Multistage Sonication Effect on the Composition and Properties of Degraded Chitosan Product

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Abstract

The sonication method randomly breaks polymer chains to produce a varied distribution of products. A scheme with multistage sonication steps is proposed to identify oligomers and low molecular weight chitosan based on changes to the degraded product. For each sonication level, 1% (w/v) chitosan in 1% (v/v) aqueous acetic acid was sonicated for 120 min at 60 °C. The products of the sonication treatment were deprotonized by adding an alkali solution, freeze dried and insoluble product re-sonicated until a low molecular weight was produced. Low molecular weight chitosan/insoluble products were characterized with Fourier-transform infrared spectra (FT-IR) and X-ray diffraction (XRD) to determine the effect of multistage sonication on degree of deacetylation (DD) and degree of crystallinity. The viscosity of the average molecular weight of insoluble chitosan was estimated by a viscometric method, while the degree of polymerization (DP) of the chitosan oligomers (COS) were determined by end group analysis. The results showed that the molecular weight of insoluble chitosan decreased and reached a limiting value, (M_{lim}). The percent yield of oligoglucosamine of the soluble products increased with the number of the sonication stages. The degree of crystallinity of insoluble chitosan increased from 19% to 34.14% while the DD decreased from 82% to 78.1%.

Abstract

Pengaruh Proses Sonikasi Bertahap terhadap Komposisi dan Sifat Material dari Produk Kitosan Terdegradasi. Metode sonikasi memiliki keterbatasan yaitu terjadinya pemutusan rantai secara random yang menghasilkan banyak variasi distribusi produk. Tujuan dilakukan proses sonikasi adalah untuk memperoleh oligomer kitosan dan kitosan berat molekul rendah yang telah berubah sifat propertiesnya di setiap tahapan sonikasi. Setiap level, kitosan 1% (w/v) dalam 1% (v/v) larutan asam asetat disonikasi selama 120 min pada 60 °C. produk dari proses diprotonasi dengan menambahkan larutan basa, dilakukan *freeze dried* dan produk yang tidak terlarut disonikasi kembali hingga memperoleh berat molekul yang konstan. Struktur rantai dari kitosan berat molekul rendah dianalisis dengan Fourier-transform infrared spectra (FT-IR) dan X-ray diffraction (XRD), untuk mengetahui pengaruh sonikasi bertahap terhadap degree of deacetylation (DD) dan derajat kristalinitas. Viskositas berat molekul rata-rata dari kitosan tidak terlarut diukur dengan metode viscometric sedangkan untuk Chitosan oligomers (COS) ditentukan melalui *end group analysis*. Hasil pengukuran menunjukkan bahwa berat molekul dari kitosan tidak terlarut berkurang nilainya dan menghasilkan nilai konstan (M_{lim}). Persen yield oligoglucosamine dari produk yang terlarut meningkat dengan bertambahnya jumlah dari tahapan sonikasi. Derajat kristalinitas dari kitosan yang tidak terlarut meningkat dari 19% menjadi 34%, sedangkan derajat deasitilasi menurun dari 82% menjadi 78.1%.

Keywords: chitosan, degraded product, multistage sonication

1. Introduction

Chitosan is a copolymer composed of N-acetyl-d-glucosamine and d-glucosamine units [1]. Chitosan obtained by deacetylation of chitin has a high molecular weight and low solubility in aqueous solvents, which limits its applications in various fields [2]. Thus, the synthesis of chitosan derivatives has recently been of great interest with more specific applications in waste-water treatment, the food industry [3], gel implants [4], and drug delivery systems [5].

Sonication systems have been used to synthesize chitosan derivatives. Upon collapse, the cavitation bubble releases its stored energy, which passes through the surrounding medium like a shock wave that is strong enough to degrade polymers by rupturing bonds in the main chain. The formation of atoms and/or radicals due to the high temperatures and pressures during adiabatic bubble compression can be used to cut the glycosidic bond of chitosan.

However, sonication systems have their limitations [6], particularly the limiting molecular weight (M_{lim}) of degradation products. M_{lim} is a unique property that is highly dependent on the experimental conditions and technique used. Non-random scission events occur near the center of the polymer chain since random scission would produce a much wider distribution of products [7].

