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Chitosan treatment and storage temperature in the retardation of fruit ripening of red guava (*Psidium guajava*)

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Abstract. *Anggarwulan E, Mudyantini W, Asiyah IJ. 2015. Chitosan treatment and storage temperature in the retardation of fruit ripening of red guava (*Psidium guajava*). Nusantara Bioscience 7: 153-159.* Guava (*Psidium guajava* L.) is a thin-skinned tropical fruit which is easy to ripe and rot. The objective of the study is to determine the effect of chitosan coating and storage temperature in restraining the ripening of guava and also to the combination of treatments which could be longest to lengthen its storage period. This study uses Completely Randomized Design, with two factors. The first factor is chitosan concentration of 0, 1, 2, and 3%, while the second factor is the storage temperature in 16°C and 28°C with three repetitions during four weeks. The quality of *Psidium guajava* which is observed during the storage is weight-loss, water content, fruit texture, reducing sugar content, vitamin C content, pigment rind (chlorophyll and carotenoid), respiration rate, and ethylene content. The treatment of chitosan concentration and the storage temperature of 16ºC are able to maintain the fruit hardness, vitamin C content, and chlorophyll content; meanwhile, it shows a decrease in weight-loss, reducing sugar, respiration rate, and ethylene content. The combination of chitosan treatment of 3% with a temperature of 16°C is able to maintain the quality and to lengthen the storage period of *Psidium guajava* up to four weeks.

Keywords: *Psidium guajava*, chitosan, temperature, fruit ripening, storage period

INTRODUCTION

Guava (*Psidium guajava* L.) is a fruit plant that belongs to the family of Myrtaceae. The fruit is round to oval with a size of about 5-10 cm and weighs about 50-300 g. Several cultivars have distinctive taste and flesh color. The fruit is high nutritional because its vitamin C content (50-300 mg/100g of fresh fruit) is 3-6 times higher than an orange (Mercadante et al. 1999). Besides, guava's flesh has high activity of antioxidant (Musa et al. 2010) because it contains many forms of carotenoid, namely *phytofluene, rubixanthin, β-carotene, β-cryptoxanthin, lycopene* and *lutein* (Thaipong et al. 2006).

The flesh of guava belongs to climacteric fruit. It means that the fruit will easily damage after it is kept for 3-5 days in a room temperature (Kumar et al. 2012). Some efforts which can be done to keep the fruit's quality are; cold storage, controlled atmosphere, the use of coating agent, and chemical substance. The temperature during the storage greatly affects the process of the fruit's physiology. High temperature will accelerate respiration process which will also speed up the fruit's damage. Cooling or storing at cold temperature is aimed at slow down respiration, minimizing microorganism attack, and water loss. Fruit storage at cold temperature is an effective and efficient effort because it will reduce respiration by restraining enzyme reaction.

The use of fruit coating agent will also slow down fruit-ripening or lengthen fruit storage period. One of the fruit coating agents which currently has a promising prospect is chitosan. Chitosan is polysaccharide derived from shrimp-shell waste which has pretty good potency as fruit coating agent. Some studies show that chitosan has potency as fruit coating agent because of its character to form film, antimicrobial, and biodegradable (Bourtoom 2008). Chitosan is a derivative product of chitin which has been widely used as thickeners, binders, stabilizers, texture forming, and gel forming. The ability of chitosan in slow down bacterial growth, molds, and yeasts can be applied as preservatives and coating agent on food products (edible coating).

Hong et al. (2012) have studied chitosan application in guava using concentration variation of 0.5, 1, and 2% which was kept at the temperature of 11°C and humidity of 90-95%. The result of the study shows that chitosan concentration of 2% is able to lower the activities of some enzymes. Based on the study, it is necessary to observe the effect of chitosan concentration and storage temperature adjusted to room temperature at the tropical area (28ºC) and refrigerator temperature (16ºC).

This study aims at knowing the effect of chitosan and storage temperature in restraining red guava's ripening. The concentration of chitosan used is 0, 1, 2, and 3%, the storage temperature are 28°C and 16°C with three repetitions for four weeks. The quality of red guava observed during the storage is weight loss, water content, fruit texture, reducing sugar content, vitamin C content, pigment rind (chlorophyll and carotenoid), respiration and ethylene content.

