

## Effect of Cold Storage Duration on the Quality of Super Red (*Hylocerus costaricensis*) and White (*Hylocerus undatus*) Dragon Fruits

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### ABSTRACT

Postharvest storage is carried out to control the metabolic rate so the quality of fruits can be maintained properly until it reaches the costumers. Cold storage can reduce metabolic reactions, such as respiration and transpiration. However, storing too long in cold temperatures can cause the quality of the fruit to deteriorate. Each type of dragon fruit has different chemical contents that will affect the quality of fruits after storage. This study aims to determine the effect of the type of dragon fruit, cold storage duration, and interaction between the two treatments. Both type of dragon fruit are packed in PP plastic and then stored at 10°C. Observation were made at different storage durations. This study used RAL, with the first being super red and white dragon fruits. The second factor was storage duration of 0, 7, 14, and 21 days. Data were analyzed using ANOVA and continued with DMRT if it had a significant effect. The result of the study showed that the treatment of fruit type had significant effect on antioxidant activity, vitamin C content, and total sugar level. Storage duration treatment had significant effect on antioxidant activity and vitamin C content.

## 1. INTRODUCTION

Storage is one of the post-harvest actions to prevent food damage until coming to the consumers. Post-harvest damage is caused by fruit still undergoing metabolic reactions, such as respiration (Murtiwulandari *et al.*, 2020) and transpiration (Kasso & Bekele, 2018). Respiration is a reaction carried out to maintain living plant tissue using glucose and oxygen with the final result of carbon dioxide, water, and ATP (Yuniarto *et al.*, 2021). Transpiration is a process of moving water in the tissue to the environment. Transpiration in a fruit depends on the surface area and conductance, as well as environmental condition (Giovannini *et al.*, 2022).

One of the factors accelerating respiration and transpiration during storage is temperature. Increasing temperature during storage cause an increase in respiration rate so that fruit quickly becomes damaged or rots (Nerd *et al.*, 1999). Optimally set storage temperature can prevent physical damage and nutrient content of the fruit (Hussein *et al.*, 2020). Super red (*Hylocereus costaricensis*) and white (*Hylocereus undatus*) dragon fruits are the most popular and easy to find in Indonesia. The largest content of dragon fruit is water. Super red dragon fruit contains 82.5 - 83 g of water (Attar *et al.*, 2022). In contrast, while dragon fruit contains 89.4 g of water (Mallik *et al.*, 2018). The high water content causes dragon fruit not to be a fruit that can be stored for a long time at room temperature. Therefore, cold storage of dragon fruit can extend the storage duration and fruit quality such as flavor, color texture, and nutritional content (Muhammad *et al.*, 2021). Saputri *et al.* (2020) stated that cold temperatures can reduce respiration activities, transpiration, aging processes, and microorganism growth.

Dragon fruit is rich in bioactive compounds, such as betacyanin, phenolic compounds, and terpenoid (Attar *et al.*, 2022). Hossain *et al.* (2021) stated that compounds have functions as antioxidant in dragon fruit are flavonoids, betalain, hydroxycinnamate, beta-carotene, linoleic acid, and vitamin C. Stability of antioxidant activities is influenced by temperature (Khotimah *et al.*, 2018). A study by Nataliani *et al.* (2018) using 2,2-difenil-1-pikrilhidrazil (DPPH) method reported that storage of natural dye solution of super red dragon fruit on the fifth day at room temperature (25°C) experienced a very rapid reduction of antioxidant activity compared to storage at refrigerator temperature (10°C). The antioxidant activity of dragon fruit stored at refrigerator temperature on the fifth day was 71.46%, while antioxidant activity at room temperature decreased by 54.23%.

