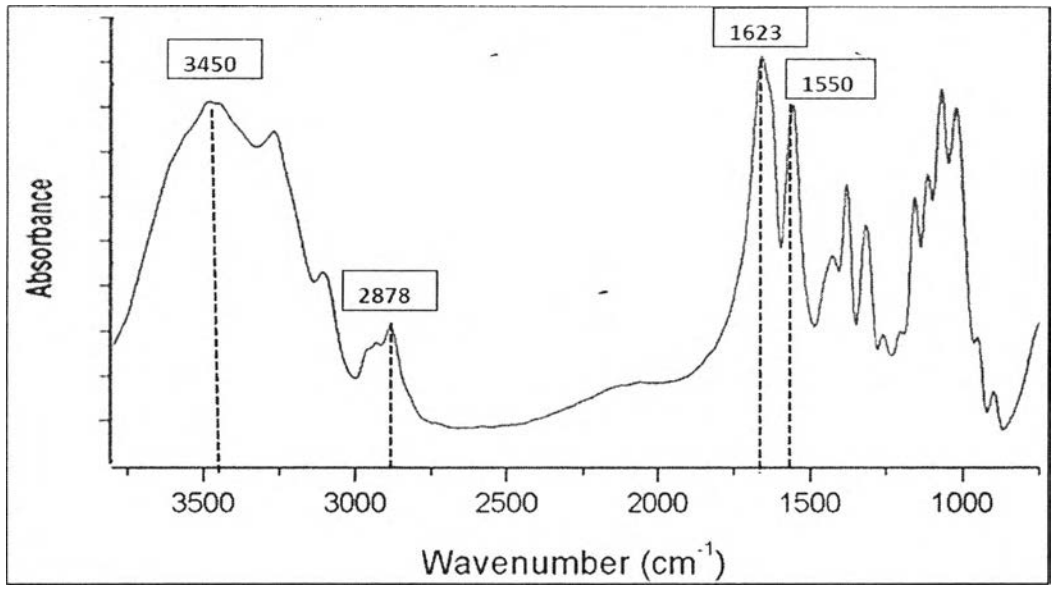


Figure 4.2 ATR-FTIR spectra of shrimp alpha chitin powder.

4.1.3 Yielding of Chitin Production

Shrimp shells are initially 100 g and decalcification for removing calcium component byHCl 1.0 N in 24 hr. and then deproteinization step and washing in distilled water and the next is dry in oven and net weight in final is 22.61 g

Table 4.1 Net weight alpha chitin in gram

Raw material	Dry Basis Weight (g)
Shrimp shells	**100.0**
Demineralized shrimp shells(decalcification)	**54.74**
Deproteinized shrimp shells	**39.17**
Chitin powder	**22.61**

4.2 Characterization of DBD Plasma-Treated Nylon-Chitin Membranes

4.2.1 Effect of DBD Plasma Treatment Time on Water Contact Angle on Nylon-Chitin Membranes

The hydrophilicity of DBD plasma-treated nylon-chitin membranes were characterized by water contact angle measurement. The effect of DBD plasma treatment time on the water contact angle of the nylon-chitin membranes wereestimated. Obviously, Figure 4.3 shows that the water contact angle drastically decreased from 88.0° to 37.4° as the DBD plasma treatment time increased from 0 s to 60 s. And after 60 s the water contact angle slightly decreased around 31.4° at the treatment time longer to120 s implied a saturation state of surface hydrophilicity. The result indicated that the DBD plasma treatment lead to an increase in the surface hydrophilicity of the plasma-treated nylon-chitin membranes. This should be mostly likely due to the presence of new polar functional groups on the plasma-treated surface induced by the active species

generated by the air plasma, especially oxygen-based species and charged species, which are very reactive such as carbonyl group (C=O) (Esena*et al.* 2008).

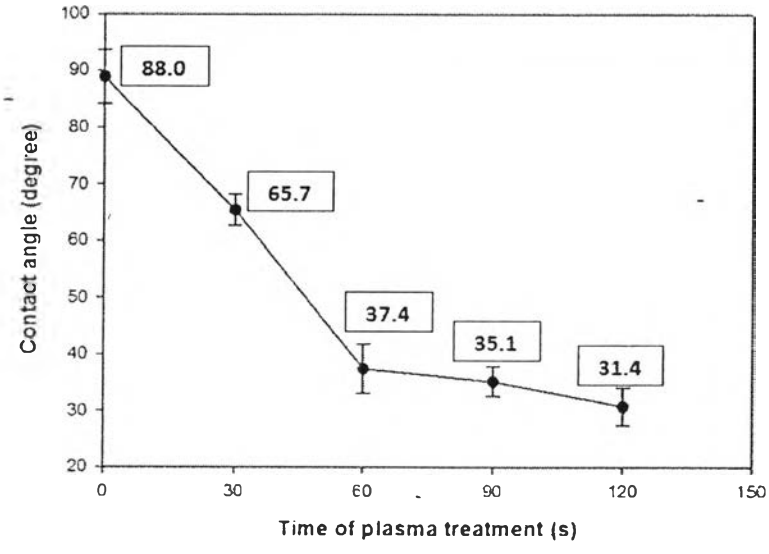


Figure 4.3 Effect of plasma treatment time for water contact angle on nylon-chitin membranes.

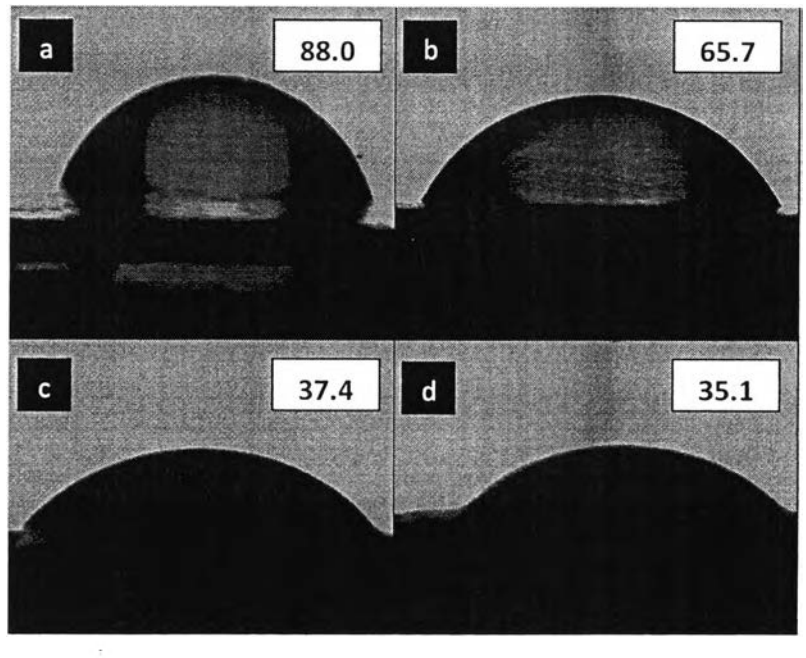


Figure 4.4 Effect of plasma treatment time for droplet shape on membranes (a) Untreated plasma (b) plasma treated 30 s (c) plasma treated 60 s (d) plasma treated 90 s.

4.2.2 Effect of DBD Plasma Treatment Time on Surface Chemical Analysis of Nylon-Chitin Membranes

The surface chemical composition of DBD plasma-treated nylon-chitin membraneswere investigated by using the ATR-FTIR technique. The ATR-FTIR spectra of the nylon-chitin membranesbefore and after the DBD plasma treatment was determined as shown in Figure 4.5. After the DBD plasma treatment, the new characteristic peaks appearing at the wavenumbers of 1720 cm^{-1} corresponding to Carbonyl, C=O stretching (Ragojanu *et al.*, 2010). The intensity of the new peaks was also found to increase with increasing the DBD plasma treatment time, implying a higher amount of the new oxygen-containing functional groups at a longer plasma treatment time and membranes after treated plasma become more polarity

