Chitosan Nanoparticle-Based Coating as Post-harvest Technology in Banana

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Abstract. Losses during post-harvest management is a common problem faced in the agricultural sector. To prolong the preservation time perishable fruits, such as banana, an edible coating is one alternative method for fruit preservation method. Aims of this research are to utilise chitosan nanoparticle as a good coating and to observe its effect on Cavendish bananas (Musa acuminata AAA group) ripening process. In this study, we synthesised and characterised chitosan nanoparticle, as well as observed chitosan nanoparticle coating effect on fruit ripening process. We showed the ionic gelation method could be used to produce chitosan nanoparticles with an average diameter of 102.4 - 370 nm and the polydispersity index of 0.251 – 0.303. Fourier Transform Infrared Spectroscopy (FTIR) analysis showed a new peak which indicated an interaction of amide groups in chitosan and tripolyphosphate groups in NaTPP. A Scanning Electron Microscope (SEM) showed a smoother skin contour on coated banana compared to control (non-coated). Observations of physical characteristics of bananas ripening showed that fruits coated with chitosan nanoparticles 0.2% has a slower skin discolouration by 2-3 days compared to control treatment.

Keywords: Cavendish, ripening process, chitosan nanoparticle, ionic gelation

1. Introduction
Bananas are favourite fruit in the world and most valuable staple food after rice, wheat and maize [1]. Bananas are climacteric fruit and have a short shelf-life after-ripening process begin [2]. The ripening process on climacteric fruit is regulated by gaseous hormone ethylene. Naturally, ethylene synthesised by the plant from the precursor of S-adenosylmethionine (SAM), then converted into ACC (1-aminocyclopropane-1-carboxylic acid) by enzyme ACC synthase (ACS). ACC is subsequently converted to ethylene when tissues get enough oxygen through ACC oxidase (ACO) catalysis process [3].
The presence of ethylene has been shown to induce ripening-related processes that occur in fruits, such as autocatalytic ethylene production, cell wall modification, and production of volatile compounds and sugars [4]. Fruits with the high nutritional value such as bananas are vulnerable to post-harvest diseases caused by microorganisms [2]. Therefore, proper post-harvest handling is required to reduce the potential for physical damage and also extends the shelf-life of bananas. The improved post-harvest management to prolong fruit shelf-life, would provide significant economic benefits for farmers, industry, and food companies [5].

A popular method to prevent fast ripening of the fruits is using edible coating [6]. The edible coating is a thin layer made of components that are safe for consumption, which applied to the fruit surface as an addition or substitute for natural waxy coating [7,8]. Edible films and coatings are applied on fruit to control moisture transfer, gas exchange (as a barrier to CO₂ and O₂), or to prevent oxidation processes [9,10]. The edible coating is a favourite choice for post-harvest treatment because of its safety and environment-friendly [6,11]. Besides, the edible coating can be used as a media to incorporate active substances, such as antimicrobial agents, antioxidants, enzymes or probiotics, and vitamins [5,9].

One of the best material for edible coating is chitosan. Chitosan (CS) is a linear polysaccharide, composed by 2000-3000 monomer N-acetyl-d-glucosamine (GlcNAc), joined by β bonds (1-4), and it is derivative of deacetylated chitin. Chitosan usage as coating material has an advantage for the non-toxic, biodegradable, biocompatible (does not cause rejection of the immune system), and has high antimicrobial and antifungal activity. Chitosan could quickly form a layer on the fruit surface and reduces the respiration rate of the fruit by controlling carbon dioxide and oxygen permeability. Based on these characteristics, chitosan widely used for many post-harvest fruits and vegetables, such as grapes [12], watermelon [5], strawberries, and cherries [13].

The nanotechnology can give an essential role in the food packaging technology using coating material such as chitosan. Nanotechnology has been successfully applied to improve the quality of packaged food. Thus, we can combine the edible coating and nanotechnology from the raw chitosan materials. To date, chitosan nanoparticles widely used as a drug carrier because of its unique biological activities due to its small size and quantum size effect. The reduction of chitosan size can reduce the permeability of the surface to water vapour and gases, while still maintaining the desired properties of the chitosan coating. As a nano-sized particle structure, chitosan nanoparticles is expected to extend the shelf life of fresh fruits [11]. So, aims of this research are to synthesise nanoparticle chitosan and to observe its potential use in extending the shelf life of bananas on the onset of maturation.

