CHAPTER IV
RESULTS AND DISCUSSION

4.1 Preparation of Chitin

Shrimp shells are composed of three major components, which are chitin, calcium carbonate, and protein. Calcium carbonate and protein can be removed by solvent extraction and chitin will be obtained as the remaining substance.

In this research, chitin was prepared from shells of *Penaeus merguiensis* shrimp by demineralization with hydrochloric acid solution and deproteinization with sodium hydroxide solution in order to remove the calcium carbonate and the protein, respectively. The yield obtained during chitin production is shown in Table 4.1.

Table 4.1 Yield of chitin production from shrimp shell

Material	Yield* (%)
Shrimp shell	100
Product after demineralization and deproteinization (chitin)	34.17

*dry weight basis

FTIR spectrum of chitin is showed in Figure 4.1. Chitin has some extent of amino groups other than acetamide groups at C2 position of N-acetyl glucosamine repeating units. The degree of deacetylation of chitin depends on the nature of chitin resources and the conditions used during deproteinization. The chitin used in this study was inevitably subjected to N-deacetylation during deproteinization process under alkaline condition and heating. The degree of deacetylation of chitin was 23.81%.

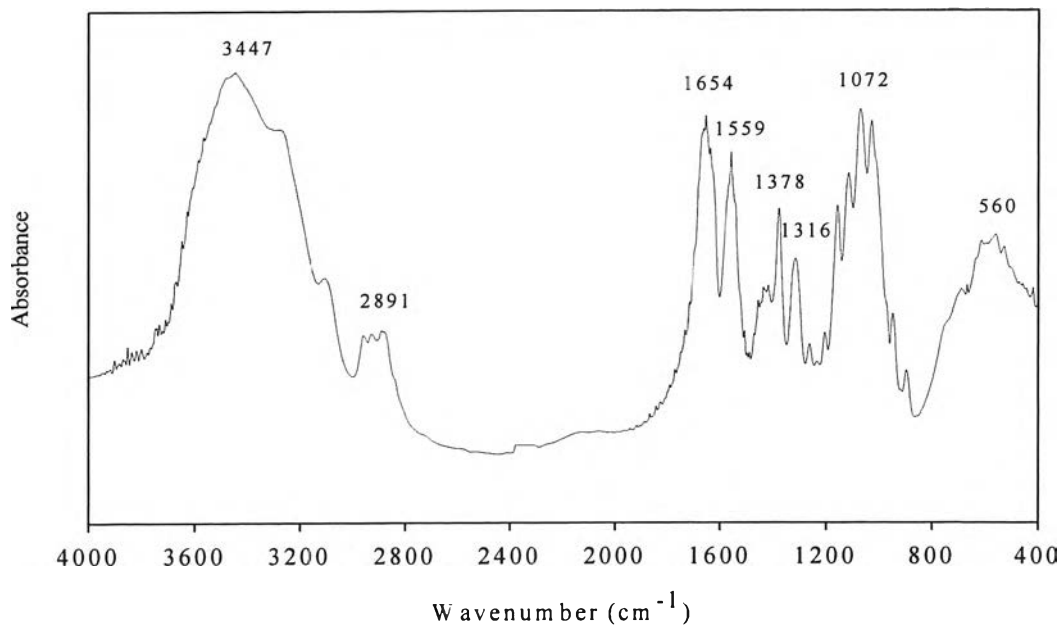


Figure 4.1 FTIR spectrum of chitin powder.

Figure 4.1 shows an IR spectrum of chitin. The characteristic absorption bands at 1654, 1559 and 1316 cm^{-1} were due to the amide I, II and III bands which are assigned to C=O, N-H and C-N stretching of acetamide groups, respectively. The sharp band at 1378 cm^{-1} was assigned to the CH_3 symmetrical deformation mode. The absorptions at 2891 and 3447 cm^{-1} were assigned to C-H and O-H stretching bands, respectively. The characteristic absorption bands of this study are similar to that of chitin, which was reported by Sannan *et al.*, (1978). The molecular weight of chitin was determined by viscometric method. According to Lee *et al.* (1974)'s study using the method, the molecular weight of chitin was derived from its intrinsic viscosity. The intrinsic viscosity of chitin was 14.80 (100 ml/g). The viscosity-average molecular weight of chitin obtained from the calculation was 8.71 $x10^5$ g/ mol.

4.2 Preparation of Chitosan

Chitin obtained was further deacetylated in 50% w/w of sodium hydroxide solution. Then chitosan with mainly reactive amino groups would be obtained. From

the step of preparation of chitosan, the deacetylation was performed repeatedly to achieve chitosan with higher degree of deacetylation.

Table 4.2 Yield of chitosan production from chitin

Material	Yield* (%)
Chitin	100
Product after deacetylation (chitosan)	85.16

*dry weight basis

The chitosan used in this study was inevitably subjected to N-deacetylation under alkaline and heating condition. The degree of deacetylation of chitosan was 82.44

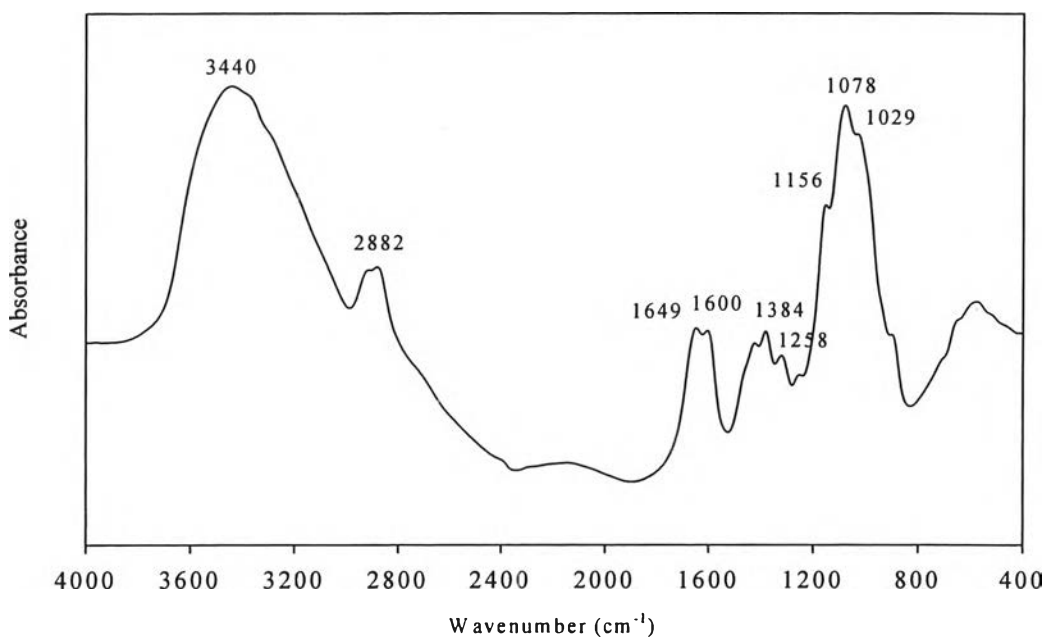


Figure 4.2 FTIR spectrum of chitosan.

Figure 4.2 shows an IR spectrum of chitosan. The strong, broad band of at 3000-3500 cm^{-1} is assigned to the hydrogen-bonded –OH and –NH bands. The absorptions peaks at 2882, 1600, 1258, 1156 and 1078 cm^{-1} are assigned to C-H

stretching, N-H bending, C-N stretching, bridge-O-stretching bands and C-O stretching vibration, respectively. The absorption due to primary hydroxyl group is appeared at 1078 cm^{-1} that is assigned to C-O stretching band. The absorption bands observed at 1649 and 1384 cm^{-1} are assigned to amide I and CH_3 vibration bands, respectively.

The molecular weight of chitosan was also determined by the viscometric method. According to the method of Kaneko (1982), the molecular weight of chitosan was derived from its intrinsic viscosity. From the step of preparation of chitosan, the deacetylation was performed repeatedly to achieve chitosan with higher degree of deacetylation. The molecular weight of chitosan in each treatment was shown in Table 4.3. This is due to the fact that the heat and alkaline condition of deacetylation of chitosan induce chain scission of polymer chains resulting in lower molecular weight. It is observed that the higher times of treatment of deacetylation used, the lower molecular weight detected.

Table 4.3 Molecular weight of chitosan after deacetylation in each treatment

No. of Treatment	Intrinsic Viscosity [η]	Molecular weight
1	14.51	1.83×10^5
2	13.00	1.64×10^5
3	11.73	1.61×10^5
4	10.46	1.32×10^5
5	8.36	1.06×10^5

4.3 Preparation of CM-Chitin

Chitin is insoluble in common solvents. However, the dissolubility of chitin can be improved by chemical modification. Chitin was modified to be CM-chitin, a water-soluble derivative, by carboxymethylation with monochloroacetic acid.

