

Study of Incubation Duration After Degreening and Storage Methods on the Quality of Tangerine "Garut" (*Citrus reticulata* B.)

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Abstract

Ripened citrus fruits with an orange rind have a higher market demand relative to unripen citrus with a green rind. This research was aimed to evaluate the combination of post-degreening incubation duration before storage with the type of storage to increase orange rind color and storage life of citrus fruit. A randomized block design with two factors was used; the first factor was post-degreening incubation duration before storage that comprised of without incubation, incubation for two and four days. The second factor was storage method that comprised of storage at room temperature ($29 \pm 1^\circ\text{C}$), at $18 \pm 1^\circ\text{C}$, waxing and storage at room temperature, waxing and storage at $18 \pm 1^\circ\text{C}$. The result shows that post-degreening incubation duration that increased orange color of citrus rind was four days. The citrus which was stored at $18 \pm 1^\circ\text{C}$ had the smallest fruit weight loss. The best treatment combination which increased orange color formation and had lower percentage of fruit weight loss was a combination of 4 days incubation after degreening and storage at $18 \pm 1^\circ\text{C}$.

Keywords: bee wax, citrus color index, cool storage, ethephon, hue values

Introduction

Indonesian citrus has a lower market demand in comparison with imported citrus. Many people assume citrus with orange rind are fresher and has sweeter taste than the other rind colors. Monica (2015) stated that citrus which has an orange rind color is more attractive to consumers than ones with a pale yellow rind. Tropical citrus cannot compete on the basis of appearance because fruits do not develop an attractive color in tropics due to temperature

requirements for developing colors (Ladaniya, 2008). The orange color of the citrus rind is formed due to a mixture of cryptoxanthin and β -citraurin (Redrigo et al., 2013). which does not occur in Indonesia lowland because β -citraurin only forms at low temperatures. The color formation of citrus rind is affected by cool temperature and temperature changes during maturation (Ladaniya, 2008).

Citrus degreening is a technology which enables the citrus fruit to change the color of the citrus rind from green to orange. This technology uses the hormone ethylene as a treatment. Exogenous ethylene can degrade green pigments (chlorophyll) on fruit rind and help to form orange pigments (carotenoids) (Zhou et al., 2010; Mayuoni et al., 2011; Rhamadhani et al., 2015; Arzam et al., 2015). The application of ethylene can stimulate carotenoid formation, such as cryptoxanthin, β -citraurin, and violaxanthin. The process of forming pigments is affected by temperature. Degreening at room temperature ($28 - 29^\circ\text{C}$) inhibits the formation of β -citraurin, this causes the citrus rind to appear yellow. Degreening at temperature of 18°C and 23°C can form β -citraurin and cryptoxanthin simultaneously to produce the orange color (Stewart and Wheaton, 1971).

Citrus is a perishable horticultural product because it has high water content. After harvesting, metabolic processes within citrus such as respiration and transpiration are still on going. This causes a physicochemical change in the fruit, especially during the duration in storage. A few ways of extending the fruit shelf life is to coat and store them in low temperature. Citrus that was not stored at cool temperature can experience weight loss and nutritional value decrease, such as vitamin C and sugar levels. The experiment conducted by Musdalifah et al. (2016) showed that the rind color of *Citrus nobilis* cv. "Pontianak" that are being stored at 10°C changed color after degreening

and also maintain a longer shelf life up to 42 days.

Waxing is coating technology which is often used for extending the fruit shelf life. Bee wax coating on sweet oranges can reduce the weight loss caused by water loss and transpiration (Shahid and Abbasi, 2011). Decreasing respiration and transpiration rates in citrus fruits can slow the process of chlorophyll degradation and carotenoid formation. Gautama's experiment (2018) shows that the tangerine fruit that was coated with a 6% or 9% bee wax can maintain the green color for 28 days. Incubation duration after degreening, storage temperature and waxing are important for tangerine quality. Therefore, this experiment aims to 1) determine the incubation duration after degreening before storage to improve orange color formation. 2) determine the appropriate storage method to maintain fruit quality. 3) determine the interaction between incubation duration after degreening before storage and storage method.

