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Application of Chitosan and Citric Acid Coating to Increase The Storage of Marigold Flowers (*Tagetes Patula* L.)

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ABSTRACT

Marigold is a flower that can be consumed (edible flower) in fresh condition. Marigold flower damage is characterized by withering and changing the color of the petals to brown (browning). Coating treatment combined with citric acid is expected to be one of the solutions to maintain quality and increase the shelf life of Marigold flowers.. This study aims to examine the effect of citric acid treatment and application of coating made from chitosan on changes in the quality of Marigold flowers as fresh edible flowers. The concentrations of chitosan (K) studied were 0.05% and 0.1%; while the concentration of citric acid (A) was 1% and 2%. Citric acid solution is sprayed first. After air dried, the flowers were sprayed with a coating solution and air-dried. All flower samples, both control and treatment, were packaged and stored at 10^{o}C*. During storage, respiration rate, moisture content, weight loss and color were measured. Organoleptic test was carried out by 35 panelists with hedonic values 0-5. The best treatment was A2K2 (2% citric acid and 0.1% chitosan) which was still accepted by the panelists (score 3) until the 6th day of storage with a moisture content of 83.04%, weight loss 21.05%, and the value of °hue 79.70°. A2K2 treatment could increase the shelf life of three days longer than the control.*

1. INTRODUCTION

Aside from being an ornamental flower, marigold flowers include flowers that can be consumed fresh or are known as edible flowers. Edible flowers have been popular since ancient times as alternative medicines and traditional culinary delights (Farishy *et al*., 2019). Compounds contained in flowers include flavonoids, lutein, alkaloids, terpenes, thiophenes, fatty acids, and carotenoids which are useful as antioxidants, gastroprotective, hepatoprotective, anti-inflammatory and free radical scavengers (Takahashi *et al*., 2019).

As an edible flower, marigold flowers have advantages over other flowers, namely, they are easy to cultivate and have a relatively long blooming period. This makes marigold flowers (*Tagetes patula* L.) easy to obtain and are often found in various restaurants as

garnishes to enhance the appearance of food. According to Gutap *et al*. (2018), adding edible flowers to food will increase visual appeal, nutritional value, taste, aroma, and soft texture of flowers will also add a unique sensation when eating them. Some examples of the use of edible flowers in food are garnishes for dishes, salads, soups, appetizers, desserts, and drinks (Kou *et al*., 2012).

The main obstacle in the agribusiness of fresh consumption flowers is their short freshness. Marigold as an ornamental flower has a freshness period of two to three days at 5 °C (Hardenburg *et al*. 1990), while as an edible flower it is faster because it is distributed or sold without stalks. Edible flowers are easily damaged, characterized by damage to the outer petals, discoloration, wilting, dehydration and browning (Fernandes *et al*., 2019). One way to prevent damage to edible flowers is to treat them with citric acid. According to Hardjono et al. (2016), the use of citric acid in food ingredients can inhibit the browning reaction. Citric acid is non-toxic so it is safe to use for coating food (Kawijia *et al*., 2017).

Based on research by Fernandes *et al*. (2019) citric acid applied to artichokes (*Cynara cardunculus* var. scolymus) is still ineffective in terms of storability because there is still microbial contamination so it needs to be coated. Edible coating is a thin layer made of edible material. The function of the coating is as a barrier so as not to lose moisture and can control the migration of water-soluble components which can cause pigment changes (Krochta *et al*., 2002). Coating application using chitosan on broccoli can increase shelf life, reduce respiration rates, and inhibit the growth of bacteria and fungi (Alvarez *et al*. 2013). Based on this, research was carried out with the aim of knowing the effect of coating applications made from chitosan and citric acid treatment so as to maintain quality and increase the shelf life of marigold flowers.

2. MATERIALS AND METHODS

2.1. Materials and Equipment

The main material used in this study was marigold flowers which were fresh and uniform in size obtained from edible flower supplier farmers in the Bandung area, West Java. Chitosan (in the form of flour), citric acid, and distilled water were obtained from chemical shops in the city of Bogor. The tools used included a continuous gas analyzer to measure respiration, a chromameter to measure color, an oven and a balance to measure water content using the OAOC 2005 method.

