IN VITRO TEST OF GASTROPOD SHELL CHITOSAN ON SAVING PERIOD AND QUALITY OF GREEN APPLE FRUIT (*Pyrus malus* L.)

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Abstract

Green apple can rot for 30 days t room temperature storage, it is necessary to use gastropod shell chitosan, which can be a natural preservative. The objectives of this study were to determine the shelf life of green apple (Pyrus malus L.) using gastropod shell chitosan and to know how the effect of variations of chitosan concentration on the shelf life and quality of green apple (Pyrus malus L.) (texture, aroma, & color) and shrinkage weight. The research method used in this study was true experimental by making variations in the solution of gastropod shell chitosan and coating of green apple. The process of making chitosan was carried out in several stages, namely deproteination, demineralization, depigmentation, and chitin deacetylation. Dyeing of green apple was done by varying the concentration of chitosan 500 ppm, 600 ppm, 700 ppm, and control solutions. This study used descriptive qualitative and quantitative data analysis. The results of this study showed that the average shrinkage weight of green apples in the variation of control concentration of 15.89% could last for 30 days, a concentration of 500 ppm of 31.74% could last for 55 days, at a concentration of 600 ppm of 32.82% could last for 60 days, and at a concentration of 700 ppm of 49.76% could last for 65 days and the variation of the best concentration of chitosan was concentration of 700 ppm which can extend the fruit shelf life.

Keywords: *Green apple, Gastropod shell chitosan, Shelf life, organoleptic*

1. INTRODUCTION

Apples (*Pyrus malus* L.) are in great demand by both children and adults. Apples are widely distributed throughout the world because they have a refreshing taste. This fruit has an essential value in the economy and good nutritional content for health (Soelarso, 1997). The increasing public knowledge of the benefits of apples for health is one of the reasons for the high demand for apples in the community. Apples *(Pyrus malus* L.) have a reasonably high water content, namely 84.1% water for every 100 grams. High water content causes apples (*Pyrus malus* L.) to rot quickly. The length of storage of apples (results in physiological damage and a decrease in quality caused by the influence of cold storage temperatures above freezing (Sihombing, 2015). Muchtadi and Sugiyono (1989) said that the fruit's storage at low temperatures (0-10°C) could be damaged because it cannot carry out normal metabolic processes. Therefore, efforts are needed to prevent rot or damage to apples by using

preservatives. The preservation method used to extend the shelf life of apples so far is by adding the chemical benzoic acid (Oktoviana *et al.,* 2012). World Food Agency (FAO), consuming fruit that contains sodium benzoate compounds in excess can cause stomach cramps numbness in the mouth for those who are tired or have skin rashes such as urticaria and eczema (Mirnawati, 2016). Consuming sodium benzoate preservative in the long term can cause lupus, cancer, and neurological diseases (Hilda, 2015).

The use of synthetic chemicals as preservatives in fruit has been prohibited because it can cause side effects to health for consumers. Therefore, it is necessary to use natural ingredients that are more effective in preventing spoilage in apples than synthetic chemicals (Sappers and Miller, 1992). One of the natural ingredients used is gastropod shell chitosan. Chitosan is antibacterial (Umarudin *et al.,* 2019). Chitosan contains the enzyme lysozyme and polysaccharide amino groups that can inhibit microbial growth. The positively charged cluster (polycation) can bind bacteria and molds that are negatively charged to inhibit the growth of bacteria and molds (Wardaniati & Setianingsih, 2014). Therefore, there is a need for further research gastropod shell chitosan as a natural preservative.

2. LITERATURE REVIEW

1.1 Snail

The snail comes from East Africa, which is the original habitat of the snail (Rukmana & Yuniarsih, 2001). Snail is one of the land snails with a shell. The shell serves to protect the mollusk group of animals from predators. The shell length of the adult snail is about 90 mm. There are a pair of short and long tentacles on the snail's head. Short tentacles function as a sense of smell. Long tentacles serve as a sense of sight. Snails can typically live about 3 years. Snail has a brown shell with stripes and a slender shell shape.

