

## PREPARATION AND ANTIFUNGAL ACTIVITIES OF CHITOSAN FROM APPLE-SNAIL (*Pila ampullacea*) SHELLS AS PRESERVING AGENT FOR POSTHARVEST PODANG MANGO (*Mangifera indica* L.) cv Podang

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**Abstract:** Podang mango, an endemic mango from Kediri, has a short postharvest shelf life that is accelerated by physiological factors and pathogenic fungal contamination. One of the commonly used strategies to preserve the shelf life of the fruit is by using the coating technique. Chitosan has been reported as an active compound for coating and it can be obtained from the apple-snail's shell. The purpose of this study was to prepare the chitosan from the shells of *Pila ampullacea* then examined antifungal activities against *Fusarium oxysporum* and *Aspergillus ochraceus*, and verified the effect of chitosan coating on shelf life and quality of Podang mango. The chitosan was prepared by transforming chitin in the snail shells to chitosan using demineralization, deproteinization, and deacetylation method. The effects of chitosan coating on fruit quality were analyzed by measuring the physical and chemical characteristics of fruit after coated with chitosan compare to the uncoated. The addition of gelatin was also tested to improve the effect of chitosan. Results showed that chitosan prepared from *P. ampullacea* had 53.56 degrees of deacetylation. Antifungal activity test of 2% chitosan had the highest inhibitory activity against *F.oxysporum* and *A.ochraceus* with 14.46 mm and 10.33 mm inhibition zone. Coating with chitosan statistically affected fruit quality for 14 days of storage (sig <0.05). Chitosan-gelatin coating is the best treatment in reducing weight loss (8.97%), maintain aroma (sweet fragrance), texture (soft), water content (84%), and vitamin C (0.182%), while the color (reddish yellow) was more influenced by chitosan coating. It can be concluded that chitosan is successfully synthesized from apple-snail shells and shows potential antifungal activities, hence, is recommended for application on Podang mangoes.

**Keywords:** chitosan; antifungal; podang mango; snail

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## 1. INTRODUCTION

Podang mango is one of the endemic plants of Kediri Regency which is one of Indonesia's export commodities (Baswarsiati & Yuniarti, 2016). The special characteristics of this mango are the sweet taste, sharp aroma, smooth fiber, reddish-yellow skin, and orange fruit flesh. The high water content of Podang mango causes a rapid rate of respiration and transpiration. Therefore, the physiological properties of the fruit are easily damaged (Pawar, 2018). That phenomenon has an impact on short shelf life after harvest, which is around 5-6 days at temperatures of 27-28 °C.

Another factor that causes decaying in Podang mangoes is microbial contamination. The research conducted by Widiastuti (Widiastuti et al., 2015) reported that the fungi *Lasiodiplodia* sp. and *Fusarium* sp causing rotten in mango fruit. Fatimah and Khot (Fatima & Khot, 2017) also succeeded in identifying 28 types of fungi infecting mangoes, the five most dominant species including *Actinodochium jenkinsii*, *Aspergillus nidulans*, *Aspergillus varicolor*, *Cladosporium herbarum*, and *Fusarium decemcellulare*. Amongst those fungi, the most frequently contaminating the mangoes are genus *Fusarium* and *Aspergillus* (Al-Najada & Al-Suabeyl, 2014). This previous study found *F. oxysporum* and *A. ochraceus* as spoilage fungi of Podang mangoes in the east java region.

Coatings using chitosan and modified chitosan are widely used methods to extend shelf life and maintain the quality of fruits and vegetables such as tomatoes (Sucharitha et al., 2018), banana (Lustriane et al., 2018), strawberry (Petriccione et al., 2015), and apple (Gardesh et al., 2016). According to (Jianglian & Shaoying, 2013), the ability of chitosan as a coating agent can be improved by modifying chitosan. The modification method that has been done is combining chitosan with organic compounds (essential oils, acids, waxes, ethanol), inorganic compounds (metals, nano-materials), biological compounds, heat treatments, hypobaric, gas fumigation, and integrated application of chitosan with modified atmosphere packaging.