2.2. Experimental Design and Data Analysis

The experimental design used was a completely randomized design with treatments A1K1 (1% citric acid and 0.05% chitosan), A1K2 (1% citric acid and 0.1% chitosan), A2K1 (2% citric acid and 0.05% chitosan), A2K2 (2% citric acid and 0.1% chitosan) and K (Control). Each treatment was repeated 3 times. The data obtained were analyzed statistically using analysis of variance (ANOVA). If the ANOVA produces a significant difference, it will be continued with the Duncan's Multiple Range Test (DMRT). Statistical tests were carried out with a 95% confidence interval ($\alpha = 0.05$) using SPSS 25 software (free edition).

2.3. Research Procedure

2.3.1. Preparation of Citric Acid Solution and Chitosan Coating

The concentrations of citric acid and chitosan studied were 1% and 2% for citric acid and 0.05% and 0.1% for chitosan, respectively. A concentration of 1% citric acid was

prepared by dissolving 1 g of citric acid in 99 ml of distilled water, while for 2%, 2 g of citric acid was dissolved in 98 ml of distilled water (w/v). Chitosan-based coating solutions with concentrations of 0.05% and 0.1% were prepared by dissolving 0.05 g and 0.1 g of chitosan respectively in 99.95 ml and 99.9 ml of distilled water to produce 100 ml of solution (w/v). For the chitosan solution, stirring was carried out using a magnetic stirrer for 60 minutes at a speed of 1,000 rpm to produce a homogeneous solution.

2.3.2. Citric Acid Applications and Coatings

The application of the two solutions (citric acid and coating) was carried out using the spraying method. Flower samples were sprayed with citric acid solution concentrations of 1% and 2% 1 time. The sample is then dried using a fan until the surface of the flower looks dry. Coating using chitosan solution was carried out by spraying on flower samples that had been treated with citric acid. The samples were again air-dried using a small fan. Treatment samples that were dry and control (without any treatment) were packed in plastic boxes (thin wall) and stored at 10 °C. During storage, measurements of respiration rate, moisture content, weight loss, color and organoleptic tests were carried out.

2.4. Observation and Measurement

2.4.1. Respiration Rate

Respiration rate was measured by closed method. The measurement procedure refers to Mannapperuma & Singh (1990). The chamber containing the sample is tightly closed and it is ensured that gas does not come in and out. Oxygen and CO_2 gases in the chamber were measured after 24 h of storage at 10 °C. Measurements are made through a hose that has been prepared in the chamber lid section. Once done, the chamber is put back in the storage room and the chamber lid is opened for 15 min to restore the gas composition in the chamber to that of the outside air. The chamber is then closed again and left for up to 24 h to be measured again the next day. Measurements are carried out every day at the same time. In this study the measurements were taken at 13.00 WIB. The chamber used has a capacity of 260 cm^3 filled with 2 flower samples for each chamber. The measured respiration rate (R) is O_2 consumption using Equation 1.

$$R = \frac{V}{W}\frac{dx}{dt} \qquad (1)$$

with R is respiration rate ($ml.kg^{-1}.h^{-1}$), x is gas concentration (%vol), t is time (h), V is free volume of respiration chamber (ml), and W is product weight (kg).

2.4.2. Moisture Content

Moisture content was measured using the AOAC 2005 method, by weighing the Marigold flower samples before and after being in the oven. The water content is calculated using Equation 2:

$$Ka = \frac{W1 - W2}{W1} \times 100\% \qquad (2)$$

where Ka is moisture content (%), W_1 is initial sample weight (g), and W_2 is final sample weight (g).

2.4.3. Lose Weight

Weight loss is calculated by measuring the difference in weight on day *n* and day *n* + 1 using Equation 3.

$$Sb = \frac{Wo - Wi}{Wo} \times 100\% \quad (3)$$

with S_b is weight loss (%), *Wo* is weight at day *n* (g), and W_i is weight at day *n* + 1 (g).

