

Synthesis of Nano Chitosan as Carrier Material of Cinnamon's Active Component

by Nor Basid Adiwibawa Prasetya

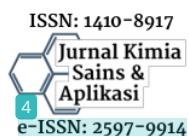
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Journal of Scientific and Applied ChemistryJournal homepage: <http://ejournal.undip.ac.id/index.php/ksa>**Synthesis of Nano Chitosan as Carrier Material of Cinnamon's Active Component**Ngadiwiyana^{a*}, Enny Fachriyah^a, Purbowatiningrum Ria Sarjono^a, Nor Basid Adiwibawa Prasetya^a, Ismiyarto^a, Agus Subagio^b^a Chemistry Department, Faculty of Sciences and Mathematics, Diponegoro University, Jalan Prof. Soedarto, Tembalang, Semarang
^b Physics Department, Faculty of Sciences and Mathematics, Diponegoro University, Jalan Prof. Soedarto, Tembalang, Semarang* Corresponding author: ngadiwiyana@live.undip.ac.id

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Abstract

Development and innovation to improve the efficacy of active ingredients of a plant can be done by using nanoparticle encapsulation of chitosan, which has dual function of protecting natural extracts degradation and delivering natural extracts to the target site. Chitosan is a natural polymer that is nontoxic, mucoadhesive, biodegradable, and biocompatible. This polymer also has a low level of immunogenicity and can be prepared into nanoparticles in mild conditions that make it suitable for natural extracts delivery systems. This paper reported synthesis of chitosan nanoparticles for cinnamon's natural extract delivery. Chitosan synthesis was carried out by chitin deacetylation isolated from shrimp shells. Chitosan characterization was done by measuring deacetylation degree by FTIR. Chitosan nanoparticles were prepared by ionic gelation method using tripolyphosphate as crosslinker. Morphology and particle size of nano chitosan were characterized using a scanning electron microscope (SEM) and a transmission electron microscope (TEM). The result found that the yield of deproteinated chitin was 62.60%. Further process of demineralization resulted a yield of 52.60%, then depigmentation with a yield of 75.56%, and deacetylation with a yield of 79.02%. FTIR analysis showed that deacetylation degree of chitin into chitosan was found of 87.78%. Characterization by SEM found that nano chitosan has a particle size of 87 nm. While TEM images showed that the nano chitosan has a uniform shape and a lower physical aggregation.

1. Introduction

The applications of natural extracts still showed less optimal results due to the lack of efficiency of absorption by the body. It is because of the relatively large particle size, low solubility and the possibility of degradation/damage during the absorption process. Those facts lead to the need to conduct modification of natural extracts to optimize their usefulness. This research will attempt to combine chitosan with natural extracts by encapsulation of this compound within nanoparticles which has function to protect degradation and facilitate the delivery of natural extracts to the target site [1]. In this study, chitosan play a role as the nanoparticles which developed as delivery systems of natural extracts. This preference is based on the abundance source of chitosan from shrimp shells [2-5]. Chitosan is a natural polymer which has characteristics

such as nontoxic, mucoadhesive, biodegradable, biocompatible, a low level of immunogenicity and can be prepared into nanoparticles in mild conditions. Therefore, it is suitable for delivery systems of natural extracts [6, 7]. Another advantage of using nanochitosan is this compound can serve as antihypercholesterol so that the combination of nano chitosan-natural extract is expected to have a synergistic effect that is reinforcing mutually.

The synthesis of chitosan can be carried out by deacetylation of chitin isolated from shrimp shells. Chitosan characterization can be performed by measuring the degree of deacetylation by using FTIR [8]. Chitosan nano particles can be prepared by ionic gelation method using tripolyphosphate as crosslinker. Obtaining nano-chitosan with dispersity level and good stability can be influenced by the preparation conditions

such as concentration of chitosan and tripolyphosphate and the volume ratio of chitosan-tripolyphosphate [9, 10].

