Effect of milling time on microstructures of nano-sized chitosan

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Abstract. Effect of milling time on the microstructures of nano-sized chitosan was investigate as the objective of this work. Variations of milling times were used 0, 120 and 180 minutes as indicated by CH0, CH120 and CH180. For as-prepared chitosan is CH0. The FTIR results showed that there are no clearly different of the function groups after ball milling treatment. The average crystallite size results of nano-sized chitosan are 20 nm. The milling process can decrease crystalline size, but significantly to increase the Polydispersity Index. The FTIR and XRD results showed the crystalline size of nano-sized chitosan changed on the milling time but did not change the chemical structure. It could be concluded that the milling time can be applied to the synthesis the nano-sized chitosan and to reduce the particle size. It also confirms that the ball mill treatment did not automatically alter the structural properties of nano-sized chitosan.

1. Introduction
Chitosan \([\text{C}_6\text{H}_11\text{NO}_4]\)n is an organic biopolymer, which has the properties of non-toxic, biocompatible, biodegradable, biofunction and hydrophilic. Chitosan consists of amine and hydroxyl [1-3]. Synthesis of nanoparticles chitosan widely attract more attention because of their nano-sized particle and small specific surface area to physical modifications, including radiation, electrical treatment and mechanical milling [4].

One kind of mechanical milling is ball mill treatment that has been proved to be an efficient to alter the particle size and crystallinity degree of several materials [5-6]. Ball mill is called high-energy milling (HEM) because of the tremendous energy dissipated in the process of collision and friction of balls with the chamber wall. It is used to get the nano-sized particles such as starch, cellulose, and chitosan [7-9]. The particle size of chitosan nano-sized powder is widely used for further research.

Milling time can reduce the crystallinity and increase the absorption of the powder produced. Because of friction, impact occurred on the jar causes the modification of the structure and properties of grain powder[10]. The milling method is a method for reducing chitosan the particle size,
physicochemical properties and structural that will change during the milling time and effect of nano-sized chitosan performance [11-12]. Milling can reduce the relative crystallinity of the powder granules which cause the phase change from polycrystalline to amorphous [13]. The length of the milling time will affect the viscosity and absorption of powder [14].

From the report of the previous researches that had been done, there are no research that reports the methods of preparation of as-prepared chitosan that lead to nanometer scale by ball milling treatment with various milling time. Some efforts for the development of as-prepared chitosan were applied by the mechanical treatment to get smaller particles, to reduce grain size and to modify the surface of chitosan through ball milling. The synthesis of nano-sized chitosan using the ball milling treatment might offer the new possibilities for the application of nano-sized chitosan because of simple and low cost. Use of ball milling treatment to prepare the nano-sized chitosan is still done with several challenges, such as the large average size range of the as-prepared chitosan. In this work, synthesis of nano-sized chitosan by ball milling treatment aims to explore the effect of milling times on the microstructures of the nano-sized chitosan.

2. Experimental
2.1. Preparation of the nano-sized chitosan
The as-prepared chitosan (CH0) was purchased from Biotech Surindo-Indonesia with a deacetylation degree (DD) about 85%. The chitosan milling results were indicated by CH60, CH120 and CH180 for milling time 60, 120 and 180 minutes, respectively. A high-energy milling machine 8000M SPEX Certiprep Mixer/Mill here was used and the jars and the milling medium were made of stainless steel. The diameter of small ball is 0.5 mm. The time for grinding operations were 60, 120 and 180 minutes. After milling, the dried samples were ground for 10 minutes and then characterized. The samples of nano-sized chitosan with milling time variations were sealed and used to the next analysis.

2.2. FTIR spectra analysis
Fourier Transform Infrared (FTIR) spectra were recorded on Shimadzu 8201 PC FTIR spectrophotometer and recorded with 1 cm⁻¹ resolution with the wavenumber from 400 to 4000 cm⁻¹. Samples were blended as KBr pellet and scanned into a blank KBr pellets before measurement.

2.3. XRD pattern analysis
The XRD patterns were performed using XRD type Rigaku 2500 V, the wavenumber of Cu-K is 1.54060 Å, 0.3 mm receiving slit and were recorded in the range of 10 – 800 at the speed of 0.0020/min.

3. Results and Discussion
The FTIR spectra of as-prepared (CH0) and nano-sized chitosan with different milling time (CH60, CH120 and CH180) were shown in Fig.1.
Fig. 1. FTIR spectra of CH0, CH60, CH120 and CH180 in the region 4000-400 cm\(^{-1}\)

For more detail about FTIR spectra for CH0, CH60, CH120 and CH180 are as shown. The absorption peaks at 3371 cm\(^{-1}\), 3417 cm\(^{-1}\), 3750-3426 cm\(^{-1}\) and 3379 cm\(^{-1}\) which can be attributed to the O-H stretching vibration. The absorption peaks at wavenumbers of 2877 - 2924 cm\(^{-1}\) and 2877 cm\(^{-1}\) are due to the methylene and methyl group of stretching vibrations. At the 1381 cm\(^{-1}\) attributed to the C-H bending. Broad absorption showed intramolecular hydrogen bonds in the structure were strong. It showed that a more amorphous phase and a disordered structure were resulted after the ball milling process. The absorption at 1651 and 1072 cm\(^{-1}\) respectively were related the C = O stretching, and C - O bending vibrations. Absorption wavenumber peaks of 1420 cm\(^{-1}\) and 1381 cm\(^{-1}\) indicated the C - N stretching which showed the acetyl group. The absorption wavenumber of 1072 cm\(^{-1}\) indicated the CN(H\(_2\)) stretching because of the amine groups was formed and 1034 cm\(^{-1}\) indicated the stretching symmetric of C – O - C group.

XRD patterns of CH0, CH60, CH120 and CH180 are shown in Fig. 2. The XRD phases of the CH0, CH60, CH120 and CH360 were indentified with the JCPDS Card No. 39-1894. The CH0 peaks were at about 20.60\(^{\circ}\) and 10.79\(^{\circ}\) due the 001, 100 and 101, respectively. The broad peak at the 10.79\(^{\circ}\) confirm the amorphous phase and the sharp peak at the 20.60\(^{\circ}\) confirms the crystalline phase formed due to intra molecular hydrogen bonds of the nano-sized chitosan. The XRD patterns of nano-sized chitosan differ the intensity and diffraction angles. The crystal lattice size and crystalline degree are (20.75 ± 9.21) nm and 49\%. Polydispersity index is 44.39\%. The peaks of CH60 are at about 20.60\(^{\circ}\) and 10.79\(^{\circ}\). The crystal lattice size and crystalline degree are (20.09 ± 7.63) nm and 36\%. Polydispersity Index is 36\%. XRD pattern of CH120 peaks are at about 20.04\(^{\circ}\). The crystal lattice size and crystalline degree are (20.21 ± 8.47) nm and 49\%. Polydispersity Index is 42\%. The peak of CH180 is at about 20.17\(^{\circ}\). The crystal lattice size and crystalline degree are (20.22 ± 9.51) nm and 47\%. Polydispersity index is 47\%. The results indicate that the crystallities are highly agglomerated in the milled powders and relatively dispersed.
4. Conclusion
The ball milling method was an method to synthesis nano-sized chitosan and can be applied to reduce the crystallite size but did not change to the functional group properties. the crystalline structures of nano-sized chitosan were destroyed. The dispersity of nano-sized chitosan increases but particle size decreases which affect its application and deserve to be further researched.

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