2.4.4. Color

Color measurements were carried out on the flower crown using a chromameter with the data obtained for brightness (*L*), a and b values. Data *a* and *b* are processed to produce °Hue values using Equation 4:

$$°Hue = \tan^{-1}\left(\frac{b}{a}\right) \quad (4)$$

with *a* is positive for red color and negative for green color, while *b* is positive for yellow color and negative for blue color.

2.4.5. Organoleptic Test

Organoleptic tests were carried out using appearance and flower color parameters based on the five human senses to determine the storage time limit with the quality of marigold flowers that are still acceptable to consumers. As many as 35 people were employed as panelists. The assessment is based on 5 hedonic test scales, namely: 1 = very dislike; 2 = dislike; 3 = neutral; 4 = like; 5 = ver like. Scores less than 3 meant to be disliked by consumers (panelists).

3. RESULTS AND DISCUSSION

3.1. Respiration Rate

The O_2 consumption rate of marigold flower samples during storage is presented in Figure 1. The O_2 consumption rate continued to increase until day 4 and then slowed down and decreased until the end of s torage. At the end of storage, the respiration

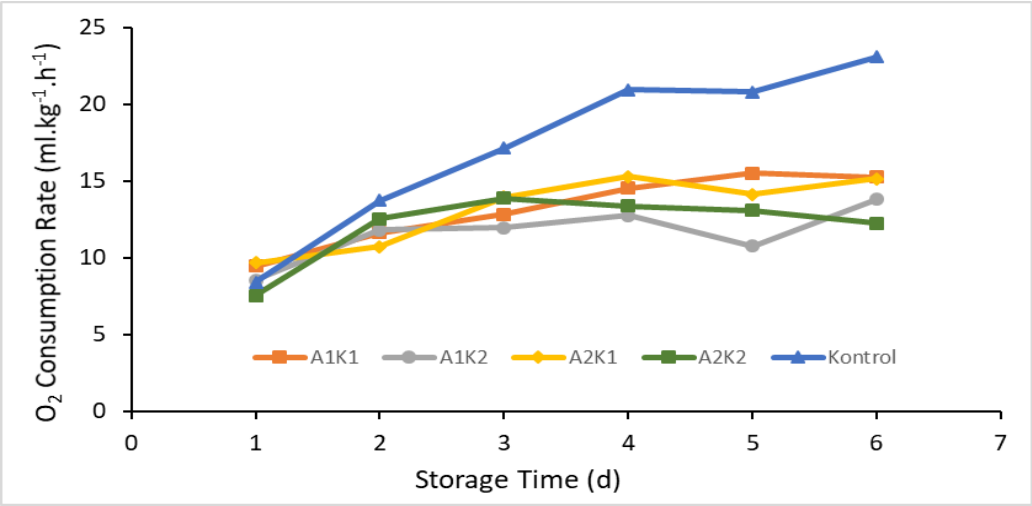

Figure 1. Oxygen consumption rate of marigold flower during storage

rate was still due to respiration carried out by all parts of the flower, namely the crown, petals, flower base and stem. Flower petals, flower base and stem still contain substrate to carry out the respiration process even though the flower crown has withered.

The respiration of the treated flower samples had a lower rate with a slow increase in rate compared to the control flower samples. The O_2 consumption rate of the control flower sample reached 23 $ml.kg^{-1}.h^{-1}$ on the 6th day of observation, while the A2K2 treatment (2% citric acid and 0.1% chitosan) measured the lowest, namely 12 $ml.kg^{-1}.h^{-1}$. This shows that the rate of O_2 consumption in marigold flowers can be reduced by giving a coating treatment.

