Characterization of chitosan nanoparticles as an edible coating material

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Abstract. Chitosan is widely used due to its non-toxic, biodegradable, and biocompatible properties. This research is conducted to study the formation of chitosan nanoparticles and to characterize this nanoparticle based on its morphology and particle size. Chitosan nanoparticle was produced using ionic gelation method. The characterization was performed using Particle Size Analyser (PSA) particle measurement while morphological exploration was performed using Fourier Transform Infra-Red (FTIR) spectroscopy. The size of this nanoparticle was found to be 0.585 µm (585 nm). Formation of this nanoparticles emulsion can be seen through FTIR analysis showing the functional bond of chitosan and TPP (tripolyphosphate). The addition of TPP and surfactant (Tween 80) had strengthen the mechanical properties of chitosan due to formation of cross ionic bond between chitosan molecules.

1. Introduction

Chitosan, a natural cationic polysaccharides obtained from deacetylation of chitin from crustacean and some fungi, is widely used as edible coating materials due to its benefits such as non-toxic, biodegradable, and biocompatible [1]. This material has been reported to have antimicrobial properties allowing the application as edible coatings and blend in polymers [2]. However, chitosan has a high molecular weight, high viscosity and low solubility in neutral and basic solutions that limit its application [3]. Therefore, the transformation of this material into nanoparticles will be potential.

Nowadays, using the nanoparticles of chemical substances has a great scientific interest as they are effectively connect between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nanoscale, size-dependent properties are often observed to be changed [1]. Nanomaterial has a wide surface area that make it potential to induce high reactivity, to be an effective catalyst for plant metabolism, to provide a better penetration into the cell, and to increase plant activity [3, 4]. Therefore, it is useful to be applied for film formula or edible coating material for foods.

This research is aimed to make chitosan nanoparticle for edible coating and evaluate its particle and structure for further application. The result is expected to be able to offer an alternative edible coating material applicable for fruits and other agricultural commodities.
2. Materials and Methods

2.1. Materials
Chitosan red crab (*Gecarcoidea natalis*) shell (powder) was purchased from CV. Chimultiguna. This chitosan powder has a light brown colour, powder mesh 200-300, degree of deacetylation 97.88%, viscosity of 8.98 mPas and molecular weight at 100 KDa – 150 KDa. Other chemicals including 1% acetic acid, 0.1% Tween 80, and 0.1% Sodium Tripoliphosphate were purchased from Panadia chemical store.

2.2. Methods

2.2.1. Preparation of chitosan nanoparticles edible coating
Chitosan nanoparticles were created following the methods used by Suptijah [5]. One concentration (0.1 %) was chosen as it has been reported to yield the best properties. To make a nanochitosan solution (0.1 %), as much as 0.5 g of chitosan powder was dissolved in 500 mL of 1% acetic acid. Homogenization using magnetic stirrer was performed for 1 hour prior to addition of tween 80 and further homogenization for 30 minutes. As much as 100 mL of 0.07% STPP solution was made separately and homogenized for 1 hour using a magnetic stirrer. The STPP solution was added drop wise to the chitosan solution and further homogenized for 30 minutes.

2.2.2. Particle size distribution and polydispersion index analysis
The particle size distribution was evaluated at room temperature using a LS Beckmen Coulter LS 13 320 particle size analyser, with laser diffraction. Nano-chitosan samples were put into dispersants (distilled water, pH 7), then placed in a 3 ml cuvette for the analysis and fraction using the principle of visible light scattering.

2.2.3. Structural and spectral measurement
Structural and spectral measurements were performed using Fourier Transformed Infrared Specstroscopy (FT-IR) 8400s Shimadzu. The spectral range was from 4000-400 cm\(^{-1}\) and measurements were carried out during 10 scans with 4 cm\(^{-1}\) resolution.

3. Results and Discussion
Nano-chitosan solution in this research was synthesized by ionic gelation method. This method had been explained as a polyelectrolyte complexation between the positively charged chitosan and negatively charged tripolyphosphate (TPP) [Jiangwan]. The final solution was milky coloured upon the cross linking between TPP solution and chitosan solution.

3.1. Particle distribution analysis
Analysis of the size and homogeneity of nanoparticles with the Dynamic Light Scattering (DLS) method is the principle of measuring particles based on diffusion of Brownian motion, namely the random motion of particles in a liquid medium due to collisions with solvent particles. Based on the results of measurements, the size of chitosan nanoparticles obtained was 0.585 μm or 585 nm as shown in Figure 1. Mohanraj [6] suggested that nanoparticles are solid particles with a size range of 10-1000 nm and the result of the current research is between the defined ranges. The size reduction using magnetic stirrer at high speed had been reported to be able to distribute energy throughout the solution and therefore the size would be more homogenous. The addition of TPP may reduce nanoparticle size while strengthening chitosan matrix bond [7]. Further addition of tween 80 as surfactant will aid in the stabilizing of the solution and prevent from agglomeration [8].
The chitosan nanoparticle solution obtained was homogenous as can be seen from the polydispersity index which has values below 0.5 shown in Table 1. Polydispersity index is related to the distribution of nanoparticles. The polydispersity index (PDI) range is 0 to 1, the PDI value which is close to 0 shows the homogeneous nanoparticle solution while the PDI value above 0.5 indicates the heterogeneous nanoparticle solution [9].