The cold temperature that is often to store fruits and vegetables is around  $\pm 10^{\circ}\text{C}$ . Some fruits and vegetables, which are tropical and sub-tropical fruits, are sensitive to temperature that are too low. Storing fruits at  $\pm 10^{\circ}\text{C}$  is sufficient to maintain fruit freshness for several days or even several weeks (Lal Basediya *et al.*, 2013). Cold storage for too long can also damage the quality of fruits. This is because fruits experience cold stress, so the production of Reactive Oxygen Species (ROS) increases, which can cause oxidative damage (Pizzino *et al.*, 2017). The effect of increasing ROS can damage DNA and proteins and can accelerate membrane lipid peroxidation, which causes tissue and organ damage (Raza *et al.*, 2023). Due to difference in chemical and nutritional content and the effect of storage, a study regarding the storage duration in cold temperature and the type of dragon fruit that can maintain fruit quality is required.

## 2. MATERIALS AND METHODS

This research was conducted from November 2023 to March 2024. The research site was at the Biological Laboratory of Plant Structure and Function, Faculty Science and Mathematics, Diponegoro University. Material used were red and white dragon fruit, 96% ethanol, distilled water, DPPH powder, and pure ascorbic acid powder. Tools used were UV-Vis spectrophotometer, refractometer, analytical balance, measuring cup, beaker glass, test tube, dropping pipet, spatula, and petri dish.

### 2.1. Sample Collection and Storage

Red and white dragon fruit were obtained from farmers of a dragon fruit plantation in Ngemplak District, Sleman Regency. Samples used were ripe dragon fruit with evenly red skin 37 days the flowers appeared and weight of 450 - 500g. Dragon fruits were stored in the refrigerator at  $10^{\circ}\text{C}$  and packaged in PP (polypropylene) plastic with a thickness of 30 microns for 7, 14, and 21 days. In the control treatment, dragon fruits were not stored at room temperature and were packaged in plastic.

### 2.2. Determining Antioxidant Activity

Measuring antioxidant activity was conducted using the DPPH method with modification (Molyneux, 2004).

#### 2.2.1. Dragon Fruit Extract Preparation

50 g of mashed dragon fruit was soaked in 100 ml of 96% ethanol. The maceration process was carried out for 2x24 hours and replaced 96% ethanol every 24 hours. The macerate was then evaporated using a rotary evaporator at  $40^{\circ}\text{C}$  until a thick extract was obtained. The 50 ppm stock solution was made from 5 ml thick extract with 100 ml of 96% ethanol.

#### 2.2.2. DPPH Preparation and Measurement

A 0.4 mM DPPH solution was made by dissolving 15.7 mg of DPPH powder in 100 ml of 96% ethanol. The absorbance of DPPH was measured by mixing 20 ml of 0.04 mM DPPH with 2 ml of 96% ethanol. And then keep the sample for 30 minutes in a dark place. The absorbance of DPPH was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm, which is the maximum wavelength of DPPH (Wahdaningsih *et al.*, 2011).

### 2.2.3. Measuring Antioxidant Activity

2 ml of dragon fruit extract with several concentration of 6, 12, 18, and 24 ppm from a stock solution of 50 ppm was added to 2 ml of 0.04 mM DPPH, homogenized, and incubated for 30 minutes in the dark place. Antioxidant activity was measured using a spectrophotometer with a wavelength of 517 nm. Antioxidant activity was measured using the formula:

$$\% \text{ antioxidant} = \frac{(A.\text{blank} - A.\text{extract})}{A.\text{blank}} \times 100\% \quad (1)$$

where *A-blank* is absorbance value for control, and *A-extract* is absorbance value of sample.

Antioxidant activity stated by Inhibitor Concentration value (IC50). IC50 value is indicates how much the concentration of compounds can reduce or prevent 50 % of free radicals. Determining IC50 value is by creating a curve of the relationship between % antioxidant and extract concentration to obtain a linear regression equation:

$$y = ax + b \quad (2)$$

where *y* is the dependent variable, *a* is the coefficient, *x* is the IC50 value to be sought, and *b* is a constant.