The encapsulation process of natural extracts in nanoparticles of chitosan was conducted by inclusion method. Nanoparticles of chitosan-natural extracts being tested to check their physical, chemical, and biological properties including the morphology, particle size, zeta potential, and stability in vitro release profiles cinnamon extract. The morphology of natural extracts nanoparticle is characterized by a scanning electron microscope (SEM) to observe the surface structure of nanoparticles. The levels of natural extracts in the supernatant is then analyzed using Fourier Transformed Infrared (FTIR) to determine the complexation reaction between the extract and chitosan nanoparticle[7].

2. Methods

Equipments

Equipments used were laboratory glasswares, furnace, oven, hot plate, magnetic stirrer, FTIR, Scanning Electron Microscope (SEM) and a transmission electron microscope (TEM).

Materials

The materials used in this study were shrimp shells, acetic acid, ammonia, HCl, NaOH, distilled water, and TPP (tripolyphosphate).

Chitin isolation from shrimp shells

Isolation of chitin from shrimp shells was carried out using NaOH solution. At first, shrimp shells was grinded to obtain a size of 120 mesh. Shrimp shell powder was added into 3.5% NaOH solution, then mixture was heated under stirred condition at 65 °C for 2 hours. The mixture was filtered and residue was washed with distilled water until neutral. The residue was then suspended into HCl and the mixture was heated under stirred at 65 °C for 2 hours. The mixture was filtered and the residue was washed with distilled water until neutral. The residue was then soaked in 20 mL of 3% H₂O₂ for 24 hours and it was repeated. The mixture was filtered and the residue was washed with distilled water and it was then dried at 80 °C in oven for 8 hours to obtain dried chitin. The chitin powder was characterized using FTIR spectrophotometer with a baseline method to determine Degrees of Deacetylation (DD).

Chitosan synthesis

Synthesis of Chitosan was done by deacetylating chitin. Chitin powder was soaked in 600 mL of 50% NaOH (1:20 w/v) and it was then stirred and heated to a temperature of 120°C for 4 hours. Residue and filtrate is separated by filtration and then the residue was neutralized by washing with distilled water until neutral pH. The residue was dried in an oven at 80°C for 24 hours. Chitosan powder was characterized using FTIR spectrophotometer to determine Degrees of Deacetylation (DD).

Nanochitosan modification

Modification of nanochitosan was carried out by ionic gelation method. At first, chitosan was dissolved in acetic acid solution in various concentrations. Afterwards, it was stirred until homogeneous. Then a solution of sodium tripolyphosphate was added slowly. The sample was then observed visually to obtain solution, aggregate and suspension. Opalescent suspension zone was then studied as a condition of making chitosan nanoparticles. In this research, influence of condition and treatment of reaction to morphological structure and size of nanoparticles of chitosan was also studied.

3. Results and Discussion

Isolation Chitosan from Shrimp Shells

Isolation of chitosan from shrimp shells was obtained with the results shown in Table 1.

Table 1. The yield of each step in chitosan isolation

Processes	Sample mass (gram)	Result mass (gram)	Yield (%)
Deproteination	25.00	15.65	62.60
Demineralization	5.00	2.63	52.60
Depigmentation	2.50	1.89	75.56
Deacetylation	1.50	1.19	79.06

Chitin isolation and chitosan synthesis from shrimp shells were carried out through five stages, namely the preparatory stage, deproteination, demineralization, depigmentation, and deacetylation.

1. Preparatory stage

The preparatory stage begins with shrimp shells washing using a warm water to remove soluble organic compounds, proteins, and other impurities in shrimp shells. The shells were boiled in water for 1 hour and afterwards it was dried under the sunlight. The dried shrimp shells were ground and sieved at 60 mesh to obtain a powder shells.

2. Deproteinization

Deproteinization aims to remove residual protein and fat contained in shrimp shells. Protein is one of the large constituents found in shrimp shells of about 20–24% of the dry material [11]. Protein binds to chitin covalently or physically. Therefore, it is necessary to break the covalent bonds between proteins with chitin, which is called as deproteinized.