Materials and Methods

The research was conducted in March - May 2018 at the Center for Tropical Horticulture Studies (PKHT) Laboratory. The tools used in this research were a degreening box, cool case, Minolta color reader CR-101865-105 and digital scales. The materials used are tangerines cv. "Garut", ethephon, bee wax, distilled water, oleic acid and triethanolamine. The research was conducted using a randomized block design (RBD) with two factors, namely the incubation duration after degreening before storage (incubation duration) and storage methods. The incubation duration factor consisted of three levels, without incubation, two days after ethephon application, and four days after ethephon application. The storage methods factor consists of four levels, namely without waxing and stored at room temperature (29 ± 1 °C), without waxing and stored at 18 ± 1 °C, waxing with 6% bee wax and stored at room temperature, and waxing with 6% bee wax and stored at 18 ± 1 °C. The total treatment combination was 12 treatments and repeated three times so that there were 36 experimental units. Each experimental unit uses 15 tangerine fruits, three fruits for non-destructive measurement and 12 for destructive measurement. The total number of tangerines used in this experiment was 540.

The tangerines were dipped in 1000 ppm ethephon for 30 seconds (Zhou et al., 2010), then air dried for three hours and treated with different incubation durations before being put into storage. For the treatment without incubation, fruits were dipped in ethephon then after being air dried for three hours

were put into the storage method treatments. For the two day incubation duration treatment, fruits were dipped in the ethephon solution, then fruits were incubated for two days at room temperature then stored at different storage method treatments. For the treatment of four days, the fruits after ethephon application were incubated for four days at room temperature, then stored at different storage method treatments. Waxing was performed by dipping citrus in a 6% bee wax emulsion for 30 seconds. The process of making bee wax emulsion refers to the research of Dhyana et al. (2014). Measurement and observation were carried out every three days during storage which included scoring of rind color, weight loss, and rotten fruit.

The scoring of rind color was done using Minolta Color Reader CR-101865-105 which applied the Hunter color notation system in which the sensor beam is fired into tangerines in three parts, the blossom-end, middle, and stem-end area of the fruit. The Hunter color notation system is characterized by three color parameters, namely "L", "a", and "b". "L" states brightness with values 0 (black) to 100 (white). The "a" and "b" are chromaticity coordinates, notation "a" stated the chromatic color of the red green mixture with the "+a" value from 0 to 60 for red and "-a" from 0 to -60 for green. The "b" stated the chromatic color of the yellow-blue mixture with the "+b" value from 0 to 60 for yellow and the "-b" value from 0 to -60 for blue.

Quantitative measurements of rind color using the calculation of the value of the citrus color index (CCI) (Jimenez-Cueata et al., 1981). The orange color in citrus rind is indicated by a high CCI value and green orange rind is indicated by a low CCI value. CCI value is calculated using following equation:

$$CCI = \frac{1000a^*}{L^*b^*}$$

The results of measuring "a" and "b" are converted into chromatic units of hue ($^{\circ}$ hue) which is an important color parameter in citrus measurements (Ramadhani et al., 2015). The value of $^{\circ}$ hue determines the degree of chromatic red, green, blue, and yellow. The high $^{\circ}$ hue value indicates the fruit is still green and the low $^{\circ}$ hue value indicates the fruit has orange color. The following equation is used to get $^{\circ}$ hue (Manera et al., 2012):

$$^{\circ}Hue = \arctan \left(\frac{b^*}{a^*} \right)$$

Data analysis in this research was conducted by analysis of variance (ANOVA) at level of 5%. If it is significantly different, it will be continued with Duncan's Multiple Range Test (DMRT) at level of 5%.