Chitosan can be prepared from the chitin of shrimp, crab, fish scale, or snail shell. *Pila ampullacea* called apple-snail in English term (GBIF secretariat, 2021) is commonly cooked as a favorite traditional food in Java. However, its shell is only thrown away as waste with no further processing. According to Kaewboonruang et al., (2016) apple snail shell contains 42,56% chitosan. Therefore, in this study the apple-snail shell (*P. ampullacea*) was used to prepare chitosan using deproteinasi, demineralisasi, deastilasi and use it as a Podang mango coating. The ability of chitosan as a coating agent will also be compared with the chitosan-gelatin combination to determine its effectiveness in extending the shelf life and maintaining the quality of Podang mango.

## 2. METHODS

### 2.2 Time and location

This research was conducted from May to August 2019 using laboratory-scale experimental methods. Preparation and characterisation of chitosan were carried out in the Bioprocess Technology Laboratory, Biomaterial Research Centre, LIPI Cibinong. The isolation of spoilage

fungi of Podang Mangoes, antifungal activity, and fruit coating were tested in the Food and Beverage Analysis Laboratory, Health Science Institute of Bhakti Wiyata Kediri, East Java, Indonesia. The 20 samples of Podang mango for each treatment were taken from the original plantation in Tarokan, Kediri within the same maturity phase.

## 2.2 Materials

Pila shells were taken from the waste of snail food industry. The chemicals include Sodium hydroxide (NaOH), hydrochloric acid (HCl) acetic acid and ninhydrin reagent were from Merck. Potato dextrose agar was from Himedia, ketoconazole 2% was used as the positive control, and bovine gelatine as the plasticizer.

### 2.1 Preparation and Characterization of Chitosan

Preparation of *P. ampullacea* shell chitosan was carried out using the following three steps, including demineralization, deproteinization, and deacetylation (Ramadani & Ningrum, 2019). In the demineralization step, the shell powder was mix with HCl 1,5 M (ratio 1:15 %w/v) then heated at 60-70 °C while stirred at 50 rpm for 4 hours. Separate the filtrate and the precipitate using centrifugation and washed using distilled water until reached neutral pH. After that, dried the precipitate at 80 °C for 24 hours and the crude chitin was obtained. In the deproteinization step, the crude chitin was reacted by NaOH 3,5% (ratio 1:10 %w/v) followed by mixing with NaOH 50% (ratio 1:10 %w/v) at 100-110 °C while stirred at 50 rpm for 4 hours in the deacetylation step. The obtained Chitosan was characterized for its solubility using dilute acetic acid (2%), the ninhydrin test to observe the forming of the amide bond. The FT-IR analysis was used to determine the functional group and the degree of deacetylation (DD). Determination of DD was carried out using the baseline method, which includes absorbance values in the following equation (Antonino et al., 2017).

$$DD = 100 - [(A_{1655} / A_{3450}) \times 115]$$

where  $A_{1655}$  is the absorbance at  $1655 \text{ cm}^{-1}$  of the amide band showing the content of the N-acetyl group and  $A_{3450}$  is the absorbance of the hydroxyl group (-OH).

### 2.2 Antifungal Activity of Chitosan from *Pila ampullacea*

The antifungal ability of *Pila ampullacea* chitosan against *F.oxysporum* and *A.ochraceus* was tested using a disk diffusion method with various concentrations of 0.5%, 1%, 1.5%, and 2% chitosan diluted in 2% acetic acid solution with 3 replications. Ketoconazole 2% was used as a positive control and distilled water for the negative control. *F.oxysporum* and *A.ochraceus* suspension were spread on PDA then the disks that were previously soaked with chitosan suspensions were placed on the surface. Incubation to observe the response of fungal growth was carried out for 5 days at room temperature (Siddik et al., 2016). Antifungal activity was determined based on the diameter of the inhibition indicated by the clear zone around the disk.

### 2.3 Coating Podang Mango Using Chitosan

Podang mango was coated by the dipping method (Sharma et al., 2019). Three treatment groups, a 2% chitosan, a combination of chitosan-gelatin (3:1), and sterile distilled water as a negative control were used. Observations were undertaken for 14 days to observe the parameters of changes in weight, water content, vitamin C, color, aroma, and texture of the fruit (Samsi et al., 2019). Changes of color was examined using browning index that classified as : 1 = no browning, 2 = less than 20%, 3 = 20-40%, 4 = 40-60%, 5 = more than 60% (Priya et al., 2014).

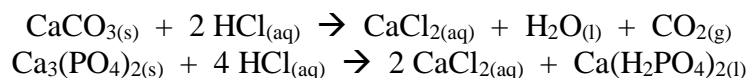
The variables of aroma and texture were scored by sensory evaluation using the seven-point hedonic scale (1= dislike very much to 7 = like very much) (Shyu et al., 2019). The results of each parameter were analyzed using the Manova test with advanced Duncan test and Chi-square test from minitab17.