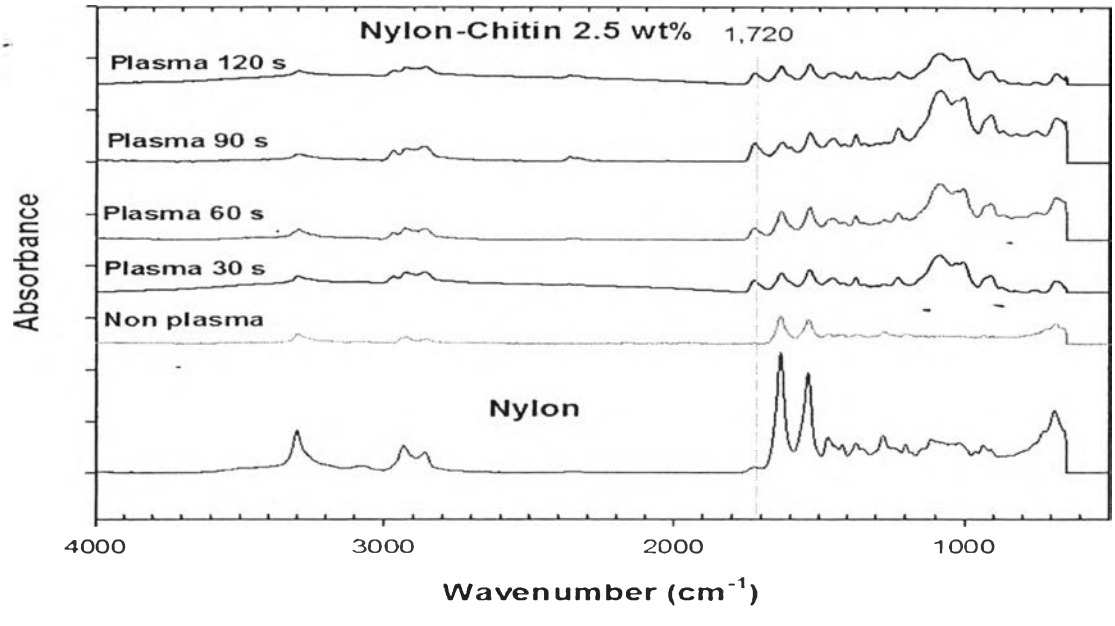


Figure 4.5 ATR-FTIR spectra of nylon-chitin membranes at different plasma treatment time.

4.2.3 Effect of DBD Plasma Treatment Time And Chitin Content on Mechanical Properties of Nylon-Chitin Membranes

The tensile strength of DBD plasma-treated nylon-chitin membranes were characterized by Lloyd, universal tensile tester. The effect of DBD plasma treatment time on tensile strength of the nylon-chitin membranes was estimated. Obviously, Figure 4.6 shows that slowly decreasing tensile strength when increasing plasma treatment time in the same chitin content because after plasma that occur porosityon membranes which can induce to and Figure 4.7 shows comparison between untreated plasma and treated plasma in different chitin content. The result demonstrates both of treated and untreatedare decreasing when chitin content is increasing and Figure 4.8 is verify chitin content and tensile strength in same treatment times and the result show the same that is decrement of mechanical properties when increment chitin content that cause may be from effect of viscosity is increase more and more in blending processwhich affects to low compatibility and become decrease of mechanical properties.

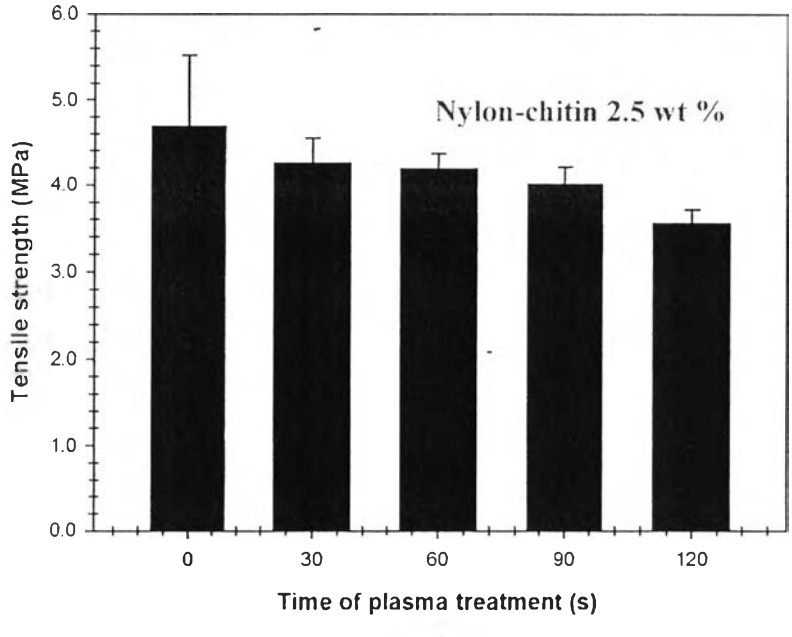


Figure 4.6 Mechanical properties of nylon-chitin membranes at different plasma treatment time.

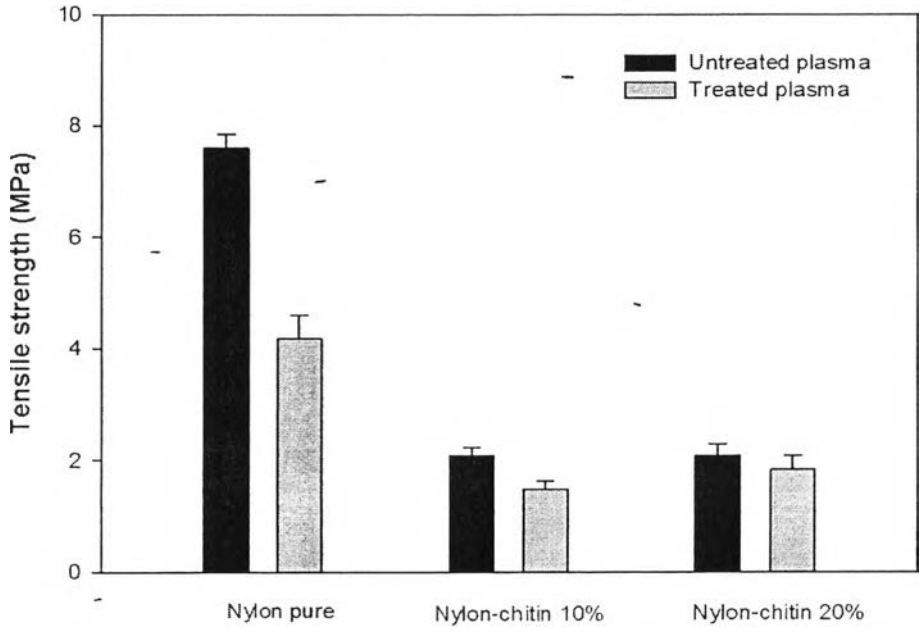


Figure 4.7 Comparison of mechanical properties on nylon-chitin membranes at different chitin content, treated and untreated plasma.

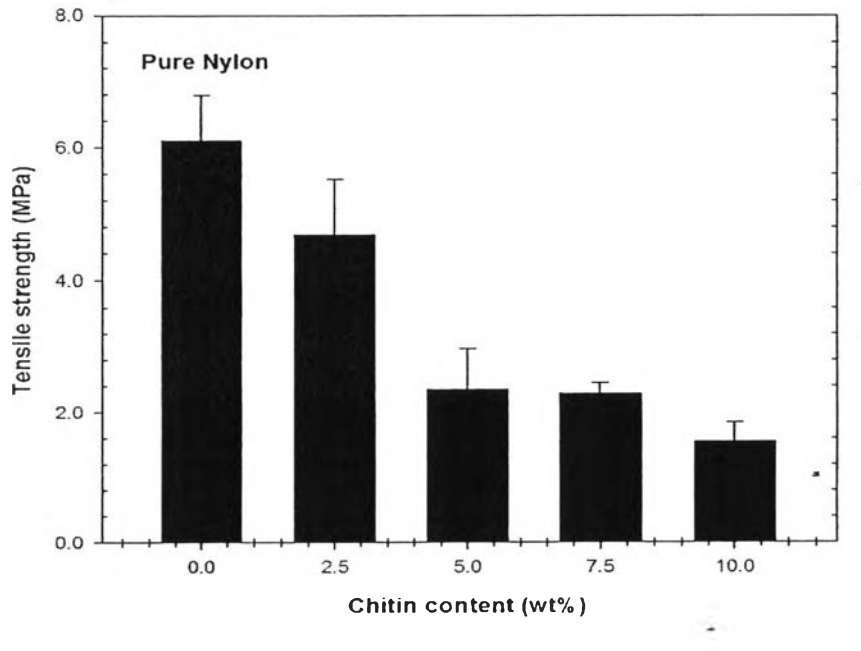


Figure 4.8 Mechanical properties on nylon-chitin membranes at different chitin content.

4.2.4 Effect of Plasma on Surface Morphology of Nylon-Chitin Membranes

Figure 4.9 (A) and (B) shows the surface morphology of 10% nylon-chitin membranes untreated and treated plasma, respectively. After treated plasma, it can be seen that the surface of membranes was more porosityand large aggregate chitin become to small spot of chitin.