2.2 Chitosan

Chitosan is an insoluble compound in water, alkaline solid solution, slightly soluble in HCl and HNO_3, and H_3PO_4, insoluble in H_2SO_4 (Rosida *et al.,* 2018). Chitosan, also called -1,4-2 amino-2-dioxy-D-glucose, is a multifunctional polymer because it contains three functional groups, namely amino acids primary and secondary hydroxyl groups. The presence of this active group causes chitosan to have high chemical reactivity.

2.3 Apple

Apples (*Pyrus malus* L.) can thrive in areas that have cold air temperatures. This plant in Europe is cultivated mainly in the northern subtropics. Meanwhile, local apples in Indonesia are famous for coming from Malang, East Java, and Mount Pangrango, West Java. In Indonesia, apples can grow and develop well if cultivated in an area with an altitude of about 1200 meters above sea level. Apple plants have tree trunks that can reach 7-10 meters high.

3. RESEARCH METHODS

Apples was obtained from Malang City, East Java, and snail shells from Doho Village, Kediri City, East Java. This research design uses experimental research (true experimental) The study isolated chitin from snail shells through several stages (deproteination, demineralization, and depigmentation) and continued with chitosan. Chitosan was applied to apples (*Pyrus malus* L.) randomly. The sample is good for the group studied (experimented and the control group). Then the two groups was carried out preliminary observations (pretest), namely measurement of weight loss, aroma, color, and texture on green apples (Pyrus malus L.), 600 ppm, and 700 ppm) for 15 minutes and dried, while the control sample was not given chitosan solution. The samples were observed and measured organoleptically (texture, aroma, & color) and shrinkage weight. After a few days, the control and experimental models were subjected to a final observation (posttest) and then compared with the initial observation results (pretest). Then the pretest results were compared between concentrations of 500 ppm, 600

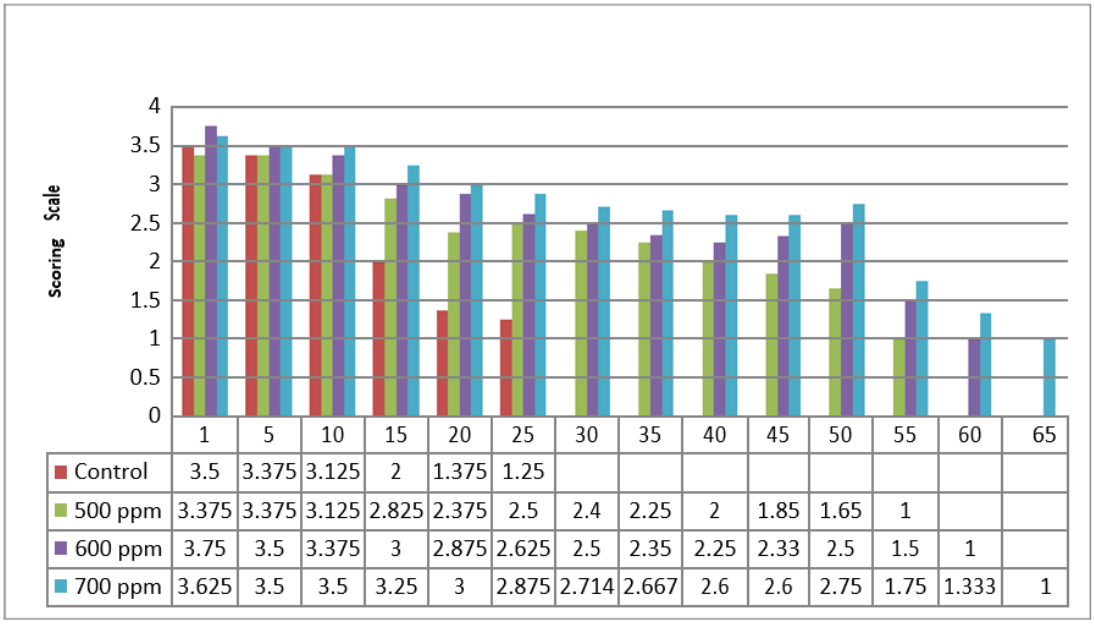
ppm, and 700 ppm. The comparison of attention can be seen by observing organoleptically, including texture, aroma, & color. The respondents' inclusion criteria for the organoleptic test included not being color blind, in good health (not sick), not deaf, and able to write. In quantitative research, data processing is generally carried out through three stages: the editing stage, the coding process, and the tabulating process. The shrinkage weight result is expressed in per cent is calculated by the equation:

$$\text{shrinkage weight} = \frac{W0-W1}{WO} \times 100$$

Information :
W0 = initial shrinkage of fruit (grams)
W1 = fruit shrinkage on day-n (grams)

4. RESULTS AND DISCUSSION

The shelf life of food products is critical information for consumers. Shelf life information is very important related to food product safety and provides quality assurance when the product reaches consumers. with the addition of gastropod shell chitosan with various concentrations can prolong the shelf life of green apples. Organoleptic testing was carried out to ensure good fruit quality. This test is carried out by the hedonic rating method. The selection of the hedonic rating method is a method to find out the most preferred product by the panelists (Tarwendah, 2017). 8 panelists carried out this test to assess green apples' texture, aroma, color and shrinkage weight. This observation was carried out to test organoleptic conducted for 65 days with a range of statements for 5 days observed with the same person. The following is an organoleptic result of texture, aroma, color and shrinkage weight. The following are the organoleptic results of green apples after being given gastropod shell chitosan by 8 panelists. Effect of variations in chitosan concentration on color of green apple.

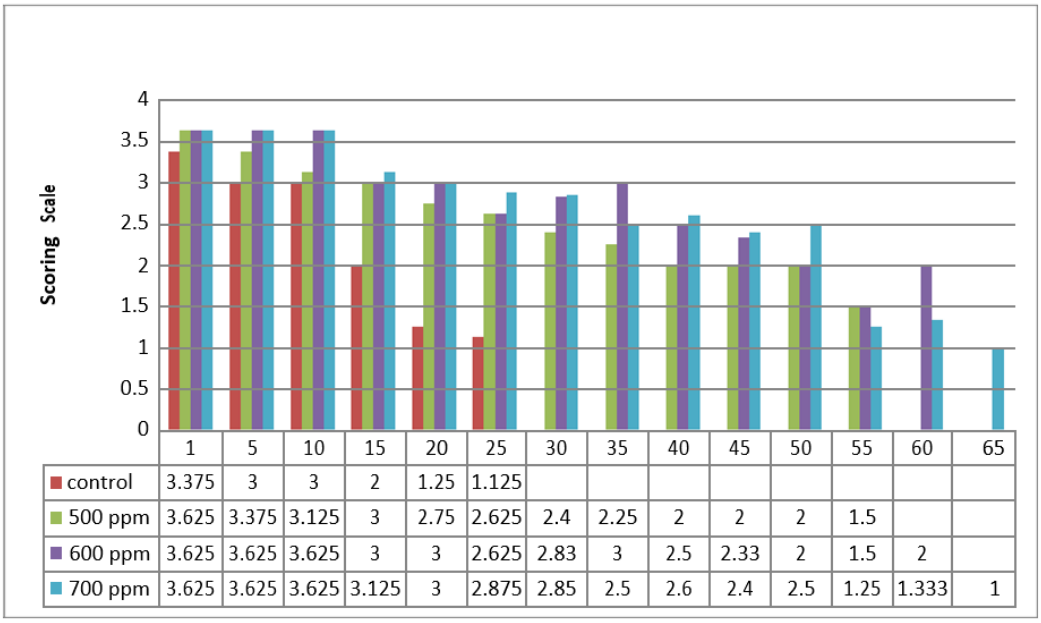


	1	5	10	15	20	25	30	35	40	45	50	55	60	65
Control	3.5	3.375	3.125	2	1.375	1.25								
500 ppm	3.375	3.375	3.125	2.825	2.375	2.5	2.4	2.25	2	1.85	1.65	1		
600 ppm	3.75	3.5	3.375	3	2.875	2.625	2.5	2.35	2.25	2.33	2.5	1.5	1	
700 ppm	3.625	3.5	3.5	3.25	3	2.875	2.714	2.667	2.6	2.6	2.75	1.75	1.333	1

Fig 1. Organoleptic value of green apple fruit green apple color

Information
Control : No chitosan coating
500-700 ppm : Coating with chitosan

Color organoleptic value of 700 ppm concentration on the 30th day was not accepted, the 600 ppm concentration on the 25th day was not accepted, the 500 ppm concentration on the 20th day was not accepted. In contrast, the panelists did not receive the control on the 15th day. This shows that the color with the most extended decreasing value is a concentration of 700 ppm. The results of organoleptic values indicate the level of preference of the panelists. In the control group that was not given gastropod shell chitosan changed color to brown to black more quickly because the control group experienced the synthesis of certain pigments such as carotenoids and flavonoids, in addition to chlorophyll degradation. Chlorophyll reshuffle causes carotenoid stains that already exist but are not natural to be visible (Nurdjannah, 2014). In the group of apples given chitosan, the snail shell provided a protective layer. Gastropod shell chitosan can extend the post-harvest storage period of fruit, especially apples, and significantly contributes to overcoming the shelf life of perishable fruit. Effect of variations in chitosan concentration on texture of green apple.