Result and Discussion

Citrus Color Index

The treatment of incubation duration showed significant effect start from initial observing to CCI values (Table 1). The highest CCI value of the incubation duration was obtained from the four-day incubation period with a value of 9.2 (indicates the color of citrus rind is orange) on the 27th day. The storage method treatment showed a significant effect starting from the third day. The storage method that produced the highest CCI value was stored without waxing at 18 ± 1 °C with a value of 10.5 (indicates the color of citrus rind is dark orange) on the 27th day.

Initial observation (Table 1) in the incubation duration treatments showed significant effect because the tangerines had changed color. The fruit had undergone the chlorophyll degradation process when the fruit was incubated at room temperature after degreening. Chlorophyll degradation in citrus rind resulted in changes in citrus rind color due to a decrease in chlorophyll content (Peng et al., 2013). Chlorophyll degradation had occurred on fruit treated with two and four days of incubation duration compared to fruit without incubation. Chlorophyll degradation occurs within 3-4 days after removal of citrus from ethylene exposure sites (Ladaina, 2008). The CCI value in the treatment incubation duration showed that the optimal duration to conduct the storage method was four days after degreening.

The most effective storage method for increasing CCI values is the non-waxed fruit and to store at 18 ± 1 °C (Table 1). Storage after degreening at 18°C and 23°C can increase the formation of orange color rind (Stewart and Wheaton, 1971). The orange color of the citrus rind occurs because of the accumulation of two carotenoid compounds, cryptoxanthin and β -citraurin (Redrigo et al., 2013). Fruits stored at 18°C and 23°C after degreening produce higher β -citraurin compounds compared to fruits stored at 29°C (Stewart and Wheaton, 1971). Fruit waxing can reduce the rate of respiration and transpiration. The increased value of CCI in wax coated fruit when stored at 18 ± 1 °C occurred slower than other storage methods due to the low respiration of the fruit. The low respiration rate inhibit the work of enzymes that affect chlorophyll degradation.

The fruit with wax application and storage at room temperature (29 ± 1 °C) started to decay on the sixth day (Table 1) after storage. Fruit without incubation after degreening experienced decay when given the application of waxing and storage at room temperature (29 ± 1 °C). We hypothesize that it occurred because the ethephon which changed into ethylene, chlorine and phosphonate reacted with bee wax emulsion at high temperatures; the results of this reaction damaged the rind of the fruit and cause fruit to decay. The interaction between incubation duration and storage methods on day 12 show that all incubation duration treatments after degreening had significant effect on CCI value in all storage methods (Table 2). The storage methods affected the CCI value in

Table 1. Effect of incubation duration and storage methods on CCI values of citrus rind

Treatments	CCI										
	BD	0 ^D	3	6	9	12	15	18	21	24	27
Incubation duration											
0 day	-1.4	-1.4c	0.1c	1.9c	3.3c	4.4c	5.2c	6.1b	6.8b	7.4b	8.3
2 days	-1.4	0.7b	2.9b	4.3b	5.2b	5.7b	6.6b	7.3a	7.8a	8.3ab	8.8
4 days	-1.4	4.8a	5.3a	5.6a	6.1a	6.4a	7.4a	7.6a	8.3a	8.8a	9.2
Storage methods											
Non-waxed, stored at 29 °C	-1.4	1.1	4.1a	5.6a	6.3a	6.7a	7.1a	7.4b	8.0b	8.3b	8.9b
Non-waxed, stored at 18 °C	-1.4	1.8	3.0b	4.7b	6.1a	7.0a	7.7a	8.5a	9.0a	9.5a	10.5a
Wax-coated, stored at 29 °C	-1.4	1.2	2.1c	3.1c	4.0b	4.7b	x	x	x	X	x
Wax-coated, stored at 18 °C	-1.5	1.2	1.8c	2.4d	3.0c	3.5c	4.4b	5.3c	5.9c	6.6c	6.9c
Incubation duration X storage methods	ns	ns	*	*	*	*	*	*	ns	ns	ns

