CHAPTER II

WATER-SOLUBLE LOW MOLECULAR WEIGHT CHITOSAN FROM HYDROCHLORIC ACID HYDROLYSIS

Abstract

The water soluble low molecular weight chitosan (LMCS) prepared by acid hydrolysis is reported. Chain degradation is proceeded to equilibrium stage and reduces the molecular weight for 94%. Degree of deacetylation is increased significantly to nearly 100% after hydrolysis time for 3 hours. FTIR and ^{1}H-NMR confirm the peaks referring to the presence of saccharide unit maintained in the structure after hydrolysis. XRD patterns show the disappearance of the peak at 9°2θ while the peaks at 20° and 22°2θ become significant comparing to the chitosan starting material. The product obtained from the hydrolysis time above 9 hours performs the water-soluble property.

Keywords: Low molecular weight chitosan, Acid hydrolysis, Hydrochloric acid hydrolysis, Chain degradation, Water-soluble low molecular weight chitosan.

Introduction

Chitin is the second most natural occurring abundant polysaccharides obtained from shells of crustaceans, insects and plant cell walls. Chitosan is a β-1,4-linked-2-amino-deoxy-D-glucopyranose (Scheme I) derived by N-deacetylation of chitin. The attractive points of chitin-chitosan can be raised as the bioactivity[1], biocompatibility[2], biodegradability[3], and nontoxicity[4]. Chitin-chitosan has received much attention to apply its bio-, chemical-, and physical- properties for practical applications. The development of chitin-chitosan for applications in medical and pharmaceutical fields provides value-added products such as drug carrier[5], functional membranes for separation[6], matrices for tissue engineering[7], and film for wound healing[8].

For more than three decades, the many approaches to apply chitin-chitosan based on its unique properties have been proposed. At present, almost all items of chitin-chitosan available in the market are achieved from the physical modification pathway as seen from the products of beads, membranes, powders, and solutions. Although, various chemical modified chitin-chitosan derivatives have been reported and known as the way to achieve the unique biopolymer products, the practical development seems to be limited. This might be due to the problem in controlling the reaction of chitin-chitosan. The main reason that obstructs the development of chitosan derivatives in industrial scale is the dissolvation in solvent and the inertness in chemical reaction.

One of the approaches to overcome the problems is to reduce the molecular weight to decrease the molecular interaction and improve reactivity. Recently, low molecular weight chitosan has received much attention because low molecular weight chitosan provides not only water-solubility, which is essential for applying in biosystem, but also the versatile functional properties such as antitumor activity[9], immuno-enhancing effects[10], antifungal activity[11], and antimicrobial activity.[11] The low molecular weight chitosan (LMCS) is known to be prepared by chemical treatment, enzymatic degradation, and γ ray-irradiation. Enzymatic hydrolysis[12] is an attractive way, since it can be achieved in natural system with mild condition. However, it requires many steps in the enzyme preparation process. Photoirradiation

is a method to propose the way to apply the high radiation energy for the peaceful and useful utilization, but the weak points are about the cost capitality and the limitation in molecular weight reduction.[13] On this viewpoint, the chemical treatment[14] is an interesting method to get low molecular weight and can be easily controlled.

Although acid hydrolysis of chitosan by using hydrochloric acid has been previously reported, most studies concerned about the depolymerization to oligomer level while the chemical structure and the change in degree of deacetylation are rarely clarified. For example, Moo-Yeal Lee *et al*[15] prepared chitosan oligomers by using 35% hydrochloric acid for 2 h at 80°C to obtain the product with DP of 5-7. Shobhan and Lawrence[16] studied the effects of hydrochloric acid in depolymerization of chitosan to conclude that acid digestion time was the main factor in the depolymerization process. The present work, thus, stands on the viewpoint of preparing low molecular weight chitosan by treating with acid at the condition that the structure of chitosan is maintained.

Experimental Section

Materials. Chitin-chitosan with a degree of deacetylation (%DD) of 87 was locally supplied from the SEAFRESH (Lab) Company Limited, Bangkok, Thailand. Acetic acid and sodium acetate were from UNIVAR, Australia. Hydrochloric acid, ethanol, and acetone were the products of BDH Laboratory Supplies, England. Deuterated oxide was purchased from Fluka Chemika, Switzerland. Methanol was purchased from J.T Baker, USA. Deuterated acetic acid was from EURISO-TOP, France. All chemicals were used without further purification.