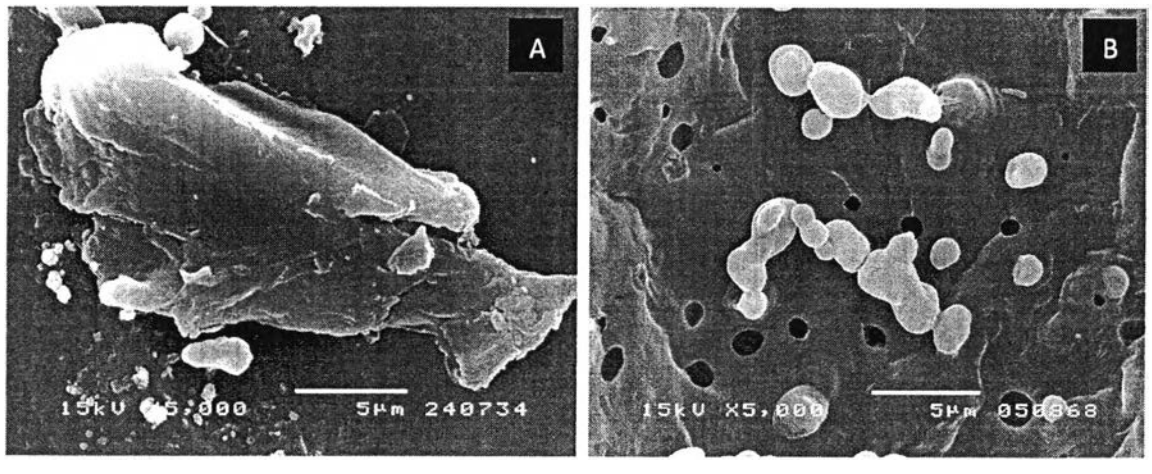


Figure 4.9 SEM images of (A) non-plasma treated nylon/chitin membrane at ratio of 90:10 and (B) plasma-treated nylon/chitin membrane at ratio of 90:10

As shown in Figure 4.10 (a), (b) and (c) the SEM image shows more porous and rough structure of nylon/chitin membranes after subjecting to DBD plasma for 120 s. An increase of chitin content in this bio-composite membrane indicated a bigger size of pores which can explain that chitin was sensitive to degrade under plasma treatment then; it facilitates to create porous structure on nylon/chitin membranes.

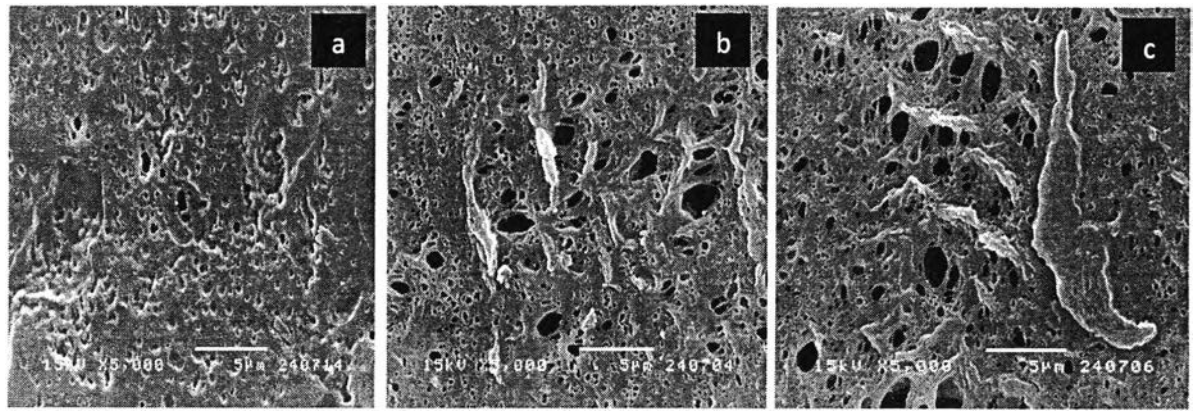


Figure 4.10 SEM images of (a) non-plasma treated nylon/chitin membrane and plasma treated nylon/chitin membranes at nylon/chitin ratio of (b) 90:10 (c) 80:20.

After lysozyme treatment, non-plasma treated nylon/chitin membrane Figure 4.11(a) shows aggregation of chitin spots spread on the surface while the plasma treated nylon/chitin membrane showed good distribution of opened-pores on the plasma treated surface. Becausechitin was susceptible to degrade with lysozyme and an increase of chitin contents showed more porous structure as shown in (b) and (c). Moreover, the lysozyme test could suggest the good distribution of porosity and compatibility between nylon and chitin after plasma treatment membrane. And figure 4.12 shows (a) before lysozyme hydrolysis and (b) after lysozyme hydrolysis that can describe chitin is digested by lysozyme from protruded chitin spots become too shallow porous.

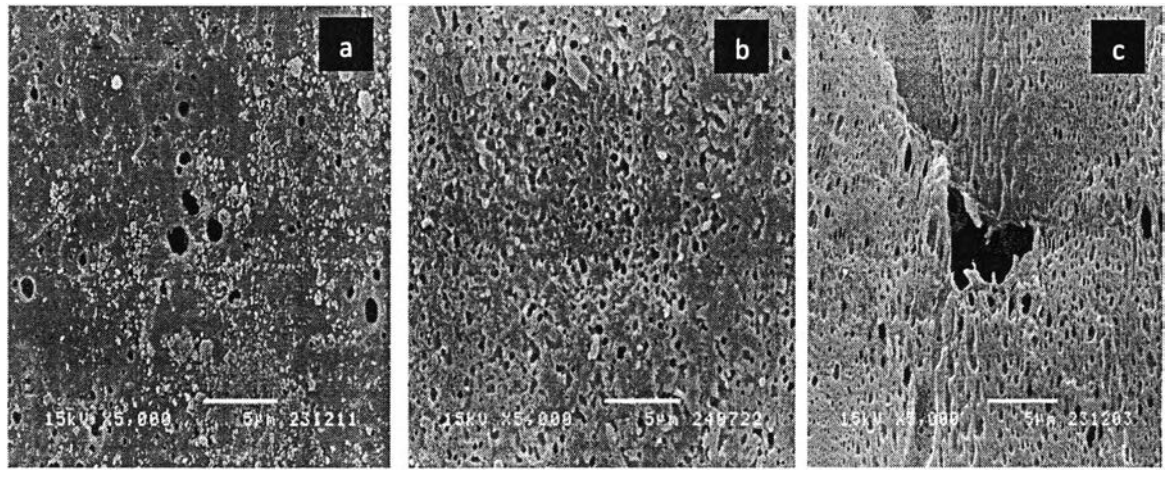


Figure 4.11 SEM images of lysozyme treatment of nylon/chitin membrane (a) non-plasma treated of 80:20 nylon/chitin and plasma treated of nylon/chitin membrane (b) 90:10 (c) 80:20

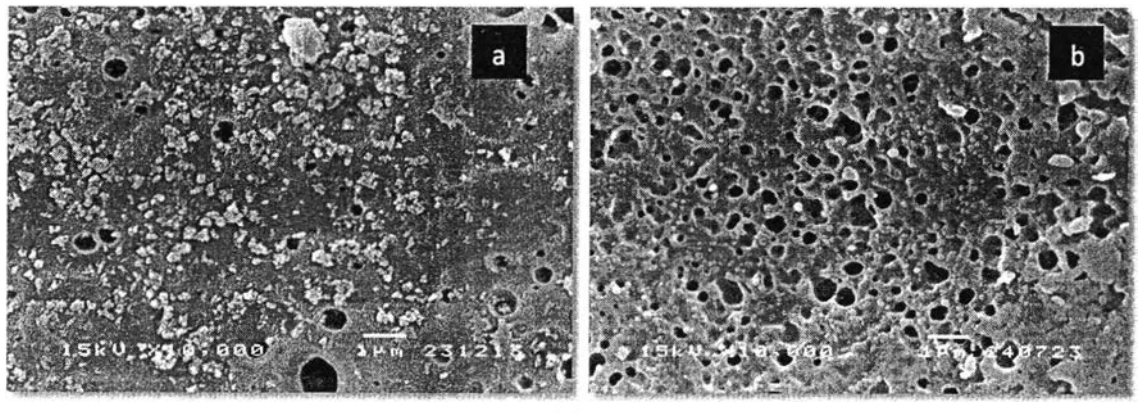


Figure 4.12 (a) membranes before lysozyme hydrolysis and (b) after lysozyme hydrolysis.

