The effect of combination treatment of inhibitor solution and beeswax coating during storage to the quality of Citrus reticulata

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Abstract. The research purposed to determine the effect of the concentration of beeswax and storage time in orange fruit, which the stalk was soaking in the AgNO₃ solution. The model of experimental design methods used in the research was a randomized block design with two factors. The first factor was the concentration of AgNO₃ 10 ppm, 8 hours and concentration of beeswax coating were 0%, 4%, 8%, and 12%. The second factor was the storage time. The levels of vitamin C, weight loss, hardness, pH and total dissolved solids of the orange fruit have investigated. The result showed that concentration of beeswax (b) was affected the weight loss and vitamin C. Meanwhile, storage time (t) was affected weight loss, vitamin C, total dissolved solids, hardness, and pH. Beeswax 8% was the best concentration according to less weight loss, total dissolved solids, and pH. On the other hand, using Beeswax 12% was the best concentration according to the levels of vitamin C and hardness.

1. Introduction
The fruit is generally a perishable commodity that requires extra careful handling after harvesting so that its quality is maintained. The post-harvest activity of fruits is critical because it will determine the quality of the fruit produced. Generally, quality plays an essential role in marketing because it will provide a significant price. Besides determining the quality, post-harvest technology will also determine the amount of loss. In the post-harvest stage, there is always loss and damage, so that it can reduce the amount and quality of production [1]. Citrus industry is the largest fruit industry in the world. Postharvest storage, transportation and shelf life are the three important processes of the citrus fresh fruit industry. The appropriate storage and transportation technology will be beneficial for the quality maintenance of citrus fruits [2]. Whereas in orange fruits due to improper harvesting and handling activities, improper sorting, packaging, and packing, transporting, and storing that are less noticed, and the presence of pests and diseases can cause damage up to around 25% [3].

The main problem faced during storage is peel shrinkage that makes the fruit becomes less attractive, weight decrease, nutritional value decrease, and decaying. Therefore proper post-harvest handling is needed so that the fruit can be stored longer [1,2]. One way to maintain the freshness of horticultural commodities is by using inhibitor materials (including silver nitrate) to inhibit growth hormone production enzymes, maturation, senescence so that the material or solution containing the inhibitor is expected to maintain the freshness of fruits. Silver nitrate (AgNO₃) with a concentration of 10 ppm to a concentration of 50 ppm is one of the most effective bactericides. Silver nitrate can increase vase life by reducing the level of blockage carried out by bacteria and also as anti-ethylene.
[2–5]. The results of the earlier study showed that the use of AgNO3 10 ppm with 4 to 12 hours of soaking time can reduce the effect of storability with the following parameters: weight decrease, hardness level, vitamin C content, and total dissolved solids (TDS) in Guava. Guava that is stored at room temperature is expected to have a storability that lasts for 29 days for the responses of weight decrease, hardness level, vitamin C, and TPI or it can reach around 2.5 to 3 times longer compared to the controls only stored for 9 days at room temperature [5].

Another approach to improve the shelf life of fruit is by applying natural material as coating agent. The coating agent is to offer a protective barrier to moisture, oxygen, flavor, aroma, etc., between the food and the environment. Additionally, coatings may act as carriers of food ingredients and help improving the handling characteristics of the food. Therefore, application of coatings to fruits is a simple technology that allows reduction in fruit moisture loss and permits regulation of respiration as a passive modified atmosphere packaging. In addition, coatings can also act as carriers for anti-microbe and fungicide agent and improve fruit quality [6]. The application of coating technique is common in post-harvest protection of orange and citrus [7]. It could improve the appearance and attractiveness of orange fruits by waxing.

Application of beeswax and chitosan in Mango at (2%) significantly reduced physiological weight loss (%), total soluble solid (brix), titratable acidity (%), pH, disease incidence (%), disease index (%), maintained firmness (N) and prolonged shelf-life of fruits compared with untreated control [8]. Other research showed that using coconut oil mixtures with beeswax coating had immense effect on retaining green colour, reducing respiration, ethylene production, weight loss and shriveling, preserving firmness and moisture content of lemon throughout the storage [9]. The using of 1% of beeswax coating in kinow orange affected the less weight loss and % spoilage better than control [10]. Therefore, the aim of the present investigation was to evaluate the effect of the concentration of beeswax and storage time in orange fruit, which the stalk was soaking in the AgNO3 solution.