## 2.3. Vitamin C Content

### 2.3.1. Determining the Wavelength of Ascorbic Acid

Ascorbic acid, as a standard solution, was made at a concentration of 10 ppm. Then, its absorbance value was measured using a UV-Vis spectrophotometer with a wavelength of 260 - 270 nm (Khafid *et al.*, 2023). The choice of wavelength that produces the maximum absorbance value in this research is because the change in absorbance for each unit of concentration is the greatest (Apriliyani *et al.*, 2018).

### 2.3.2. Creating Calibration Curve

Ascorbic acid solution was prepared by mixing distilled water as a solvent. In this study, several concentrations of ascorbic acid solution were made such as 2, 4, 6, 8, and 10 ppm. And then the absorbance value is measured using the highest wavelength that has been obtained. After that make a graph with the x- axis is the concentration of ascorbic acid and the y-axis is the absorbance value, then it was obtained the equation of the line with the formula of  $y = bx + a$ , where *y* is the absorbance value, *x* is vitamin C concentration, *b* is a coefficient, and *a* is constant.

### 2.3.3. Determining Vitamin C Content

0.25 g of mashed dragon fruit flesh was added with 5 ml of distilled water. Then, it was centrifuged for 15 minutes at 3500 rpm. Samples were then filtered and added with 12 ml of distilled water. The absorbance value was measured using the highest wavelength that had been obtained previously. The concentration of vitamin was obtained by entering the absorbance value of samples into the linear equation of  $y = bx + a$ .

## 2.4. Total Sugar Level

This measurement used a refractometer by dripping dragon fruit extract onto the light plate calibrated with distilled water. The reading was made on a scale located in the center of the plate through a lens.

## 2.5. Water Content

Dragon fruit sample was weighed at 2 g, then placed in a petri dish whose empty weight is known. Then, the sample was dried in the oven at 103°C. Drying was carried out until constant dry weight was obtained. The percentage of water content was calculated using the formula:

$$\text{Water content \%} = \frac{B-C}{B-A} \times 100 \quad (3)$$

where *A* is dry weight of dish (g), *B* is dry weight of dish and initial sample (g), and *C* is dry weight of dish and sample after drying (g)

## 2.6. Weight Loss

This measurement was carried out by calculating the weight difference before and after treatment with formula:

$$\text{Weight loss \%} = \frac{B_0 - B_n}{B_0} \times 100 \quad (4)$$

where B<sub>0</sub> is initial storage weight (g), and B<sub>n</sub> is weight on day *n* (g).

## 2.7. Research Design and data Analysis

The research design used a completely randomized design (CRD) with a 2x4 factorial design. The first factor was the types of super red and white dragon fruit, and the second factor was storage duration of 0, 7, 14, and 21 days at 10°C. Data obtained were then analyzed using ANOVA at a 95% confidence level, and if there is a significant effect, further tests will be carried out using the DMRT Test.

## 3. RESULTS AND DISCUSSION

### 3.1. Antioxidant Activity

Based on the results of ANOVA, the type of fruit and storage duration showed a significant effect on antioxidant activity. The type of dragon fruit with storage duration showed no interaction with antioxidant activity. The total antioxidant activity of white dragon fruit was 39.67 µg/ml, which was significantly different and greater than super red dragon fruit, which was 43.89 µg/ml (Figure 1). Both types of dragon fruit had an IC<sub>50</sub> value of less than 50, so they were included in a group with very strong antioxidant activity (Badarinath *et al.*, 2010). The difference in IC<sub>50</sub> value between the two dragon fruits was caused by the difference in the ability of genes to express enzymes that function as biocatalysts, which convert substrates into different products (Bulley *et al.*, 2009). White dragon fruit is presumed to have more enzymes that have functions to convert substrates into antioxidant products. Bioactive compounds in dragon fruit that act as antioxidants are flavonoids, betalains, hydroxycinnamates, carotenoids, lycopene, vitamin C, linoleic acid, and linolenic acid (Hossain *et al.*, 2021). The results of antioxidant activity in this test were directly proportional to the results of vitamin C content analysis. Figure 2 shows that white dragon fruit has the highest vitamin C content of 10.130 ppm compared to super red dragon fruit of 8.338 ppm. These results support that white dragon fruit has higher antioxidant activity than super red dragon fruit.