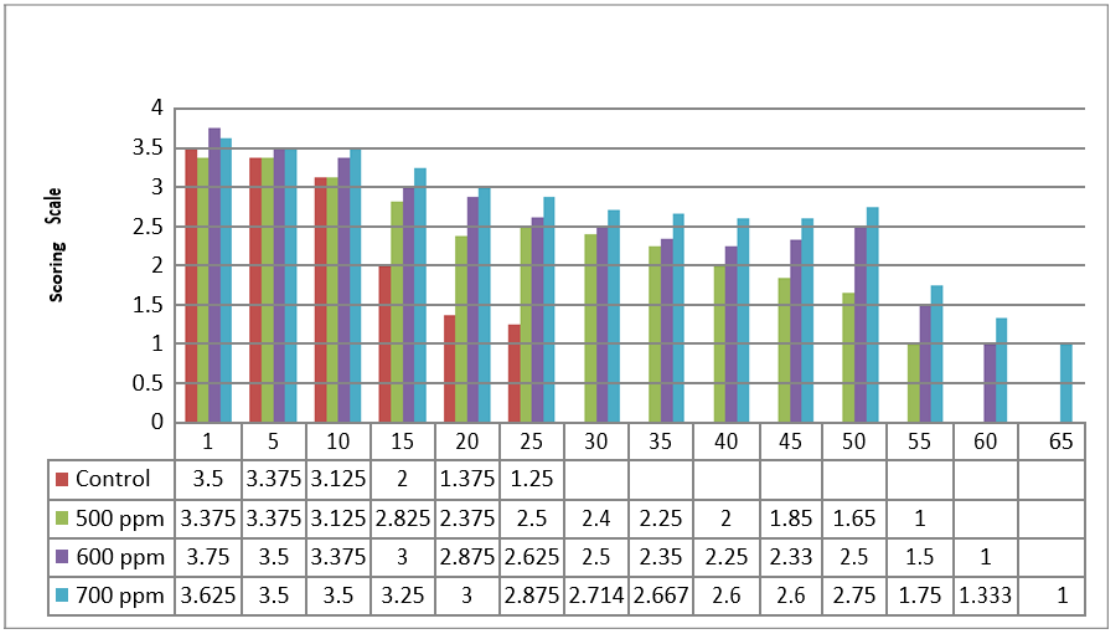


	1	5	10	15	20	25	30	35	40	45	50	55	60	65
control	3.375	3	3	2	1.25	1.125								
500 ppm	3.625	3.375	3.125	3	2.75	2.625	2.4	2.25	2	2	2	1.5		
600 ppm	3.625	3.625	3.625	3	3	2.625	2.83	3	2.5	2.33	2	1.5	2	
700 ppm	3.625	3.625	3.625	3.125	3	2.875	2.85	2.5	2.6	2.4	2.5	1.25	1.333	1

Fig 2. Organoleptic value of green apple fruit green apple texture

Information
Control : No chitosan coating
500-700 ppm : Coating with chitosan

The texture organoleptic value of 700 ppm concentration on day 25 was not accepted, the concentration of 600 ppm on day 25 was not obtained, the concentration of 500 ppm on day 15 was not accepted. In contrast, the panelists did not receive the control on day 10. The skin texture of green apples in the control group softens faster due to water loss which causes the cell wall composition to change, causing a decrease in cell turgor pressure (Nurdjannah, 2014). Apples given snail shell chitosan can form a layer that prevents changes in the composition of the cell walls in green apples.. Effect of variations in chitosan concentration on aroma of green apple.



	1	5	10	15	20	25	30	35	40	45	50	55	60	65
Control	3.5	3.375	3.125	2	1.375	1.25								
500 ppm	3.375	3.375	3.125	2.825	2.375	2.5	2.4	2.25	2	1.85	1.65	1		
600 ppm	3.75	3.5	3.375	3	2.875	2.625	2.5	2.35	2.25	2.33	2.5	1.5	1	
700 ppm	3.625	3.5	3.5	3.25	3	2.875	2.714	2.667	2.6	2.6	2.75	1.75	1.333	1

Fig 3. Organoleptic value of green apple fruit green apple aroma

Information
Control : No chitosan coating
500-700 ppm : Coating with chitosan

The organoleptic value of aroma concentration of 700 ppm on the 25th day was not accepted. The 600 ppm concentration on the 20th day was not obtained. The 500 ppm concentration on the 15th day was not carried. In contrast, the panelists did not receive the control on the 15th day. This