### 3. RESULTS AND DISCUSSION

#### 3.1 Preparation and Characterization of Chitosan

This research used apple-snail shell as a basic material in chitosan synthesis. This species is redundant spread in paddy fields and becomes a pest for agriculture activities. But these animals are also widely consumed by Indonesians as local dishes. Consumption of the apple-snail body makes many side wastes like shell products thrown into the environment. The further impact is become pollutant of soil and water.

Studies by Kaewboonruang et al. (2016) reported that the content of chitosan in golden apple-snail shells was 42.56%. To prepared the chitosan from chitin in the apple-snail shells, the three stages method includes demineralization, deproteination, and deacetylation was employed. The demineralization stage using HCl 1.5 M to remove minerals in the form of CaCO<sub>3</sub> (calcium carbonate) and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (calcium phosphate) or other inorganic compounds contained in the shell. The reaction is initiated by an acid reagent such as HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, CH<sub>3</sub>COOH, and HCOOH, but the preferential reagent is HCl (hydrochloric acid) (Younes & Rinaudo, 2015). The reactions that occur at the demineralization stage are as follows:



The calcium chloride (CaCl<sub>2</sub>) formed is a salt that water-soluble so it can be easily separated by filtration of the chitin solid phase followed by washing using distilled water. The gas formed in this process was carbon dioxide (CO<sub>2</sub>). The demineralization stage using NaOH 3.5% solution causes bonding between the protein and chitin to form brownish-yellow crude chitin. The deproteination stage may be performed before or either after the demineralization step of the solid material (Younes & Rinaudo, 2015). Isolated chitin can be transformed into chitosan through the deacetylation reaction. At this reaction, a 50% NaOH reagent was used to break the bond between the carbon atoms in the carboxyl group and the nitrogen atoms in chitin. Several factors influence the deacetylation step including the type of alkaline reagent used, the concentration of alkali, temperature, reaction time, the use of successive baths, atmospheric conditions, and the use of sodium borohydride as a reducing agent. Chitosan obtained from this deacetylation process was a white bone powder. Overall, the results of chitin and chitosan isolation are shown in table 1 (Ramadani & Ningrum, 2019). This study obtained low biomass of chitosan (28.5%) compared to Kaewboonruang et al., (2016) with a stirring technique combined with heating. The difference in heating temperature is likely to be a determining factor for the amount of chitosan biomass produced from the deacetylation process. This is supported by Pambudi et al., (2018) that adjusting a high temperature will accelerate the degradation of the polymer in the deacetylation reaction. The temperature should be optimized up to 150 °C.

Table 1 Mass of chitin and chitosan of each step preparation from *Pila ampullacea* shell

Shell powder (g)	Demineralization (crude chitin)	% Crude chitin	Deproteination (chitin)	% Chitin	Deacetylation (chitosan)	% chitosan
110	50.8521 g	46.229	39.740 g	36.127	31.444 g	28.585

Source: primary data

The purpose of the solubility test was to confirm the formation of chitosan. Chitosan will dissolve in dilute acetic acid while chitin is insoluble due to the hydrogen bond formed between the carboxyl group from acetic acid and the amine group from chitosan. Factors affecting the level of solubility of chitosan are the degree of deacetylation (DD). The greater DD chitosan the higher the solubility and closely to be pure chitosan (He et al., 2016).

The ninhydrin test was used to prove the presence of primary amine groups in amino acids, protein derivatives, and chitosan. The reaction between ninhydrin and the primary amino group will form a purple complex called Ruhemann's purple (Boros et al., 2016). In this study, chitosan reacted with ninhydrin reagents gave positive results in the form of purple color. FT-IR spectroscopy test on chitosan aimed to analyze the deacetylation degree of chitosan. The results of chitosan and chitin can be seen in Figure 1.

The degree of deacetylation is a parameter that shows the low acetyl group in chitosan. The higher the DD value, the better the quality of chitosan. Chitosan obtained from this study had a DD of 53.86%. According to (Kaczmarek et al., 2019), polymers that have DD > 50% are called chitosan while those < 50% are called chitin. DD results were not too high due to the lack of optimal deacetylation reaction. DD can be adjusted by the elevated temperature of the reaction, extend the reaction time, and the most relevant increased alkali reagent concentration (NaOH). However, increment in alkali concentration means more pollution and serious corrosion (He et al., 2016).