Table 4.4 Yield of CM-chitin production from chitin

Material	Yield* (%)
Chitin	100
Product after carboxymethylation (CM-chitin)	94.5

*dry weight basis

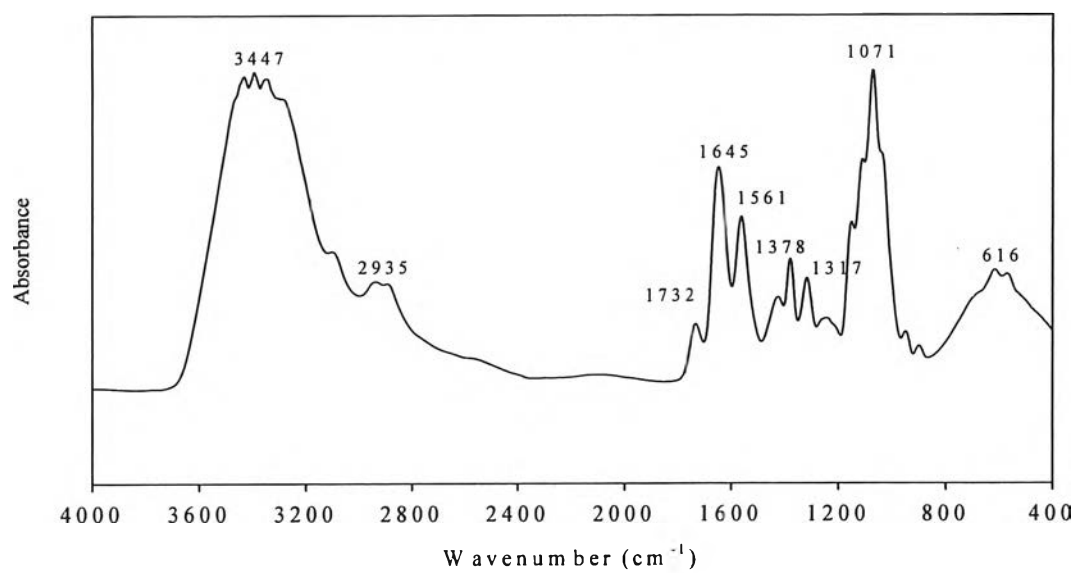


Figure 4.3 FTIR spectrum of H-form of CM-chitin.

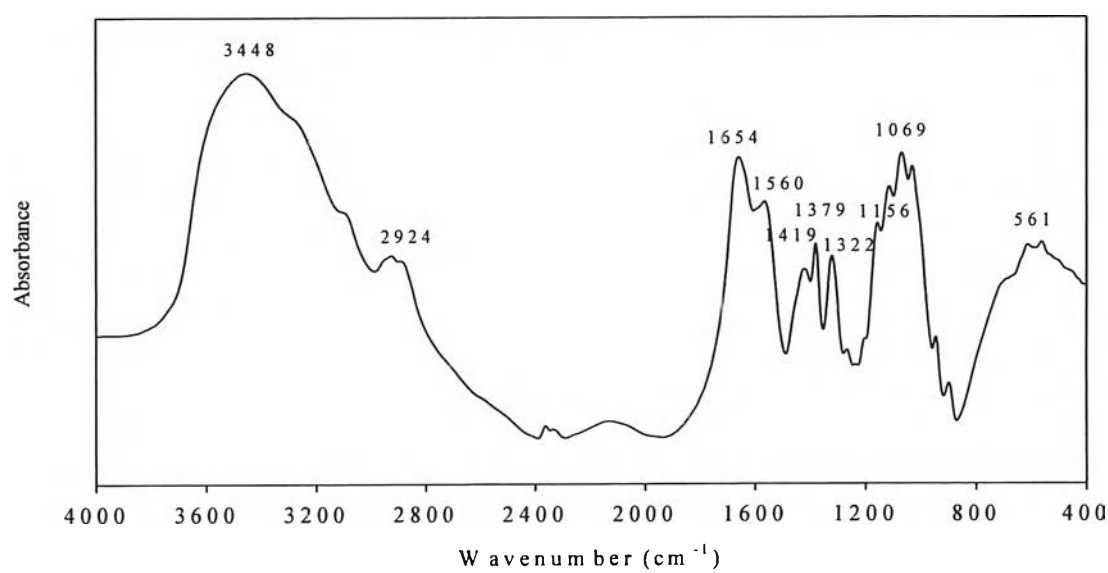


Figure 4.4 FTIR spectrum of Na-form of CM-chitin.

Figure 4.3 shows an IR spectrum of H-form of CM-chitin. The characteristic absorption band at 1732 cm^{-1}, attributed to carbonyl stretching of carboxyl group, was increased by carboxymethylation procedure. The absorption due to primary hydroxyl group was appeared at 1071 cm^{-1} assigning to the C-O stretching band. The characteristic absorption bands appeared at 1423, 1645, 1561, 1317 and 1378 cm^{-1} were due to symmetric stretching vibration of COO^-, indicating a successful substitution of carboxymethyl groups at amide I, II, III and CH_3 vibration bands, respectively. The strong, broad band of spectrum at 3000-3500 cm^{-1} were assigned to the hydrogen-bonded –OH and –NH groups. The secondary amide vibration band at 1561 cm^{-1} appeared in the spectrum conformed the NH deformation. Figure 4.4 depicts an IR spectrum of Na-form of CM-chitin. The absorption bands appeared at 1419, 1654, 1560, and 1322 are owing to symmetric stretching vibration of COO^-, amide I, II, and III, respectively.

The molecular weight of CM-chitin was determined by the viscometric method. According to the method of Kaneko (1982), the molecular weight of CM-chitin was derived from its intrinsic viscosity. The intrinsic viscosity was 7.38 (100 ml/g). The viscosity-average molecular weight of CM-chitin obtained from the calculation was 9.32×10^4 g/ mol. The degree of substitution of CM-chitin was 0.65.

4.4 Preparation of CM-Chitosan

CM-chitosan was obtained by deacetylation of CM-chitin in alkaline and heat condition.

Table 4.5 Yield of CM-chitosan production from CM-chitin

Material	Yield* (%)
CM-chitin	100
Product after deaceylation (CM-chitosan)	86.62

*dry weight basis

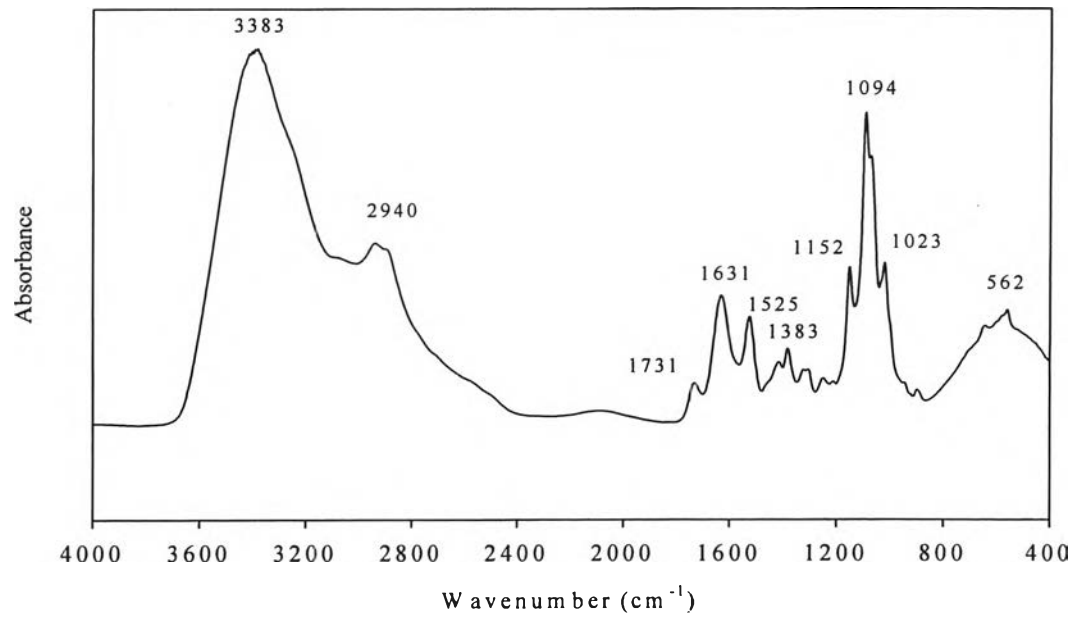


Figure 4.5 FTIR spectrum of H-form of CM-chitosan.

