



## Potential of Chitosan From Local Crab (*Portunus Pelagicus*) to Enhance Storability of *Musa Paradisiaca L.*

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### Abstract

Potential waste of local crab carapace (*Portunus pelagicus*) as a source of chitosan as an active layer that can protect bananas has been studied. The process in this study consists of three stages. The first stage was the isolation of chitin through deproteinization process using 2.0 N NaOH solution with a ratio of 1:6 w/v and demineralization process using 1.5 N HCl solution with a ratio of 1:12 w/v. The second stage is the deacetylation stage using 50% NaOH solution with a ratio of 1:20 w/v. Fourier Transform Infra-Red (FTIR) Spectroscopy is used to determine the degree of deacetylation. The third stage is the banana coating application using chitin solution to determine the shelf life of bananas with variations in levels of 2, 2.5, 3 and 3,5 % w/v by immersion method for one hour. It was found that carapace crab, a part that was underutilized from crab, gave rise to chitin deacetylation with a deacetylation rate of 62.11%; pH 8.9 and water content of 7.677%. Chitosan-based coatings are applied to fresh bananas and are found to increase fruit firmness, and inhibit browning. The results show that chitosan-coated bananas have a longer storage time. The application of chitin deacetylated (chitosan) as fruit banana coater found that higher coater levels extend the shelf life of bananas with the best coater content is 3% b/v. It results in a shelf life of bananas for up to 12 days, this is longer than bananas without chitosan layer which only has a shelf life of four days. Increased coating rates have a positive effect on the shelf life of bananas. This study shows that waste from carapace crabs can be used to form active layers that can preserve fruit.

### INTRODUCTION

Banana fruit is the most consumed fruit in the community. Indonesia has the most banana varieties from the other countries and meets 50% of the needs of bananas in Asia. the export value of banana fruit decreases because it has a short shelf life and it is easily damaged during storage. This damage is characterized by browning (Sholihati et al., 2015). Bananas must have a layer to prevent damage. The edible layer is a method of giving a thin layer to the surface of the fruit to inhibit the release of gas, moisture and avoid contact with oxygen. It will slow down fruit damage. The coating material used must be safe for the body. The use of chitosan coater is the right alternative, it is

effective and safe for health (Hardjito, 2006). Chitosan is famous for its properties that can be used as adsorbents (Darmadi et al., 2018; Rahayu & Purnavita, 2017), coater (Khalifaa et al., 2017), antimicrobials (Friedman & Juneja, 2010) etc.

Based on several previous studies, it was shown that adding 2.5% chitosan layer to strawberries increased microbial growth inhibition (Harianingsih, 2010). Provision of 2.5% chitosan coating on Lansium Domestic Corr fruit has a shelf life of six days (Trisnawati et al., 2013). Giving 3% chitosan layer on red guava fruit can increase the age of up to eight days (Sitorus et al., 2014). Research on the ability of chitosan layer to increase the age of fruit storage that has been studied in several fruits such as apples, apricots, mangoes,

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avocados, guava, strawberries, etc. (Silva et al., 2018; Khalifaa et al., 2017; Silva et al., 2017; Tesfay and Magmawa, 2017; Wang & Gao, 2013; Zhang et al., 2018) with various types of chitosan-based coatings. The research about banana coating has never been studied. Chitosan can be made from the chitin contained in crustacean shells such as shrimp (Sofia et al., 2016), shells of *Portunus pelagicus* (Rahayu & Purnavita, 2017), crabs (Harianingsih, 2010), fish (Kumari and Rath, 2014) and its manufacture is still being studied from the cell wall of fungi.

Processed products from *Portunus pelagicus* which are sold in the form of frozen crab or canned packaging, leave the shell waste reaching around 40-60%. It has the potential to be used as chitosan base material. This study has examined the use of crab carapace waste as a banana coater that has never been done before.