Instruments and Equipment. Qualitative and quantitative FTIR spectra were obtained from Bruker Equinox 55/S Spectrometer with 32 scans at a resolution of 4 cm^{-1}. A frequency range of 4000-400 cm^{-1} was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^*, of $1x10^9$ $cm.Hz^{1/2}w^{-1}$. A DuPont Thermal Gravimetric Analyzer was used to study thermal properties of the samples under a N_2 flowing rate of 20 mL/min and heating rate was 20°C/min from 30°C to 600°C. ^{1}H-NMR was obtained from a 500 MHz JEOL (JNM-A500) at 70±1°C using deuterated acetic acid (CD_3COOD) and deuterated oxide (D_2O) with a trace amount of tetramethylsilane (TMS). X-ray diffraction patterns were obtained from a RIGAKU RINT 2000, using CuK_α (λ = 0.154 nm) as an X-ray source with 2θ of 5-50° operating at 40 kV, 30 mA with Ni filter. Intrinsic viscosity [η] was measured with a calibrated viscometer Connon-Ubbelohde (No.2, A149) in 0.1 M sodium acetate/ 0.2 M acetic acid aqueous solution at 30±0.05°C. Molecular weight was calculated using the Mark-Houwink equation with $K=1.64\times10^{-30}\times DD^{14}$ and $a=(-1.02\times10^{-2}\times DD)+1.82$ as proposed by Wang *et al.*[17]

Preparation of Low Molecular weight Chitosan (LMCS). Chitosan sample (1.5 g) was refluxed with 1.7 N hydrochloric acid solution (100 mL) under nitrogen atmosphere. The solution was cooled to room temperature and neutralized with 4 N sodium hydroxide. The product was washed with deionized water for several times.

Results and Discussion

Molecular Weight Reduction. In the present work, chitosan flake (%DD = 87, M_v = 3.7x10^5) was hydrolyzed by acid hydrolysis for various time to observe changes in molecular weight and chemical structure. Figure 1 shows a significant decrease in molecular weight for 94% in the first three hours of hydrolysis. The molecular weight was maintained at 1-2x10^4 dalton even after 12 hours.

Structural Characterization. FTIR and ^{1}H- NMR techniques were applied to clarify the changes in structure of the products obtained. The saccharide peaks at 895-1200 cm^{-1} of low molecular weight chitosan confirm that the chitosan backbone is maintained (Figure 2). However, the peaks at 1655 and 1550 cm^{-1} of amide I and II are slightly changed. This might be due to the changing of degree of deacetylation after hydrolysis. The effect of acid hydrolysis on the degree of deacetylation was evaluated by curve fitting quantitative FTIR technique using the peak intensity ratio of 3450 cm^{-1} (hydroxy group) and 1655 cm^{-1} (amide I). Figure 3 shows the decrease of amide I after hydrolysis as compared to the starting chitosan. This implied that the number of N-acetyl groups was reduced as a result from acid depolymerization. Figure 4 shows ^{1}H-NMR of chitosan after hydrolysis for 3 hrs. comparing to the starting chitosan. The methyl protons at 2.3 ppm and methine protons at 3.4 ppm were applied to calculated degree of deacetylation as shown in the equation (1). Figure 3 shows that the degree of deacetylation calculated from ^{1}H-NMR is increased to nearly 100% after 3 hours of the hydrolysis time

$$\text{Degree of Deacetylation (\%DD)} = [1 - \{I_{CH3} / (3 \times I_{CH} \text{ at } C_2)\}] \times 100\ \% \qquad (1)$$

Speculated Depolymerization Mechanism. It is important to note that ^{1}H-NMR clarified some minor changes in the structure as evidenced from the peaks at 2.26-2.28 ppm. Recently, Moo-Yeal Lee *et al.*[15] reported the result similar to ours, however, the structural characterizations were not detailed. In the past, Varum *et al*[18] proposed the depolymerization of chitosan using hydrochloric acid hydrolysis while the structure related to the mechanism was not reported. Here, we speculated the depolymerization based on ^{1}H-NMR as follows. Considering the mechanism proposed by Varum, the related structure to the proton peak at 2.24-2.28 cannot be

assigned. However, if the saccharide ring cleavage occurred at C_1-C_4 position, the species of the open ring structure would be obtained (Scheme II). The methine proton generated should give multiplet peaks which could be assigned to the multiplet peaks at 2.24-2.28.