In this research, a multistage sonication process aims to classify products from the degradation process. A scheme can then be made from the degradation product. A scheme of multistage sonication was based on the polymer molecular weight, DD, degree of crystallinity and percent yield oligoglucosamine at every stage of sonication.

2. Experimental

Materials. Chitosan was made from a crab shell with a molecular weight (Mw) = 3700 kDa and deacetylation degree (DD) = 87%. The chitosan was a production of Biotech Surindo-Cirebon, Indonesia. Ethanol, acetic acid, sodium hydroxide, potassium ferricyanide, and sodium cromat were produced by Merck. purified by flash column chromatography on silica gel to yield **CYT** with 95% yield.

Experimental. At each level, 1% (w/v) chitosan in 1% (v/v) aqueous acetic acid was sonicated for 120 min at 60 °C at 20 kHz. The products of the sonication treatment were deprotonized by adding an alkali solution. The composition of degraded products was divided into insoluble and soluble chitosan. Insoluble products were freeze dried and re-sonicated until the lowest molecular weight was attained. The schema of the experiment is shown in Figure 1.

The experiments were conducted using an ultrasonic source (VCx-500, Sonics, and Materials, 500W). The probe tip diameter was 19 mm with 50% amplitude. The temperature was monitored with a thermocouple as in Figure 2.

The intrinsic viscosity of the polymer [8]**.** The intrinsic viscosity of a polymer solution is related to the polymer molecular weight (M). The polymer molecular weight is calculated using Equation (1) from the Mark–Houwink–Sakurada (MHS) equation.
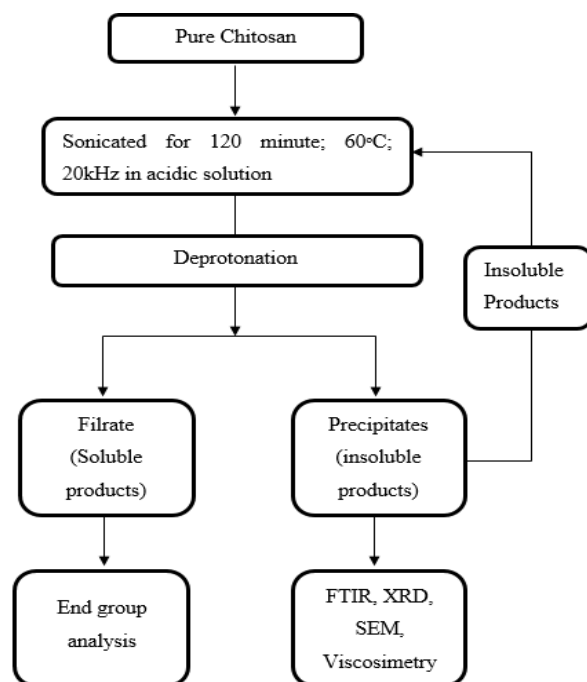
$$[\eta] = k.M^{\alpha} \tag{1}$$
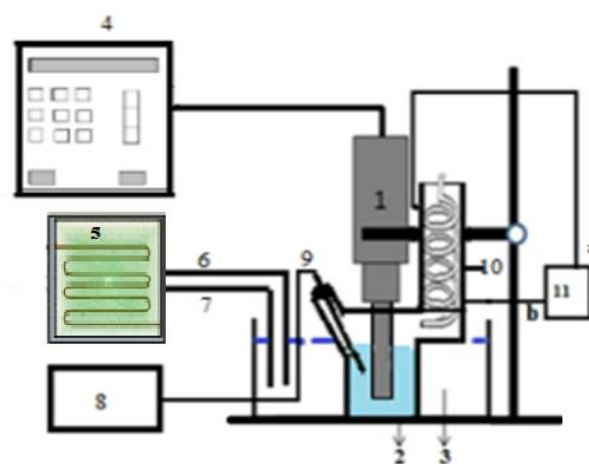