2. Materials and Methods

2.1. Plant Material

Cavendish bananas from PT. Sewu Segar Nusantara was treated using ethylene for 24 hours and collected at mature green stage (Scale 2). The maturity scales based on the standard quality of PT. Sewu Segar Nusantara (Table 1). The banana hands were separated from the bunch and carefully selected to be uniform in appearance (weight, colour, and physiological age), and have no injury or physical damage as mentioned in Dwivany et al. (2016) [14].

| Scale | Colour                                      |
|-------|--------------------------------------------|
| 1     | Fresh green colour                         |
| 2     | Bright green colour, early colour change in the ripening process |
| 3     | Yellow-green colour (dominant green)       |
| 4     | Green-yellow colour (dominant yellow)      |
| 5     | Yellow colour with a slight green colour at both ends of the banana, and already edible |
| 6     | Entirely yellow, with good flavour          |
| 7     | Yellow colour with brown freckle has a sweet aroma and flavour |

Table 1. Cavendish maturity scale after treated using ethylene (PT. Sewu Segar Nusantara, 2014)
2.2. Chemical Materials
We used two types of chitosan in this research: 1) Analytical grade chitosan (Low Molecular Weight, deacetylation degree = 80%) purchased from Wako Japan (Catalogue No. #034-16091), and 2) Food grade chitosan (High Molecular Weight, deacetylation degree = 85%-89%) purchased from PT. Biotech Surindo Indonesia. Sodium Tripolyphosphate purchased from Sigma (Catalogue No. #72061) and food grade from BrataChem Indonesia.

2.3. Chitosan nanoparticle preparation
We made chitosan nanoparticles chemically (ionic gelation) based on the previous work with modifications [15]. Chitosan (0.2% w/v) was dissolved in acetic acid (0.5% v/v) and homogenized using a magnetic stirrer at room temperature. Tween 80 (0.1% v/v) was added to improve solution wettability.

The tripolyphosphate (TPP) solution made by dissolving NaTTP (sodium tripolyphosphate) in deionised water (1 mg/ml). Nanoparticles solutions prepared by mixing chitosan solutions containing Tween surfactant and TPP droplets with a volume ratio 5:1 in a beaker glass under stirring for 30-60 minutes at room temperature. Nanoparticles will spontaneously form after the addition of TPP into chitosan solutions. The mixture was homogenised in a high-speed homogeniser to reduce the particle size. For control, we used chitosan (0.2% w/v) in acetic acid (0.5% v/v) with pH of 5.6.

2.4. Chitosan Nanoparticles Characterizations
FTIR (Fourier Transform Infrared Spectroscopy) spectra of nanoparticles were measured using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy, recorded on Bruker Alpha FTIR Spectrometer with a wave number of 4000 – 750 cm⁻¹. Dynamic Light Scattering (DLS) method using PSA (Particle Size Analyzer) was used to determine particle size and polydispersity index.

2.5. Bananas Coating with Chitosan Nanoparticles Solution
Bananas were washed with tap water, dipped into organic fungicide water (Hermawaty, 2014) for 3 minutes at room temperature and air-dried. Chitosan nanoparticle (CS nano) and chitosan solution (CS) applied through the use of spraying techniques. Bananas without coating used as a control. The fruit stored at 22 ± 1°C. We observed changes in the fruit skin colour during six days of storage and micrographic analysis of banana peel using Scanning Electron Microscope (SEM).

3. Results and Discussion
We observed the effect of chitosan molecular weight and deacetylation degree of polymers to the size of nanoparticles. Ionic gelation method can synthesise chitosan LMW nanoparticles with the size 102.4 nm in 10 mL solution, and 143.4 nm in 100 mL. On the contrary, chitosan HMW produced larger nanoparticles in size: 370 nm. Both solutions have a good-quality homogeneity as shown by the polydispersity index (PI < 0.5, Table 1). PI associated with nanoparticles size distribution. PI greater than 0.5 indicates a relatively independent nanoparticles suspension, while PI less than 0.3 indicates a homogeneous nanoparticles suspension [16].

The differences in the nanoparticles size obtained from the two types of chitosan polymer (LMW and HMW) because of the amount of monomer and by polymer size. Nanoparticles will quickly form on LMW chitosan because the polymer chain is shorter than chitosan HMW, so the nanoparticles formed will smaller (Crysanto, Unpublished data).