The results of ANOVA and DMRT showed that the respiration rate of the control samples was significantly different from all treatments starting on day 3 of storage, while the samples among treatments were not significantly different with a significance of 95% from day 1 to the end of storage. According to the statement of Vina *et al.* (2007), that coating with edible coatings is able to inhibit the rate of respiration and suppress the occurrence of damage. Jiang et al. (2012) stated that the use of chitosan as a coating can control damage, reduce O_2 diffusion, and slow down the rate of respiration, so that it is able to maintain the freshness of flowers longer. Respiration rate can cause water loss in the material while water loss in flowers causes withering and wrinkling so that the quality of the flowers decreases (Miskiyah *et al*., 2011). Jiang *et al*. (2012) stated that the use of chitosan as a coating can control damage, reduce O_2 diffusion, and slow down the rate of respiration, so that it is able to maintain the freshness of flowers longer. The use of chitosan as a coating as well as antimicrobial. Chitosan applied to mangoes as a coating with a concentration of 1.5% was able to inhibit microbial growth better than beeswax with a concentration of 6% (Yulianti *et al*., 2022).

3.2. Moisture Content

According to Pires et al. (2017) water represents more than 80% of flower composition. Changes in the water content of Marigold flower samples during storage are presented in Figure 2.

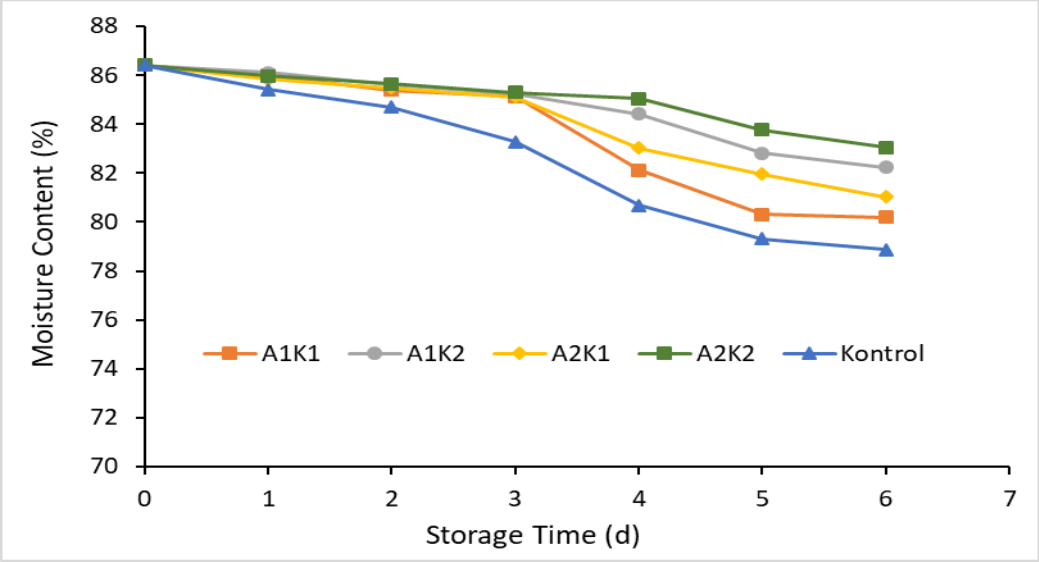

Figure 2. Moisture content changes of marigold flower during storage

The results showed that the water content of the Marigold flower samples decreased with storage time. This happened to all samples that received coating or control treatment. The water content value of Marigold flowers with citric acid and coating treatment after 6 days of storage ranged from 80.20% -83.04%, while the water content in the control sample was 78.86%. This significant difference indicates that the coating treatment is able to withstand water loss during storage, because the coating functions as a barrier to prevent moisture loss (Krochta et al., 2002).

Based on the results of the ANOVA, it is known that the coating has an effect on the decrease in water content and this effect is visible from the 4th day until the end of storage. Marigold flower samples treated with chitosan coating with 0.1% concentration were significantly different from 0.05% chitosan treatment and both were also significantly different from control flower samples. While the citric acid treatment factor did not show a significant difference, meaning that the citric acid treatment did not affect the decrease in water content. Dhyan *et al*. (2014) explained that coating treatment can inhibit metabolic processes, both respiration and transpiration rates so that the reduction in water content can be suppressed which can maintain flower quality and a longer shelf life.