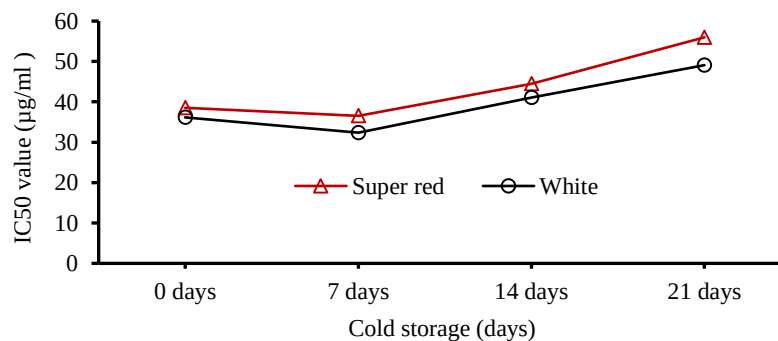


Figure 1. Antioxidant activity of dragon fruit with cold storage.

Storage duration showed results that affect antioxidant activity. Treatment without storage (0 days) had an antioxidant activity of 37.33 µg/ml. Antioxidant activity had increased on storage day 7 by 34.45 µg/ml. IC<sub>50</sub> value in no storage treatment and day 7 was included in the group of very strong antioxidant activity. An increase in antioxidant activity on day 7 was caused by an increase in the content of biochemical compounds that have a role in antioxidants. This study analyzed one of the biochemical compounds that have a role in the antioxidant, namely vitamin C. The results of the study showed that the highest vitamin C content was on storage day 7 at 10.861 ppm (Figure 2).

However, after the storage day 7, the antioxidant activity decreased. Antioxidant activity on day 14 was 42.79  $\mu\text{g/ml}$ , and on day 21 was 52.54  $\mu\text{g/ml}$  (Figure 1). The decrease in antioxidant activity on storage days 14 and 21 was presumed to be due to the dragon fruit experiencing environmental stress due to storage duration at cold temperatures. The signals emitted by dragon fruit due to environmental stress cause the amount of Reactive Oxygen Species (ROS) to increase. Yang & Lian (2020) stated that in normal amounts, ROS has a significant role in homeostasis involving metabolism, immunity, growth, and differentiation. The large amount of ROS production must be balanced with the large amount of antioxidant activity of dragon fruit to neutralize ROS. Valenzuela *et al.* (2017) mentioned that one of the antioxidant mechanisms to neutralize ROS is through the Foyer-Halliwell-Asada cycle, which uses ascorbate to reduce hydrogen peroxide by the enzyme ascorbate peroxidase (APX). The use of antioxidants is to neutralize ROS, which is presumed to decrease IC<sub>50</sub> value in dragon fruit on days 14 and 21. The increasing amount of ROS is not comparable to the biosynthesis of compounds that have a role in antioxidants, called oxidative stress (Guo *et al.*, 2022). This can damage cells that will affect the fruit quality, such as nutrition, color, and taste. The dragon fruit damage due to cold storage duration is called chilling injury. Chilling injury in the fruit can be indicated by brown spots in the fruit peel.

### 3.2. Vitamin C Content

In testing vitamin C content, ascorbic acid was used as a standard solution that has a function to determine the vitamin C level of dragon fruit. Line equation obtained from the calibration curve of ascorbic acid was used to obtain the vitamin C content of dragon fruit according to absorbance value. Based on the result of testing, a calibration curve was obtained with a line equation of  $y = 0.0735x - 0.0634$  (Figure 2). Moreover, the researcher entered the absorbance value of dragon fruit, where variable  $y$  is the absorbance value and variable  $x$  is the vitamin C content of dragon fruit.