Figure 1. The Experimental Flow Chart

Figure 2. Experimental Set Up for Sonication: 1. Ultrasonic probe, 2. Stainless steel reactor, 3. Cooling water, 4. Probe system display, 5. Chiller, 6. Water inlet into chiller, 7. Water outlet from chiller, 8. Data taker, 9. Thermocouple, 10. Condenser, 11. Water inlet (a) and water outlet (b)

Chitosan samples were prepared in 1% HAc (acetic acid) at 30 °C, with:

k = 0,0474 mL/gr
α = 0,723

End group analysis. The molecular weight of chitosan oligomer was calculated using an end group analysis

method [9]. A total of 0.75 mg/mL potassium ferricyanide was dissolved in 0.3 mol/L Na_2CO_3 as a color-producing reagent and 10.0 mg/mL of a D-glucosamine-hydrochloride (GAH) solution was used as a standard solution. Different concentrations of the GAH solution were prepared by diluting with distilled water, then the solutions were reacted with potassium ferricyanide in a boiling water bath for 15 min. The absorbance of color reducing reactions at 420 nm was recorded to obtain the standard curve of absorbance and concentration of GAH. The concentration of GAH of oligoglucosamine were calculated by Equation (2):

$$A = XW + C \qquad (2)$$

where:
A is the absorbance value.
W is the amount of GAH (gram).
Thus, the percent yield of chitosan oligomer was calculated by Equation (3):

$$\% \text{ Yield} = \frac{W_a}{W_b} \text{ x100\%} \qquad (3)$$

where:
Wa   = the weight of chitosan oligomer (gram)
Wb   = the weight of initial chitosan (gram)

The degree of deacetylation. The deacetylation degree (DD) of the products was calculated by Equation (4):

$$DD = 100 - \left[\frac{1 - A_{1655}}{A_{3450}} \times \frac{100}{1.33} \right] \qquad (4)$$

where:
A_{1655} was the absorption peak of amide at the wavelength of 1655 cm^{-1}.
A_{3450} was the absorption peak of NH at the wavelength of 3450 cm^{-1}.

Degree of crystallinity. XRD measurements were carried out on a PANalytical PW 3373/00 X'Pert X-ray diffractometer at a Cu Kα ray (λ=1.54 Å) in the range $10°{\sim}90°$ 2θ at a scan rate of $2°$ 2θ per minute. The spectra were recorded at 40 kV-30 mA.

3. Results and Discussion

Effects of multistage sonication on the molecular weight of water-insoluble chitosan. The effects of the multistage sonication on insoluble product are shown in Figure 3. The molecular weight chitosans decreased rapidly during the first sonication stage, then slowed during resonication. The molecular weight of pure chitosan decreased from 3700 kDa to 248 kDa when chitosan in 1% (v/v) aqueous acetic acid was sonicated for 120 min at 60 °C at 20 kHz (Stage 1). The molecular weight of insoluble chitosan decreased at a slower rate to 242 kDa, 233 kDa, and 216 kDa after chitosan was re-sonicated at the same condition in Stages 2, 3, and 4. Then, the molecular weight in Stage 5 decreased to 115 kDa but increased to

138 kDa in Stage 6. Thus, in ultrasonic degradation, the degradation proceeds more effectively at high molecular weight with the degradation rates decreasing with decreasing molecular weight. No further degradation occurs when the molecular weight is below a limiting value, M_{lim} [10]. In this process, the limiting value was approximately 115 kDa.

Effects of multistage sonication on the number molecular weight of chitosan oligomers (COS) The end group analysis used a color-producing reagent. The hemiacetal hydroxyl of the reductive sugar can change color during the reaction with basic potassium ferricyamide. As both GAH and oligoglucosamine have hemiacetal hydroxyls in their molecule, they can be colored by basic potassium ferricyamide [9]. The regressive equation was A=379.1W +0.575, where A is the absorbance value, W is the amount of GAH (g), and the correlation accuracy (r) of this regressive equation is 0.999.

Table 1 shows that the percent yield oligoglucosamine of the soluble products increased with the number of sonication stages. The process of degradation by resonication would be faster because chitosan had been depolymerized, which is similar to longer exposure. Thus, the degradation also increased with longer sonication [11].