## RESEARCH METHODOLOGY

### Materials

The basic ingredients in this study were carapace of *Portunus pelagicus* obtained from the crab processing industry in Rembang, Central Java, Indonesia. After being separated from the remaining meat, the crab carapace is washed with water until it is clean and then dried in the sun. In the preliminary stage of this study, a chemical analysis of the basic ingredients of crab carapace includes water, protein, and ash. Banana fruit (*Musa paradisiaca L.*) was obtained from the local market in Semarang, Central Java, Indonesia. And the sampling technique uses random sampling.

### Methods

This study consisted of three stages, first isolation of chitin from crab carapace waste (*Portunus pelagicus*), secondly deacetylation of chitin to chitosan, and third layer test for banana.

#### *Isolation of Chitin*

There are two steps of chitin isolation, namely deproteinization and demineralization. Crab carapace is dried in the sun, mashed and sieved with a 100 mesh sieve. Deproteinized using 2.0 N 1:6 (w/v) NaOH solution heated in an oven at 80°C for one hour. Then separated and powder washed with aqua distilled until neutral. It dried using an oven at a temperature of 100°C to a constant weight and calculate the yield. The next

stage is demineralized with 1.5 N HCl solution 1:12 (volume) (Rahayu & Purnavita, 2017). After being filtered, dried using an oven at a temperature of 100°C to a constant weight, the yield was calculated to obtain chitin powder.

#### *Deacetylation of Chitin becomes Chitosan*

The deacetylation process is done by boiling chitin in NaOH 50% (w/v) solution with a ratio of 1:20 (gram/mL) at 90°C, with a boiling time of 120 minutes. then the solution is filtered, and it is added with distilled aqua to neutral. the solid is dried at a temperature of 70-80°C in an oven for 24 hours. The product produced by this process is called chitosan, and chitosan characterized using Fourier Transform Infra Red (FTIR) spectroscopy method (Rahayu & Purnavita, 2017). In this study, the calculation of deacetylation degree (DD) was carried out using the Sabnis and Block's methods, it's based on the results of FTIR analysis using the equation below (Roberts, 1992).

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

with,

$$A (\text{Absorbance}) = \log(P_0/P) \quad (2)$$

Where,  $A_{1655}$  is the absorbance at a wavelength of 1655  $\text{cm}^{-1}$  for the absorption of amide/acetamide groups ( $\text{CH}_3\text{CONH-}$ ),  $A_{3450}$  is the absorbance at a wavelength of 3450  $\text{cm}^{-1}$  for the absorption of hydroxy groups ( $-\text{OH}$ ) and factor 1.33 is a value ratio of  $A_{1655}/A_{3450}$  for chitosan with full deacetylation.

#### *Banana Coating by Chitosan*

Chitosan is dissolved in acetic acid solution (1%V). Variations in chitosan levels (2, 2.5, 3 and 3.5% w/v) were prepared for banana coatings. chitosan solution was stirred at 40°C for one hour, then it was filtered and prepared in nine containers. bananas are dipped for one hour with three replications. After drying, coated bananas are stored at room temperature. The observations were made on banana peels during storage.

## RESULTS AND DISCUSSION

The results of physical analysis of carapace base material and chitin used in this study are presented in Table 1.