2. Materials and methods

2.1. The placed of research

The research activities were conducted in the Research Center for Appropriate Technology-Indonesian Institute of Sciences, Subang, West Java-Indonesia.

2.2. Materials collection and preparation

The sample of orange was Garut Siamese Orange (Citrus reticulata) aged 5-6 months, taken out from Wanaraja, Garut-West Java. The material used consisted of distilled water, AgNO3, beeswax, thiosulfate (Na2S2O3), potassium iodate (KIO3), 1% starch, Tryetanaleamine (TEA), oleic acid, iodine 0.01 N and potassium iodide (KI).

2.3. Research methods

Preliminary research was conducted to determine the concentration and immersion time of orange stalks in an optimal AgNO3 solution. The concentrations of AgNO3 solution were 10 ppm, 30 ppm and 50 ppm for soaking time were 4 hours, 8 hours and 12 hours [5]. Then an analysis of vitamin C, hardness, and weight loss were carried out on the 7th and 14th days. The primary research aimed to determine the combination of the concentration of AgNO3 solution and beeswax and the storage time to obtain orange fruits with excellent characteristics based on weight loss, hardness, total dissolved solids, acidity (pH), and vitamin C levels. The treatment design consists of two factors. The first factor was the concentration of AgNO3 selected in the preliminary study with the concentration of beeswax (B) which consists of 4 levels and the second factor was the storage time (T) which consists of 5 levels. Factor 1, namely Beeswax concentration (B), consists of: b1 = beeswax concentration 0%, b2 = beeswax concentration 4%, b3 = beeswax concentration 8%, b4 = concentration of beeswax 12%. Whereas Factor 2, namely Storage Length (T), consists of: t1 = 0 days, t2 = 4 days, t3 = 8 days, t4 =
12 days, and $t_5 = 16$ days. The experimental design used in the main study was factorial in the interaction of Randomized Block Design (RBD). The factorial pattern used was $4 \times 5$ with two replications. The design of the response carried out in this study were a chemical, physical, and physico-chemical response. The chemical response carried out was the level of vitamin C by the iodimetry method [5]. The physical response includes testing hardness and weight loss [10]. The weight loss is carried out by weighing and texture analysis, namely hardness using a penetrometer. Physico-chemical responses include testing total dissolved solids (TDS) using a refractometer, and the degree of acidity (pH) using a pH meter [10].

3. Results and discussions

3.1. Preliminary research

Preliminary research carried out was the immersion of orange stalks in inhibitor solutions (AgNO$_3$ solution) 10 ppm, 30 ppm and 50 ppm with an immersion time of 4 hours, 8 hours, and 12 hours. Then chemical and physical analysis were carried out. The analysis was carried out on the 7th and 14th days. The treatment of immersion of orange fruit in an inhibitor solution (AgNO$_3$ solution) affected on inhibiting or even delayed the ripening process for up to 14 days at room temperature ($26 \, ^\circ\text{C} \pm 1 \, ^\circ\text{C}$). According to Baret et al. (2010) [11], in the maturation process, there are color changes from green to yellow or red; the formation of taste from acid becomes sweet; texture becomes soft; the formation of vitamins; the distinctive aroma arises because of the formation of volatile compounds.

Based on the results of the analysis, the chosen treatment was the immersion of orange fruit in an inhibitor solution with a concentration of 10 ppm and soaking time of 8 hours because it has the lowest increase in weight loss, namely shrinkage weight on day 7 of 6.31% and day 14 of 9.22%. The lowest decrease in vitamin C level was on the 7th day of 42.07 mg gram / 100 grams of material and the 14th day was 38.25 mg / 100 grams of material, and the lowest level of violence was on the 7th day at 19 mm/50 g/10 seconds and the 14th day is 18.31 mm/ 50 g/ 10 seconds. Ethylene is a compound that is at the temperature of a gas-shaped chamber, which functions to stimulate fruit ripening. Giving ethylene is closely related to maturation. Ethylene hormones can accelerate the senescence process so that an anti-ethylene material is needed to inhibit the wilting process in orange fruits. One ethylene effective inhibitor such as Ag$^+$ can delay or even prevent maturation.