3.3. Weight Loss

Alsuhendra *et al*. (2011) stated that the increase in weight loss was one result of the loss of water and other volatile components that easily evaporated at refrigerator temperature (10 °C). Loss of weight can affect the quality of marigold flowers which indicates the level of freshness. According to Hardenburg *et al*. (1990), reduced flower weight makes the material look less attractive, the texture becomes ugly, and the quality decreases. The increase in weight loss of marigold flower samples during storage can be seen in Figure 3.

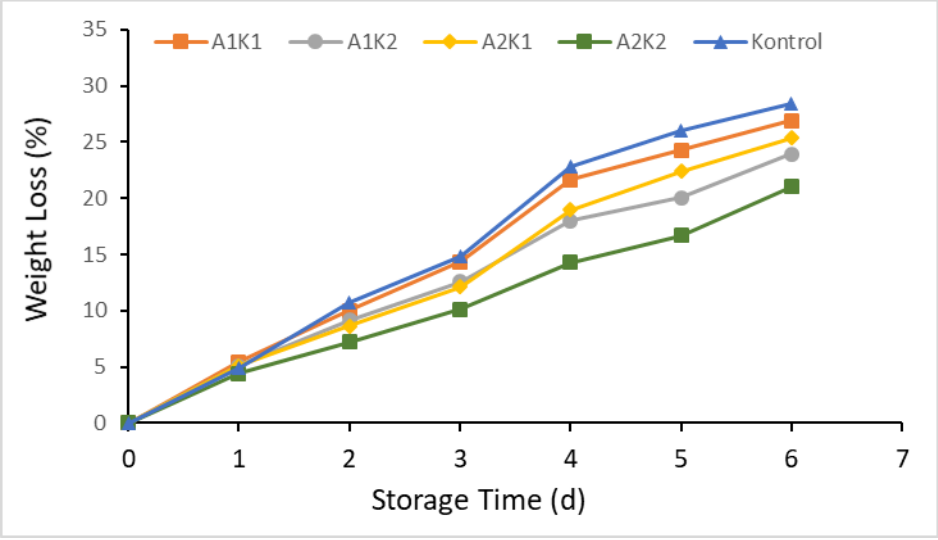

Figure 3. Changes in weight loss during storage

The weight loss of the marigold sample without treatment (control) reached 28.43% after the 6th day of storage, while the A2K2 treatment sample had the lowest weight loss of 21.05%. This shows that coating as a barrier can prevent water loss in flowers. The results of the ANOVA test stated the same thing as the water content, namely the

chitosan coating treatment showed an effect on day 4 until the end of storage while the citric acid treatment showed no significant difference. Chitosan coating with a concentration of 0.1% can reduce the weight loss of Marigold flower samples better than a concentration of 0.05%. This was shown in the DMRT test results for the chitosan coating factor on day 4 where the chitosan concentration of 0.1% was significantly different (sig <5%) with the chitosan coating treatment with a concentration of 0.05% and the control.

3.4. Color

Color is the most important factor in terms of acceptance of a product by consumers. If the product looks unattractive, consumers will reject the product regardless of other factors (Miskiyah *et al*. 2011). Edible flowers are used to beautify a dish, so the color on edible flowers is very important as a visual attraction. The *L* (lightness) value represents the brightness or darkness of the marigold flower petals. Changes in the *L* value of Marigold flowers during storage are presented in Figure 4.

The measurement results showed that the accumulated change in the *L* value of the marigold flower sample increased with the length of storage time, meaning that the change in the brightness level of the marigold flower decreased. A decrease in the brightness level during the storage period occurred in all sample flowers with both the coating and control treatments.