Protein was removed by reacting 60 mesh of shrimp powder with 4% NaOH at 80 °C for 1 hour. Deproteinization can be done under basic conditions followed by heating process for a certain time. NaOH is widely used because it is not only effectively work, but also relatively inexpensive and readily available. Giving bases are meant to denature the protein into sodium proteinic which soluble in NaOH solution.

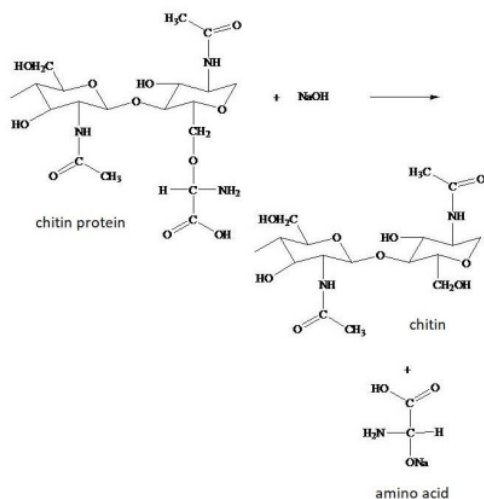


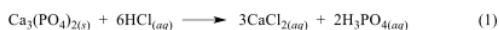
Figure 1. Deproteinization reaction

Deproteinization process was not conducted at a high temperature and a long period to avoid damaging of acetyl groups. Filtering and washing with distilled water until a neutral pH was done to remove sodium hydroxide that may still remain in the residue. Then drying was carried out to obtain a dry brown powder. The yield obtained from deproteinization stage is about 62.60%.

3. Demineralization

Demineralization is a process to remove minerals contained in the shrimp shells, especially calcium carbonate. Demineralization is generally carried out using HCl, HNO₃, H₂SO₄, CH₃COOH and HCOOH. Among these acids, a dilute hydrochloric acid is most commonly used [12].

Demineralization was conducted by reacting 5 gram of the residue obtained from the deproteinized process with 75 mL of 1 N hydrochloric acid under stirring for 1 hour at 80°C. Hydrochloric acid plays a role in dissolving the calcium salts. Mineral dissolution reactions is shown in equation (1) and (2) as follows:



Carbon dioxide generated can be seen from the scum formed on the process of demineralization. It showed that there has been a mineral separation process on the shrimp waste. After solution became homogen, then filtering and washing were done using distilled water until obtaining a neutral pH. This washing process aims to avoid mineral re-attached to chitin surface and to dissolve molecules into water. Then, the residue was dried for 24 hours. The yield obtained from the demineralization phase is 52.60%.

4. Depigmentation/Decolorization

Decolorization is a stage of removal of pigment (dye) such as astaxanthine in shrimp waste. Pigment

contained in chitin does not bound to the mineral or protein, resulting in a brownish chitin. Decolorization aims to provide a better appearance to chitin obtained [12]. In this step, the demineralized product was reacted with bleaching agents such as sodium hypochlorite (NaOCl) or peroxide. The demineralized chitin was reacted with NaOCl under stirring for 1 hour to obtain a white-colored chitin. Then, the solution was neutralized to obtain a white mixture, which indicates that the pigment has been separated from chitin. The drying process was carried out in an oven at a 60°C for 1 hour to obtain a white clean chitin of 1.62 gram with yield of 75.56%.