According to Qin et al. (2010) [4], Ag$^+$ ions were one of the inhibitors of ethylene action by deactivating ethylene receptors. The Ag$^+$ ion was suspected to deactivate the coding gene in ACC synthase or ACC oxidase, which causes the ripening of orange fruits to be inhibited. During ethylene biosynthesis, ATP-methionine-S-adenosyltransferase converts methionine to SAM (Sadenosylmethionine). ACC synthase (S-adenosyl-L-methionine methylthioadenosine-liase) converts SAM to ACC (1 aminocyclopropane-1-carboxylic acid). ACC is then converted to ethylene by ACC oxidase. Ethylene biosynthesis can thus be derived by deactivating the methionine-S adenosyltransferase coding gene, ACC synthase, ACC oxidase, or introducing the SAM hydrolase coding gene or ACC deaminase.

3.2. Main Research

In the primary research, the process of immersion of orange fruit in an inhibitor solution (AgNO$_3$ solution) with a concentration of 10 ppm for 8 hours which resulted from the preliminary study, then the orange fruit was coated with beeswax with a concentration of 0%, 4%, 8%, and 12%. The orange fruit which had been soaked and coated with beeswax then was stored at room temperature ($26 \, ^\circ\text{C} \pm 1 \, ^\circ\text{C}$). The first study conducted aimed to determine the effect of wax coating on orange fruits which immersed in an inhibitor solution (AgNO$_3$ solution) and then stored at room temperature ($26 \, ^\circ\text{C} \pm 1 \, ^\circ\text{C}$) for 16 days. Orange fruits which had been immersed in an inhibitor solution (AgNO$_3$ solution) and coated with beeswax were then analyzed for vitamin C levels, total dissolved solids, pH, weight loss, and hardness on day 0, day 4, day 8th, 12th day, and 16th day.
Measurement of weight loss was carried out using the scales carried out on day 0, day 4, day 8, day 12, and day 16. The results of the analysis of weight loss in orange fruits showed in table 1.

| Treatment | Average % Weight Loss |
|-----------|-----------------------|
| b3        | 5.73a                 |
| b2        | 6.19a                 |
| b4        | 7.26b                 |
| b1        | 10.02c                |

b1 = beeswax 0%, b2 = beeswax 4%, b3 = beeswax 8%, b4 = beeswax 12%

Table 1. Effect of beeswax concentration (B) on weight loss of orange

Description: The average followed by different letters showed significant differences according to Duncan's Advanced Test at the level of 5%.

Table 1 showed that b3 treatment (immersion in 10 ppm AgNO₃ solution and 8% beeswax) has the lowest weight loss and the weight loss of non-coated fruit was higher than that of the fruit given coating; this was due to 8% beeswax could cover the pores of the fruit skin evenly so that it could suppress water loss due to respiration and transpiration. According to a previously published paper (9), the success of coating wax for fruits and vegetables depends on the thickness of the layer. Too thin coating does not affect the reduction of respiration and transpiration rates, while those that are too thick can cause damage, odour, and distorted taste due to air in vegetables and fruits that contain too much CO₂ and a little O₂ [9]. The result of the Eshetu et al. (2009) [8] study also showed that the beeswax coating was able to suppress the percentage weight loss only 12.92% during storage. Coating with beeswax can inhibit weight shrinkage better than control and chitosan; this was because the fruit pores coated with beeswax were more closed compared to the controls and chitosan so that fruit transpiration could be suppressed.

Table 2. Effect of storage time (T) on weight loss of orange

| Treatment | Average % Weight Loss |
|-----------|-----------------------|
| t2        | 4.07a                 |
| t3        | 6.58a                 |
| t4        | 8.43b                 |
| t5        | 10.14c                |

t2 = 4 days, t3 = 8 days, t4 = 12 days, and t5 = 16 days

The average followed by different letters showed significant differences according to Duncan's Advanced Test at the level of 5%.

According to table 2, the longer the storage, the higher the weight loss of orange fruit, this is the same as that delivered by Eshetu et al. (2009) [8], the decrease in fruit weight increased with increasing storage time. According to an earlier study (2), the orange fruits stored at room temperature experience more significant loss of weight than fruit at low temperatures. According to Eshetu et al. (2009) [8], weight loss in mango fruit also increased during storage 30 days. Depreciation of fruit weight during storage is caused by water loss so that it will have an impact on quality degradation and trigger damage. Water loss is caused by some of the water in the material tissue undergoing evaporation or called transpiration. The impact of high water loss will cause flooding and cause shrinkage on the surface of the fruit so that the appearance of the fruit becomes unattractive and not suitable for marketing. During the storage period, the fruit physiologically process will continue to change and significantly will affect the quality of the fruit, both the colour and texture (hardness) of the fruit; this happened because orange fruits are a group of non-climatic fruits which are characterized by a decrease in respiration rate shortly after harvest until the senescence phase [11].