4.2.5 Effect of DBD Plasma Treatment on Crystallinity of Nylon-Chitin Membranes

The crystallinity of nylon-chitin membranes was determined with the use of wide angle x-ray diffraction (WAXD) analysis. Figure 4.13 (a) shows the characteristic diffractions of nylon membranes that can be seen showed the four diffraction peaks of 2θ at 13.7, 17.1, 20.2 and 24.3 degreebut nylon-chitin membranes that can be observed both samples showed the three diffraction peaks 2θ at 13.5, 20.4 and 24.3. It may be implied that addition of chitin become to more amorphousand crystallinity index decreases. Thismaybe indicate to the intermolecular reaction between nylon and chitin, which causes membranes molecular chains difficult to move because of viscosity effect while blending together or good enough compatibility of composite membranes. Figure 4.13 (b) will compare nylon-chitin membranes untreated-plasma and 30s treated plasma. The characteristic diffractions of untreated and 30 s DBD plasma-treated nylon-chitin

membranes can be seen that both samples showed the diffraction peak 2θ at 13.7, 20.4, 24.1 that may be implied that the DBD plasma modification does not change the degree of crystallinity, does not eliminate crystalline domains or form new crystalline species (Calvimontes et al., 2011).

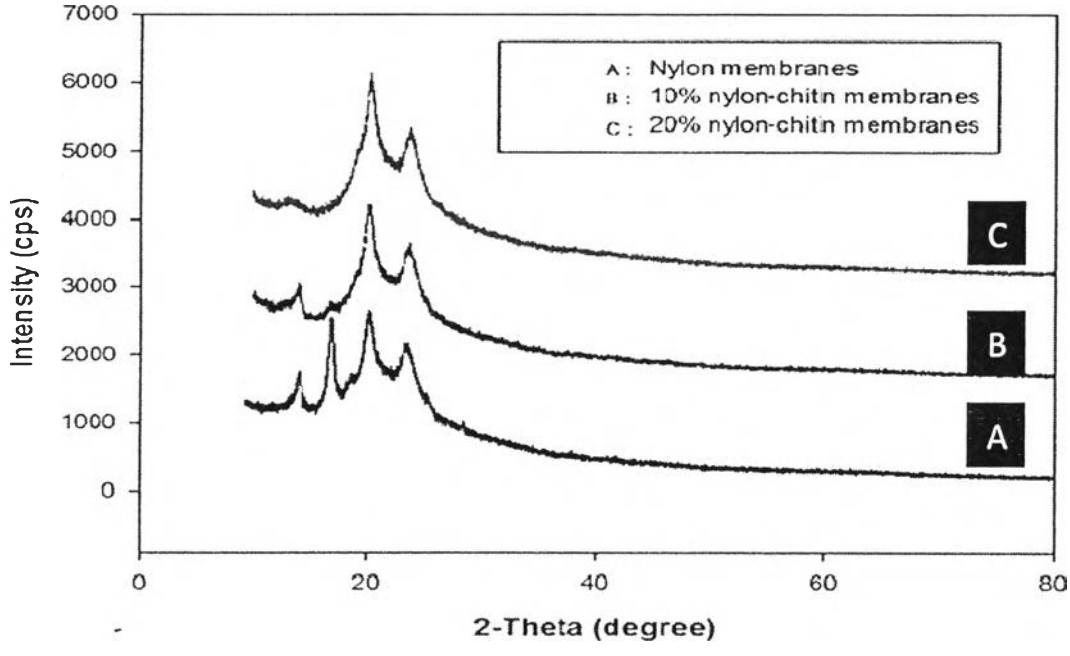


Figure 4.13(a) XRD pattern of nylon and nylon-chitin membrane.

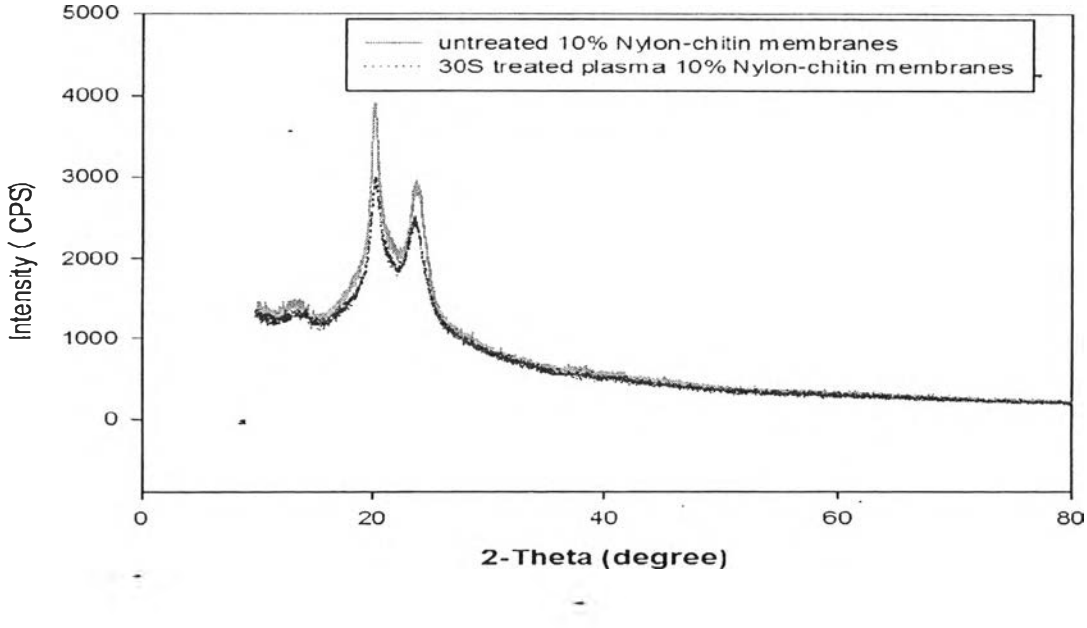


Figure 4.13(b) XRD pattern of untreated and 30 s DBD plasma treated nylon-chitin membranes

4.2.6 Effect of DBD Plasma Treatment on Thermo-Gravimetric Analysis of Nylon-Chitin Membranes

Thermo-Gravimetric Analysis (TGA) is a continuous process,involving in accordance with increasing temperature in the form of programmed heating. The thermal degradation behavior of the nylon-chitin membranes was determined andillustrated in Figure 4.14; the TGA of nylon membranes showed the temperature of degradation at 442 °C and observed 10% and 20% nylon-chitin membranes that have 2 steps. The first region in the temperature regime of 31°C to 104°C is due to the evaporation of weakly boundof water and the second step, the 10% nylon-chitin membranes were degraded at about 436 °C and 20% nylon-chitin membranes degraded at 425 °C. The results indicated that chitin content can induce fast temperature of decompositionand nylon-chitin membranes are rather lower temperature of degradation and far more absorptive moisture than nylon membranes.

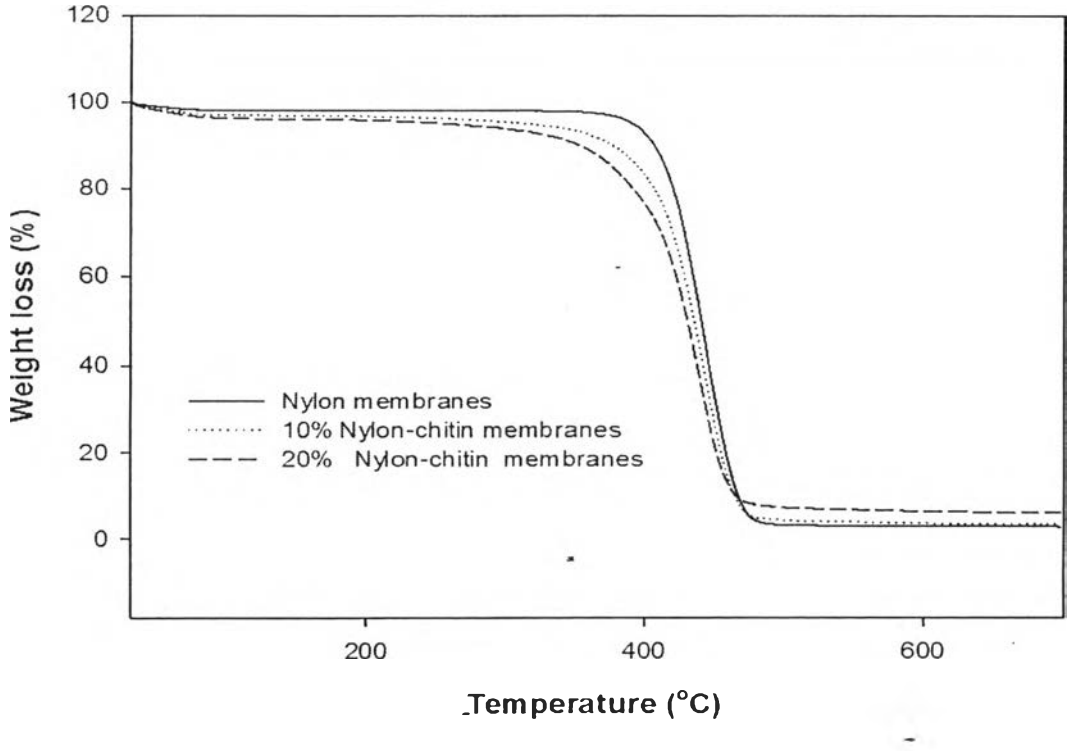


Figure 4.14 Thermal-Gravimetric Analyses of Nylon-Chitin Membranes.