Note: values followed by the same letters in the same column are not significantly different at the 5% DMRT test, ns = not significantly different, * = significantly different at the 5% level, BD = before degreening, ^D = days after storage, x = rotten fruit. Range citrus color index (CCI): CCI \leq -5 (dark green), -5 < CCI \leq 0 (green), 0 < CCI \leq 3 (yellowish green), 3 < CCI \leq 5 (greenish yellow), 5 < CCI \leq 7 (yellowish orange), 7 < CCI \leq 10 (orange), and CCI > 10 (dark orange) (Jimenez-Cueata et al. 1981).

all incubation duration treatments. In treatments without incubation, non-waxed fruit stored at room temperature had the highest CCI value compared with the other storage methods. Fruit from waxing treatment which stored at $18 \pm 1^\circ\text{C}$ had the lowest CCI value. In two, and four days incubation duration, non-waxed fruit had higher CCI value compare to the fruit with waxing treatment, both at room temperature and $18 \pm 1^\circ\text{C}$. This is because the waxing treatment can decrease the respiration and transpiration rate which lead to slowing of chlorophyll degradation and carotenoid formation. The incubation duration had no effect on CCI value in non-waxed fruit stored at room temperature (Table 2), however incubation duration affected the CCI value in non-waxed fruit stored at $18 \pm 1^\circ\text{C}$, wax-coated fruit stored at room temperature and wax-coated stored at $18 \pm 1^\circ\text{C}$. The smallest CCI value was found in fruit without incubation which wax-coated treatment and stored at $18 \pm 1^\circ\text{C}$. In general, fruits with incubation duration (two and four days) had higher CCI value compared with fruit without incubation. Fruit with wax-coated treatment and stored at $18 \pm 1^\circ\text{C}$ had higher CCI value in four days incubation duration rather than in two days incubation duration treatment. This is because the ethylene had already reacted on incubated fruits which lead to chlorophyll degradation and carotenoid formation. The treatment of incubation duration showed that the $^{\circ}\text{hue}$ values produced had significant effects at day zero (Table 3); $^{\circ}\text{hue}$ value in all treatments incubation duration has decreased during storage. This shows the fruit changes color from green to orange. The lowest $^{\circ}\text{hue}$ value occurred at the 4 days incubation duration treatment which was 65.6 on the 27th day after storage. The treatment of storage methods













began to show a significant effect on the third day. The wax coated fruits which were stored at $18 \pm 1^\circ\text{C}$ have slower $^{\circ}\text{hue}$ reduction than other types of storage. The storage method that produces the lowest $^{\circ}\text{hue}$ value is the storage method at $18 \pm 1^\circ\text{C}$ without waxing with a value of 63.0 on the 27th day.

The treatment of incubation duration showed that the $^{\circ}\text{hue}$ value were significantly different starting from initial observations (Table 3). $^{\circ}\text{hue}$ value in all incubation duration after degreening treatments has decreased during storage. This showed that the fruit changes color from green to yellowish orange. The four days incubation duration had relatively lower $^{\circ}\text{hue}$ value than the other treatments. The incubation treatment allowed the ethylene to work on the tangerine rind in order to transform color from green to orange. The application of exogenous ethylene could increase the degradation rate of chlorophyll and helps the formation of carotenoids (Rodrigo and Zacarias, 2007).

Non-waxed citrus fruit that stored at 18°C had the lowest $^{\circ}\text{hue}$ value on 27th day compared to other storage treatments. β -Citraurin formed in citrus rind during low temperature storage could change the rind color into orange. The experiment conducted by Rodrigo et al. (2013) showed that sweet orange cv. "Navelina" stored at 20°C had a higher β -citraurin than the fruit stored at 37°C . The orange color of citrus rind was caused by the decrease of chlorophyll and also the increase of β -citraurin content.