The results of ANOVA showed that there was an effect of citric acid treatment and chitosan coating on the *L* value from the 4th day to the end of storage (6th day). The citric acid factor was significantly different from the control, but the concentrations of citric acid were not significantly different. The results of the coating treatment test showed that the chitosan coating concentration of 0.1% was significantly different from the 0.05% chitosan coating, and both were significantly different from the control where the best L value at the end of storage was in the A2K2 treatment. According to Jiang *et al*. (2000), chitosan coating can slow down the formation of beta carotene and chlorophyll degradation so that the color change will be stable.

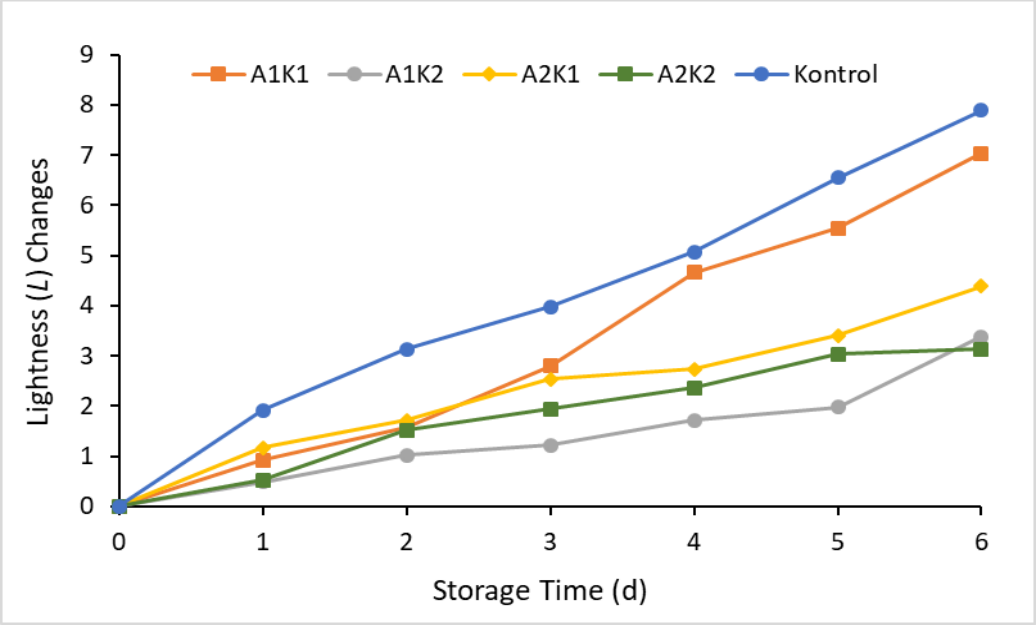

Figure 4. Accumulated changes in the *L* value of marigold flowers during storage