MATERIALS AND METHODS

Plants materials

The material used in this study includes red guava fruit (*Psidium guajava* L) aged 1.5 months after flowering, weighs about 200-300 g and free of fruit disease. The fruit is obtained from guava fruit plantation at Kemuning Village of Ngargoyoso Subdistrict in Karanganyar District, Central Java, Indonesia.

The making of chitosan edible coating

Edible coating of chitosan 1% is made by dissolving 1g of chitosan in 100 mL of acetic acid 1%. The treatment is given by dipping the guava fruit into the chitosan solution with concentration of 1, 2, and 3% for 10 minutes in room temperature. The fruit which has been given chitosan treatment is wind-dried at room temperature, until chitosan forms film and then kept in refrigerator room (16°C) and room temperature (28°C) for four weeks.

The measurement of weight loss

The measurement of weight loss is taken out for four weeks. The decreasing of the weight loss is stated in percent.

The measurement of water content

Water content is the same as wet-weight substracted by dry-weight, divided by wet-weight and multiplied by 100% (Sudarmadji et al. 1976). The measurement is taken out every week for four weeks.

The measurement of the fruit texture

Guava fruit is placed just under Electric Penetrometer needle BI-235. The testing is repeated in five different points and then taken its average (Trenggono 1992; Paramita 2010).

The measurement of reducing sugar content

Sugar content is analyzed using DNS method. A total of 10mg anhydrate glucose is dissolved in 10 mL of aquadest. This solution is used as a stock. A standard solution is made with concentration of 0.2-1 mg/mL. a total of 10g softened fruit is dissolved in 100 mL of aquadest. The solution is taken 1 mL and the added by 2 mL DNS reagent of 1%, and then vortexed. The next step is that it is put into boiling water for five minutes, and then cools down in the water. Rochelle salt of 1 mL is added into the reaction tube to be measured its absorbance at a wavelength of 540nm with spectrophotometer UV Vis Lamba 25 Perkin Elmer (AOAC 2005).

The measurement of vitamin C content

The main solution of vitamin C of 100 ppm is made by weighing ascorbic acid of 50 mg and then put into 500 mL flask and dissolved into aquabidest up to the limit sign. The calibration curve is made of vitamin C 100 ppm solution in the pipette and then put into the flask, each for 2, 4, 6 and 8 mL (4, 8, 12, 16 ppm). Then, add 50 mL of aquabidest which is then homogenized, and measure its absorbance at a wavelength of 265 nm. A total of 5g samples are mashed. Its filtrate is put into the flask and then adds 10 mL of aquabidest and the homogenized (Wardani 2012). The measurement of vitamin C content of the sample of guava fruit is measured with spectrophotometer UV Vis Lambda 25 Perkin Elmer at a wavelength of 265 nm (Monalisa et al. 2013).

The measurement of pigment rind

The fruit pigment is calculated using spectrophotometric method by Hendry and Grime (1993). A total of 1g sample is dissolved into 10 mL of acetone 80%, then filtered, and measured its absorbance at a wavelength of 480, 645, and 663 nm using spectrophotometer UV Vis Lambda 25 Perkin Elmer.

The value of chlorophyll and carotenoid content is calculated by:

Total clorophyll mg/g fruit weight
$= 8{,}02 \times A663 + 20{,}2 \times A645 \times 10^{-1}$ [1]

Carotenoid µmol/g fruit weight

$$= \frac{A480 + 0{,}114 \times A663 - 0{,}638 \times A645 \times V \times 10^{3}}{112{,}5 \times 0{,}1 \times 10}$$ [2]

V = extract volume

The measurement of oxygen content

The fruit which has been coated by chitosan is put into a jar and closed until there is no air from outside flows into the jar. The upper part of the jar is equipped with a valve to put the probe of oxygen meter. A total sample of 0.5kg is put into the jar. The sealed jar is put into the fridge according to the treatment. Oxygen concentration is measured with oxygen meter Lutron 5510. The measurement of O_2 gas is stopped when the measurement has been stable (Arda 2010).

The measurement of ethylene content

The ethylene hormone rate is analyzed in the first week by taking gas sample from the jar containing guava fruit of 0.5 kg. The sample of ethylene gas is injected for 0.5 mL into the chromatographic gas of Shimadzu GC 9AM series, alumina column length of 1m and inside diameter of 3mm. The column temperature is 100 ^oC, with carrier gas N_2 0.5 mL, pressure of 1 kg/cm^2. The FID Detector (*Flame Ionization Detector*) is 230 ^oC, injector 230^oC (Ryohei et al. 2003).