4.3 Characterization of DBD Plasma-Treated Chitin Coated Nylon Mesh

4.3.1 Surface Morphology of Chitin Coated on Nylon Mesh via DBD Plasma

For the coated method, chitin was coated on the plasma treated nylon mesh in Figure 4.15 (A) The increase an amount of chitin from 0.5 % to 1.0 % w/v showed an increase the amount of chitin coated on plasma treated nylon mesh as shown in Figure (B) and (C), respectively. However, the chitin coated on nylon mesh trended to decrease when the % chitin concentration increase to 2.0% w/v as shown in Figure (D) which might be due to the high viscosity of 2.0% w/v chitin solution than other conditions.

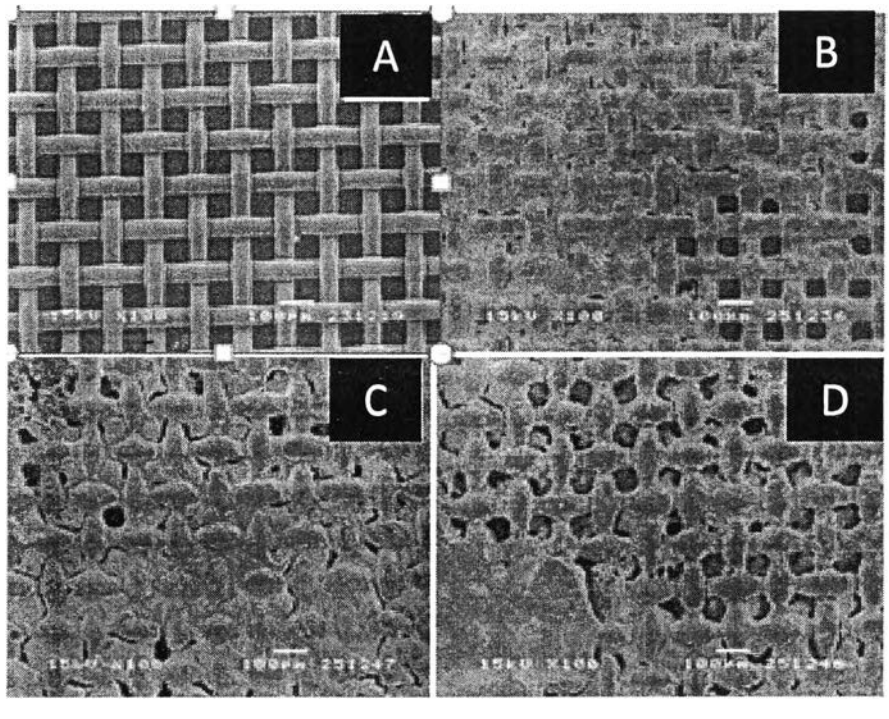


Figure 4.15 SEM images of (A) nylon mesh and plasma treated nylon mesh coated (B) 0.5% w/v of coated chitin (C)1 % w/v of coated chitin and (D) 2 % w/v of coated chitin.

4.3.2 Surface Chemical Analysis of Chitin Coated Nylon Mesh

The surface chemical composition of DBD plasma-treated nylon-chitin membranes were investigated by using the ATR-FTIR technique. The ATR-FTIR spectra of the nylon-chitin membranes before and after the DBD plasma treatment were determined as shown in Figure 4.5. After the DBD plasma treatment, the new characteristic peaks appearing at the wavenumbers of 1720 cm-1 corresponding to -Carbonyl, C=O stretching (Ragojanu et al., 2010). The intensity of the new peaks was also found to increase with increasing the DBD plasma treatment time, implying a higher amount of the new oxygen-containing functional groups at a longer plasma treatment time and membranes after treated plasma become more polarity.

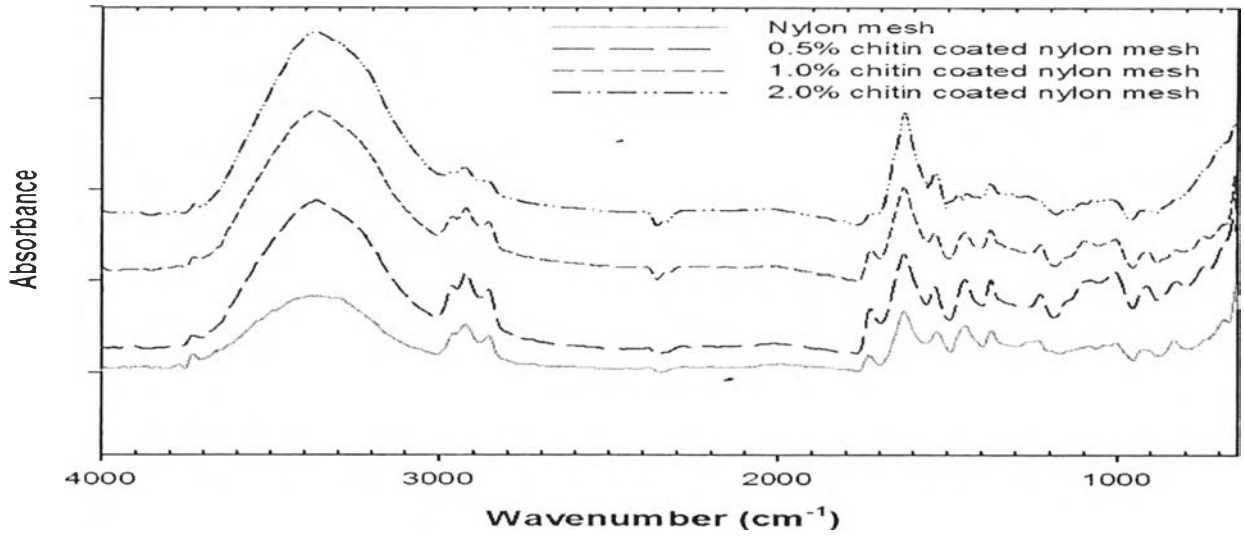


Figure 4.16 FT-IR images of nylon mesh, 0.5%,1.0 % and 2.0 % w/v of coated chitin.

4.3.3Mechanical Properties of Chitin Coated on Nylon Mesh via DBD Plasma

Tensile strength and elongation at break of the plasma-treated nylon mesh films with different chitin concentration are shown in Figure 4.17(A) and (B)after the plasma

for coated chitin on nylon mesh, both of the tensile strength and the elongation at break of the chitin coated nylon mesh films slightly decreased from that of the untreated one. Consequently, when the plasma treatment time was prolonged to 120 s.