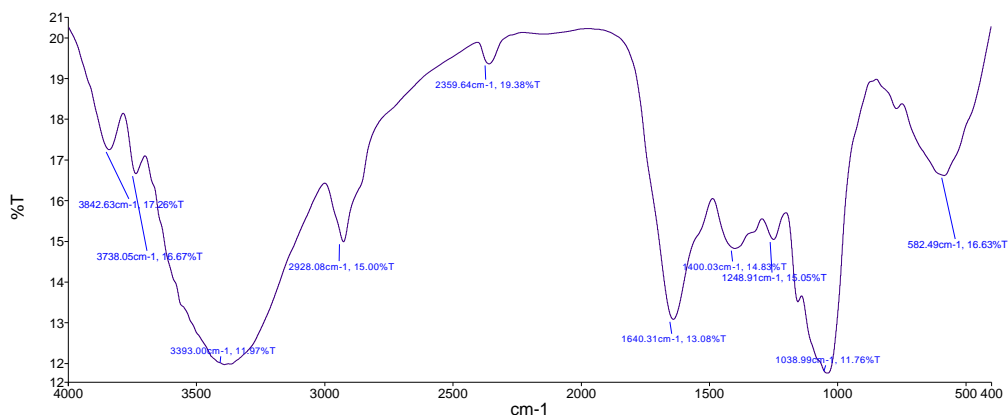


Figure 1. Chitosan FTIR Spectra

Table 1. The results of physical analysis of carapace and chitin

Parameter	Carapace	Chitin
Form	Powder	Powder
Colour	Brownish	Brownish
pH	white	white
Moisture content (%)	7.6	7.2
	6.836	6.421

**Isolation of Chitin**

In the chitin-isolation phase consists of deproteinization and demineralization processes. The sequence of processes, both deproteinization and demineralization processes did not show significant differences seen from chitosan produced (Purwanti, 2014). The yield of chitin isolation from each process is shown in Table 2.

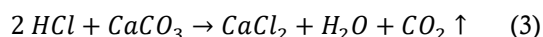
Table 2. The results of physical analysis of carapace and chitin.

Process	Yield (%)
Deproteinization	81
Demineralization	66.25

Deproteinization process to break the bond between powder and protein contained in the sample using 2 N NaOH solution with a ratio of 1:6 (w/v), it was heated in an oven at 80°C for one hour. A yield of 81% was obtained, this indicates that the amount of protein bound by Na<sup>+</sup> ions forms sodium proteinate which dissolves water by 19%. There is a little bubble above the surface of the solution and it's rather thick, it indicates the protein content taken. This result is similar with previous research on deproteinization using 4% NaOH 1:10 (w/v) at a temperature of 100°C and it was stirred using a magnetic stirrer for 12 hours, obtained a deproteination yield of 82.13% (Sukma et al., 2014).

The demineralization process to remove inorganic salts or minerals in the crab carapace. The

content of noble minerals is CaCO<sub>3</sub> and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> in small quantities, the minerals contained in this carapace are easier compared to proteins because they are only physically bound (Kumari et al., 2017). Demineralization is generally performed by acid treatment using HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, CH<sub>3</sub>COOH, and HCOOH (No and Hur, 1998; Percot et al., 2003). Among these acids, the preferential reagent is dilute hydrochloric acid. Demineralization is easily achieved because it involves the decomposition of calcium carbonate into the water-soluble calcium salts with the release of carbon dioxide as shown in the Eq. 3 (Younes & Rinaudo, 2015).



The chitin isolation process is carried out without the decolorization process and gets a large enough yield so that it is immediately followed by the deacetylation process.

**Deacetylation of Chitin becomes Chitosan**

Deacetylation process with the addition of 1:20 (w/v) 50% NaOH solution was heated at 90°C for two hours. This process produces 86.00%. This shows CH<sub>3</sub>COO<sup>-</sup> bond to Na<sup>+</sup> obtained 14% of bonding. Previous studies used 70% 1:20 NaOH (w/v) solution at temperature 100°C for 24 hours, obtained 46.25% (Sukma et al., 2014). The chitin deacetylation produced in this study has a physical character: the form is powder, the color is brownish white, has a pH of 8.9 and a moisture content of 7.677%. At this stage, chitosan product has alkaline pH. The results of chitosan FTIR spectra showed in Figure 1.

Chitin deacetylated (chitosan) from crab carapace contains O-H, N-H amine, C-H, C = O amide, CH<sub>3</sub>, C-C, C-O, C-O-C, and C-OH groups.

Table 3. Chitosan FTIR Spectra Data

Fungsional Group	Wave Number(Cm <sup>-1</sup> )	
	Chitosan Standard	Chitosan sample
O-H	3363.24	3393
N-H	3363.24	3393
C-H	2902.12	2928.08
C=O	1658.48	1640.31
CH <sub>3</sub>	1434.77	1400.03
C-C	1350-1470	1396.00
C-O	1275	1248.91
C-O-C	1064.51	1058.99
C-OH	1050	1038.99

Based on the results of FTIR analysis, the degree of deacetylation can be determined. Calculation of deacetylation degree (DD) is done by line base method (Roberts, 1992). The chitosan constituent group is known according to the wave number (cm<sup>-1</sup>) shown in Figure 1. Chitosan FTIR spectra data is presented in Table 3.