Data analysis

The data is analyzed using Anova at a significance level of 5%. If there is a significant difference, it will be followed by *Duncan Multiple Range Test* (DMRT).

RESULTS AND DISCUSSION

Weight loss

The treatment of chitosan concentration and the storage temperature affect significantly in decreasing the weight of red guava fruit for two weeks of storage (Table 1). The

biggest fruit weight loss occurred in the fruit which is not coated with chitosan whether in the temperature of 28°C or 16°C, while in chitosan of (1, 2, and 3%) in both room temperature and cold temperature has lower weight loss than control (chitosan 0%). The result of Hewajulige et al. (2009) study in papaya fruit coated with chitosan in a room temperature proves to keep its weight loss for 14 days. The weight loss data of the red guava fruit and the other quality parameters can be seen from Table 1. While the decreasing of the red guava for four weeks can be seen from Figure 1. Based on the figure, it is obtained that the value of R^2 is 0.012 and the regression model is $Y=25.552-0.593X_1-0.078X_2$.

The treatment combination in guava fruit by giving low chitosan coating and storage temperature is able to restrain the increase of weight loss as seen in the first week of observation up to the fourth weeks (Figure 1). It is in line with Jayaputra and Nurrachman (2005) result-study that chitosan can maintain the freshness of mango because the coating materials of chitosan could cover the whole part of the fruit that O_2 gas which will flow into the fruit can be restrained and causes the retardation of respiration process. It is supported by Hofman et al. (1997) who states that weight loss caused by the on-going biological process, namely respiration process, which made reducing sugar diffused into CO_2 and H_2O which are easy to evaporate.

Weight loss after harvesting time is caused by physiological processes like respiration and transpiration process, and also other reactions caused by high temperature (Trenggono and Sutardi 1989). The water loss will also be faster at high temperature rather than at low temperature. Based on the data from Table 1, it is clearly seen that chitosan 2% and temperature of 16 °C has the least weight loss. The higher chitosan concentration given, the lower weight loss obtained. The temperature of 28°C has lower weight loss than the temperature of 16°C. According to Dong et al. (2004), weight loss in a fruit which is kept is mainly caused by the water loss as a result of evaporation process and carbon (CO_2) loss during respiration. The water loss during the storage not only loses weight but also reduces the quality and causes damage.

Table 1. The changes of several parameters of red guava fruit in distinctive chitosan concentration treatment and storage temperature

Parameter of quality	**Temperature**	**Chitosan concentration (%)**			
Time (week-)	**°C**	**0**	**1**	**2**	**3**
Weight loss (g) *)					
2	28	35.38^{b}	25.57^{ab}	26.28^{ab}	26.74^{ab}
	16	50.38^{c}	31.22^{b}	17.54^{a}	27.49^{ab}
Total water content (mg/100g)					
2	28	88.00	92.26	93.98	85.69
	16	80.00	85.56	86.70	87.05
Hardness rate (mg/100 g) *)					
2	28	0.10^{a}	0.18^{b}	0.20^{cb}	0.29^{c}
	16	0.42^{cd}	0.55^{e}	0.55^{e}	0.55^{e}
Reducing sugar content (mg/100 g) *)					
2	28	0.99^{abcd}	0.65^{ab}	1.00^{abcd}	0.59^{a}
	16	1.68^{d}	1.47^{cd}	1.36^{bcd}	0.88^{abc}
Vitamin C (mg/100 g) **)					
2	28	73.88^{a}	78.87^{b}	74.01^{a}	78.05^{b}
	16	78.44^{p}	80.38^{p}	82.79^{q}	79.87^{p}
4	16	70.17^{x}	68.35^{x}	75.46^{y}	70.91^{x}
Total chlorophyll (mg/L) **)					
2	28	1.80^{a}	1.90^{a}	1.87^{a}	2.43^{a}
	16	8.47^{p}	12.29^{q}	13.09^{q}	21.91^{r}
4	16	2.71^{x}	3.25^{x}	7.76^{y}	8.29^{y}
Carotenoid (µmol/g)					
2	28	0.01	0.01	0.01	0.02
	16	0.02	0.02	0.02	0.03
4	16	0.01	0.00	0.01	0.01
Ethylene content (ppm) **)					
1	16	0.87^{ab}	1.84^{b}	0.60^{a}	0.21^{a}