Packing Structure. X-ray diffraction technique was used to confirm the change in packing structure of chitosan after acid hydrolysis. Generally, chitosan shows three major peaks at 9° , 20° , and 22°2θ . The crystallinity of chitosan is known to be based on the intermolecular and intramolecular hydrogen bondings. The former peak refers to the long d spacing which might reflect the intermolecular hydrogen bonding while the last two peaks might be based on the intramolecular hydrogen bonding. Figure 5 exhibits the changes in morphology after acid hydrolysis. The peak at 9°2θ decreases significantly while the peak at 20° and 22°2θ are slightly shifted but becomes sharp. This might be related to the fact that the acid hydrolysis loses the intermolecular hydrogen bonding network. The sharp peak at 20° and 22°2θ implies the significant intramolecular hydrogen bond in the short chain chitosan.

Thermal Stability and Solubility. The TGA study of chitosan shows the degradation temperature of chitosan at 311°C (Figure 6a). When hydrolysis time increased, the degradation temperature is decreased for more than 50°C (Figure 6b). This supports the changes in packing structure of low molecular weight chitosan especially the reduction of hydrogen bonding.

One of the most important points to concern for depolymerization is the improvement of solubility. Figure 7 shows that the solubility of the product obtained in acetic acid is increased when the hydrolysis time increased. This implies the improve in dissolvation between acetic acid and chitosan molecules when chitosan becomes low molecular weight. It should be noted that in the case of the hydrolysis time for 9 hours, the product was soluble in water. This informs that the water molecules can form hydrogen bond with chitosan at the level of 1.1×10^4.

Conclusions

Depolymerization of chitosan by hydrochloric acid hydrolysis was achieved for above 90% while the degree of deacetylation was increased upto nearly 100%. ^{1}H-NMR clarified the minor open ring structure of saccharide while the FTIR informed that the major structure of chitosan is maintained. WAXD patterns showed that the packing structure was drastically changed which could be referred to the decrease of intermolecular hydrogen bonding.

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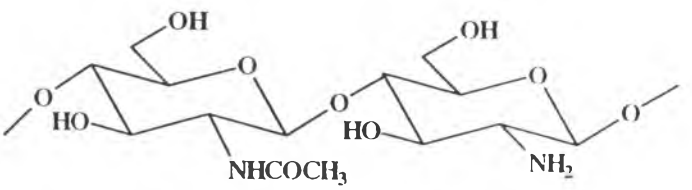
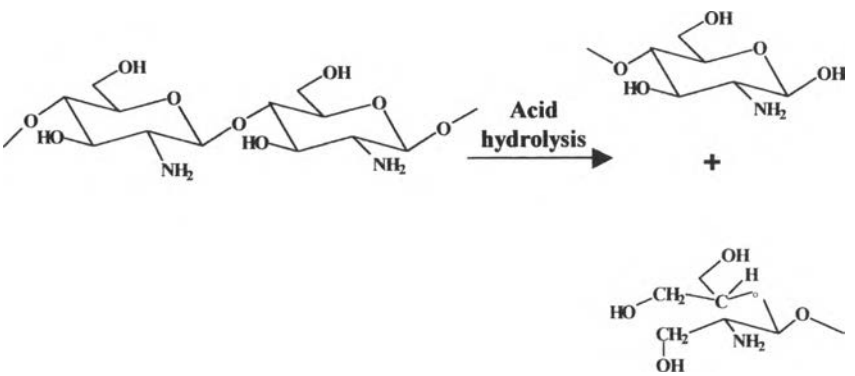


Chitin **Chitosan**

Scheme I. (Kosum et al.)