Based on the results calculation, the deacetylation degree value is 62.11%. It showed that chitin acetamide group was converted to amine group 62.11%. In this study only using carapace waste as a base material for making chitosan, and another study using all crab shells obtained % DD of 66.11-79.65% (Rahayu & Purnavita, 2017; Sartika et al., 2016). The basic ingredients determine the value of % DD chitin. Deacetylation was investigated using seven factors: the alkali reagent, its concentration, temperature, reaction time, the use of successive baths, atmospheric conditions and the use of sodium borohydride as a reducing agent (Younes et al., 2014). For that purpose, a fractional factorial design was applied and a mathematical model was established to allow optimizing experimental conditions for chitosan of desired DA (degree of acetylation). Results clearly revealed a significant effect of temperature and the alkali reagent nature (NaOH treatment is much more efficient than KOH). It has been found that DA is significantly affected by the use of successive baths, reaction time and alkali concentration. (Younes et al., 2014).

In the deacetylation process of chitin into chitosan, the response parameters were measured only by the deacetylation degree of chitosan, while the values of water, protein/nitrogen, and chitosan ash were not analyzed. The deacetylation level of chitosan produced is in accordance with trade standards, which is more than 60% (Srijanto, 2003). It can be used for the next banana coating process.

#### *The effect of chitosan concentration on the shelf life of bananas*

The banana coating process uses a chitosan solution in acetic acid one percent volume with variations in chitosan concentrations of 2, 2.5, 3 and 3.5% w / v. The correlation between the concentration of chitosan coating and banana shelf life is shown in Figure 2.

The results showed that the higher the coating level, the longer the shelf life of the banana (*Musa paradisiaca* L.) until three percent of chitosan concentration. The bananas without coater decay on the fourth day. Banana with chitosan coater concentration 2, 2.5, 3, 3.5% decomposes on the seventh, ninth and twelfth days. More than three percent the shelf life of the banana has barely unchanged. Chitosan layers on the surface of the fruit are used to inhibit the release of gas, moisture and avoid contact with oxygen. The addition of chitosan coater on the fruit surface can prevent fruit damage (Silva et al., 2018) and can also be used as an antibacterial with the higher antimicrobial activity (Friedman & Juneja, 2010).

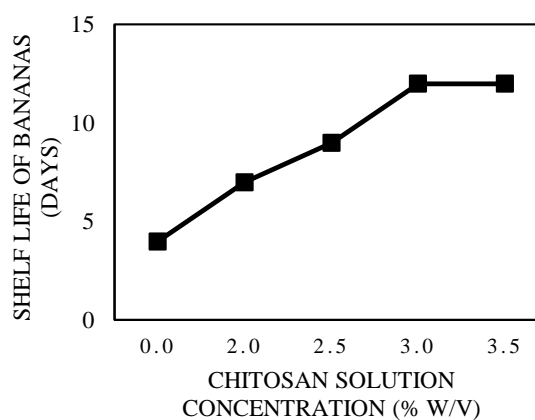


Figure 2. Correlation between the concentration of chitosan coating and banana shelf life.

## CONCLUSION

In this study, local carapace waste (*Portunus pelagicus*) was very potential as a coating of banana fruit (*Musa paradisiaca* L.). The degree of deacetylation (DD) from chitosan is 62.11%. The best coater content for bananas is three percent in acetic acid solution (1% V) which has a storage capacity of twelve days compared to uncoated ones that only have a shelf life of four days. Increased coating rates have a positive effect on the shelf life of bananas and a maximum content of chitosan is three percent. Addition of chitosan levels more than three percent to banana coatings does not increase its shelf life. This study shows that chitosan from carapace waste can be used for fruits coating.

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