The interaction of incubation duration and storage methods to $^{\circ}\text{hue}$ value was occurred at 3 to 21 days

Table 2. Interaction of incubation duration and storage method for CCI values

Storage method	Incubation duration		
	0 (days)	2 (days)	4 (days)
Non-waxed, stored at 29°C	7.19A 	6.69A 	6.61AB 
Non-waxed, stored at 18°C	6.23Bb 	7.56Aa 	7.31Aa 
Wax-coated, stored at 29°C	3.39Bb 	4.81Ba 	5.96Ba 
Wax-coated, stored at 18°C	0.87Cc 	3.99Bb 	5.70Ba 

Note: Values followed by the same capital letters in the same column show no significant difference according to DMRT at 5%; the values followed by the same lowercase letters in the same row show no significant difference according to DMRT at 5%. Range of citrus color index (CCI): CCI $5 - 5$ (dark green), $-5 < \text{CCI} \leq 0$ (green), $0 < \text{CCI} \leq 3$ (yellowish green), $3 < \text{CCI} \leq 5$ (greenish yellow), $5 < \text{CCI} \leq 7$ (yellowish orange), $7 < \text{CCI} \leq 10$ (orange), and $\text{CCI} > 10$ (dark orange) (Jimenez-Cueata et al. 1981).

Table 3. Effect of incubation duration and storage methods on °hue value on orange rind

Treatment	°hue value										
	BD	0 ^D	3	6	9	12	15	18	21	24	27
Incubation duration											
0 day	86.8	86.8a	85.5a	82.7a	80.2a	77.7a	75.3a	73.2a	71.6a	70.1a	68.6
2 days	86.8	87.0a	82.1b	77.9b	75.6b	74.5b	71.7b	70.1b	68.8b	67.7ab	67.0
4 days	86.8	76.3b	74.8c	73.9c	72.6c	71.5c	69.4c	68.6b	67.2b	66.3b	65.6
Storage methods											
Non-waxed, stored at 29 °C	86.8	84.0	78.6c	74.0c	72.1c	71.1c	70.1b	69.1b	68.0b	67.3b	66.2b
Non-waxed, stored at 18 °C	87.0	82.4	79.8b	76.1b	72.7c	70.2c	68.4c	66.9b	65.7b	64.5c	63.0c
Wax-coated, stored at 29 °C	86.7	83.5	82.4a	80.9a	79.0b	77.2b	X	x	x	x	x
Wax-coated, stored at 18 °C	86.7	83.6	82.5a	81.7a	80.9a	79.7a	77.9a	75.8a	74.0a	72.3a	71.9a
Incubation duration X storage methods	ns	ns	*	*	*	*	*	*	*	ns	ns

Note: Values followed by the same letters in the same column are not significantly different according to DMRT at 5%; ns = not significantly different; * = significantly different at the 5% level; BD = Before degreening; ^D = days after storage; x = rotten fruit.

after treatment. Table 4 shows that storage method had significant effect on °hue value in all incubation duration treatments. Fruit of non-waxed treatment which stored both at room temperature or 18 ± 1°C had significant lower °hue value compared to wax-coated fruit in every incubation duration. This shows that fruit without wax-coated treatment had more orange color than fruit with wax-coated treatment. In 2 days incubation duration, non-waxed fruit stored at 18 ± 1 °C had the lowest °hue value compared with the other storage method treatment. Fruit after degreening at 18 °C and 23°C can increase orange color on its rind because *β-citraurin* compound was increased (Stewart and Wheaton, 1971).











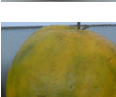

The incubation duration treatment has a significant effect on the °hue value of the non-waxed fruits stored at 18 ± 1 °C, the wax-coated fruits stored at room temperature, and the wax-coated fruits stored at 18 ± 1 °C (Table 4). The incubation duration did not affect the value of °hue in the non-waxed fruits stored at room temperature. Non-waxed fruits stored at 18 ± 1 °C which had incubation duration of two days and four days after degreening had the lowest °hue value. Wax-coated fruit, both stored at room temperature or at 18 ± 1 °C, the value of °hue decreases if the incubation duration is longer. This shows that the degreening will effective if the fruits are incubated 2 or 4 days before being given a wax application.