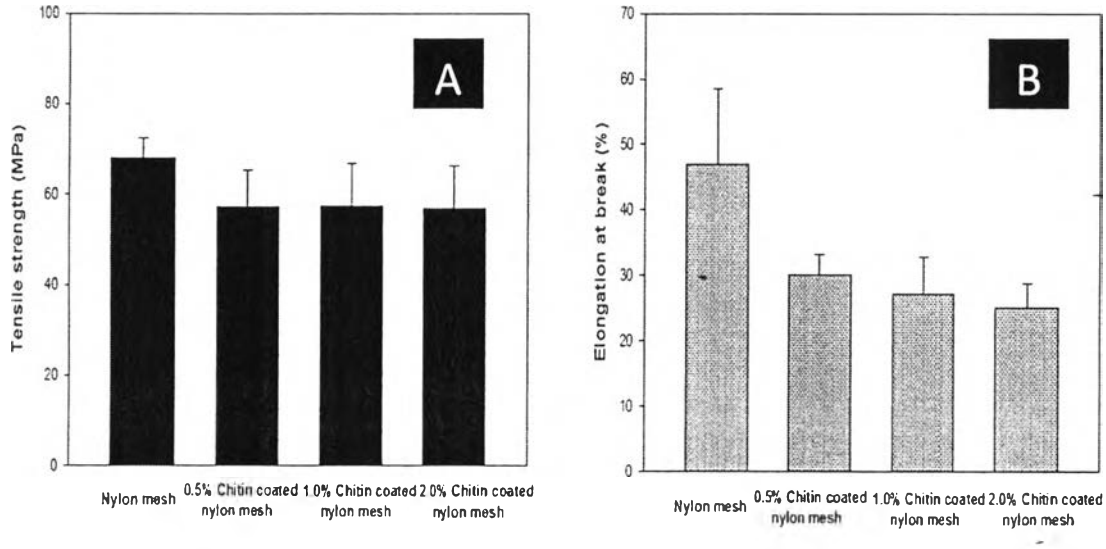


Figure 4.17 Effect of plasma treatment and chitin concentration on mechanical properties (A) tensile strength (MPa) and (B) elongation at break (%)

4.3.4 Thermo-Gravimetric Analysis of Chitin Coated on Nylon Mesh via DBD Plasma

TGA of native chitin coated nylon mesh showed the weight loss in five stages. The Thermo-gravimetric analysis (TGA) is a continuous process, involving in accordance with increasing temperature in the form of programmed heating. The thermal degradation behavior of the nylon-chitin membranes was determined and illustrated in Figure 4.14; the TGA of nylon membranes showed the temperature of degradation at 442 °C and observed 10% and 20% nylon-chitin membranes that have 2 steps. The first region in the temperature regime of 31°C to 104°C is due to the evaporation of weakly bound of water and the second step, the 10% nylon-chitin membranes were degraded at

about 436 °C and 20% nylon-chitin membranes degraded at 425 °C. The results indicated that chitin content can induce fast temperature of decomposition and nylon-chitin membranes are rather lower temperature of degradation and far more absorptive moisture than nylon membranes.

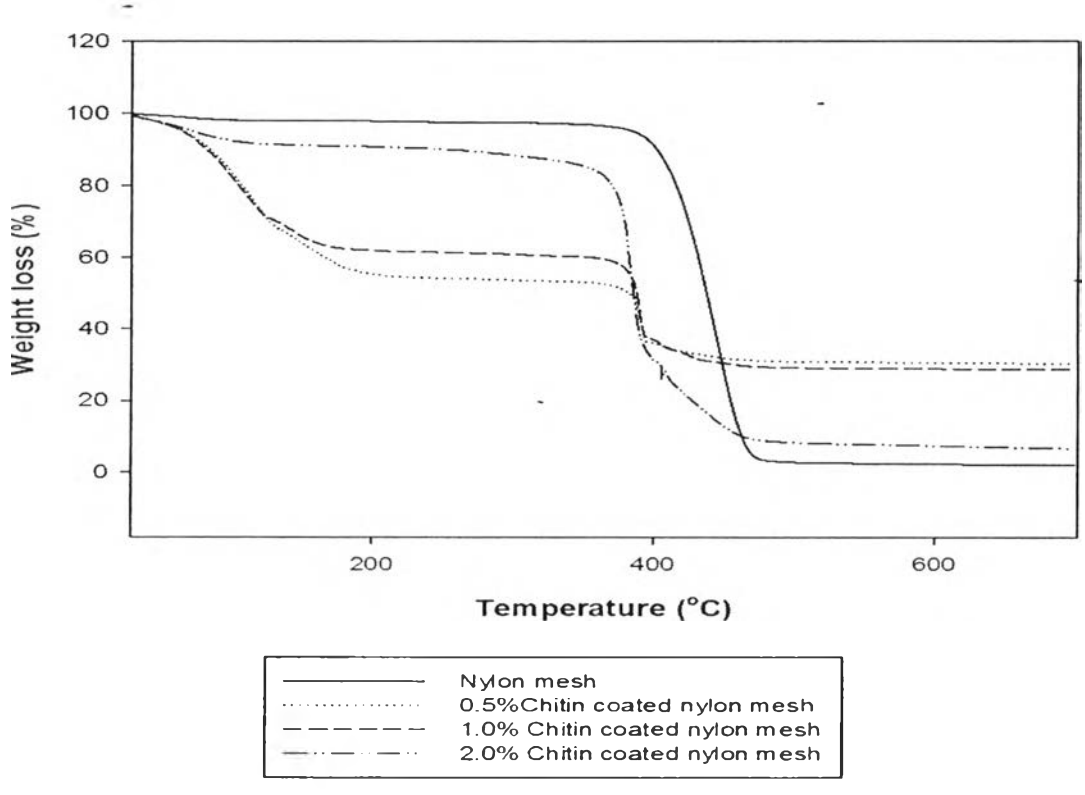


Figure 4.18 Thermal-Gravimetric Analysis of Chitin Coated Nylon Mesh Series.

4.3.5 Crystallinity of Chitin Coated Nylon Mesh Series

The crystallinity of nylon-chitin membranes was determined with the use of wide angle x-ray diffraction (WAXD) analysis. Figure 4.13 (a) shows the characteristic diffractions of nylon membranes that can be seen showed the four diffraction peaks of 2θ at 13.7, 17.1, 20.2 and 24.3 degree but nylon-chitin membranes that can be observed both samples showed the three diffraction peaks 2θ at 13.5, 20.4

and 24.3. It may be implied that addition of chitin become to more amorphous and crystalline index decreases. This may be indicate to the intermolecular reaction between nylon and chitin, which causes membranes molecular chains difficult to move because of viscosity effect while blending together or good enough compatibility of composite membranes. Figure 4.13 (b) will compare nylon-chitin membranes untreated-plasma and 30 s treated plasma. The characteristic diffractions of untreated and 30 s DBD plasma-treated nylon-chitin membranes can be seen that both samples showed the diffraction peak 2θ at 13.7, 20.4, 24.1 that may be implied that the DBD plasma modification does not change the degree of crystallinity, does not eliminate crystalline domains or form new crystalline species (Calvimontes et al., 2011).

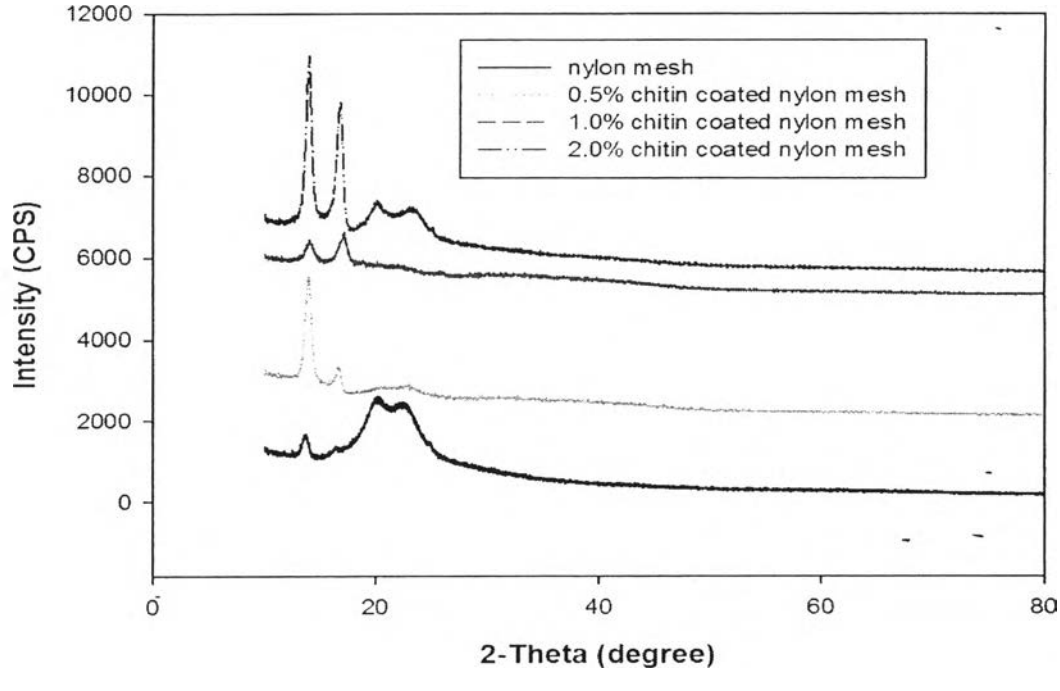


Figure 4.19 The XRD pattern of nylon mesh and chitin coated nylon mesh.