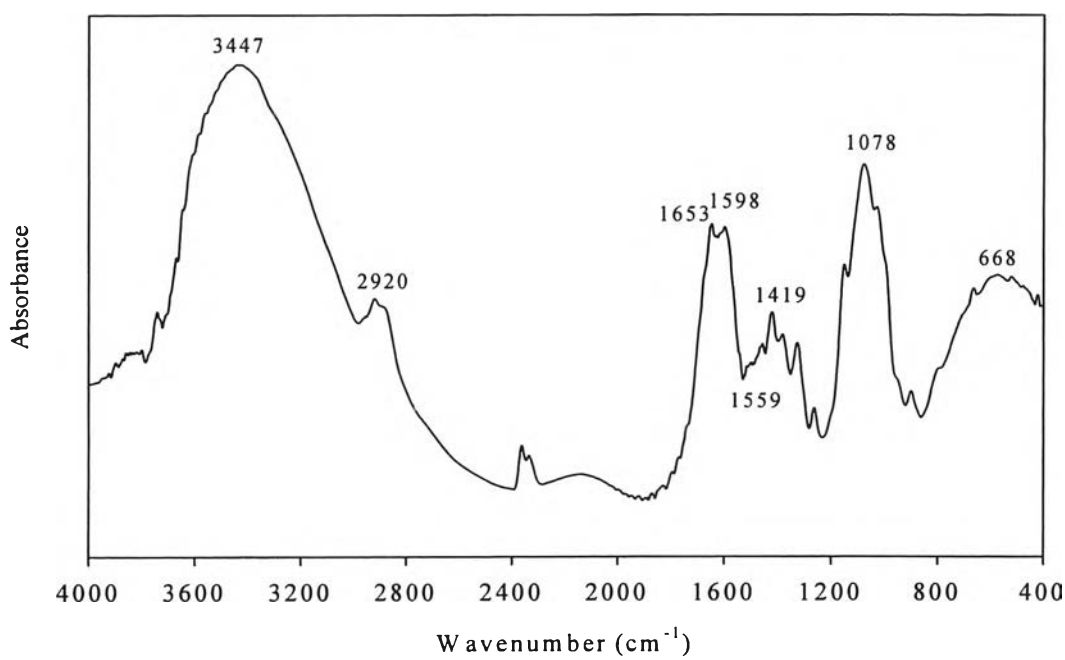


Figure 4.6 FTIR spectrum of Na-form of CM-chitosan.

Figures 4.5 and 4.6 showed IR spectra of two forms of CM-chitosan which are H-form and Na-form of CM-chitosan. For the H-form of CM-chitosan, the characteristic absorption band at 1731 cm^{-1}, attributed to carbonyl stretching of carboxyl group, was increased by carboxymethylation procedure. 1631 and 1525 cm^{-1} ($-NH_3^+$) were the characteristics of O-CM-chitosan (Liu *et al.*, 2001). The absorption peak at 1383 cm^{-1} ($-CH_3$ symmetrical vibration) of CM-chitosan has smaller absorption than this peak of CM-chitin, showing the occurrence of deacetylation. For the Na-form of CM-chitosan, the characteristic absorption band appeared at 1419 cm^{-1} was due to symmetric stretching vibration of COO^- which is indicated a successful substitution of carboxymethyl groups. The absorption bands at 1653 and 1598 cm^{-1} were owing to amide I and N-H bending. The strong, broad band of spectrum at 3000-3500 cm^{-1} are assigned to the hydrogen-bonded –OH and –NH groups.

The molecular weight of CM-chitosan was determined by the viscometric method. According to Kaneko (1982)'s study using the method, the molecular weight of CM-chitosan was derived from its intrinsic viscosity. The intrinsic viscosity of CM-chitosan was 3.16 (100 ml/g). The viscosity-average molecular weight of CM-chitosan obtained from the calculation was 4 x 10^4 g/ mol. The degree of substitution of CM-chitosan was 0.92.

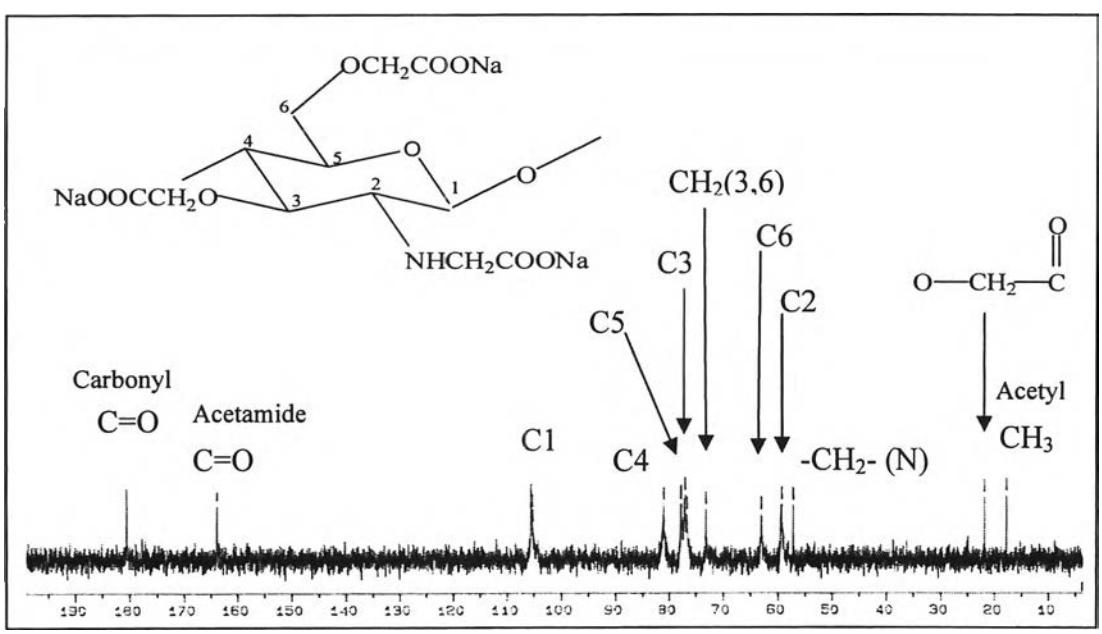


Figure 4.7 ^{13}C-NMR spectrum of CM-chitosan.

Figure 4.7 shows ^{13}C-NMR spectrum of CM-chitosan in DSS. It shows signals at180.50 (C=O(-O)), 163.67 (C=O(-N)), 105.26 (C1), 80.81 (C4), 77.56 (C5), 76.61 (C3), 62.89 (C6), 59.18 (C2). The resonance at 73 ppm was referred to be the $-CH_2-$ groups of both C-3 and C-6 (Hjerde *et al.*, 1997). Moreover, the spectrum indicates the presence of $-N-CH_2-COO^-$ at 57.07 ppm (Chen *et al.*, 2002). From FT-IR and ^{13}C-NMR spectra, we can deduce that the carboxymethylation of chitosan took place at OH (C3 and C6) and NH_2 to produce N,O-(3,6)-CM-chitosan.

4.5 Characterization of Scaffolds

4.5.1 Coloration after Steam Treatment

Steam treatment was used to promote crosslinking of chitosan, CM-chitin, and CM-chitosan scaffolds. The scaffolds were autoclaved at different temperature, i.e., 110°C, 115°C, and 121°C, for 15 min. After steam treatment in the autoclave, colors change of chitosan scaffold from yellow to brown was observed where as the color of CM-chitin and CM-chitosan scaffolds were changed from white to pale yellow. The higher the temperature used, the darker the color change was observed. It is known that heat can induce chain scission of polymers. In case of chitosan and its derivatives, the main chain of chitosan and its derivatives consists of pyranose rings where heat may possibly induce the opening of pyranose rings, resulting in the formation of aldehyde group at the chain end. As a consequence, aldehyde groups can involve in coloration and crosslinking reaction. Amino groups of chitosan react with aldehyde group to form amide linkage. Coloration in the steamed scaffolds due to the presence of aldehyde groups may be attributed to the Maillard reaction (Rizzi, 1994). Coloration by steam treatment was also reported in the study of Lim *et al.* (1999) who indicated that the coloration of chitosan was developed by steam treatment.

4.5.2 FTIR Analysis of Non-Steamed and Steamed Chitosan Scaffolds

Figure 4.8 shows FT-IR spectra of chitosan scaffolds with and without steam treatment at 121°C for 15 min. The FT-IR spectrum of non-steamed chitosan

scaffold (Figure 4.8 (a)) shows typical absorption peaks of chitosan at 3440 cm^{-1} (O-H stretching), 1649 cm^{-1} (-CONH-), 1600 cm^{-1} (N-H bending), 1156 cm^{-1} (bridge -O- stretching), and 1078 cm^{-1} (C-O stretching). On the other hand, the FT-IR spectrum of steamed chitosan scaffold is shown in Figure 4.8 (b). The absorption peaks at 1645 and 1560 cm^{-1} assigned to amide I and II, respectively, were observed. In addition, it was found that the degree of deacetylation, based on quantitative infrared spectroscopic technique (Sannan *et al.*, 1978), of steamed chitosan scaffold (64%) was lower than that of non-steamed chitosan scaffold (82%), indicating the reduction of amino groups of chitosan during steam treatment. This result is in good agreement with the study of Lim *et al.* (1999) who suggested that the amino groups of chitosan may involve in crosslinking reaction during heat treatment, resulting in the reduction in the number of amino groups.