Scheme II. (Kosum et al.)

Figure Captions

Figure 1. Molecular weight of low molecular weight chitosan as a function of time.

Figure 2. FTIR spectra of: (a) chitosan and chitosan obtained after hydrolysis time for: (b) 3, (c) 6, (d) 9, and (e) 12 hrs.

Figure 3. Degree of deacetylation as a function of time determined by: (a) FTIR: integral ratio of amide I band (1655 cm^{-1}) and hydroxyl band (3450 cm^{-1}), and (b) ^{1}H-NMR.

Figure 4. ^{1}H-NMR of: (a) chitosan and (b) low molecular weight chitosan.

Figure 5. X-ray diffractograms of: (a) chitosan and chitosan obtained after hydrolysis time for: (b) 3, (c) 6, (d) 9, and (e) 12 hrs.

Figure 6. (a) TGA and DTA diagrams of chitosan, and (b) Degradation temperature of the products obtained from various hydrolysis times.

Figure 7. Solubility of low molecular weight chitosan in 1% acetic acid as a function of time.

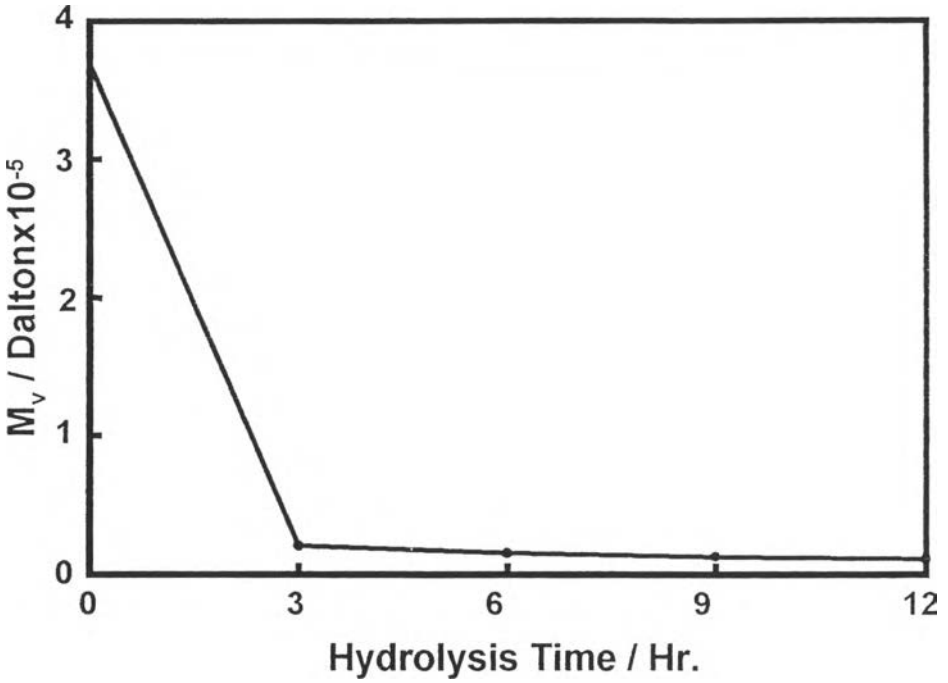


Figure 1. (Kosum et al.)