5. Deacetylation

Chitosan is obtained by performing chitin deacetylation process. Deacetylation is a process of converting the acetyl group (-NHC(=O)CH₃) in chitin, through addition of high concentrations of NaOH into an amine group (-NH₂) to be a chitosan. Chitin deacetylation reaction is based on an amide hydrolysis reaction of α-(1→3)-2-acetamido-2-deoxy-D-glucose by a base. Chitin acts as an amide and NaOH as a base. At first, it starts by an addition reaction, wherein hydroxyl group (-OH) entering into NHC(=O)CH₃ followed by CH₃COO⁻ group elimination to produce an amide product, which called chitosan. Hydrolysis with a strong base is explained as follows:

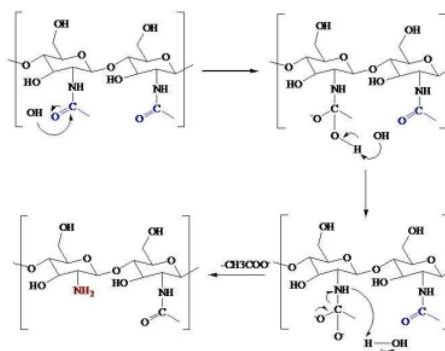


Figure 2. Deacetylation reaction

Chitin deacetylation process was done using 4% NaOH under stirring for 1 hour at 80°C. This condition is needed because chitin has thick cell structures and has a strong intramolecular hydrogen bond between hydrogen of amine group and oxygen of carbonyl group. Deacetylation using hot strong bases will lose acetyl group in chitin through break the bond between carbon of acetyl group and nitrogen of amine group. However, if the temperature applied is too high and carried out in a too long reaction time, it will result in depolymerization of chitosan, which obtain a smaller molecular weight chitosan [13]. From the experimental results obtained chitosan of 1.19 grams with a yield of 79.06%.

Characterization

Analysis by FTIR spectra provided information about the functional groups of the products. The spectra showed that the compound has a functional group as expected compound. FTIR spectra of chitin showed some vibration patterns, which are in 3490 cm^{-1} from NH, 3310 cm^{-1} from -OH, 1650 cm^{-1} from stretching vibration of C=O, and 1560 cm^{-1} from stretching vibration of NH. The stretching vibration of NH is a characteristic for chitin due to a NH group in -NH-CO- (acetylated amine group). Vibration of CH_3 from chitin at 1310 cm^{-1} coincides with C-N amide vibration at 1400 cm^{-1} . Vibration of amine group of chitin in the 3490 cm^{-1} is coincided with OH vibration because hydrogen bond in amine is weak and less polar. It makes N-H bond vibration becomes less intense than OH. Other vibration at 2970 cm^{-1} is a stretching from aliphatic C-H. This absorption is weak because chitin structure is dominated by $\text{R}_3\text{C-H}$ (methyn) which has a weak absorption. The presence of an absorption band at $1010\text{--}1150\text{ cm}^{-1}$ showed a vibration of C-O. Absorption pattern appears to indicate that the residue is a chitin as expected.

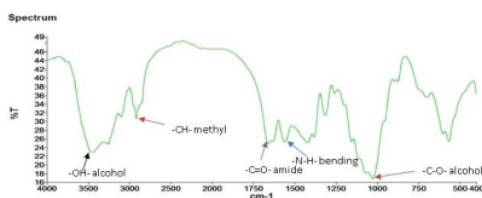


Figure 3. FTIR Spectra of Chitosan

FTIR spectra of chitosan residues showed a broad absorption band at 3490 cm^{-1} indicating hydrogen bond of OH stretching vibration. Absorption band at 2950 cm^{-1} and 1050 cm^{-1} are responsible for stretching vibration of C-H methylene group and C-O group, respectively. Chitosan absorption band are similar to that of chitin. The differences are occur after deacetylation step, in which there are changes in absorption spectrum at 1690 cm^{-1} from stretching of C=O. The spectra (Fig 3) showed a shift of C=O absorption from 1650 cm^{-1} to 1690 cm^{-1} and a decrease of N-H absorption band in CONH group at 1560 cm^{-1} in chitosan. It also showed a new appearance of a weak absorption at 1555 cm^{-1} .