4.3.6 Water Sorption of Chitin Coated Nylon Mesh Series

Water sorption isotherms were measured according to the procedure described by mesh samples werecut into small pieces (2.0mm×2.0mm) and dried in a vacuum and the dried samples were weighed to into pre-weighed and then immerse in 1 hour and then dried and weighed until value is constantly which were obtained for each type of film withindividually prepared films as replicated experimental samples,and three specimens from each sample.The effects of chitin on the water sorption isotherm in chitin coated nylon meshcan be concluded as follows coating of chitin via plasma that is mainly and slightly increase of water sorption in figure 4.20and can reflect the relationshipbetween the water contact angle and the wettability of the surface by water.

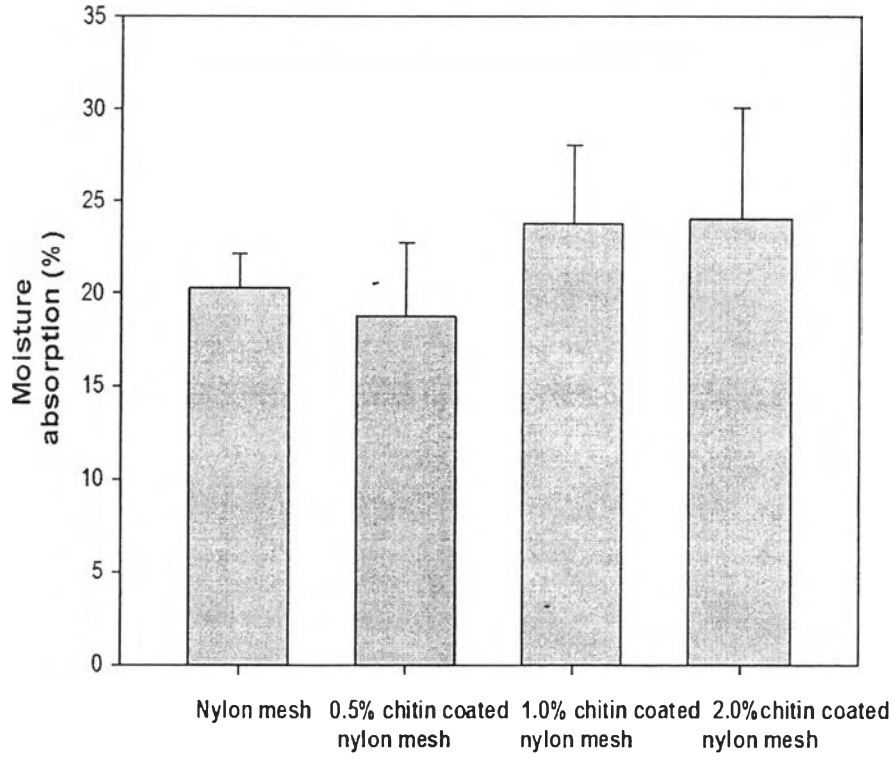


Figure 4.20 Water sorption of nylon mesh and chitin coated nylon mesh.

4.4 Characterization of Calcium Content

4.4.1 Calcium Content of (80:20) Nylon: Chitin Membranes

The amount of calcium can be calculate from Atomic absorption spectrometer (AAS) that can be create the standard calibration curve of calciumconcentration in ppm (part per million or mg/L) in order to find calcium content in next step. The result shows three times measuring absorbance and converts to calcium concentration by using calibration curve of calcium concentrationwhich shows in appendix E. Finally, quantity of calcium in 20% nylon-chitin membranes is 0.80 ppm in table 4.2 .

Table 4.2 Calcium content of (80:20) nylon:chitin membranes

Sample Nylon-chitin membranes	Mean absorbance	concentration (ppm)
Round I	0.059 ± 0.0010	0.82
Round II	0.056 ± 0.0005	0.78
Round III	0.057 ± 0.0005	0.80
Mean calcium concentration (ppm)		**0.80 ± 0.020**

4.4.2 Calcium Content of 2.0% Chitin Coated on Nylon Mesh

The result shows three times measuring absorbance and converts to calcium concentration by using calibration curve of calcium concentration which shows in appendix E. Finally, quantity of calcium in 2% chitin coated on nylon meshis 3.01 ppm in table 4.3 .

Table 4.3 Calcium content of 2% chitin coated on nylon mesh

Sample Chitin coated on nylon mesh	Mean absorbance	concentration (ppm)
Round I	0.205 ± 0.0010	2.98
Round II	0.212 ± 0.0023	3.07
Round III	0.204 ± 0.0015	2.96
Mean calcium concentration (ppm)		**3.01± 0.058**

4.5 Characterization of Sensitivity for Plasma Treatment between Nylon and Chitin

The result shows that both nylon and chitin are sensitive with plasma treatment together because increment of treatment time increases hence, the tensile strength decreases moreover, chitin has more sensitive than nylon 6,6 because the decrement effect of mechanical properties is higher than nylon and more chitin content also affects

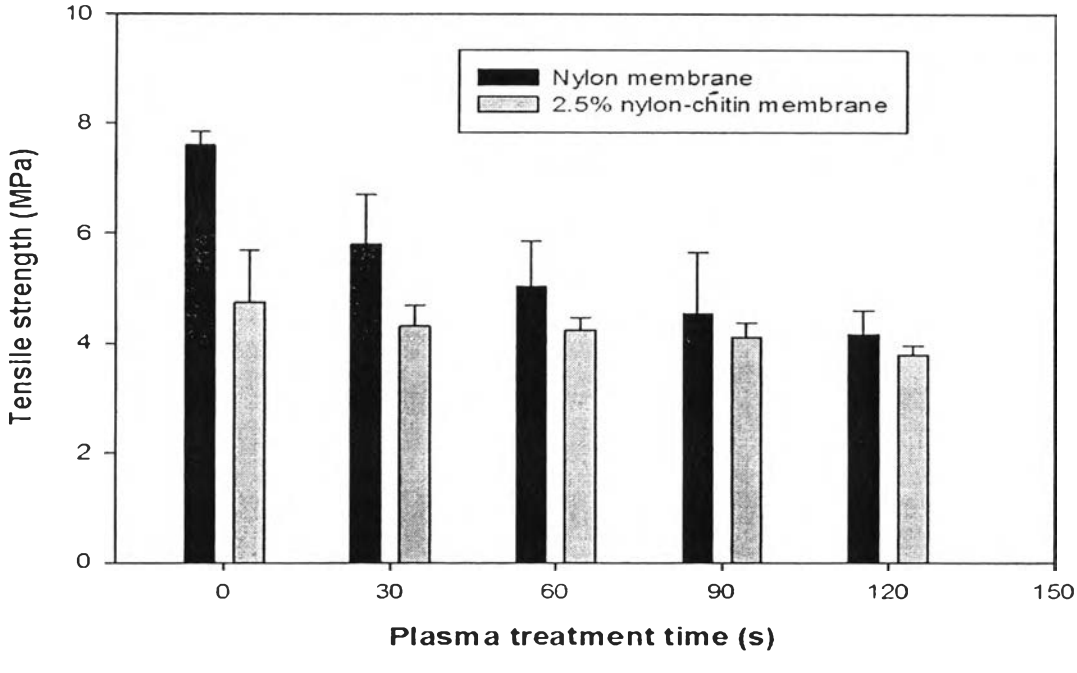


Figure 4.21 Comparison mechanical properties between nylon membrane and nylon-chitin membrane in differential plasma treatment times.

4.6 Characterization of chitin content

4.6.1 Chitin content on nylon-chitin membranes

TGA measurements provide valuable information that can beused to find compositional analysis ofmulti-component materials orblends. In this case for measuring chitin content from nylon-chitin membranes from blending method and the result shows in figure 4.22 the first, pure nylon membranes does not have chitin so chitin content is zero percentage and the next is nylon-chitin membranes in other ratios that are increasing chitin ratio to blend respectively in 95:5 (nylon:chitin), 90:10 (nylon:chitin) and 80:20 (nylon:chitin), chitin content are 12.25% , 18.77% and 29.10% (in % w/w unit) the result reveals the increment ofchitin content when more adding ratio of chitin.