Hardness measurements of orange fruit were carried out using a penetrometer, in three sections, namely the end, middle, and base of the fruit. The value of hardness was the penetration distance of the
penetrometer needle against the fruit within 10 seconds with a load of 50g. The hardness unit is mm/50g/10 seconds. The results of the analysis of the level of violence in orange showed in Table 3.

Table 3. Effect of storage length (T) on hardness of orange

| Treatment | Average of Hardness |
|-----------|---------------------|
| t1        | 10.92               |
| t2        | 13.87               |
| t3        | 15.59               |
| t4        | 15.70               |
| t5        | 16.60               |

\( t1 = 0 \text{ days, } t2 = 4 \text{ days, } t3 = 8 \text{ days, } t4 = 12 \text{ days, and } t5 = 16 \text{ days} \)

Description: The average followed by different letters showed significant differences according to Duncan's Advanced Test at the level of 5%.

Table 3 showed that the hardness of orange fruits storage for 12 days increased and that decreased for 16 days storage. The findings of this study were following the results of an earlier study [12]. The hardness of orange storage at 18 °C and room temperature shows a decrease and then increases during storage; this is due to that in the ripening process of fruit, hydrolysis of pectin and hemicellulose occurs. Pectin and hemicellulose are components that formed the cell wall structure so that this change causes the fruit become soft when it is ripe. According to a previously thesis paper [13] at the beginning of storage, the mangoes teen fruit is not too hard. Still, along with increased shelf life, an increase in fruit skin hardness is due to the loss of the ability to bind water, so the commodity becomes hard. Pectin content indicates the hardness (texture) of the fruit. During ripening, pectin will be readily hydrolyzed into water-soluble components so that the total pectin substances will decrease and the water-soluble components will increase, and resulting in soft fruit. During the development and maturation process, cell turgor pressure is changing because of the occurring of the transformation of the cell wall composition. The existence of this change affects the hardness of the fruit during ripening. A decrease in protopectin that is not soluble in water and an increase in the amount of water-soluble pectin will cause the squeezing of fruits. According to the earlier study [12], cell turgor pressure always changes during the ripening process. This change happens because the composition of the cell wall that is plastic and caused the contents of the cell can be enlarged because it absorbs water from its surroundings. Therefore cell turgor pressure affects fruit hardness. If the water in the cell decreases, the cell will become soft and weak.

The total dissolved solids were measured using a refractometer. The scale displayed on the refractometer indicated the value of the Brix degree. Measurements were carried out on day 0, day 4, day 8, day 12, and day 16. Table 4 showed the results of the analysis of total dissolved solids (TDS) of orange fruits.

Table 4. Effect of storage duration (T) to TDS orange fruit

| Treatment | Average of Total Dissolved Solids (TPT) |
|-----------|----------------------------------------|
| t1        | 9.13\textsuperscript{a}               |
| t5        | 9.91\textsuperscript{b}               |
| t2        | 10.04\textsuperscript{b}              |
| t3        | 10.93\textsuperscript{c}              |
| t4        | 11.35\textsuperscript{c}              |

\( t1 = 0 \text{ days, } t2 = 4 \text{ days, } t3 = 8 \text{ days, } t4 = 12 \text{ days, and } t5 = 16 \text{ days} \)

Description: The average followed by different letters showed significant differences according to Duncan's Advanced Test at the level of 5%.
Table 4 showed that the total dissolved solids of orange fruits continued to increase during the storage of 12 days, then decreased on the 16th day even though only slightly and very slowly. According to the earlier published paper Sembiring (2014) [14], the total dissolved solids contained in fruit will increase faster when the fruit experiences maturity and will continue to decline along with the length of fruit storage. The process of ripening and decay will change the carbohydrate and sugar content. A decrease in the total dissolved solids is possible because dehydration occurs, and the sugar content decreases so that the total value of dissolved solids also decreases. Besides, it is also because during storage occurs the activity of enzymes and microbes that damage and decompose nutrients, resulting in a decrease in total dissolved solids. Oranges are a group of fruits with a non-climatic respiration pattern where changes in sugar levels occur slightly and slowly. Referring to changes in sugar levels in fruit are classified into two groups, namely fruits with high and low starch content. In harvesting, the fruit contains very few starches, and so that an increase in sugar levels during storage cannot be expected [15].