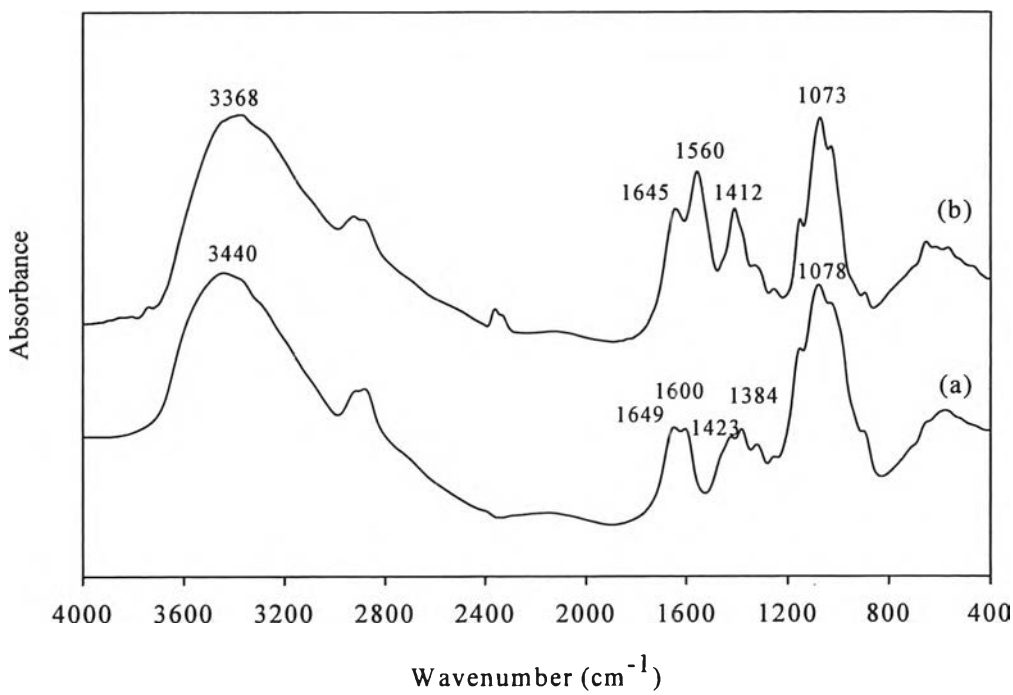


Figure 4.8 FTIR spectra of (a) non-steamed and (b) steamed chitosan scaffolds.

4.5.3 FTIR Analysis of Non-Steamed and Steamed CM-chitin Scaffolds

FT-IR spectra of CM-chitin scaffolds with and without steam treatment at 121°C for 15 min are illustrated in Figure 4.9. For non-steamed CM-chitin (Figure 4.9 (a)), the absorption bands at 1654, 1560, and 1419 cm^{-1} are assigned to amide I, amide II, and symmetrical stretching vibration of COO^-, respectively. Similarly, the spectrum of the steamed CM-chitin scaffold (Figure 4.9 (b)) shows absorption peaks at 1650, 1563, and 1421 cm^{-1}. The result revealed that there was no significant change in both FT-IR spectra of non-steamed and steamed CM-chitin scaffolds.

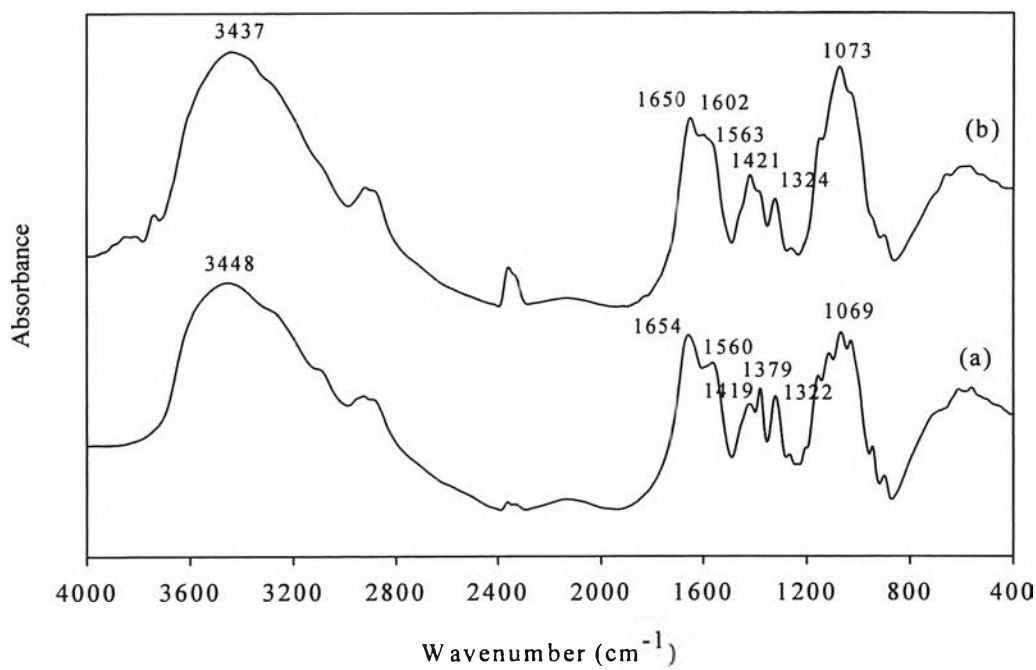


Figure 4.9 FTIR spectra of (a) non-steamed and (b) steamed CM-chitin scaffolds.

4.5.4 FT IR Analysis of Non-Steamed and Steamed CM-chitosan Scaffolds

Figure 4.10 depicts FT-IR spectra of CM-chitosan scaffolds with and without steam treatment at 121°C for 15 min. The absorption bands of non-steamed CM-chitosan (Figure 4.10 (a)) were appeared at 1419, 1653, and 1598 cm^{-1} owing to symmetric stretching vibration of COO^-, amide I, and N-H bending, respectively. For steamed CM-chitosan, the FT-IR spectrum (Figure 4.10 (b)) shows absorption

bands at 1650 and 1567 cm^{-1} which are assigned to amide I and amide II, respectively. No absorption peak at 1598 cm^{-1} (N-H bending), that has been observed in non-steamed CM-chitosan in the FTIR spectrum of steamed CM-chitosan scaffold, was detected. It is possible that amino groups of CM-chitosan might involve in crosslinking reaction. Janvikul *et al.*, (2003) reported that crosslinking by amide linkage could occur due to the reaction between $-NH_2$ and –COONa groups of CM-chitosan.

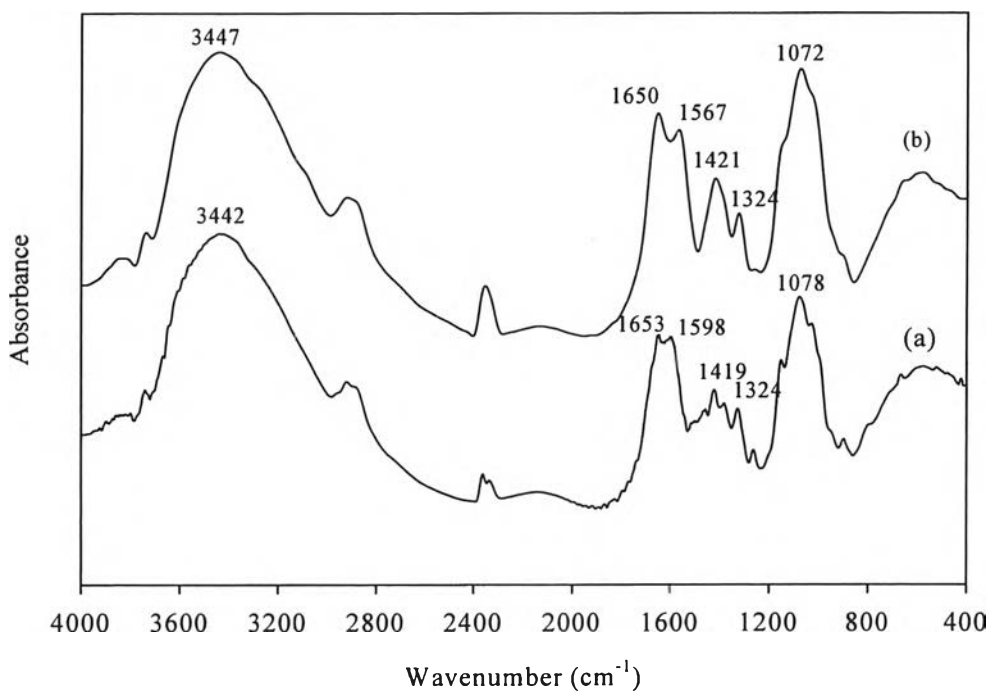


Figure 4.10 FTIR spectra of (a) non-steamed and (b) steamed CM-chitosan scaffolds.