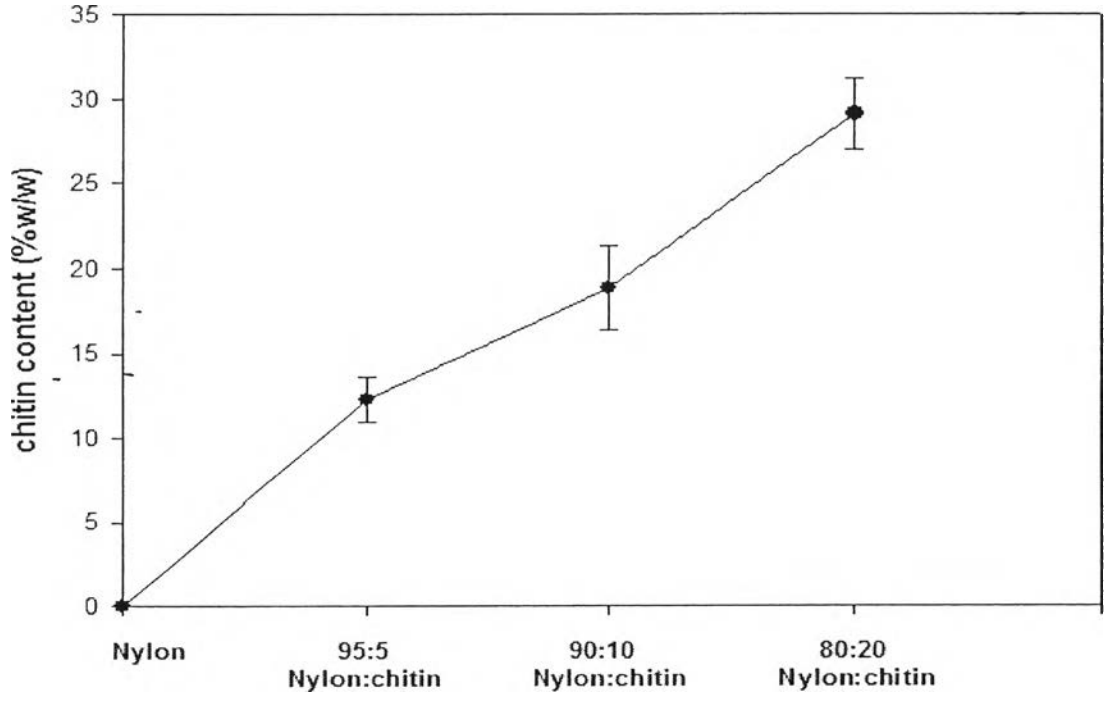


Figure 4.22 Chitin content (%w/w) of nylon-chitin membranes (Blending).

4.6.2 Chitin Content on Nylon Mesh

In this case for measuring chitin content from chitin coated on nylon mesh from coating method and the result shows in figure 4.23 the first, pure nylon meshthatuncoated chitin so chitin content is zero percentage and the next is coated chitin on surface of nylon mesh in other different concentration of chitin solution and verify solution for immersing in0.5% , 1.0% and 2% chitin concentration respectively and the results reveal the increment of chitin content and values are 73.53% , 77.25% and 77.78% (in % w/w unit) when immerse in more concentration.Moreover, in coating method there are a lot of chitin attach on surface that can observe in SEM images that correspond high chitin content on sample than blending method.However, when immerse in high chitin concentration that is slightly increase chitin content because of viscosity effect from chitin solution that prohibits the adhesion of chitin on nylon mesh surface.

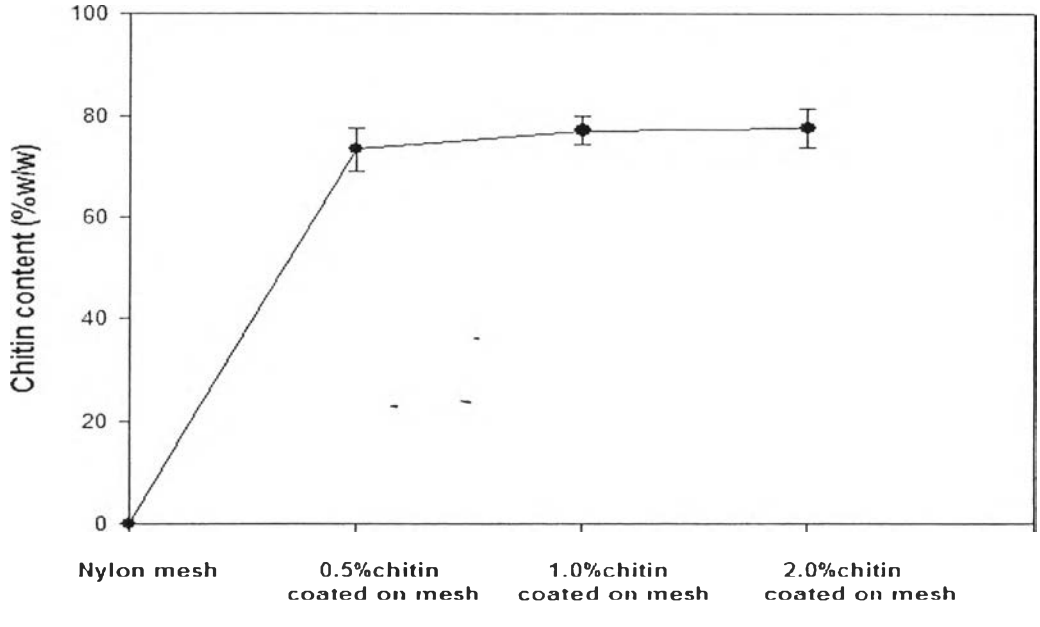
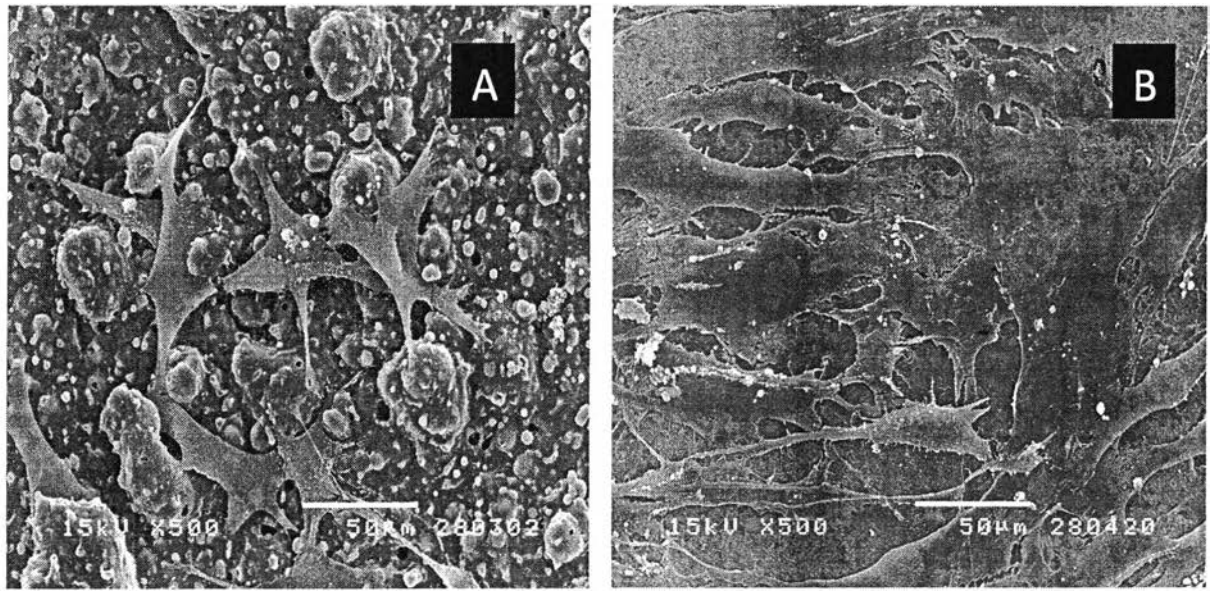


Figure 4.23 Chitin content (%w/w) of chitin coated on nylon mesh.

4.7 Characterization of Biocompatibility Cytotoxic

4.7.1 Surface Morphology of Nylon-Chitin Membranes

The human skin dermal fibroblast cells attachment on pure nylon membranes and nylon-chitin membranes were seeded on surface. After 48 h of incubation, there were many cells attached on 5% nylon-chitin membranes in Figure 4.21 (A) and most of the cells remained in round-shape. But for nylon-chitin membranes, cells adhered and almost completely spread on the surface in Fig. 4.21 (B). They had many pseudopodia and formed a layer on the surface. These results indicated thatthe cells stretched their morphology and were proliferating. This preliminary experiment suggests that the nylon-chitin membranes have better biocompatibility compared with nylon membranes in terms of fibroblast cell culture. It would have potentials to be used as wound dressing materials or tissue regeneration scaffold in vitro. Further investigation such as cellular proliferation and differentiation assays are underway.



Figurê 4.24(A) Fibroblast cell attachments of nylon membranes (B) and 5% nylon-chitin membranes of 48 h seeding the cells.