The treatment between the fruit type and cold storage duration showed an interaction with vitamin C content. The highest vitamin C content was obtained in white dragon fruit stored for 7 days of 11.629 ppm, while the lowest vitamin C content was obtained in super red dragon fruit stored for 21 days of 5.584 ppm (Figure 3). If looking at the average type of dragon fruit in terms of vitamin C content, there were significant differences. This shows that white dragon fruit has a higher vitamin C content. The large difference in vitamin C content between the two types of dragon fruit was presumed due to the difference in genes that can express enzyme biosynthesis of vitamin C. This is in accordance with the opinion by Bulley *et al.* (2009) that *Actinida eriantha* has more enzyme GDP-D-mannose 3'.5'-epimerase (GME) that has a role in the biosynthesis of vitamin C by converting glucose into ascorbic acid compared to other two *Actinida* species. This causes vitamin C content in *Actinida eriantha* to be higher.

Increased vitamin C content of the two types of dragon fruit occurred on day 7 due to an increase in the synthesis of vitamin C. Fruit, after being harvested, still undergoes metabolic reactions, one of which is the biosynthesis of ascorbic acid. Hancock *et al.* (2003) explained that the L-galactose pathway, also called as Smirnoff-Wheeler pathway, is the main biosynthesis pathway for ascorbic acid. The enzymes in the L-galactose pathway are GDP-D-mannose pyrophosphorylase (GMP), L-galactose dehydrogenase (GDH), GDP-D-mannose 3'.5'-epimerase (GME), and L-galactono-1.4-lactone dehydrogenase (GLDH), which are used to convert glucose into ascorbic acid. Tsaniklidis *et al.* (2014) stated that storage conditions at 10°C can increase the expression of gene-encoding enzymes of ascorbic acid biosynthesis, such as L-galactono-1.4-lactone dehydrogenase (GLDH) and GDP-D-mannose 3'.5'-epimerase (GME).

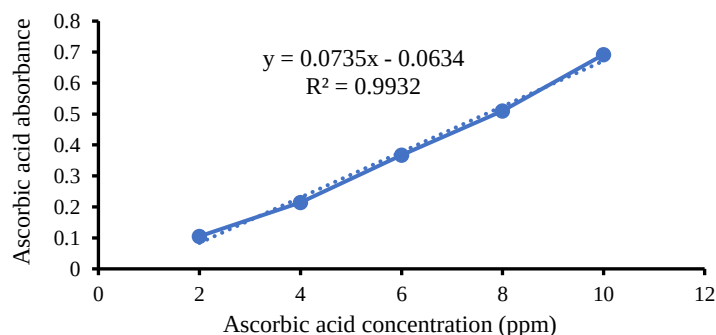


Figure 2. Calibration curve of ascorbic acid concentration.

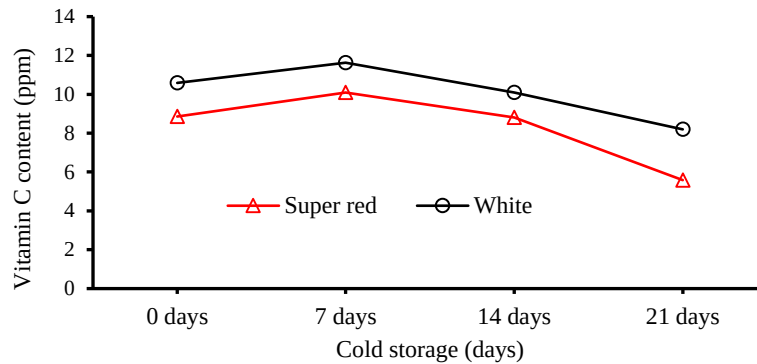


Figure 3. Vitamin C content of dragon fruit with cold storage.