4.6 Swelling Study

4.6.1 Weight Loss

Crosslinking of polymers in the scaffolds by steam treatment was investigated by determination of weight loss after immersion in good solvents. It was found that the non-steamed scaffolds of all polymers could not retain their shapes and were completely disintegrated within 5 hours after immersing in water. However, the scaffolds after steam treatment could retain their shapes longer than 24 hours. During steam treatment, the saturated steam in an autoclave was employed to crosslink or enhance stability of scaffolds (Lim *et al.*, 1999;Muramatsu *et al.*, 2003). Table 4.6 shows the weight loss of scaffolds after steaming at 110°C, 115°C, and 121°C for 15 minutes followed by immersing in water or 0.2 M acetic acid (for chitosan scaffolds) at 25°C for 24 hours. The results revealed that the percent weight losses of the scaffolds were in the range of 8-20% on dry weight basis, depending on the types of polymer and polymer concentrations. Due to the decrease in solubility of the steamed scaffolds, it may be inferred that the saturated steam promoted the crosslinking of polymers. In term of polymer concentration, it was found that the weight loss of chitosan (I-H) scaffold was lower than that of chitosan (I-L) scaffold, and the weight loss of CM-chitosan (H) was lower than that of CM-chitosan (L) scaffold. It may be said that the higher concentrations used, the lower weight loss detected. For the certain area of a mould, the polymer contents of the scaffolds increased with the increasing of the polymer concentrations proportionally. The higher the polymer concentrations, the denser the scaffolds obtained. As a result, the denser the scaffolds were, the more the possibility to promote crosslinking in the scaffolds. In addition, it was found that the weight loss of chtiosan (I-L) scaffold was almost similar to the weight loss of chitosan (II) scaffold although both of these polymers were different in molecular weight and viscosity. For all studied polymers, the weight losses of scaffolds treated at different steaming temperature from the highest to the lowest was in the order as follows: 110°C > 115°C > 121°C. The higher steaming temperature used, the higher degree of crosslinking obtained, and the lower percentages of weight loss detected. It is indicated that the steaming temperature affected to the weight loss of polymeric scaffolds. It may be concluded

that saturated steam could promote the crosslinking in the scaffolds, resulting in decrease of solubility of the scaffolds.

fect of steaming temperatures on weight losses of steamed scaffolds

ple	M_v	Conc. (% w/w)	Viscosity[a] (cp)	Weight loss[b] (%) Steaming temperature[d]			Weight loss[c] (%) Steaming temperature[d]		
				110°C	115°C	121°C	110°C	115°C	121°C
san H)	1.06×10^5	6[e]	7,800	16.25 ±1.31	14.39 ±0.75	13.95 ±0.24	13.24 ±0.32	12.04 ±0.75	8.42 ±0.91
san L)		2.5[e]	820	29.37 ±0.53	24.93 ±0.62	18.06 ±1.75	19.15 ±1.23	14.40 ±0.53	10.46 ±0.87
san)	5.54×10^4	2.5[e]	480	29.58 ±0.78	26.11 ±1.25	20.20 ±1.44	18.36 ±0.70	16.99 ±0.49	10.65 ±0.49
I- in	9.3×10^4	2[f]	8400	34.23 ±0.78	30.83 ±0.82	12.72 ±0.35	N/A	N/A	N/A
I- san)	4.0×10^4	3[f]	8600	21.09 ±1.26	16.58 ±0.82	14.49 ±0.51	N/A	N/A	N/A
I- san)		2.5[f]	7000	24.15 ±0.11	18.80 ±0.81	14.99 ±0.87	N/A	N/A	N/A

iscometer

n water

n 0.2 M acetic acid

15 minutes

0.2 M acetic acid

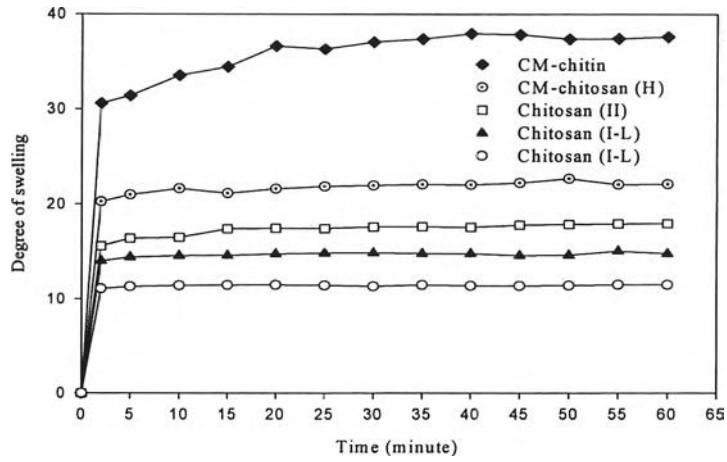
water

4.6.2 Swelling Behavior

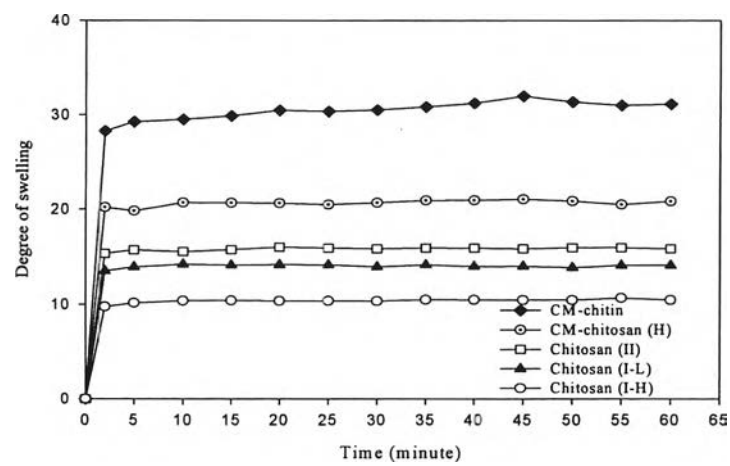
The effects of steaming temperatures and the types of material on crosslinking could be indirectly observed from the swelling behavior of the steamed scaffolds. It is known that the lower the degree of swelling detected, the higher the crosslinking density was.

4.6.2.1 Effect of time on the degree of swelling

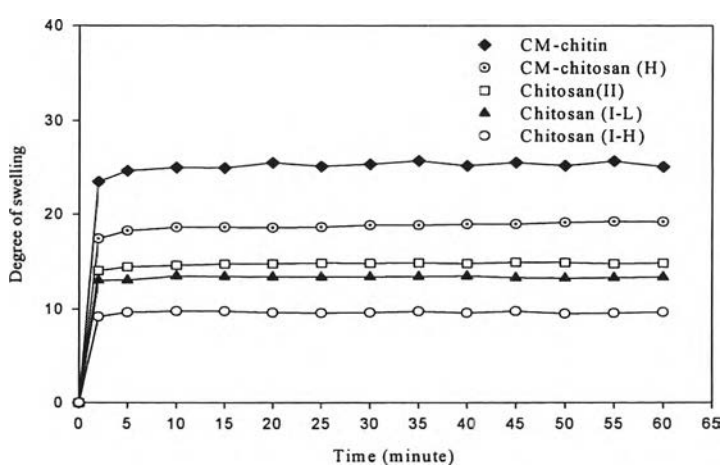
Figure 4.11 illustrates the degree of swelling of chitosan (I-L), (II), and (I-H), CM-chitin, and CM-chitosan (H) scaffolds at 110°C, 115°C and 121°C as a function of immersion time. For all studied polymers, it was found that the degree of swelling of the steamed scaffolds increased remarkably within 5 minutes after immersing the steamed scaffolds in distilled water, indicating the rapid increase in water contents in the steamed scaffolds. Subsequently, the degree of swelling of the steamed scaffolds was gradually increased when the immersion time increased and became rather constant within 15 minutes.



(a)



(b)



(c)

Figure 4.11 Degree of swelling of steamed scaffolds at (a) 110°C, (b) 115°C, and (c) 121°C. ▲ chitosan (I-L), □ chitosan (II), ○ chitosan (I-H), ♦ CM-chitin, and ⊙ CM-chitosan (H) scaffolds.

4.6.2.2 Effect of steaming temperature on the degree of swelling

The degrees of swelling at equilibrium of chitosan (I-L), (II), and (I-H), CM-chitin, and CM-chitosan (H) scaffolds at 110°C, 115°C and 121°C are shown in Figures 4.12. It was observed that the degrees of swelling of samples at different steaming temperatures from the highest to the lowest degree of swelling was in the order as follows: 110°C > 115°C > 121°C. This is in good agreement with the result of weight loss, as shown in Table 1. In general, the higher steaming temperature used resulted in the higher degree of crosslinking taken place, and the lower degree of swelling obtained.