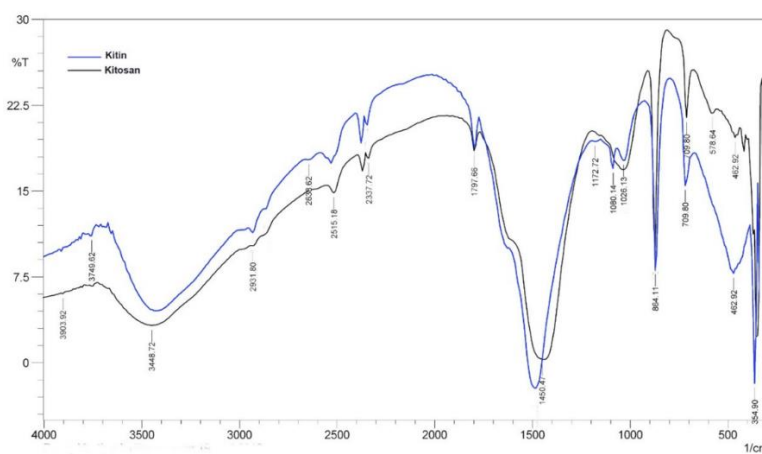


Figure 1 Chitosan IR Spectra (black) and Chitin (blue)

### 3.2 Antifungal activity of chitosan from *Pila ampullacea*

Species of fungi that were used in this research were isolated directly from rotten fruit after week of storage. *Fusarium* is a well-known genus that frequently contaminates postharvest fruits during the storage phase (Widiastuti et al., 2015). Reciprocally for *Aspergillus*, Sharma &

Verma, (2013) stated that this genus is capable to infect fresh fruit in postharvest time. Our study found that *F.oxysporum* infected Podang mango is new information as it commonly found in banana or Australian mango (Liew et al., 2016; Hasan & Zanuddin, 2018). The habitat of this fungus especially in the soil.

Antifungal activity of different coatings on the *F.oxysporum* and *A.ochraceus* growth tested by in vitro method is shown in Table 2. This data described that the chitosan alone from *P. ampullacea* shell has antifungal activity. Antifungal activity of chitosan was strengthened by the results in gelatin where it is proven that this plasticizer is not able to inhibit the growth of mango fruit rot fungi. The absence of the halo zone in gelatin was also reported by Lakshminarayanan et al., (2014). The loss of antifungal activity of gelatin as a justification to make a combination among chitosan-gelatin for coating application on fruit. Chitosan was able to inhibit the growth of *F. oxysporum* and *A.ochraceus* characterized by the presence of inhibitory zones. Chitosan concentration of 2% was the most effective in inhibiting fungi *F. oxysporum* (14.46 mm) and *A.ochraceus* (10.33 mm).

The inhibition zone of chitosan was more less than positive control caused by the characteristics of chitosan which didn't directly inhibit fungal growth. This result showed that chitosan tends to be a fungistatic than fungicidal (da Silva et al., 2018). Chitosan causes more damage to morphological tissue and changes the ultrastructure of the pathogenic fungus *F.oxysporum* and *A. ochraceus*. But, the changes more visible and proven in *F.oxysporum* (Lopez-Moya et al., 2019). Changes in fungal structure due to chitosan exposure include suppressing mycelia growth both radially and growth in liquid media, inhibiting sporulation and spore germination, hyphae structural abnormalities in the form of thickened, malformed (twisted rope-like structure), incomplete septae, and formation of inclusion bodies in the cytoplasm (Al-Hetar et al., 2010; Xing et al., 2016). The mechanism of chitosan antifungal activity through 3 mechanisms, (1) the interaction of the positive charge of the chitosan molecule with the negative charge of the surface of the fungi cell wall which is in the phospholipid layer causing damage to the fungi cell membrane, (2) the destruction of the cell membrane makes it easier for the chitosan molecule to enter the cells and interact with DNA then block the synthesis of mRNA and protein, (3) chitosan emits a chelating effect on the spore elements and nutrients needed by fungi (da Silva et al., 2018; Al-Hetar et al., 2010; Xing et al., 2016)