Vitamin C content decreased on days 14 and 21 in both types of dragon fruit. The vitamin C content of super red dragon fruit on days 14 and 21 was 8.813 ppm and 5.584 ppm, respectively, while white dragon fruit was 10.101 ppm and 8.196 ppm, respectively (Figure 3). Decreased vitamin C content in days 14 and 21 was caused by a chilling injury. Chilling injury is fruit damage during storage at cold temperatures. [Tsaniklidis \*et al.\* \(2014\)](#) stated that cold storage can cause stress in dragon fruit, resulting in excessive ROS production. The increasing amount of ROS is not comparable to the biosynthesis of compounds that have a role in antioxidants, which is called oxidative stress ([Raza \*et al.\*, 2023](#)). ROS can damage DNA and proteins and accelerate membrane lipid peroxidation, which causes tissue and organ damage and inhibits the synthesis of vitamin C ([Sharifi-Rad \*et al.\*, 2020](#))

### 3.3. Total Sugar Level

According to ANOVA, the treatment of fruit type showed a significant effect on total sugar level. The highest total sugar level in the treatment of fruit type was in super red dragon fruit treatment of 11.61°Brix (Figure 4). Moreover, storage duration did not show a significant effect on total sugar level.

The total sugar level of the super red dragon fruit was 11.61°Brix, which was higher than white dragon fruit, which was 10.57°Brix (Figure 4). The difference in total sugar level is presumed to be related to vitamin C content between the two types of dragon fruit. According to [Perla \*et al.\* \(2016\)](#), glucose and galactose are the basic materials used in the biosynthesis of ascorbic acid. According to Table 2, the highest vitamin C content was in white dragon fruit. High vitamin C requires more glucose, so the glucose level of white dragon fruit is lower. Glucose will be used in the Smirnoff-Wheeler pathway, with the final synthesis result of ascorbic acid.

There was no significant difference in total sugar level because the sugar content in dragon fruit is only used a little in respiration. Respiration involves glucose and oxygen, producing carbon dioxide, water, and energy. Dragon fruit includes non-climacteric fruit, which is a fruit that does not experience many changes in respiration after harvesting

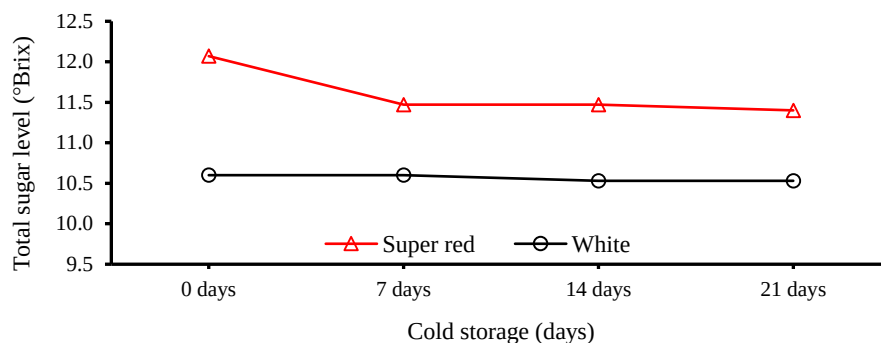


Figure 4. Total sugar level of dragon fruit with cold storage

(Sutrisno & Purwanto, 2011). Moreover, low temperature during storage also suppresses respiration rate, causing differences in sugar content to not be significantly different. Imamah *et al.* (2016) mentioned that food commodities stored at low temperatures can suppress respiration activity so they can have a longer storage period.

### 3.4. Water content

According to the results of ANOVA, the treatments of fruit type and storage duration showed no significant difference and did not show interaction with water content. In the treatment of dragon fruit types, super red dragon fruit had a water content of 94.17% and white dragon fruit of 92.5% (Figure 5). Dragon fruit is known for its high water content (Hazarika *et al.*, 2019). In the storage treatment until day 21, dragon fruit still had a water content of 90% (Figure 5).