Effects of the multistage sonication on the ß-1,4-glycoside linkage. Chitosan has a characteristic IR spectrum. The 3500~3200 cm^{-1} peak is O-H, N-H stretching; 2874~2878 cm^{-1} is C-H stretching; 1639~1663 cm^{-1} is the amide I band; 1591~1597 cm^{-1} is the N-H bending of NH_2; 896 cm^{-1} is the glucose ring stretching; 1154~1156 cm^{-1} is asymmetric bridge oxygen (C-O-C) stretching and 1623 cm^{-1} is C=O [12],[13].
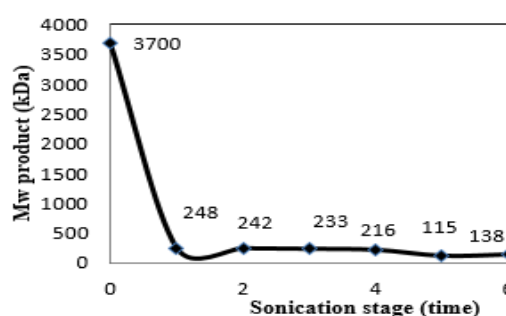

Figure 3. Average Molecular Weight of Chitosan in Every Degradation Product

Table 1. Number Molecular Weight of Soluble Chitosan at Every Stage

Stage (time)	%yield oligoglucosamine (%)
1	0.247
2	4.183
3	4.240

4	4.369
5	4.825

The characteristic IR spectrum of the glucose ring can be observed in Figure 4. The spectrum is valued at 896 cm^{-1} (on pure chitosan), indicating the presence of ß-1,4-glycoside linkage. Then, it disappears and changes the value to 895 cm^{-1} (stage 1,2,4,5) and 894 cm^{-1} (stage 3). According to Min Larng Tsaih and Rong Huei Chen [14], the energy of cavitation may preferentially react at the ß-1,4-glycosidelinkage.

Effects of the multistage sonication on the degree deacetylation of water-insoluble chitosan. In pure chitosan, the value of the DD was 87%, while the results of degradation products decreased, though not significantly, at every stage of sonication. The DD values at Stage 1, 2, 3, 4, and 5 of 86.59%, 85.06%, 79.87%, 79.49%, and 78.1% decreased 0.02%, 1.78%, 7.77%, 8.22%, and 9.33% from the initial chitosan as Figure 5.

Insignificant reduction of the N-acetyl group occurred because of degradation in the acidic reaction system. The amino groups on C-2 of chitosan facilitated a site-specific fragmentation of the glycosidic linkages while the N-acetyl group slowed the rearrangement of radicals during ß-cleavage.

Figure 6 is the XRD spectra of pure chitosan, water-insoluble product from sonication Stage 1, water-insoluble product after resonication from product Stage 1 (i.e., Stage 2), Stage 3 until Stage 5.