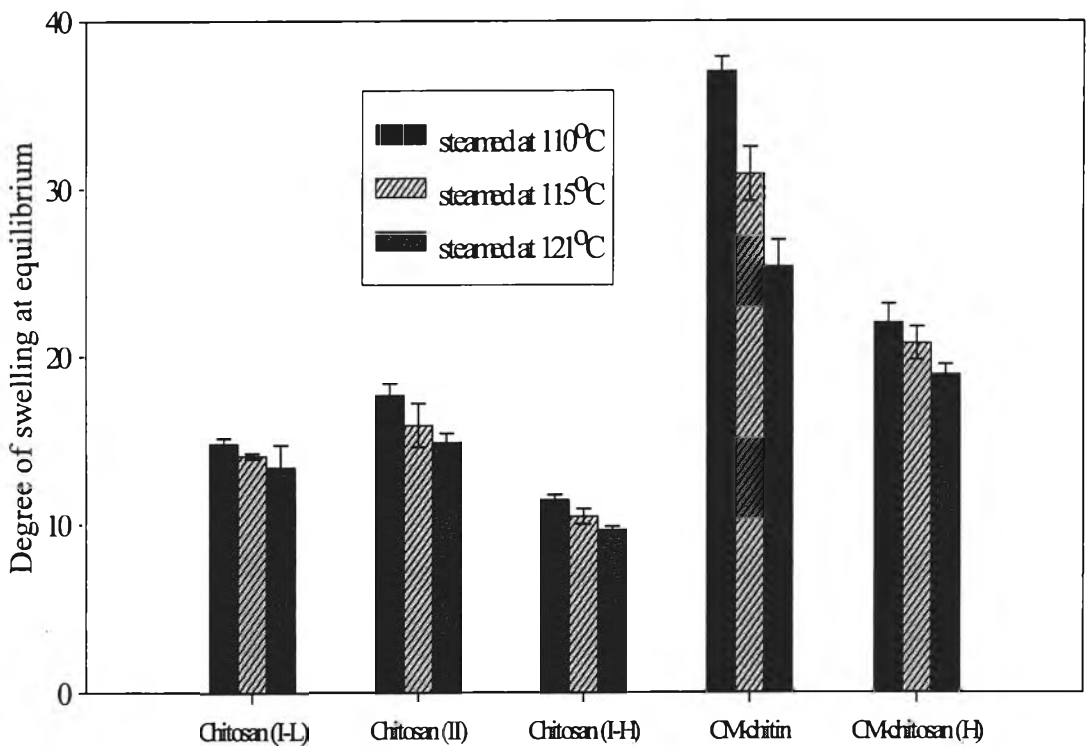
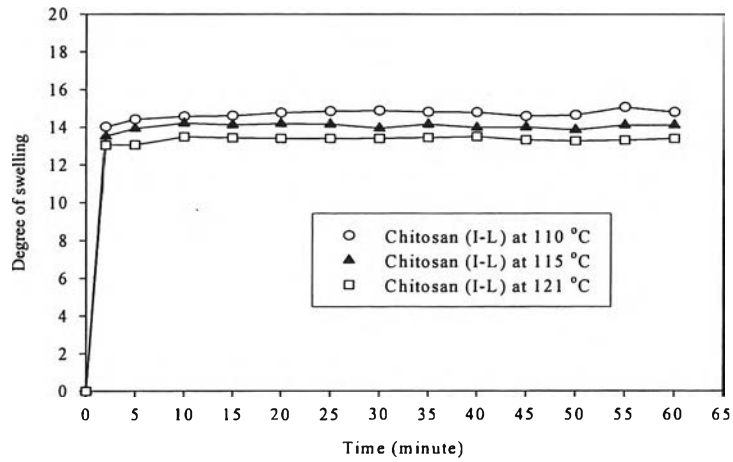


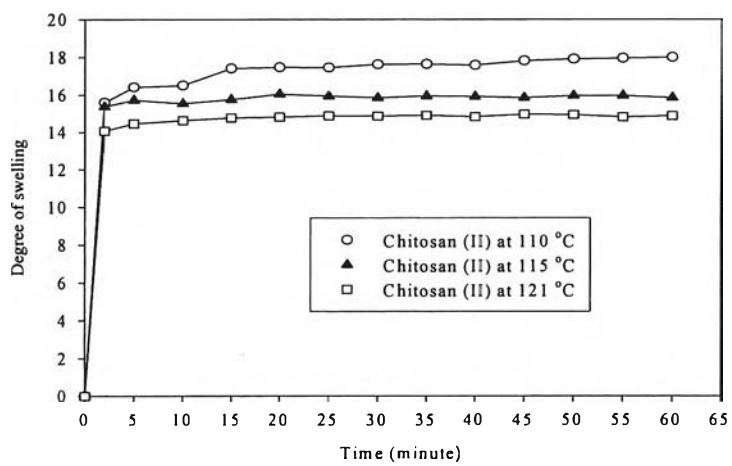
Figure 4.12 Degree of swelling at equilibrium of chitosan (I-L), chitosan (I-H), chitosan (II), CM-chitin, and CM-chitosan (H) scaffolds at 110°C, 115°C, and 121°C.

4.6.2.3 Effect of type of polymer on the degree of swelling

In case of chitosan, the degrees of swelling of the steamed chitosan scaffolds as a function of immersion time are shown in Figure 4.13. It was found that the concentration of chitosan solution affected to the degree of swelling of the scaffolds. Chitosan (I-L) scaffold had a higher degree of swelling than chitosan (I-H) scaffold. It can be implied that the denser polymer content was, the more possibility of crosslinking during steam treatment occurred, resulting in the lower degree of swelling. In addition, the molecular weight of polymer and viscosity of polymer solution also affected to the degrees of swelling of the scaffolds. The degree of swellings of chitosan (II) scaffold was higher than chitosan (I-L) scaffold. As shown in Table 4.6, chitosan (II) (M_v=5.54x10^{4}g/mol, viscosity = 480 cp) had the lower molecular weight and viscosity than chitosan (I) (M_v=1.06x10^5 g/mol, viscosity = 820 cp). The lower the molecular weight or viscosity of chitosan solution used, the higher the degree of swelling observed. It can be explained by the fact that the lower molecular weight or viscosity which means the shorter polymer chains has higher mobility, and they absorb higher the amount of water higher than the longer ones.



(a)



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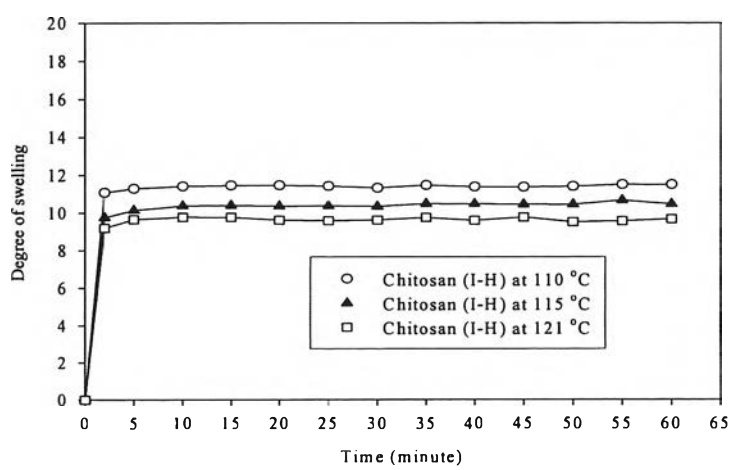
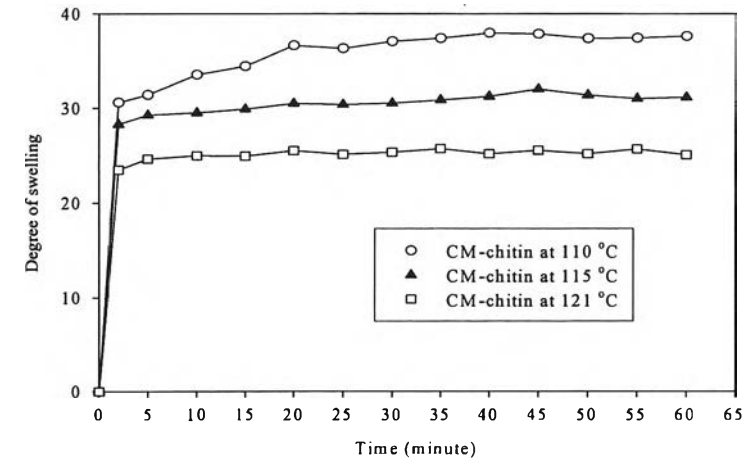


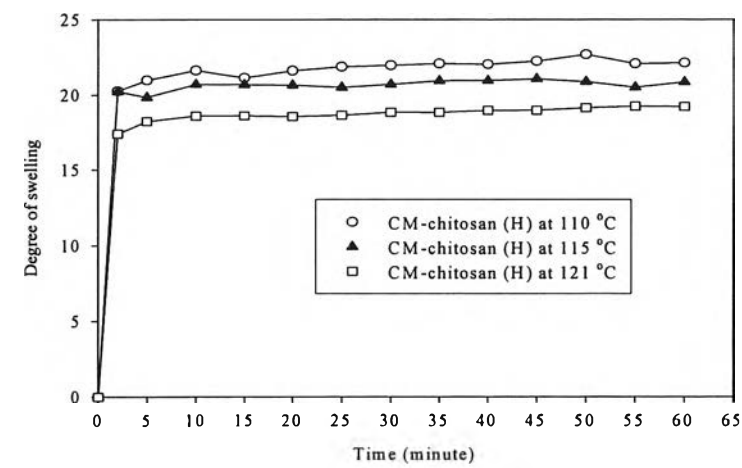
(c)

Figure 4.13 Degree of swelling of (a) chitosan (I-L), (b) chitosan (II), and (c) chitosan (I-H) scaffolds at ○110°C, ▲115°C, and □ 121°C.