Fruit Weight Loss

Citrus fruits, like fruits in general, has high water content, so it will experience physical changes due to respiration and transpiration during storage. The loss of water in the fruit will cause the fruit to shrink thus reducing the quality of the fruits. Incubation duration did not affect the fruit weight loss (Table 5), because incubation treatment after degreening was only long enough for the ethephon to work so that the color on the citrus rind turned orange. The ethephon application did not affect the weight loss of tangerines (Nacing, 2017).

The storage method has a significant effect on the citrus fruit weight loss (Table 5). The fruit without waxing and that were stored at room temperature had the highest weight loss. The fruits without waxing and stored at room temperature experienced much higher respiration and transpiration than non-waxed fruits stored at 18 ± 1 °C, the wax coated fruits stored at room temperature and the wax-coated fruits stored at 18 ± 1 °C. A high transpiration rate results in a decrease of water content in the fruit which will reduce the weight of the fruits. High weight loss will reduce fruit quality. Non-waxed fruit stored at 18 ± 1 °C and wax coated fruit stored at 18 ± 1 °C on the 27th day has lower percentage of weight loss compared to non-waxed fruit stored at room temperature, 15.01% and 15.39% lower, respectively. This result is in accordance with the Gautama experiment (2018)

Table 4. Interaction of incubation duration and storage methods on the value of °hue on day 12

Storage methods	Incubation duration					
	0 (days)		2 (days)		4 (days)	
Non-waxed, stored at 29 °C	70.5C		71.0B		70.6B	
Non-waxed, stored at 18 °C	72.2Ca		69.2Cb		69.2Bb	
Wax-coated, stored at 29 °C	81.3Ba		77.4Ab		72.9Ac	
Wax-coated, stored at 18 °C	86.7Aa		79.1Ab		73.4Ac	

Note: values followed by the same capital letters in the same column have no significant differences according to DMRT at 5%; values followed by the same lowercase letters within the same row have no significant differences according to DMRT at 5%.

Table 5. Effect of incubation duration and storage methods on the weight loss of citrus fruits.

Treatment	Percentage of fruit weight loss (%)								
	3 ^D	6	9	12	15	18	21	24	27
Incubation duration									
0 day	2.323	4.72a	6.930	8.381	10.787	12.643	14.66a	16.370	18.195
2 days	2.350	4.70a	6.480	8.367	10.128	11.918	13.74ab	15.709	17.379
4 days	2.284	4.08b	5.990	7.514	9.256	10.890	12.37b	14.141	15.637
Storage methods									
Non-waxed, stored at 29 °C	2.87a	5.49a	7.71a	10.01a	12.28a	14.46a	16.60a	18.65a	20.81a
Non-waxed, stored at 18 °C	1.87c	3.75c	5.42b	7.07bc	8.70b	10.29b	11.95b	13.68b	15.01b
Wax-coated, stored at 29 °C	2.29b	4.57b	6.58bc	8.29b	x	X	X	x	x
Wax-coated, stored at 18 °C	2.24b	4.19bc	6.15c	6.99c	9.19b	10.70b	12.22b	13.89b	15.39b
Incubation duration X storage methods	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Values followed by the same letters in the same column show no significant difference from the 5% DMRT test, ns = non significant, ^D = days after storage, x = rotten fruit.

that "Garut" tangerine waxed fruits and non-waxed fruits stored at 18°C did not significantly affect the percentage of fruit weight loss on the 28th day of storage. Low temperatures can suppress respiration and transpiration rate. "Selayar" tangerine which was stored at 18-20°C have lower fruit weight loss than those stored at room temperature (Arzzam and Baba, 2018). Waxing functions as a barrier between fruit and the environment which will reduce fruit transpiration (Shahid and Abbasi, 2011).