Table 1. Characterization of chitosan nanoparticle solutions based on particle size and polydispersity index.

| Chitosan Concentration          | Ratio Chitosan:STPP | Volume of solution | size (nm) | Polydispersity Index |
|---------------------------------|---------------------|--------------------|-----------|---------------------|
| 0.1% in 1% acetic acid solution | 5:1                 | 500mL              | 585       | 0.3                 |

Factors that can influence the formation of chitosan nanoparticles are chemical factors, physical factors, and characteristics of chitosan. The concentration of chitosan used in this study was 0.1% with a deacetylation degree of 85%. The increase in concentration will increase the size of the nanoparticles. Another factor that influences the formation of chitosan nanoparticles is mechanical treatment. Size reduction with a magnetic stirrer can produce more stable particles with a more even size, below 1000 nm [9]. Therefore, homogenization plays an important role in the gel formation of nanochitosan.

3.2. FTIR analysis

Determination of nanochitosan structure and deacetylation degree were carried out by FTIR spectrophotometer. This is required to ensure the formation of nanoparticle solution by evaluating the presence of functional groups of chitosan and STPP in the solution of chitosan nanoparticles. The FTIR spectra of nanochitosan solution evaluated in this study was represented in Figure 2.
FTIR provides information about the functional properties of nanochitosan which correlate its functional group and structure. Figure 2 shows that the chitosan nanoparticles have a peak at wavenumbers 3385.60 cm\(^{-1}\) indicating the stretching vibration of the \(-\text{NH}_2\) and \(-\text{OH}\) groups. A wide peak between 3650-3200 cm\(^{-1}\) areas was attributed to hydrogen-bonded (O-H) stretching vibration [10], while overlapping of N-H stretching from primary amine and type II amide occur in the same region [11]. The peaks formed at 1659.43 cm\(^{-1}\) and 1597.71 cm\(^{-1}\) indicates the interaction between NH\(^+\) groups. This is in accordance with Silverstein et.al [12] who reported that wave number 1650-1580 cm\(^{-1}\) shows the presence of a functional NH amine primary group with bending vibration type. Wavenumber 1107.83 cm\(^{-1}\) indicates C-O-C while the IR spectrum of chitosan nanoparticles appears at 1044.18 cm\(^{-1}\) as a sharp peak indicating a P=O group. It has been previously reported by Silverstein et.al [12] that the functional group P=O with stretching vibrations is found at 1040-910 cm\(^{-1}\). The formation of this functional group P=O shows the cross linkage between phosphate groups of STPP and chitosan by ionic bond. The results of this analysis indicate that the nanoparticle solution has been formed in the solution.

Addition of tripolyphosphate (TPP) and surfactants (Tween 80) may enhance the mechanical properties of chitosan that are naturally fragile and enhanced formation if ionic crosslinking between chitosan molecules. This method had been reported to improve mechanical properties, thermal properties, barrier properties, and physicochemical properties of the nanomaterials in comparison to the micro-scale materials [13]. The chitosan became more stable and can increase the adsorption capacity of chitosan in fruit cell tissue [3]. This characteristic will offers advantages in the application of nanochitosan as an edible coating material.

4. Conclusion
Chitosan nanoparticles was created and analysed to evaluate its size and structure. The result showed that the chitosan nanoparticles had a size of 0.585 μm (585 nm), still between the ranges of defined
nanomaterials at 10-1000 nm. Structural analysis of nanoparticle solution had shown the formation of nanochitosan due to the presence of functional groups of chitosan and STPP characterized by the P = O bonds. Further research will need to be carried out to apply the nanochitosan produced to a food product in order to evaluate its effectivity and usefulness in increasing product’s shelf-life.

References

[1] Luo Y, Wang Q 2013 Recent advances of kitosan and its derivatives for novel applications in food science J. Food Proces. Beverages 1 1-13.
[2] Kong M, Chen X G, Xing K, Park H J 2010 Antimicrobial properties of chitosan and mode of action: A state of the art review Int. J. Food. Microbiol. 144 51-63.
[3] Gouda M, Elayaan U, Youssef M M 2014 synthesis and biological activity of drug delivery system based on chitosan nanocapsules Adv. Nanoparticles 3 148-158.
[4] Eshghi S, Hashemi M, Mohammad A, Badii F, Mohammadhoseini Z, Ahmad K 2014 Effect of nanochitosan-based coating with and without copper loaded on physicochemical and bioactive components of fresh strawberry fruit (Fragaria x ananassa duchesne) during storage Food Bioprocess Technol. 7 2397–2409.
[5] Suptijah P, Jacoeb M A, Rachmania D 2011 Karakterisasi nano kitosan cangkang udang vannmei (Litopenaeus vannamei) dengan metode gelasi ionic (Characterisation of nanochitosan from vannmei shrimps (Litopenaeus vannamei) using ionic gelation method) Jurnal Pengolahan Hasil Perikanan Indonesia 14 78-84. [In Indonesian]
[6] Mohanraj V J, Chen Y 2006 Nanoparticles-a review Trop. J. Pharm. Res. 5 561-573.
[7] Du W L, Niu S S, Xu Y L, Xu Z R, Fan C L 2009 Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions J. Carbohydr. Polym. 12 385-389.
[8] Xu Y, Du Y 2003 Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles Int. J. Pharmaceut. 250 215-226.
[9] Avadi M R, Sadeghi A M M, Mohammadpour N, Abedin S, Atyabi F, Dinarvand R, Tehrani M R 2010 Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method. Nanomed-Nanotechnol. 6 58–63.
[10] Sastrohamidjojo H 2001 Spektroskopi inframerah Liberty Yogyakarta 12 – 37
[11] Yu J H, Du Y M, Zheng H 1999 Blend films of chitosan gelation Wuhan Univ. J. Nat. Sci. 45 440-444.
[12] Silverstein R M, Webster F X, Kiemle, D J 2005 Spectrometric identification organic compounds 7th John Wiley & Sons, Inc USA.
[13] Sorrentino A, Gorrasi G, Vittoria V 2007 Potential perspectives of bionanocomposites for food packaging applications Trends Food Sci. Technol. 18 84–95.