Note: *) the number accompanied by the same letter in the same column/ row shows that there is not significant difference. **) the number accompanied by the same letter in the same row, shows no significant difference

Water content

The combination of chitosan concentration treatment and storage temperature don't affect the water content of red guava in the second week of storage (Table 1). However, in the storage temperature of 16°C, there is a tendency of higher water content; in accordance with the increasing of chitosan concentration. Fruit coating using chitosan is expected to take a role in restraining transpiration process (Trung et al. 2011). Chitosan coating in various concentrations causes the fruit's rind pore to cover, that the activity of respiration and transpiration of the fruit restrained or reduced. In accordance with what is stated by Anggrahini and Suwedo (1988), fruits and vegetables keep on losing water after being harvested. Evaporation and changes cause the water loss on fruit occurred in the fruit which is caused by respiration process during the storage. The data of water content changes every week for four weeks with the result of regression analysis R^2= 0.584 and regression model $Y=100.140 + 3.517X_1-21.266X_2$ can be seen from Figure 2. The fruit's water content parameter has a positive correlation with the parameter of fruit's weight loss, where fruit loss occurs as a result of water reduction in the fruit. As what has been explained at the parameter of fruit's weight loss, respiration and transpiration process of the fruit is done through the fruit's rind surface.

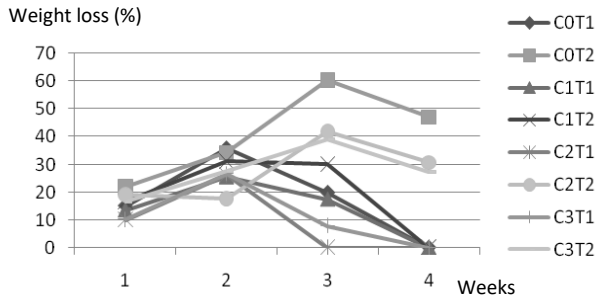

Figure 1. Weight loss of red guava in the variation of chitosan concentration and storage temperature for four weeks. Note: C0 = chitosan concentration 0%, C1 = chitosan concentration 1%, C2 = chitosan concentration 2%, C3 = chitosan concentration 3%, T1= temperature of 28°C, T2 = temperature of 16 °C.

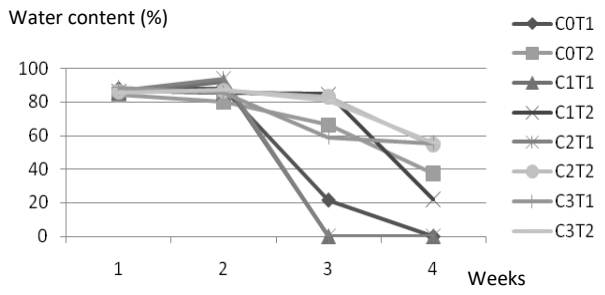

Figure 2. The water content of red guava in the variation of chitosan concentration and storage temperature. Note: C0 = chitosan concentration 0%, C1 = chitosan concentration 1%, C2 = chitosan concentration 2%, C3 = chitosan concentration 3%, T1= temperature of 28 °C, T2 = temperature of 16°C.

Fruit's hardness

The treatment of chitosan combination and temperature for two weeks storage is able to maintain the hardness of red guava fruit (Table 1). The increasing of chitosan concentration will improve fruit's hardness, especially in storage temperature of 28ºC. In the storage of 16ºC, there's a significant difference between control and chitosan coated fruit**;** however, among chitosan concentration levels, it doesn't show significant effect. During fruit ripening, the activity of degradable cell-structured enzyme will improve, so that it may lower fruit hardness. The application of chitosan into fruits restraints the process of gas exchange CO_2 and O_2 inside the fruit; that press out metabolism process (Trenggono 1992). Therefore, the application of chitosan is able to press the activity of degradable cell-structured enzyme, so that it may give better contribution during fruit storage (Li et al. 2006) and maintain fruit hardness (Hanani et al. 2012). Ethylene arranges fruit ripening by coordinating genes expressions which are responsible in various processes, among others; the increasing of cell breaking enzymes which is synthesized inside the fruit, namely, cellulase to break cellulose, polygalacturonase (PG) and pectin methylesterase (PME) which degrading pectin. The fruits soften caused by depolymerization and polysaccharide dissolve of cell-wall belonged to pectin, hemicelluloses, and cellulose. During the fruit's soften pectin and hemicelluloses are depolymerized and dissolved. Hydrolysis in pectin is catalyzed by polygalacturonase enzyme which hydrolyzes glycosidic linkage in the polygalacturonic acid chain of pectic (Pua and Davey 2010).