Table 2. Characterization of CS and CSnano solution based on particle size and PI with ionic gelation method

| Type of chitosan       | Deacetylation degree | Volume: 10 mL | Volume: 100 mL |
|------------------------|----------------------|---------------|----------------|
| Analytical grade (LMW)| 95%                  | 102.4 nm      | 143.4 nm       |
|                        |                      | 0.251         | 0.303          |
In addition to polymer weight, nanoparticles synthesis also influenced by the pH. Acidity level of chitosan solution can affect the level of amine group ionisation in chitosan. At a high pH level, the amount of protonated amine group is low, so the interaction between chitosan and polyanion TPP is not strong enough causing the loose formation and large nanoparticles. On the contrary, at low pH level, the amine group is more protonated, which increases the positive charge density of chitosan. High charge density induce chitosan cross-linking with TPP is getting stronger, so nanoparticles formed at low pH will be smaller [17].

In this research, we used chitosan solution at pH 4.54 to form a stable suspension as the degree of protonated amines in the chitosan enough to interact with TPP, forming nanostructure complex. At pH 4.5, chitosan (pKa, degrees of dissociation = 6.5), the ratio between the protonated amine group and non-protonated amine group is 100: 1 (Crysnanto, Unpublished data). However, the 100 nm particle is obtained only at LMW chitosan, while in HMW chitosan the particle obtained is above 300 nm. Therefore, it is necessary to re-optimise the physical factors such as the optimisation of the stirring speed and the addition method of NaTPP to obtain chitosan nanoparticles. In addition to pH, surfactants, and the characteristics of used chitosan, the physical factors such as the type of mechanical treatments, mechanical treatment speeds, and the duration of mechanical treatment also influences the formation of chitosan nanoparticles [18]. CS nano solution then was used for FTIR analysis and the coating solution for banana storage.

The nanoparticles formulation with chitosan polymer-based material classified into two methods: 1) Electrostatic interaction, the formation of nanoparticles based on electrostatic interaction as ionic gelation. This method has used for biological and pharmaceutical application because of its simplicity, and it does not require organic solvents which are more secure for the living organism, and its particle size could be controlled [17], and 2) Nanoparticles formed by covalent cross-linking, this method creates particles with chemical bond that is stronger than the electrostatic crosslinking method. However, the method use of toxic solvents such as formaldehyde or glutaraldehyde, making it less suitable for biological applications [19].

Ionic gelation method is the formation of polymer chains crosslinking to form a three-dimensional web. Chitosan on ionic gelation method serves as polycation that carry multivalent positive ions, and reacts with TPP carries multivalent negative ions. During the interaction between chitosan and polyanion, the system gave mechanical disturbances until the nanometer-sized complex formed. Besides, we did the constant stirring using a stirrer and a high-speed homogeniser to reduce the particle size [20]. The drawbacks of this method are the high level of polydispersity of nanoparticles and the low stability of nanoparticles on storage [17].

Sodium tripolyphosphate is a polyanion used for forming chitosan nanoparticles. NaTPP commonly used as a crosslinking agent in the formation of nanoparticles because of its non-toxic and multivalent character. NaTPP is also a food additive and used for forming texture. When dissolved in water, NaTPP will ionise and carry a negative charge with high density so that the interaction between electrostatic and chitosan could vigorously form, which will affect the mechanical strength of nanoparticles [17].

**3.1. Characterization of chitosan-NaTPP crosslink**

FTIR spectra of chitosan (Figure 1) have specific peaks at wave number 3452 cm⁻¹ (OH group stretching vibration) and peak for primer amide laid at 1671 to 1622 cm⁻¹ (C=O stretching). Another band of chitosan was observed at 1457 cm⁻¹, coming from -CH₂ vibration coupled with OH in-plane deformation. Band 1155 cm⁻¹ showed a C-O-C bending [21].

The formation of chitosan-NaTPP crosslink observed from IR absorption. The changes in transmittance intensity showed the existence of the interaction between chitosan and TPP from NaTPP solution. FTIR spectrum of chitosan nanoparticles possessed some differences compared to chitosan spectrum, i.e. the emergence of two new absorbance peak at wave number 1018 cm⁻¹ (related to -PO₃⁻), and at 1071 cm⁻¹ (associated with asymmetrical C-O-C stretching). Peaks at 1401 and 2894 cm⁻¹
attributed to symmetrical –CH₂ bending and stretching, respectively. Also, an increase in peak intensity at 1623 cm⁻¹ and the shifting of amide II peak to 1557 cm⁻¹ might attribute to the protonation of chitosan and subsequent ionotropic cross-linking by TPP ions. We observed the absorbance level at wave number 1557 cm⁻¹ resulted from an interaction between amide group from chitosan and triplyphosphate group from NaTPP [21].