The changing of FTIR spectra of chitin and chitosan can be ascertained that deacetylation was successfully transformed chitin into chitosan. However, the carbonyl group absorption band at 1690 cm^{-1} showed that chitosan is not perfectly deacetylated due to that reason, it is a need to calculate the degree of deacetylation (DD) based on the baseline method [14], according to the following equation:

$$A = \log \frac{P_0}{P}$$

with

A = absorbans

P_0 = % transmitans at baseline, and

P = % transmitans at minimum peak

$$\% DD = 1 - \left[\frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \right] \times 100\%$$

with

A_{1655} = absorbance at 1655 cm^{-1} wavelength number (amide absorption)

A_{3450} = absorbance at 3450 cm^{-1} wavelength number (hydroxyl absorption)

1.33 = A_{1655} for chitin which is fully deacetylated (100%)

Based on chitosan spectra (Fig 3), it is resulted a degree of deacetylation of 87.78%. It means only about 87.78% residual chitin which is deacetylated into chitosan. The degree of acetylation of commercial chitosan is between 20–25%. In other words, the degree of deacetylation is between 70–90% [15]. It can be concluded that deacetylation done in this research is in a good range.

To determine the size of chitosan synthesized, it is necessary to measure the chitosan particles using SEM. SEM-EDS of chitosan showed that chitosan has a particle size of 87 nm.

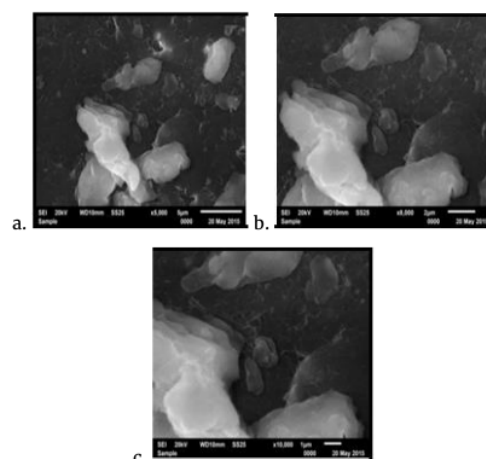


Figure 4. SEM 3,000×; 8,000×; and 10,000×

SEM analysis of chitosan was done to determine the morphology of the surface that will be coated in nanosilika. A magnification of 3,000×; 8,000×; and 10,000× showed that the particle size are 97 nm, 75 nm, and 45 nm, respectively. These data indicating that the chitosan has a nano sized particles. These sizes also indicated that chitosan nanoparticle will be coated well on silica. The observation from transmission electron microscope (TEM) gave information on the particle shape. In the present study, TEM images showed that the nano chitosan has a uniform shape. It also showed a lower physical aggregation of the chitosan nanoparticles. Typical TEM micrograph of the chitosan nanoparticles was shown Fig. 5.below:

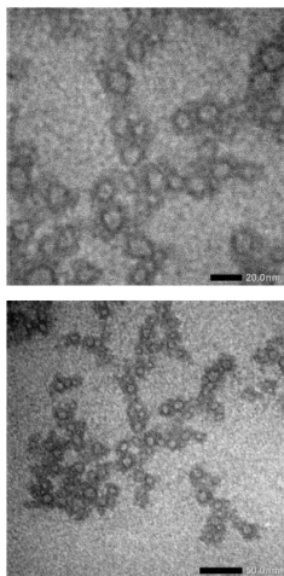


Figure 5. TEM of the chitosan nanoparticles

4. Conclusion

It can be concluded that the Chitin has been isolated successfully from shrimp shells. Deproteinized chitin was obtained with yield of 62.60%, and then demineralization with a yield of 52.60%. The depigmentation of chitin providing a yield of 75.56%. Deacetylation of chitin into chitosan obtaining a yield of 79.02% with the degree of deacetylation value of 87.78%. Characterization by SEM found that nano chitosan has a particle size of 87 nm. While TEM images showed that the nano chitosan has a uniform shape and a lower physical aggregation.

5. Acknowledgement

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