Figure 6 shows that broad diffraction peaks at $2\theta = 20°$ symbolized semi-crystalline pure chitosan. Generally, chitosan has two types of crystals. The form I crystal is orthorhombic with a unit cell of a = 7.76, b = 10.91, and c = 10.30 Å. The strongest reflection appears at $2\theta = 11.4°$, which is assigned to the (100) reflection. The form II crystal is also orthorhombic with a unit cell of a = 4.4, b = 10.0, and c = 10.3 Å (fiber axis). The strongest reflection appears at $2\theta = 20.1°$, which corresponds to the (100) reflection [12]. In this case, pure chitosan is like form II.
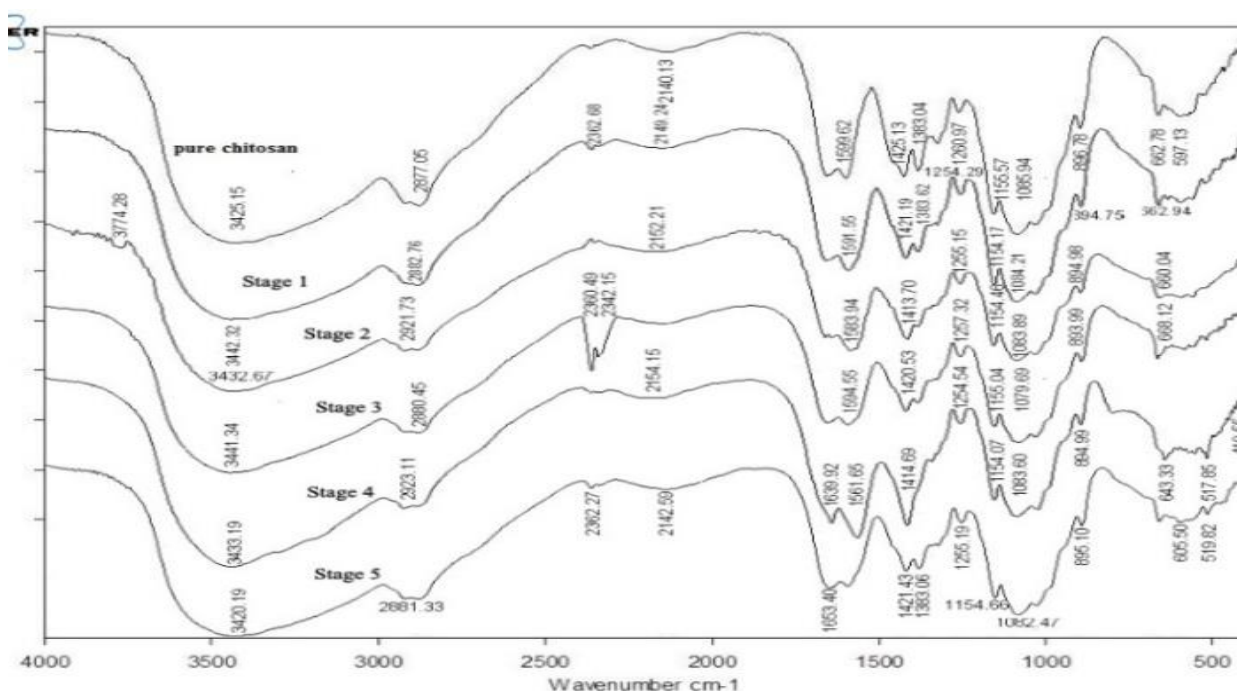

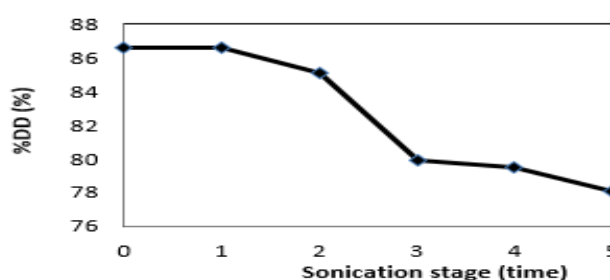**Figure 4. IR Spectra Water-Insoluble Chitosan at Various Stages**