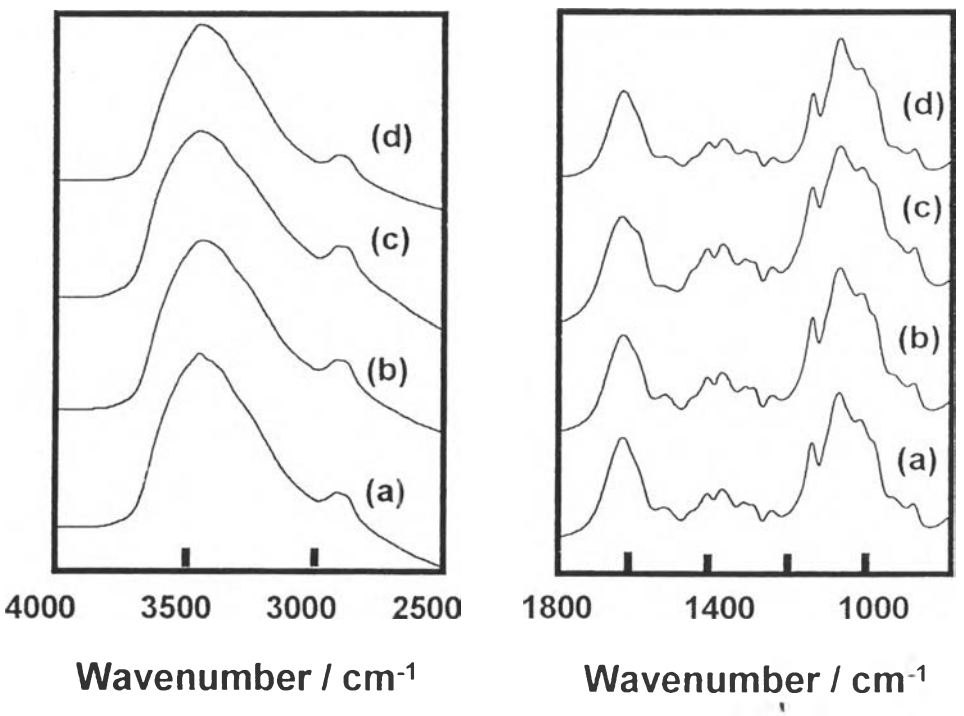


Figure 2. (Kosum et al.)

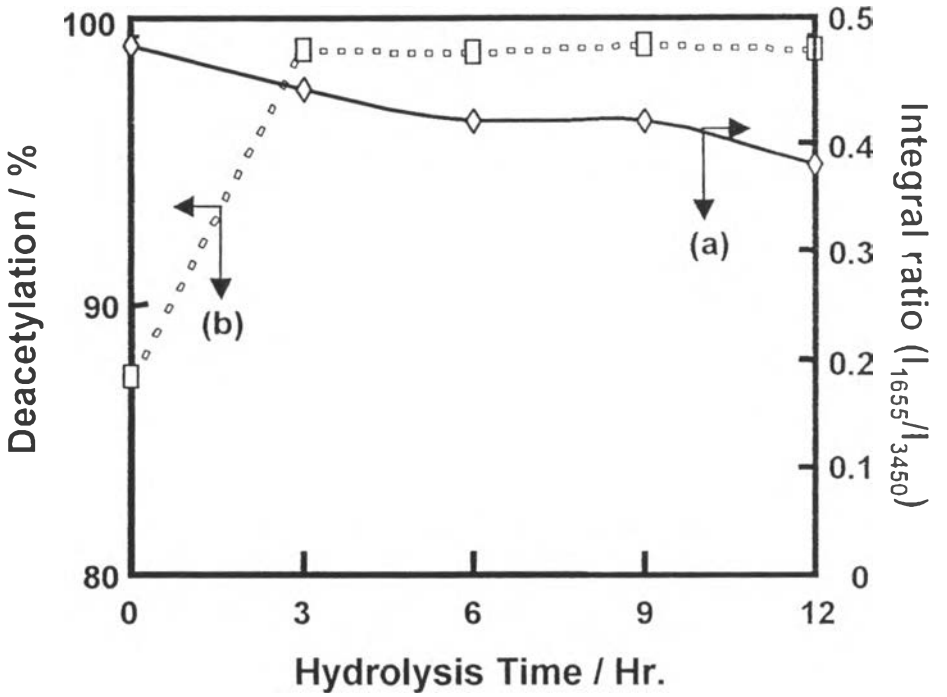


Figure 3. (Kosum et al.)