For CM-chitin and CM-chitosan scaffolds, it was found that the degree of swelling of CM-chitin scaffold was higher than CM-chitosan (H) scaffold as illustrated in Figure 4.14. Since, CM-chitosan has more amino groups than CM-chitin and this functional group is supposed to involve in the crosslinking formation during steam treatment, CM-chitosan thus has higher possibility of crosslinking than CM-chitin, resulting in lower degree of swelling. Moreover, the degrees of swelling of chitosan (I-L), (II), and (I-H) scaffolds were lower than the degrees of swelling of CM-chitin and CM-chitosan (H) scaffolds. CM-chitin and CM-chtiosan are water-soluble derivatives containing carboxymethyl groups which are hydrophilic group being able to absorb water. Khor *et al.* (1997) suggested that the ability to absorb water of CM-chitin and CM-chitosan was attributed to the introduction of carboxymethyl groups distributing along chitin and chitosan chains on the glucosamine residues. It is known that chitosan had low degree of swelling in water because chitosan could not dissolve in most organic solvents including water (David and Hon, 1996) due to its rigid crystalline structure and high polarity (Zong *et al.*, 2000). In addition to polymer concentration, viscosity, molecular weight, and the chemical structure of polymers also affected to the swelling behavior of chitosan and its derivatives scaffolds.



(a)



(b)

Figure 4.14 Degree of swelling of (a) CM-chitin scaffold and (b) CM-chitosan (H) scaffold at ○110°C, ▲115°C, and □ 121°C.

4.7 Mechanical Properties

4.7.1 Tensile Strength

The tensile strengths of the scaffolds with and without steam treatment at 121°C for 15 min are shown in Figure 4.15. The tensile strength of the steamed chitosan (I-L) and (II) scaffolds could not be monitored by a Lyod tensile tester because of their brittleness. For the non-steamed chitosan scaffolds, it was found that chitosan concentration greatly affected to the tensile strength of the scaffolds. The chitosan scaffold with higher polymer concentration had higher tensile strength than those with lower concentration owing to the higher mass content in the scaffold. It was observed that the non-steamed scaffolds showed higher tensile strengths than the steamed scaffolds. This may be due to the porous structure of scaffolds that became more brittle after crosslinking by the steam treatment. However, the overall tensile strengths were rather low at around 0.12 MPa. In general, scaffolds prepared by freeze-drying technique have porous morphology with low degree of crystallinity and low tensile strength (Korey *et al.*, 1989).

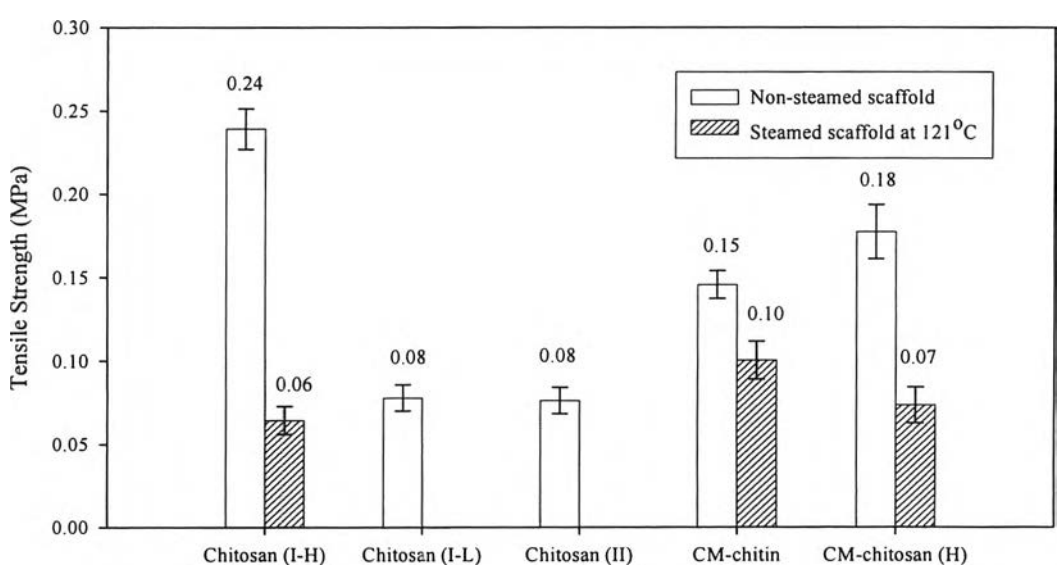


Figure 4.15 Tensile strengths of chitosan (I-H), chitosan (I-L), chitosan (II), CM-chitin, and CM-chitosan (H) scaffolds.

4.7.2 Elongation at Break

Figure 4.16 shows the elongation at break of the scaffolds without steam treatment and with steam treatment at 121°C for 15 min. The elongation at break of the steamed chitosan (I-L) and chitosan (II) scaffolds could not be determined because of the brittleness of the scaffolds. The steam treatment of the scaffolds resulted in the reduction of elongation of the scaffolds. This can be another evidence to show that crosslinking might be formed in the scaffolds by steam treatment. It is seemed that the scaffolds with the higher solid mass content had better mechanical properties than those with the lower solid mass content. However, the increase of the solid mass content of the scaffolds was limited by the solubilities of chitosan and its derivatives. In this study, the solutions of chitosan, CM-chitin, and CM-chitosan were prepared to obtain as high concentration as possible while the complete dissolution could still be achieved. Moreover, it is likely that the polymer content in the scaffolds had more effect on mechanical properties than the molecular weight of polymer.

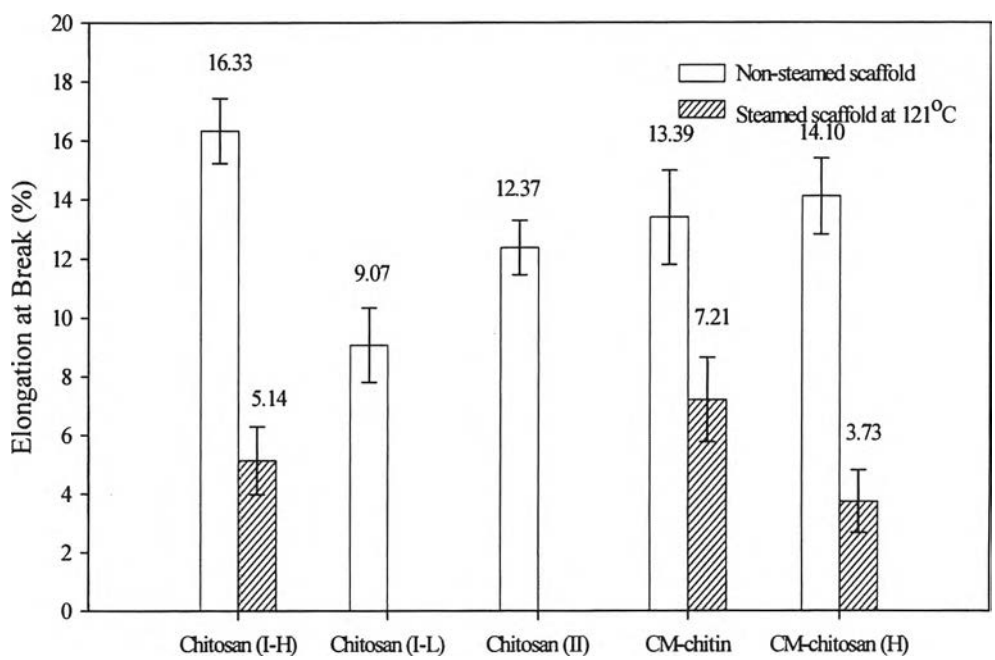


Figure 4.16 Elongation at break of chitosan (I-H), chitosan (I-L), chitosan (II), CM-chitin, and CM-chitosan (H) scaffolds.

4.8 Morphology

Comparison of scanning electron micrographs of the surfaces of the non-steamed and steamed scaffolds is illustrated in Figure 4.17. It was found that the pore sizes of chitosan (I-H), CM-chitin, and CM-chitosan (H) scaffolds were 69.13μm ± 21.20, 130μm ± 35.67, and 136μm ± 31.02, respectively. Chitosan (I-H) scaffold had smaller pore size in the horizontal direction than CM-chitin and CM-chitosan (H) scaffolds due to the high concentration of 6% chitosan. The pores of chitosan (I-H) were not well distributed over the scaffold, however, the pore sizes of CM-chitin and CM-chtiosan (H) scaffolds were well distributed over the scaffolds. Figure 4.18 shows scanning electron micrographs of cross-sectional structures of the scaffolds. There were no specific differences in the cross-sectional structures observed for chitosan (I-H), CM-chitin, and CM-chitosan (H) scaffolds. The pore structures were open pore at the surface and columnar shape along the vertical direction. It was observed that the pore walls on the vertical direction were thicker than those on the horizontal direction, indicating that the pore enlarged in the vertical direction during freezing. It is known that the porosity of scaffolds can be controlled by size of ice crystals formed during freezing. Kang *et al.* (1999) suggested that the scaffold obtained by freezing at -80°C had combined orientations between random ice crystallization and ununiform enlargement of the pore during freezing of ice.