Figure 5. Degree of Deacetylation of Chitosan After Sonication at Various Stages of Sonication
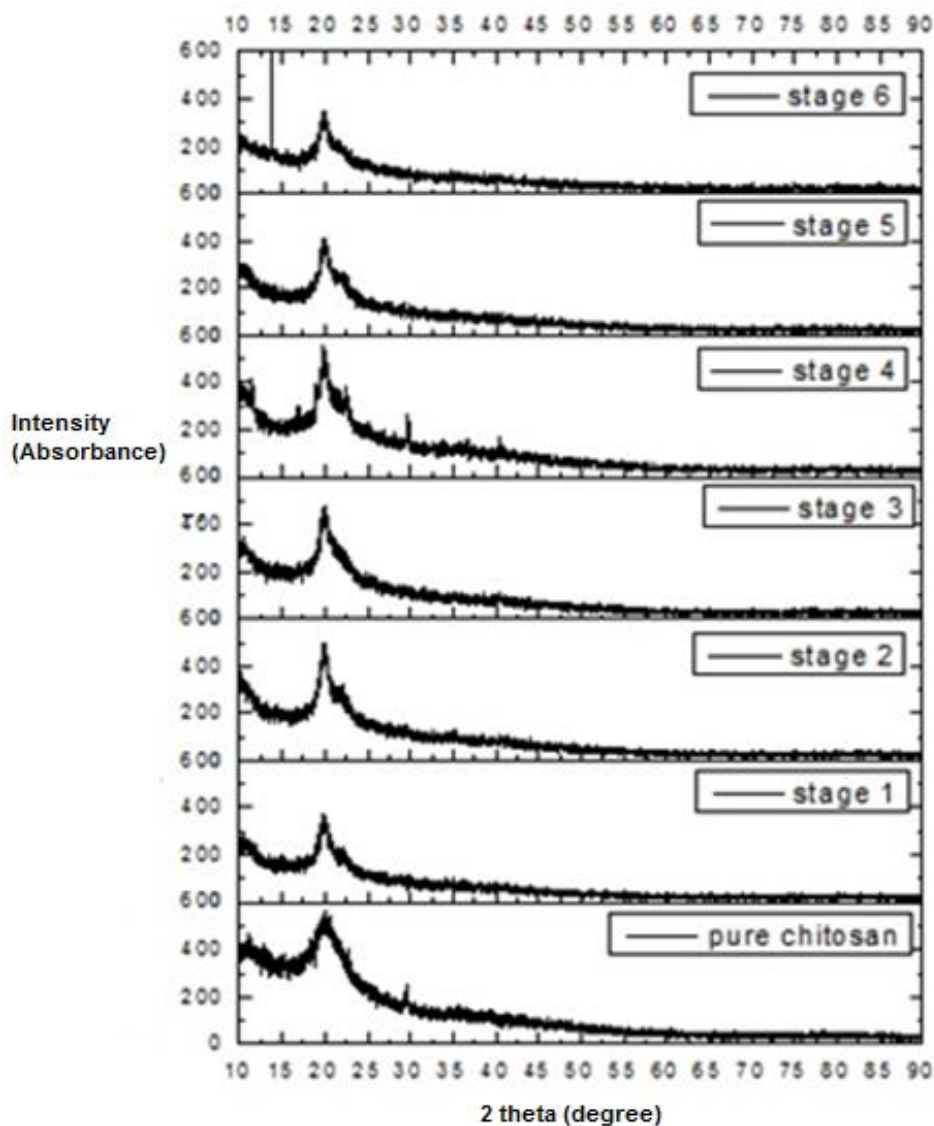

Figure 6. XRD Spectra of Water-Insoluble Chitosan at Various Stages

The degree of crystallinity of pure chitosan was 19.53%. The degree of crystallinity of degraded product increased more than pure chitosan. In insoluble chitosan, the degree of crystallinity of the degraded produce was Stage 1: 20.2%; Stage 2: 30.2%; Stage 3: 30.6%; Stage 4: 34.14%; Stage 5: 32.65%; and Stage 6: 30.25%. The amorphous part of chitosan was preferentially degraded and the crystal part was temporally maintained. Therefore, the degradation products were part of the crystalline phase. The improvement in crystallinity at each stage was also impacted by the decrease in DD as shown in Figure 5. At lower DDs, more acetyl groups remained attached to the

amino groups on the C2 position of the glucosamine residue.

Their carbonyl groups could contribute to the formation of intermolecular and intramolecular hydrogen bonds. Not only did they make the chitosan more rigid and extended, but they also facilitated the formation of a liquid-crystalline mesophase [15].

Effect of the Morphology of Water-Insoluble Chitosan.
Chitosan has a inhomogeneous and non-smooth surface with straps and shrinkage. As shown in Figure 7, the surface structure of the product became torn with more

sonication stages due to the effects of cavitation during the sonication process.