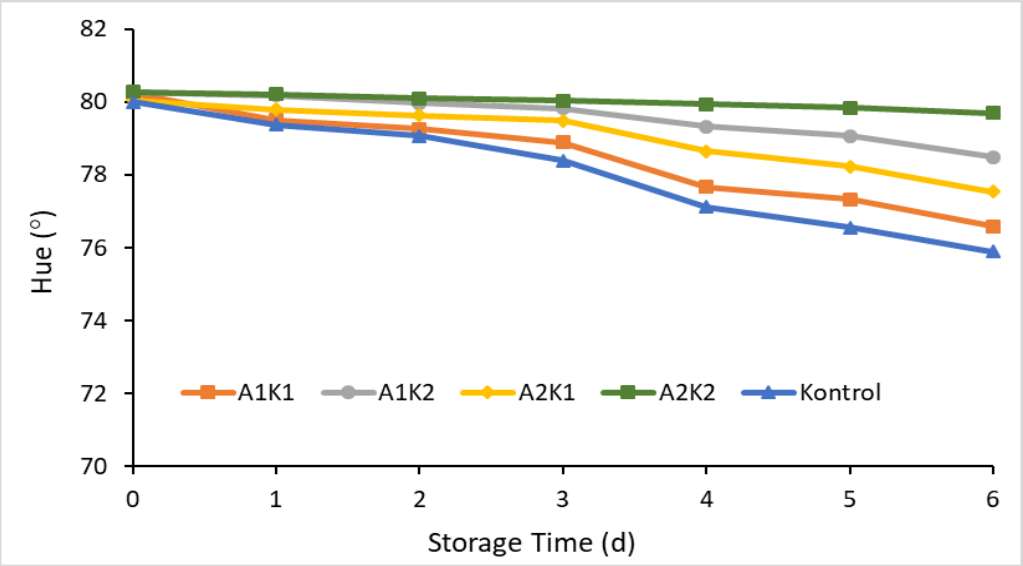

Figure 5. Changes in the °hue value of marigold flowers during storage

In addition to decreasing the *L* (brightness) value, the color of Marigold flower petals also decreased the °hue value. The change in the °hue value corresponds to the *L* value due to a change in the color of the petals. Changes in the °hue value of Marigold flowers during storage time are presented in Figure 5.

The initial °hue value of marigold flowers is in the range of 80.00° - 80.28°. At the end of storage, the highest °hue value (79.70°) was found in sample flowers treated with 2% citric acid and 0.1% chitosan coating (A2K2), while the °hue value in the control sample was 75.90°. The high decrease of °hue values in the control was due to high respiration rate. Marigold flowers have a main pigment, i.e. lutein (a carotenoid usually found in plants). Carotenoid synthesis was triggered by increasing concentration of O_2, ethylene, and increasing storage temperature (Alotaibi *et al*., 2021).

The results of ANOVA showed that there was an effect of citric acid and chitosan treatment on the °hue value. The results of the DMRT test on the citric acid factor showed that citric acid at a concentration of 2% was significantly different from citric acid at a concentration of 1%, and both were significantly different from the control. In contrast to the results of the DMRT test on the chitosan coating factor on the 4^{th} day of observation, the sample flowers treated with 0.05% chitosan coating were not significantly different from the control, but both were significantly different with a concentration of 0.1%. Based on these data it can be stated that the concentration of the coating solution at a certain limit will have an effect on changes in the ohue value. The results showed that chitosan with a concentration of 0.1% had a visible effect in suppressing the discoloration of marigold flower petals.

3.5. Organoleptic Test

Organoleptic testing of a product is important because it can determine consumer interest in the product. The criteria assessed in the organoleptic test included the appearance and color of the Marigold flowers.

The results of the panelist's assessment of the appearance of the flower samples from day 0 to day 6 of storage are shown in Figure 6, while based on the color of the flower petals are shown in Figure 7. The results of the organoleptic test showed that the A2K2 treatment sample had a hedonic score of 3 both for the appearance and

color of the flowers up to the 6_{th} day of storage, while the control samples up to the 3^{rd} day of storage. The A2K2 treatment can extend the shelf life longer by three days with the condition that the flower is still acceptable to consumers compared to no citric acid or coating treatment. The appearance of the control flower sample and the sample that was given the best treatment (A2K2 formulation) on day 0 and day 6 is shown in Figure 8.