pH measurements on orange fruits using a pH meter tool and carried out on day 0, day 4, day 8, day 12, and day 16. The results of pH analysis on orange data were obtained as follows:

| Beeswax (B) | Time of Storage (T) |
|-------------|---------------------|
|             | t1      | t2      | t3      | t4      | t5      |
| b1          | 3.32    | 3.62    | 3.66    | 3.70    | 3.80    |
| b2          | 3.58    | 3.85    | 3.86    | 4.09    | 4.26    |
| b3          | 3.37    | 3.43    | 3.67    | 3.74    | 3.82    |
| b4          | 3.62    | 3.66    | 3.92    | 3.95    | 3.94    |

Table 5 showed that the pH value of orange during storage tended to increase (decrease in acidity). Results of earlier research [12] also show that orange has an increase in pH value; this is due to the increase of sweetness (Total Dissolved Solids) during storage. Another earlier finding [16] states that the decrease in pH in storage is related to bacteria that can catalyze sugar into acetic acid and lactic acid. In cold storage, the decrease and increase in pH take place slower than that in ambient temperature storage; this difference is caused by a decrease in temperature, causing the microbial growth rate to decrease so that the pH decrease is slight. The increase in the pH of the mangosteen fruit is caused by decreasing acidity in the cooking process. Further decomposition of the organic acid content by the enzyme into CO₂ and H₂O causes the H⁺ ions in the fruit to decrease so that the pH value will rise. The increase in pH is proportional to the increase in sugar levels in orange fruits. The longer the storage, the sweeter the fruit will be, so the pH value will increase, because the sugar will cause the atmosphere to be more neutral. The closer to pH 7, the acidity decreases because the pH 7 states that the material is classified as neutral. While the pH below seven approaches 0 is classified as acid and shows a strong acidity level. The Selected Beeswax Concentration was determined on the results of the overall chemical response, physical response, and physicochemical response to vitamin C levels, weight loss, hardness, total dissolved solids, and pH value of orange fruits during storage. The following table showed the best concentration of beeswax.
Table 6. Results of selected beeswax concentration

| Beeswax (B) | Value of Analysis Result Average |  |
|-------------|---------------------------------|--|
|             | Chemical response | Physic Response | Physicochemical Response |
|             | Vitamin C | Weight Loss | Hardness | Total Dissolved Solids | pH |
| b1 (0%)     | 43.81 | 10.02 | 13.77 | 10.61 | 3.62 |
| b2 (4%)     | 47.71 | 6.19 | 15.26 | 10.43 | 3.93 |
| b3 (8%)     | 49.10 | 5.73 | 14.94 | 9.88 | 3.60 |
| b4 (12%)    | 50.59 | 7.26 | 14.17 | 10.16 | 3.82 |

Table 6 showed that the concentration of 8% beeswax (B3) was the best concentration for weight-loss parameters because it had the lowest weight loss percentage, the lowest total dissolved solids value, and the lowest pH value. The concentration of 12% beeswax is the best concentration for vitamin C parameters with the highest value of vitamin C compared to the concentration of another beeswax, and also the best concentration for the parameters of the level of hardness. The hardness level at a concentration of 12% was chosen because it tended to raise the value of hardness during storage when compared with the concentration of wax 4% and 8%. While 0% of waxing treatment has the lowest hardness value because it has begun to shrinkage.

4. Conclusion
The results of the preliminary study obtained the concentration of selected AgNO\(_3\) solution was 10 ppm, and the immersion time was 8-hour. Determination of the concentration of 10 ppm AgNO\(_3\) solution and 8-hour immersion time was selected based on the lowest decrease in vitamin C levels, the lowest increase in weight loss percentage, and the lowest decrease in hardness during 14 days of storage. The results of the main study found that coating using beeswax (B) significantly affected a weight-loss and vitamin C. While the storage time (T) significantly affected weight loss, vitamin C, total dissolved solids, and the level of hardness of orange fruits. The 8% concentration of beeswax was the best concentration for weight-loss parameters, total dissolved solids, and pH, while the concentration of 12% beeswax is the best concentration for the parameters of vitamin C levels and hardness.

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