shows that the aroma value decreasing the longest is the concentration of 700 ppm. This rotting of apples is caused by a fungus that causes the aroma of apples to be unpleasant and the distinctive aroma of apples to be damaged. Effect of Variations in Chitosan Concentration on Shelf Life and shrinkage weight of Green Apple.

Table 1. Percentage of shrinkage weight of Green Apple Fruit

Percentage Of Shrinkage Weight Of Green Apple				
day	Chitosan Concentration			
	control	500%	600%	700%
	% shrinkage	% shrinkage	% shrinkage	% shrinkage
1	0.00	0.00	0.00	0.00
5	2.64	3.13	2.49	3.17
10	4.07	4.24	3.65	4.44
15	8.15	7.76	10.20	11.93
20	10.99	10.44	10.35	15.27
25		12.11	11.82	17.20
30		13.76	11.97	17.93
35		14.87	12.83	18.49
40		16.42	14.24	18.85
45		19.16	14.68	20.54
50		20.34	17.29	21.87
55		31.74	20.38	26.48
60			32.82	37.97
65				49.76

Information
Control : No chitosan coating

500-700 ppm : Coating with chitosan

The table shows that the weight loss of green apples that showed the most decreased weight loss were the control concentration of 15.89%, 500 ppm of 31.74%, and 600 ppm of 32.82%, and the concentration of 700 ppm of 49. 76%. Chitosan solutions of 500 ppm, 600 ppm, and 700 ppm can maintain weight loss because chitosan can form a film layer in the tissue (Rosida et al., 2018). This decrease in weight loss occurs due to respiration and transpiration in the fruit (Kusumiyati *et al.,* 2017). Nur'aini and Apriyani (2015), chitosan can inhibit the rate of respiration and transpiration, which can reduce fruit quality and affects shelf life. The higher concentration of gastropod shell chitosan given to apples could affect the storage time of green apples compared to controls without gastropod shell chitosan. Green apples have a short shelf life due to green apples

Table 2. Shelf life of green apples (*Pyrus malus* L.)