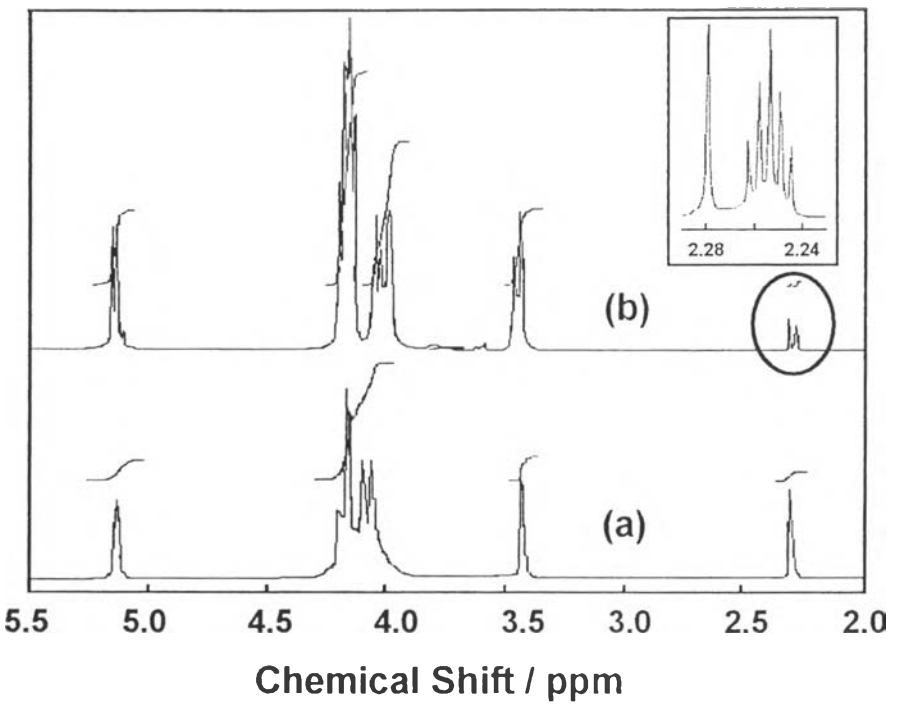


Figure 4 (Kosum et al.)

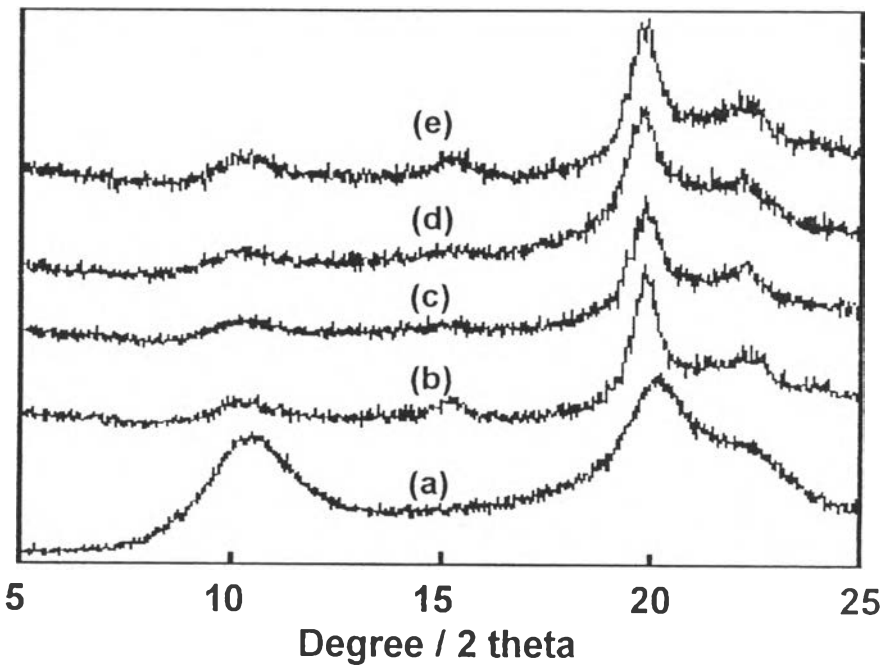


Figure 5. (Kosum et al.)