The two treatments did not show a significant effect. This was presumed because dragon fruit did not experience much respiration and transpiration. Dragon fruit includes non-climacteric fruit (Widodo *et al.*, 2020). Respiration in non-climacteric dragon fruit is very slow, so water from respiration is low. Cold storage and packaging also inhibit the metabolic reactions of dragon fruit, such as respiration and transpiration, so that water loss is relatively not significantly different. Packaging fruit with plastic can reduce respiration and transpiration because fruit does not interact directly with the environment. Setyaputri & Kurnia (2019), mentioned that packaging causes atmospheric modification, so it can regulate the levels of O<sub>2</sub>, CO<sub>2</sub>, and water vapor.

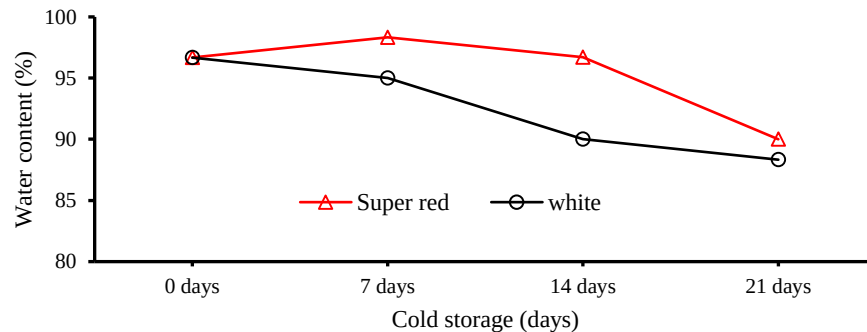


Figure 5. Water content of dragon fruit with cold storage.

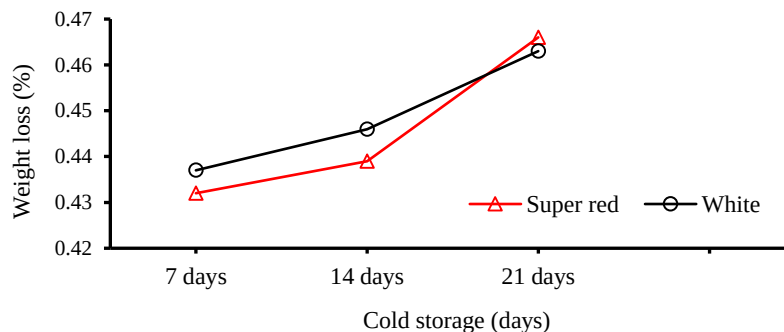


Figure 6. Weight loss of dragon fruit with cold storage.

### 3.5. Weight Loss

Based on ANOVA, the treatments of the dragon fruit types and storage duration did not show a significant effect after storage. The two treatments also showed no interaction with weight loss. This is presumed due to low carbohydrate catabolism during storage. Dragon fruit includes non-climacteric fruit, which does not experience much respiration after harvesting. The results of the study showed that the average weight loss of the type of dragon fruit based on treatment conditions was not much different. Super red dragon fruit experienced weight loss of 0.446%, while white dragon fruit was 0.449% (Figure 6).



The low weight loss in this study was also due to low fruit transpiration. In this study, dragon fruits were stored in a refrigerator at 10°C and packed in plastic. Fruit storage at low temperatures can reduce transpiration (Wucher *et al.*, 2021). Packaging causes fruit to not be directly connected with the environment, so water in the fruit does not immediately move to an environment with low humidity.

#### 4. CONCLUSION

The conclusion of this study is that the type of fruit shows an effect on antioxidant activity, vitamin C, and total sugar level. White dragon fruit has the highest antioxidant activity and vitamin C, while super red dragon fruit has the highest sugar level. In addition, cold storage has an effect on antioxidant activity and vitamin C. Antioxidant activity and vitamin C increases on day 7 in both types of dragon fruit. The antioxidant activity in super red and white dragon fruits on day 7 is 36.53 µg/ml and 32.37 µg/ml. Moreover, the vitamin C content in super red and white dragon fruit on day 7 10.092 ppm dan 11.629 ppm, respectively.

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