Reducing sugar content

Chitosan concentration and storage temperature give significant effect to the reducing sugar content of red guava for two weeks of storage (Table 1). Chitosan treatment at the temperature of 16ºC is significantly able to maintain the reducing sugar content in the second week. There's a tendency that the higher chitosan concentration applied will cause a lower reducing sugar content. However, in the storage temperature of 28ºC, the increasing of chitosan coating concentration is not followed by the reducing sugar content. Temperature treatment shows a strong effect in controlling the reducing sugar content. Reducing sugar is the sugar form resulted from polysaccharide decomposition, which is glucose and fructose having reactive group to do reaction with oxidizing agents (Stryer and Tymoczko 2002). According to Baldwin (1994), starch substance is wholly hydrolyzed into sucrose which then turns into reducing sugar as substrate in respiration. The speed of respiration product depends on the storage temperature and the existence of oxygen for respiration. The more oxygen used, the more active the respiration is (Nelson and Cox 2012). The content of reducing sugar in fruit generally increases up to the sixteenth days, and will lower down then (Islam et al. 2008).

The result of the study of the content of reducing sugar in red guava fruit after chitosan coating and various storage temperatures every week for four weeks is displayed at

Figure 3. The value of R^2 is 0.713, while the equation model of the regression is $Y=1.782 + 0.044X_1-0.461X_2$.

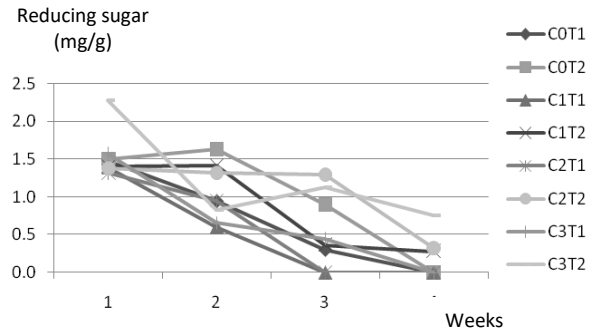

Figure 3. The total of reducing sugar of red guava in chitosan concentration variation and storage temperature. Note: C0 = chitosan concentration 0%, C1 = chitosan concentration 1%, C2 = chitosan concentration 2%, C3 = chitosan concentration 3%, T1= room temperature (28 °C), T2 = cold temperature (16°C)

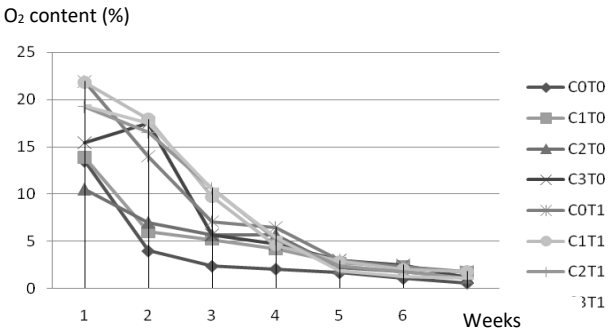

Figure 4. Daily oxygen content of red guava fruit with chitosan treatment and storage temperature for seven days. Remark: C0 = chitosan concentration 0%, C1 = chitosan concentration 1%, C2 = chitosan concentration 2%, C3 = chitosan concentration 3%, T1= temperature of 28 °C, T2 = temperature of 16 °C.