(a)

(b)

(c)

(d)

(e)
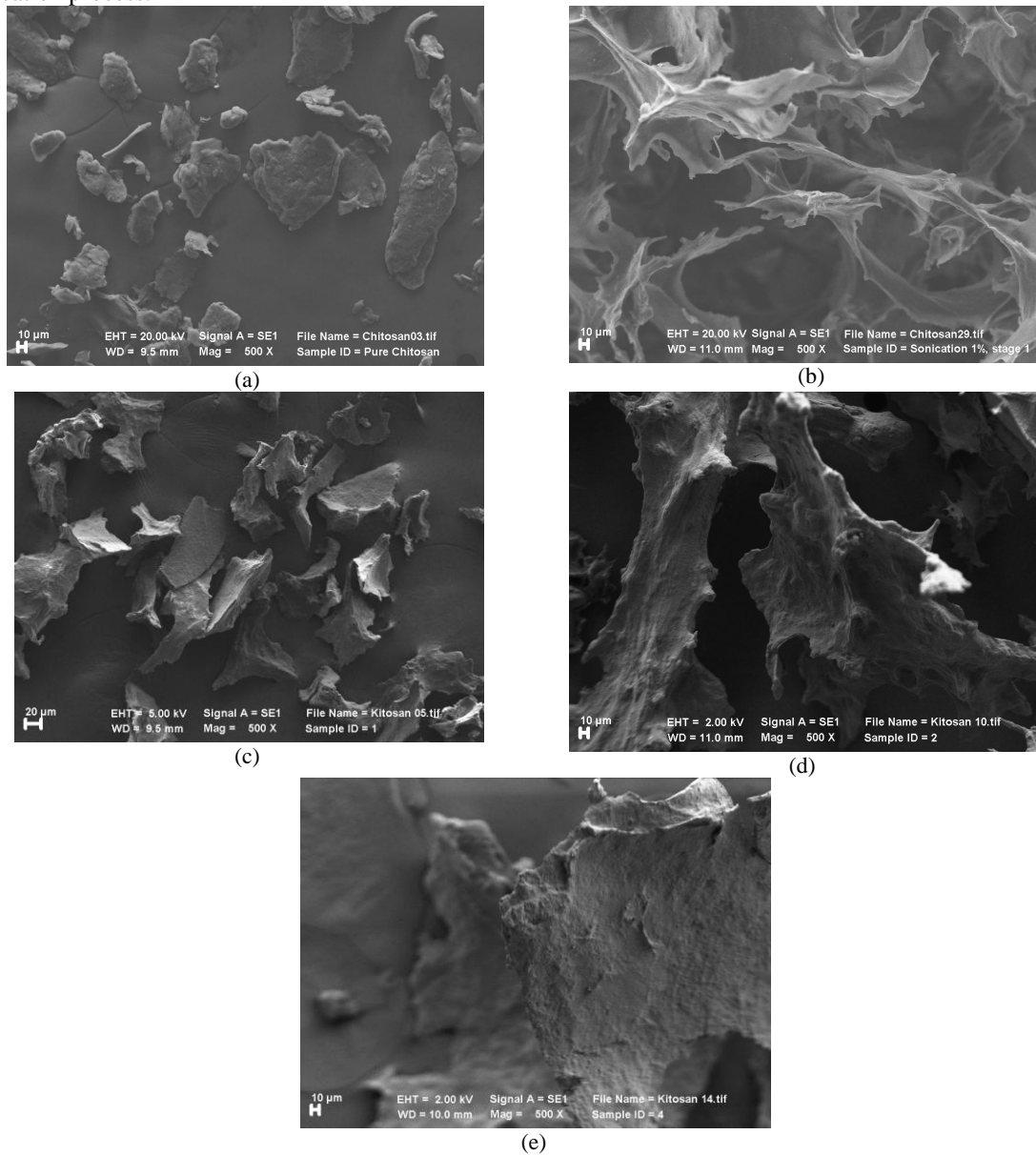
Figure 7. SEM Analysis Results of (a) Pure Chitosan, (b) Sonication at Stage 1, (c) Sonication at Stage 2, (d) Sonication at Stage 3, (e) Sonication at Stage 4, and (f) Sonication at Stage 5

4. Conclusions

The multistage sonication of chitosan in acidic conditions is an interesting process to produce oligomers and low molecular weight chitosan. The composition of degraded products was divided into insoluble and soluble chitosan. The results showed that the molecular weight of insoluble chitosan decreased and reached a limiting value, (M_{lim}). The percent yield of oligoglucosamine of the soluble products increased with the number of sonication stages. The degree of crystallinity of insoluble chitosan increased from 19% to 34.14%, while the DD decreased from 82% to 78%.

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