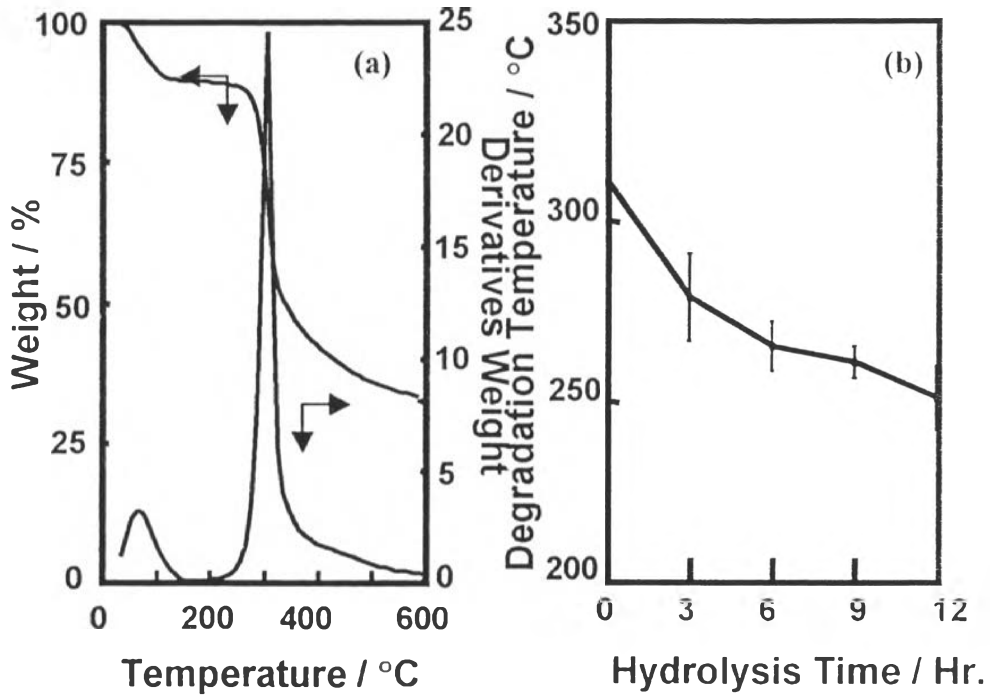


Figure 6. (Kosum et al.)

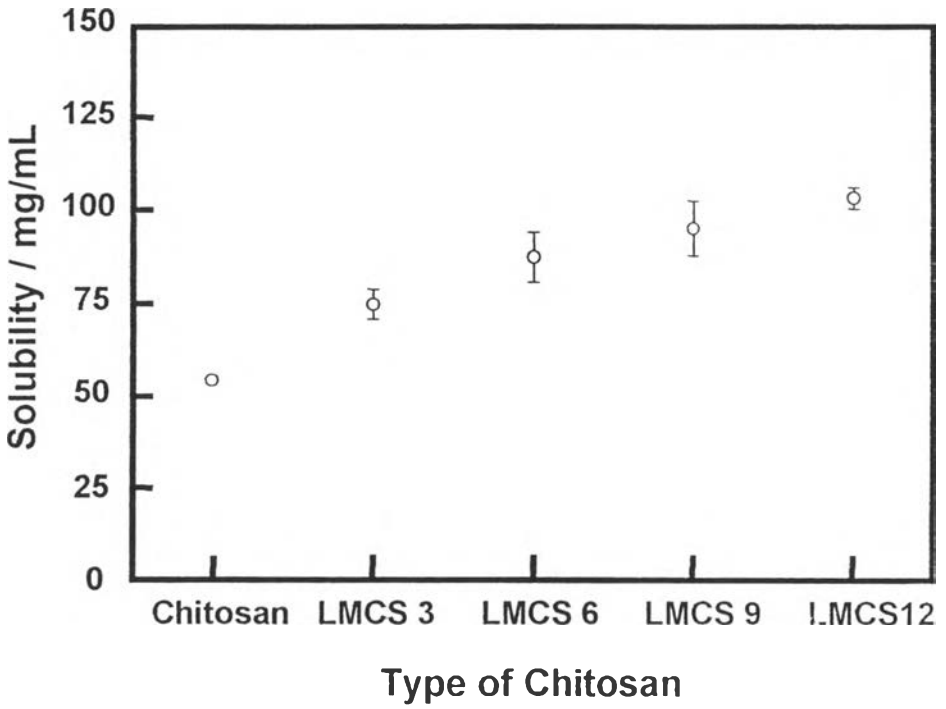


Figure 7. (Kosum et al.)