Table 2 Inhibition zone of *P. ampullacea* shell chitosan

Treatment group	Mean of inhibition zone (mm)	
	<i>F. oxysporum</i>	<i>A.ochraceus</i>
Control (-) : aquadest	6 ± 0.01 <sup>a</sup>	6 ± 0.01 <sup>a</sup>
Control (+) : ketoconazole 2%	22.1 ± 3.74 <sup>c</sup>	17.33±0.58 <sup>c</sup>
Gelatin 25%	6.08 ± 0.06 <sup>a</sup>	6.06± 0.04 <sup>a</sup>
Chitosan 0.5%	6.10 ± 0.11 <sup>a</sup>	6.04 ± 0.01 <sup>a</sup>
Chitosan 1%	6.12 ± 0.02 <sup>a</sup>	6.12 ± 0.02 <sup>a</sup>
Chitosan 1,5%	9.60 ± 1,31 <sup>a</sup>	6.19 ± 0.02 <sup>a</sup>
Chitosan 2%	14.46 ± 3,38 <sup>b</sup>	10.33±0.57 <sup>b</sup>

Remarks: \*Mean values within a column followed by the same letters are not significantly different at p < 0.05 according to Duncan's Multiple Range Test

The chitosan antifungal activity was determined by the degree of deacetylation and pH of chitosan and the concentration of chitosan tested (Shiekh et al., 2013). A high degree of deacetylation will provide a high resistance capability. The pH of chitosan determines the activity of cationic polymers as antifungals which will form at low pH (<6.5). This cationic activity firstly causes the inhibition mechanism. Chitosan concentration affects the number of chitosan molecules that are exposed to targeted fungi, the higher the concentration, the more chitosan molecules within a solution. However, the concentration of chitosan will not always show results that are directly proportional to the ability to inhibit fungi. Fungi can tolerate and form resistance to chitosan with a certain concentration. Research of Klerk et al., (2016) and da Silva et al., (2018) stated that *Aspergillus* group has resistance to chitosan. This is the scientific reason why the antifungal activity of apple-snail chitosan in *A. ochraceus* conducted in this study did not show highly inhibition zones as *F. oxysporum*. A significant inhibition zone of *A.ochraceus* began to appear at a concentration of 2% and only  $10.33 \pm 0.57$  mm in diameter. While the optimum concentration of *F.oxysporum* in the range of 1.5-2% chitosan with a diameter of  $9.6 \pm 1.31$  and  $14.46 \pm 3.38$  mm. This concentration was also reported as the optimum concentration to inhibit *Fusarium sp* from bananas (Martinez et al., 2016). *Aspergillus* spp., resistance occurs due to their natural characteristic structure of glucosamine and chitosan content in its cell walls so that it can reduce wall damage. Another factor is the ability of *Aspergillus* to produce the chitosanase enzyme. This enzyme can degrade and reduce chitosan toxicity to *Aspergillus* cells (da Silva et al., 2018). However, there are efforts that we can do to maximize the antifungal power against *A. ochraceus* that is incubating at low temperatures (below 8<sup>0</sup>C) or high temperatures (higher than 40<sup>0</sup>C). This fungus species proved unable to grow in such temperature range in culture media (Pandit et al., 2014).

### 3.3 Coating Podang Mango Using Chitosan

Chitosan 2% solution was used as a coating agent on Podang mangoes because it showed maximum inhibition to *F. oxysporum* and *A.ochraceus*. Chitosan was applied to mangoes by dipping method. The dipping method was chosen because it is easiest to coat fruit and vegetables, broadly used for fleshy fruit, get the same surface thickness, reach all surface folds, and avoid missing uncoated certain surfaces of fruit skin (Sharma & Chaudhary, 2019).

Table 3 Effect of chitosan coating to quality of podang mangoes

Treatment	Mean		
	colour	texture	aroma
control (-)	5.2±0.7 <sup>a</sup>	4.2±0.9 <sup>ab</sup>	4.4±0.08 <sup>ac</sup>
chitosan	4.7±0.3 <sup>ab</sup>	3.0±0.5 <sup>b</sup>	3.8±0.9 <sup>b</sup>
chitosan-gelatin	4.1±0.7 <sup>c</sup>	2.8±0.2 <sup>c</sup>	2.5±0.4 <sup>c</sup>

Remarks: \*Mean values within a column followed by the same letters are not significantly different at  $p < 0.05$  according to Duncan's Multiple Range Test; The number in colour, texture, and aroma showed quantification scale (see the method).