The absorbance similarity between chitosan and cross-link chitosan is the presence of overlapping between stretching vibration -OH and N-H group (3425-3449 cm⁻¹). The absorption band on bending vibration of N-H primary amide appears at wavenumber 1623 cm⁻¹, whereas the absorption band at wavenumber 1557 cm⁻¹ arise due to the interaction between the ammonium ion with phosphate ion [22].

3.2. Physical observations of the bananas ripening process
We observed the visual changes in fruit skin colour from green to yellow. We used ripe green bananas as described in Material and Methods. Figure 2 shows the visual observation result of the three treatment groups: (1) Bananas control without coating, (2) Bananas coated with CS (0.2% v/v), and (3) Bananas coated with CS nano (0.2% v/v). We observed the fruit for six days, denoted by T1, T2, T3, T4, T5, and T6. During observations, the control group had early ripening speed than the other two groups; the fruit colour turned yellow (scale 3) at T3, and entirely yellow at T6 (scale 6). In CS 0.2% group, the fruit colour at maturity level scale three observed at point T3 to T4. In CS nano group, the colour of the fruit was green until T4, and turn to yellow at T5 (scale 3). Besides, CS nano influenced bananas ripening process.

The colour changes the pattern on banana peel occurs due to chlorophyll decomposition during the ripening process, so the skin colour changes from green to yellow. The decomposition caused by chlorophyllase enzyme lead to decreasing chlorophyll concentration in fruit skin, so the carotenoids pigments in thylakoid membranes of unripe fruits become exposed. During the ripening process, an amount of chlorophyll will be reduced gradually to near zero [4]. Slower of bananas colour changes coated with CS nano indicates the ripening process delays in fruits compared to the control treatment. The ripening delay because of a film layer that covers the surface of the fruit, affecting gas transport on fruit peel. According to Hosain and Iqbal (2016), chitosan forms a semipermeable film and therefore could modify the internal atmosphere condition (through permeability distraction to water, oxygen, and carbon dioxide) and thus reduce the transpiration loss, reducing transpiration rate, and delay fruit ripening process [2]. According to Jianglian et al. (2013), the edible coating could prevent loss of
moisture and aroma, as well as inhibit the oxygen penetration into plants tissues and prevents the microbial growth [12]. Oxygen in plants assists in the conversion of ACC into ethylene with the aid of ACC oxidase [23].

![Image](image_url)

**Figure 2.** Changes in bananas colour during the six days of storage (T1-T6). Control (A), Banana coated with CS (0.2% v/v) (B), Bananas coated with CS nano (0.2% v/v) (C). A red box indicates the observation point when the fruits' colour begins to turn yellow.

We hypothesise that the limited oxygen availability suppresses ACC conversion into ethylene, therefore affecting the fruit ripening process. To verify that chitosan nanoparticles coat affected gas traffic in banana, we performed SEM analysis (Figure 3). Micrographic analysis at the skin surface of bananas with and without chitosan nanoparticles solution using SEM observed in two magnification: magnification of 1000x and 2000x. As a result, banana fruit coated with chitosan nanoparticles have more subtle contours closed due to the polymer chitosan film covering it. Besides, it is clear that the nanoparticles are well dispersed and embedded in the skin surface of fruits (Figure 3). Similarly, another research on the chitosan-glycerol coating on bananas (*Musa sapientum*) [8]. Chitosan polymer coating on the skin surface of fruits acts as a selective barrier to the gas transport [24]. Since the oxygen played a role in ACC synthesis conversion into ethylene, the presence of a chitosan nanoparticles barrier thought to be the cause of the deceleration in the fruit ripening process by disrupting the ethylene biosynthesis process.
4. Conclusion

Chitosan nanoparticles (142.4 nm in 100 mL) successfully made from chitosan LMW with the ionic gelation method with a polydispersity index of 0.303. FTIR analysis showed the presence of -PO$_4$ group in CS nano, as well as new peaks, resembled interaction between amide groups from chitosan and tripolyphosphate groups from NaTPP. From the SEM results, bananas coated with CS nano showed a uniformly smoother skin compared to control. Visual observation on bananas showed that Banana coated with CS nano had a slower rate of ripening process (2-3 days) compared to the control. These results are feasible to be used as a platform for further research to investigate the effect of nanoparticles coating on physiological, biochemical, and molecular characters in the fruit ripening process. Furthermore, the optimisation of the nanoparticles produced in solution needs to be improved to get the best result on post-harvest practices.

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