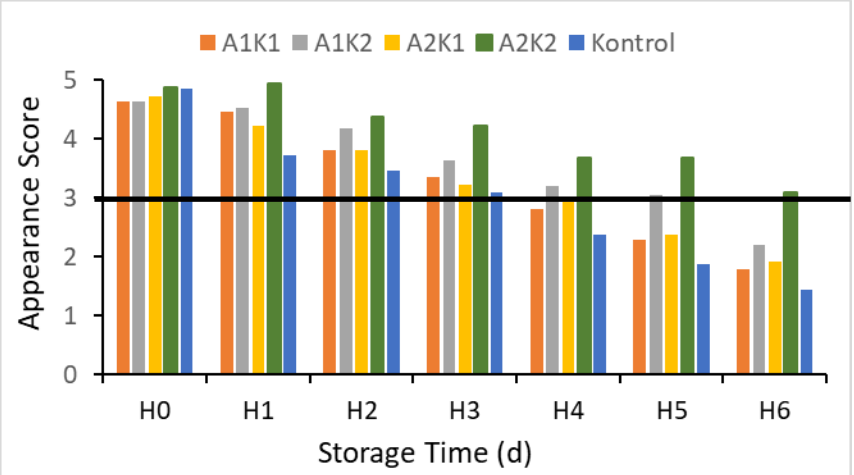

Figure 6. Changes in marigold flower appearance scores during storage

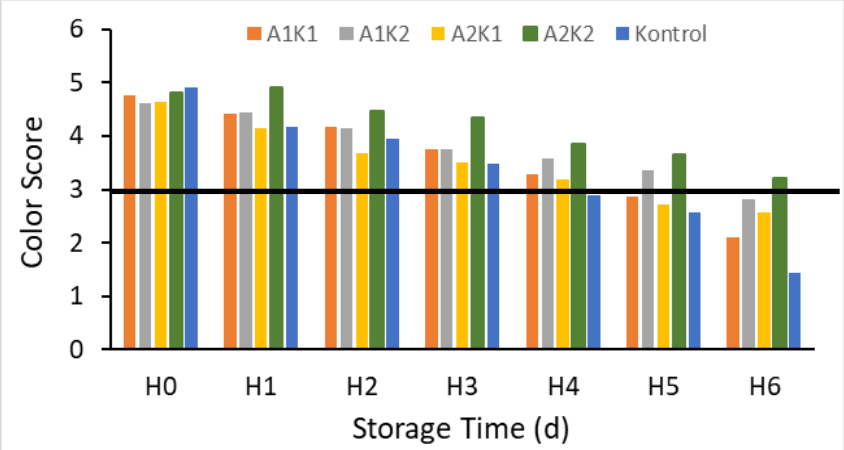

Figure 7. Changes in marigold flower color assessment scores during storage

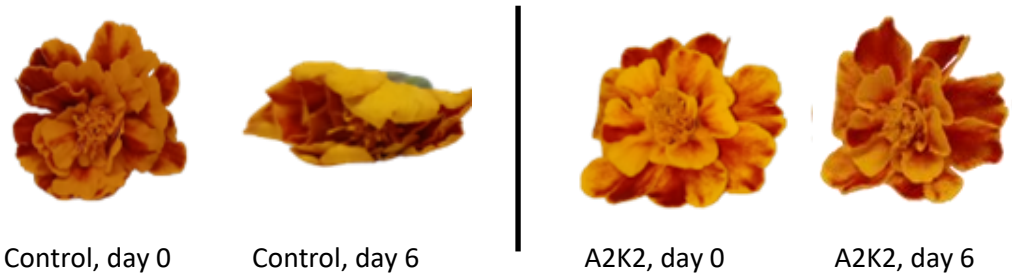

Figure 8. Changes of marigold flower on day 0 and day 6 of control (left) and A2K2 treatment (right)

4. CONCLUSIONS

4.1. Conclusion

Citric acid treatment and chitosan coating on Marigold edible flowers had an effect on maintaining quality during the shelf life. Chitosan coating application with a concentration of 0.1% is a coating solution that is more effective in maintaining quality than a concentration of 0.05%. The citric acid treatment played a role in inhibiting the decrease in °hue values with the best concentration being 2%. The combination of 0.1% chitosan concentration and 2% citric acid (A2K2) as a whole produced the best value on the quality parameters analyzed. The A2K2 treatment was able to maintain a moisture content of 83.04%, a weight loss of 21.05%, and a °hue value of 79.70° on the 6th day of storage, with panelist acceptance of a score of 3 (0-5) both appearance and color. The A2K2 treatment can extend the shelf life of Marigold flowers three days longer than control (no treatment)

4.2. Suggestions

The results showed that the treatment with the highest concentration (0.1% for chitosan and 2% for citric acid) provided the best degradation inhibition. The higher the concentration of the two treatments does not guarantee better inhibition, so further research is needed to find a combination of the two concentrations (chitosan and citric acid) that gives optimum results.

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