Observation of fruit quality parameters was carried out for 14 days using browning index for colour parameter also used hedonic scale for texture and aroma. Statistical test results stated

that coating treatment and storage time affected the color, texture, aroma, and weight of Podang mango (Table 3). This statistical test was also applied to the combination of the two treatments. The color was more influenced by the chitosan-gelatin coating treatment and the changes were most visible on 3 to 10 days. Texture parameters were sensitive to the treatment of chitosan and chitosan-gelatin coating (Table 3). In addition to texture, the parameters responding to both treatments were the aroma and weight of the fruit. Changes caused by the treatment on fruit texture appeared during storage on day 3, 4, 5, 6, 9, 12, 13, 14. The aroma of mango began to change at the time of storage on day 4 and 7. While the weight of the fruit began to change on the first day.

Changes in fruit weight in each treatment had a different trend (Figure 2). The greatest decrease in fruit weight on negative control (20.17%) was followed by 2% chitosan coating treatment (17.15%). Chitosan-gelatin coating treatment prevented the most effective weight loss with a percentage of 8.97%.

The highest decrease in water content also occurred in the negative control (28.14%) followed by 2% chitosan coating treatment (19.94%) as described in Figure 3. Chitosan-gelatin coating gave the most effective prevention on water content increasing with a percentage of 17.28%. Shyu et al., (2019) also showed that combination of chitosan and fish gelatin treatment gives a slight impact change in apple weight loss. The evaporation stimulated by water vapor pressure gradient inside the fruit flesh as a respiration activity induces weight loss. The respiration process is closely related to the ripening stage on mangoes. Paul & Pandey, (2014) state that mangoes are classified as climacteric fruit, when they reach the ripening phase, there a significant increase in respiration rate and followed by rising ethylene production. This is also has contributed to carbon release to the atmosphere in CO<sub>2</sub> gases.

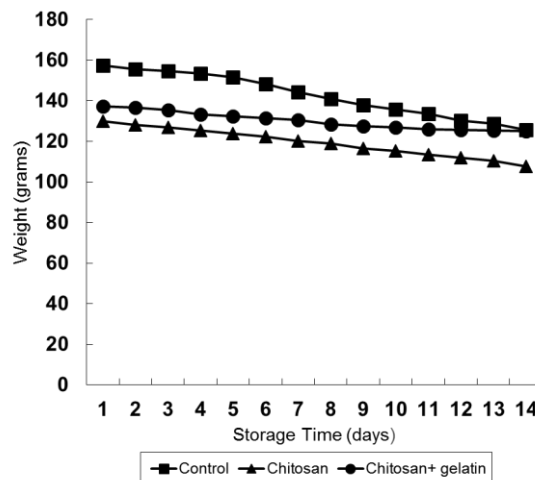


Figure 2. Weight loss of podang mangoes from control, chitosan 2%, and chitosan+gelatin treatment

The vitamin C level test showed that negative control has the greatest decrease (0.055%) followed by 2% chitosan coating treatment (0.034%). The lowest decrease in vitamin C level occurred in the chitosan-gelatin coating treatment with a percentage of 0.021% (Figure 3). Decreasing of vitamin C content in Podang mango is not much different for each test group also



reported by Shyu et al., (2019). This is a manifest of organic acid decreasing and increasing respiration rate following the senescence of fruit (Shyu et al., 2019; Song et al., 2013).