Vitamin C content

The combination of chitosan treatment and temperature are able to maintain vitamin C content of red guava for two weeks of storage (Table 1). So is the treatment of chitosan concentration combination and temperature of 16ºC in the fourth week. At the fourth week under the temperature of 28ºC, the fruit has been rotten, so the vitamin C content was not analyzed. The vitamin C content of red guava which is stored at the temperature of 16ºC is higher than the temperature of 28ºC. The declining of vitamin C content can be slowed down in cold temperature storage (Njoku et al. 2011). Fruit coating with various concentrations of chitosan causes the process of fruit's rind surface covered, so the gas in-and-out is restrained that the respiration rate declines. Chitosan concentration of 2% is optimally able to restraint the exchange (the in-and-out) of the gas compared to chitosan concentration treatment of 1% and 3%. Chitosan concentration of 3% is the highest chitosan solution thickness in coating or fruit's surface covering. Fruit's coating with chitosan in various concentration causes fruit's surface pores covered, so that the oxygen in-and-out is restrained (Zhang et al. 2011). Therefore, the oxidation process of vitamin C can also be hampered (Wahab and Rashid 2012). The declining occurs by the oxidation of ascorbic acid to dihydroxy ascorbic acid and will have further change that will form diketogluconic acid (Winarno 1990).

Pigmentation of the fruit rind

The treatment of chitosan combination and temperature effects in maintaining chlorophyll content of the fruit's rind of red guava in two and four weeks of storage (Table 1). Low temperature causes chlorophyll degradation process during the storage becomes slow. In the storage for 4 weeks under the temperature of 28ºC, the fruit has been damaged (rotten). However, in the storage temperature of 16ºC, chlorophyll still exists with the increasing rate as the increasing of chitosan content. It shows that the combination of chitosan treatment and cold temperature is able to restrain the activity of chlorophyllase enzyme in degrading chlorophyll. The change in the fruit's rind color is the most prominent change during the process of fruit ripening. The chlorophyll content of ripening fruit is slowly reduced. Generally, a number of green substances still exists in the fruit, especially in the inside tissue of a fruit (Ai and Banyo 2011). Instead of having chlorophyll change, it also occurs in this process a certain pigment synthesis, namely carotenoid (Li et al. 2006). The data of carotenoid in Table 1 shows that chitosan treatment and storage temperature for 2 and 4 weeks doesn't show significant difference.

Respiration

The combination of chitosan treatment and storage temperature lower the oxygen content of red guava fruit. In Figure 4, it is seen that the biggest decreasing of oxygen content during seven days is in control; while in the chitosan treated fruit and temperature of 16ºC shows lower decrease. It shows that chitosan coating is mechanically stronger, reducing transpiration, and also restraining gases (O_2, CO_2, and ethylene) from getting into the fruit, so that respiration restraint (Hawa 2005). The data of oxygen content for seven days in red guava fruit after chitosan treatment and storage temperature shows R^2 is 0.011, with regression equation $Y=56.043+2.324X_1-4.924X_2$. Chitosan coating will decline respiration, so that the metabolic activity during the fruit storage restraint (Jitareerat et al. 2007). By giving chitosan concentration, the increasing of O_2 for respiration process is restrained, resulting in lower respiration and pectate degradation process, lignin, cellulose, and hemicellulose by the activity of pectin methyl esterase enzyme and polygalacturonase in the process of restraining fruit ripening (Malmiri et al. 2011). On the second day, respiration started to lower down, and from the second day until the fourth day, there was a sharp decline. On the fourth day until the seventh day, there was a little decline. The products having thin rind layer like guava has high respiration.

Ethylene content

The result of ANOVA analysis shows that chitosan concentration treatment and storage temperature of 16ºC can lower ethylene production (Table 1). Chitosan coating increases the content of CO_2 and lower O_2 inside the fruit tissue that also lower the respiration rate. The lower respiration rate is followed by the decreasing of ethylene content that will restraint fruit ripening (Ghaouth et al. 1992). According to Musa et al. (2010), chitosan coating can lower the production of CO_2 and ethylene. The higher concentration of chitosan coating will lower ethylene content. The lower ethylene content will restraint fruit ripening due to the decreasing of genes expressions which are responsible in various processes including respiration rate, autocatalytic production of ethylene, chlorophyll degradation, carotenoid synthesis, conversion of starch into sugar, and the activities of cells-breaker enzymes (Winarno 2002).

In conclusion, chitosan concentration treatment and storage temperature maintain fruit hardness, vitamin C content, and chlorophyll content; meanwhile, reducing sugar content, respiration rate and ethylene content show a decreasing. Chitosan treatment of 3% and the temperature of 16ºC is able to lengthen the storage period up to four weeks and maintain its quality.

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