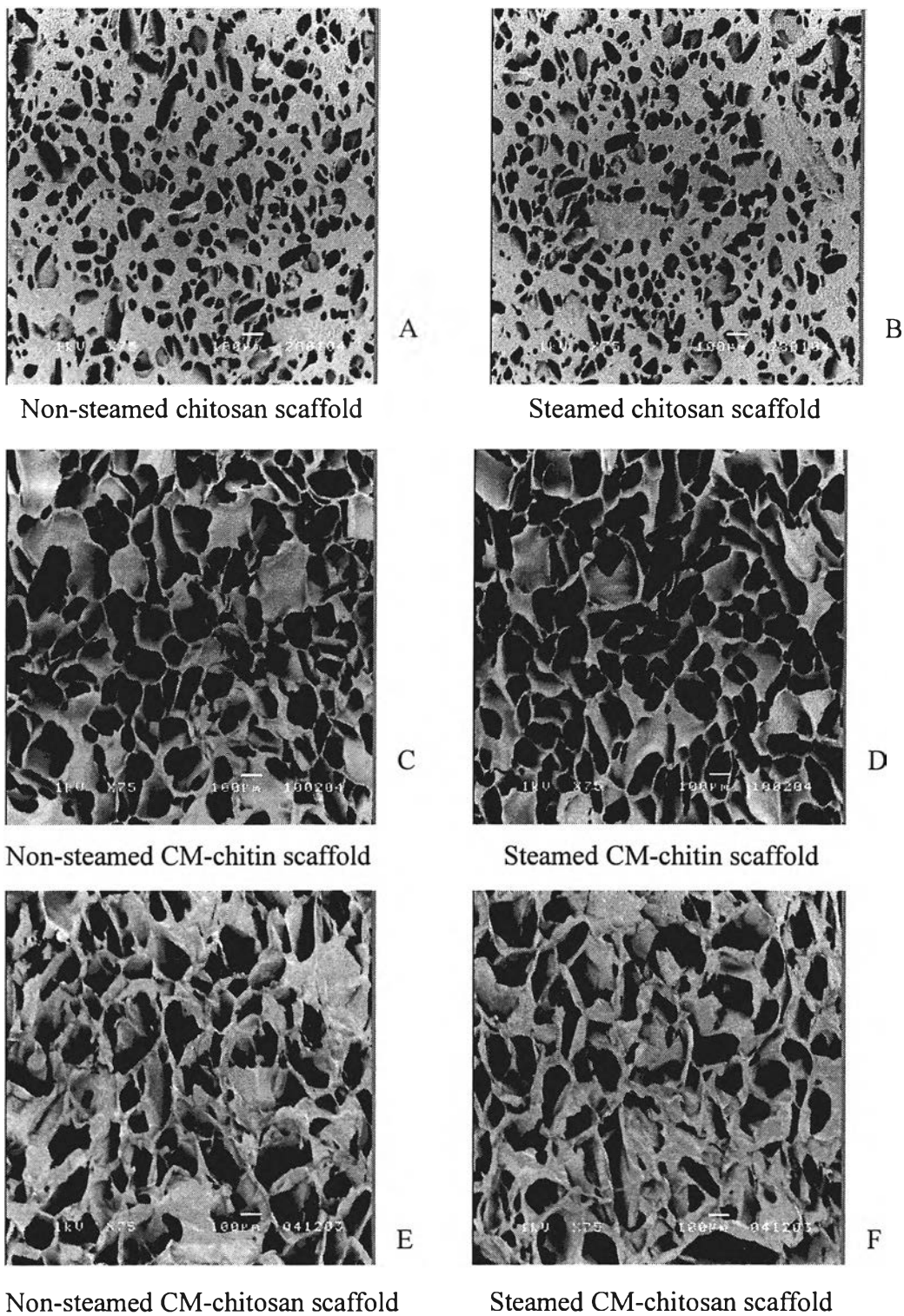


Figure 4.17 Comparison of scanning electron micrographs of surfaces of non-steamed and steamed scaffolds (A) non-steamed chitosan (I-H), (B) steamed chitosan (I-H), (C) non-steamed CM-chitin, (D) steamed CM-chitin, (E) non-steamed CM-chitosan (H), and (F) steamed CM-chitosan (H) scaffolds.

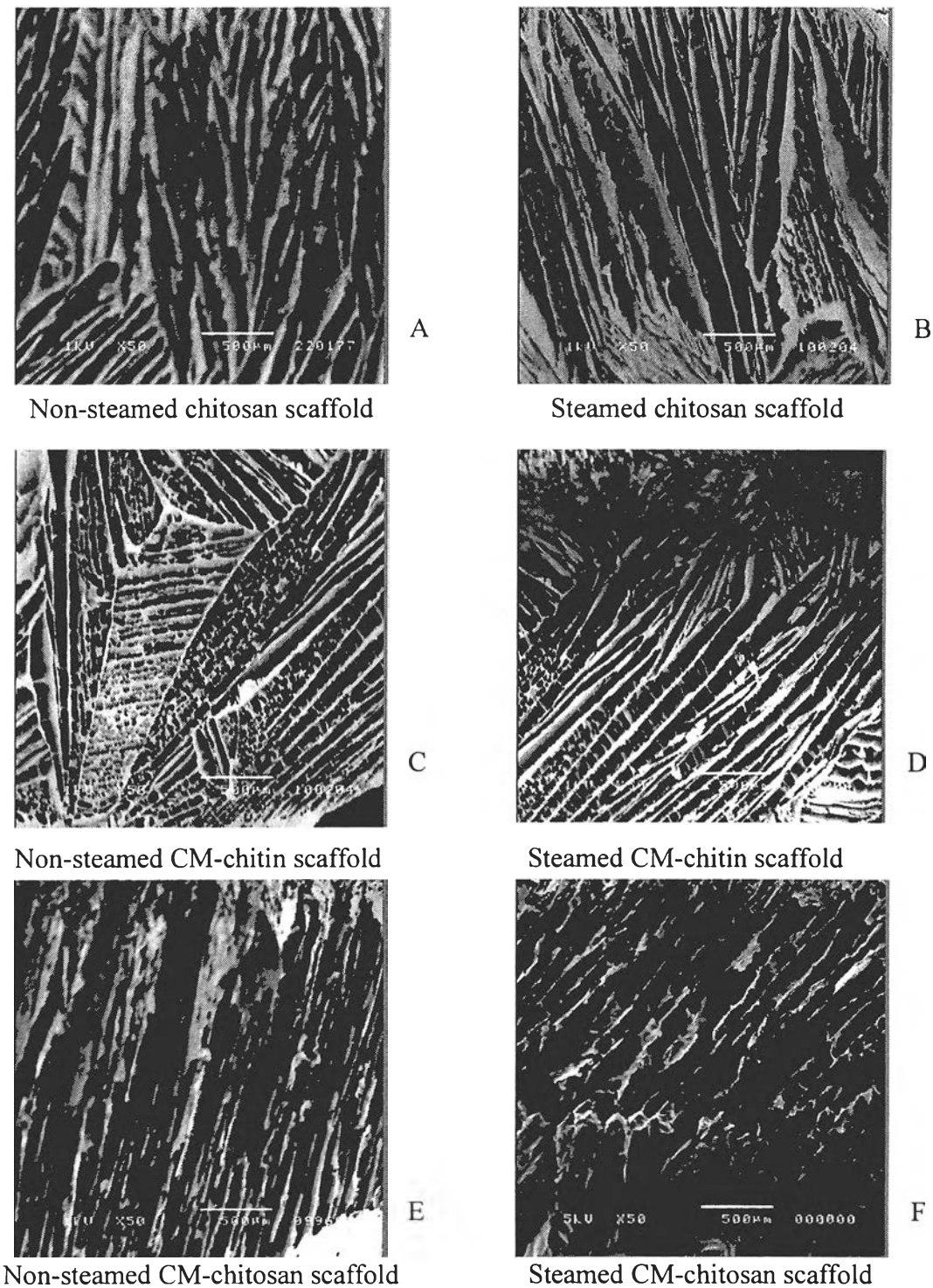


Figure 4.18 Comparison of scanning electron micrographs of cross-section of non-steamed and steamed scaffolds (A) non-steamed chitosan (I-H), (B) steamed chitosan (I-H), (C) non-steamed CM-chitin, (D) steamed CM-chitin, (E) non-steamed CM-chitosan (H), and (F) steamed CM-chitosan (H) scaffolds.