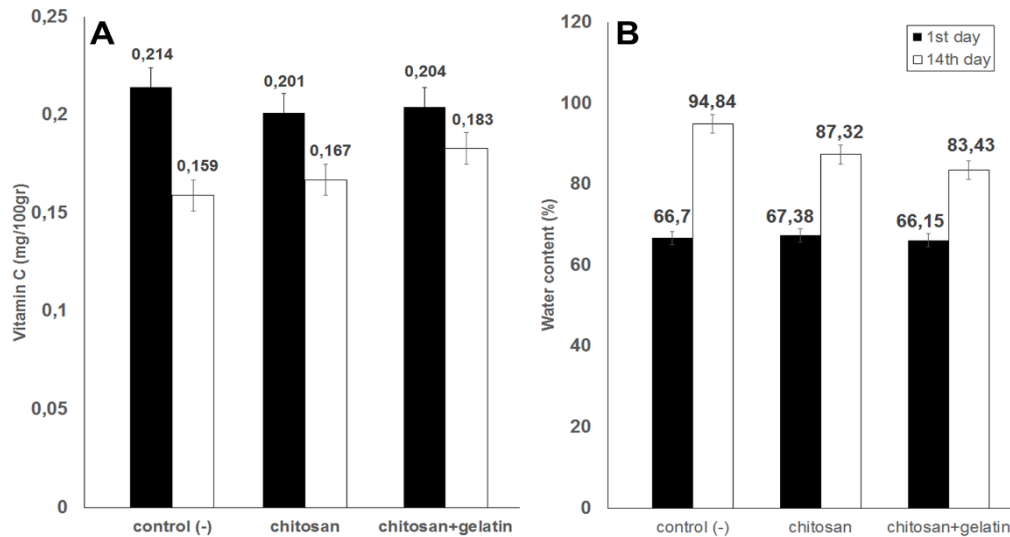


Figure 3. Percentage of vitamin C (a) and water content (b) in podang mango for 1<sup>st</sup> day storage and after 14<sup>th</sup> day storage

Based on observations for 14 days the results of the ripe Podang mango give a darker appearance of the fruit skin (originally greenish-yellow to dark yellow), the texture of the fruit skin is increasingly shriveled, the fruit aroma is sharper, the mass and levels of vitamin C are reduced and levels the water content is increasing. According to (Appiah et al., 2011), during the ripening process, carbohydrates will be hydrolyzed into fructose, sucrose, and glucose so that it will increase the osmotic pressure from peel to the pulp. As a result, the texture of the Podang mango skin will wrinkle (the texture begins to shriveled) and the water content increases. High water content can dissolve various types of substances, such as sugar, which greatly affect the taste and aroma of Podang mangoes. Therefore, the higher the level of fruit maturity, the fruit aroma will be sharper and the fruit tastes sweeter. Sugar levels in Podang mangoes are inversely proportional to vitamin C levels, where the higher the sugar content, the lower the vitamin C level.

Increased osmotic pressure from peel to pulp will also result in plasmolysis so that the integrity of the cell membrane will be lost or damaged. Damage to the integrity of the cell membrane will affect the decrease in fruit mass and trigger browning (Shyu et al., 2019). Browning is an oxidation process in phenols whose effects can kill several cells. This is the reason why fruits or vegetables that are more mature (ripe) become increasingly shriveled.

The chitosan can be used as a coating agent in maintaining the quality of Podang mangoes. Sucharitha et al., (2018) noted that when a mango was coated with chitosan, a semi-permeable film will be formed which can modify the surrounding internal tissue so that the ripening process can be delayed. Chitosan is also able to inhibit the evaporation of water levels, O<sub>2</sub>, and CO<sub>2</sub> exchange which can affect the levels of vitamins and minerals in fruit. Antimicrobial activity possessed by chitosan is also able to protect the fruit from microbial contamination (fungi or bacteria) that cause rot. (Tezotto-Uliana et al., 2014) also proved that chitosan can reduce respiration and ethylene production, so extend their postharvest life.

Coating using the chitosan-gelatin combination was more effective in maintaining the quality of the Podang mango than the 2% chitosan. The composition of this material was similar to the study of Poverenov et al., (2014) which blended chitosan and gelatin as the most effective coating to inhibit the growth of microbes in *Cucumis melo*. This is because coating using a combination of chitosan-gelatin produces a thicker film layer than coating using chitosan without gelatin (Shyu et al., 2019). Gelatin plays a vital role to cover all the surface skin of mango to reinforce its function as a major barrier of gaseous exchange for horticulture fruit (Paul and Pandey, 2014). In addition, gelatin as a plasticizer is also able to improve mechanical and barrier properties of the resulting films (Ramos et al., 2016) so that the film's ability to protect the fruit against factors that can affect fruit quality during storage is also greater.

#### 4. CONCLUSION

Chitosan is successfully prepared from apple-snail shells (*P. ampullaceae*) by demineralization, deproteinization, and deacetylation methods. The prepared chitosan has an antifungal activity against *F. oxysporum* and *A. ochraceus*. Moreover, it showed the potential to preserve the quality of Podang manggo in the term of shelf life, weight loss, texture, color, and aroma. The addition of gelatin increases the effectiveness of chitosan as a coating agent. Based on these results, the chitosan prepared from apple-snail are potential to be used as a coating agent for the preservation of postharvest Podang mangoes.

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