Consentration	Shelf life (day)
Control	30
500 ppm	55
600 ppm	60
700 ppm	65

Information :
Control : No chitosan coating
500-700 ppm : Coating with chitosan

The table shows that the most extended shelf life of green apples is at a concentration of 700 ppm for 65 days, 600 ppm for 60 days, 500 ppm for 55, and a control concentration of 30 days. Green apples (*Pyrus malus* L.) have a short shelf life of green apples because they have a high enough water content that can accelerate the decay process. Therefore, to prolong the shelf life, use a natural preservative gastropod shell chitosan. The results showed that green apples treated with gastropod shell chitosan could prolong the shelf life of 55-65 days compared to control without chitosan for 30 days based on quality parameters texture, aroma, color and shrinkage weight. This is because snail shell chitosan can maintain fruit quality. After all, chitosan has an amine group that acts as an anti-microbial so that it can prolong the shelf life of the fruit (Sarwono, 2010). Illustration of the mechanism of chitosan against bacteria in fig 2.

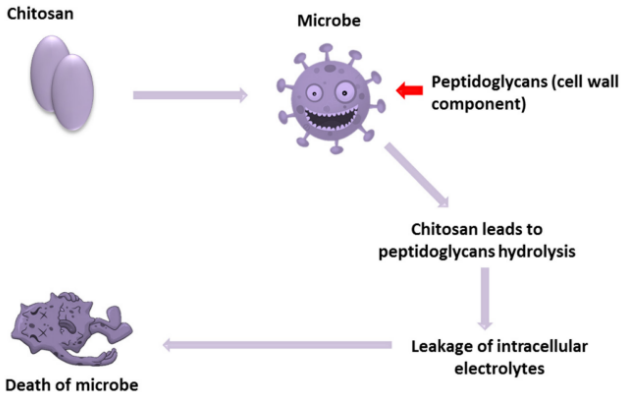


Fig 4. Chitosan antimicrobial mechanism illustration (El-Hack *et al*., 2020)

Gastropod shell chitosan can be applied to green apples (*Pyrus malus* L.) as a natural preservative. Nur'aini and Apriyani (2015), the shelf life of duku (*Lansium domesticum*) is 6 days using 1.5% chitosan in 30 seconds of immersion. This means that chitosan can inhibit the growth of bacteria and fungi. The mechanism of chitosan compounds can kill bacteria by damaging cell membranes (Hui, 2004). Because chitosan has an amine functional group ($–NH_2$) that is positively charged, it can bind to the negatively charged bacterial cell wall. This bonding occurs at electronegative sites on the surface of the bacterial cell wall. In addition, because -NH_2 also has a lone pair of electrons, this group can attract Ca^{2+} minerals found in the bacterial cell wall by forming a coordinating covalent bond. Gram-negative bacteria with lipopolysaccharide in the outer layer have a negative pole that is very sensitive to chitosan. Then the cell membrane undergoes lysis to inhibit metabolic activity and eventually die.

CONCLUSION

In this research. The results of this study showed that the average shrinkage weight of green apples (*Pyrus malus* L.) in the variation of control concentration of 15.89% could last for 30 days, a concentration of 500 ppm of 31.74% could last for 55 days, at a concentration of 600 ppm of 32.82% could last for 60 days, and at a concentration of 700 ppm of 49.76% could last for 65 days and the variation of the best concentration of chitosan was concentration of 700 ppm which can extend the fruit shelf life.

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