Percentage of Rotten Fruit

Treatment of incubation duration affected the percentage of rotten fruits (Table 6). Fruits that were not incubated and incubated for less duration (2 days) had the highest percentage of rotten fruit. The fruit that was incubated for 4 days had the lowest percentage of rotten fruit. The wax-coated fruit and the stored at room temperature has a high percentage of rotten fruit compared to fruit in other storage methods (Table 6). The non-waxed fruit which was stored at 18 ± 1 °C had the lowest percentage of fruit rot, which is 5.93%

on day 27 after storage. Low temperatures can reduce fruit decay factors such as, microorganism activity, respiration, transpiration, and inhibit enzyme activity.

Interaction of incubation duration and storage methods on the percentage of fruit rot began to display differences on the 6th day. Table 7 showed that the treatment of storage methods had a significant effect on the percentage of fruit rot at all incubation durations. The treatment without incubation, in non-waxed fruit and wax-coated fruit stored at 18 ± 1°C has a smaller percentage of rotten fruit and was significantly different than the fruit stored at room temperature. This shows that low temperatures can reduce the percentage of fruit rot. Incubation duration of two or four days in wax-coated fruit stored at room temperature has the highest percentage of rotten fruit and it was significantly different than the fruit of other storage methods.

Incubation duration has an effect on the percentage of rotten fruit only in the storage method of waxing which is stored at room temperature (Table 7). Fruits with longer incubation duration will produce a smaller

percentage of fruit rot in wax-coated treatment stored at room temperature. This means that incubation duration that conducted after degreening prior to storage could reduce the percentage of rotten fruit in wax-coated treatment which stored at room temperature.

Conclusion

The best incubation duration after ethephon application that can increase the orange color and had the smallest percentage of rotten fruit is four days. The best storage method for increasing the orange color of citrus rind, having low fruit weight loss and the lowest percentage of rotten fruit is storage at 18 ± 1°C. Four days incubation duration combined with non-waxed and storage at 18 ± 1°C was determined as the best treatment indicated by the high rate of orange color of citrus rind and the low percentage of fruit weight loss and rotten fruits.

Table 6. Effect of incubation duration and storage methods on percentage of rotten fruit

Treatment	Percentage of rotten fruit (%)							
	6 ^D	9	12	15	18	21	24	27
Incubation Duration (days)								
0	8.9a	12.a	14.4a	15.6a	20.0a	23.3a	24.4a	28.3a
2	7.8a	10. 0a	12.2a	16.7a	17.8a	21.1a	27.2a	28.9a
4	1.1b	1.1b	4.4b	6.1b	9.4b	11.6b	15.0b	16.1b
Storage methods								
Non-waxed, stored at 29 °C	2.2b	2.2b	2.2b	4.4b	8.9b	12.6b	17.0b	22.2b
Non-waxed, stored at 18 °C	0.7b	0.7b	1.5b	1.5b	2.2c	3.0c	5.9c	5.9c
Wax-coated, stored at 29 °C	19.3a	26.7a	35.6a	42.2a	48.2a	54.1a	58.5a	60.0a
Wax-coated, stored at 18 °C	1.5b	1.5b	2.2b	3.0b	3.7bc	5.2c	7.4c	9.6c
Interaction	*	*	*	*	*	*	*	*

Note: Values followed by the same letters in the same column show no significant difference according to DMRT at 5%; * = significantly different at 5%; D = days after storage

Table 7. Interaction of incubation duration and storage methods on percentage of rotten fruit on 27th day after storage

Treatment	Incubation duration (days)		
	0	2	4
Non-waxed, stored at 29 °C	24.44B	26.67B	15.56B
Non-waxed, stored at 18 °C	8.89C	2.22B	6.67B
Wax-coated, stored at 29 °C	73.33Aa	68.89Aa	37.78Ab
Wax-coated, stored at 18 °C	6.67C	17.78B	4.44B

Note: values followed by the same capital letters in the same column show no significant difference; values followed by the same lowercase letters in the same row show no significant difference according to DMRT at 5%.

Acknowledgement

We thank the Center for Tropical Horticulture Studies of IPB University for the facilities provided throughout the study.

Reference

- Arzam, T.S., and Baba, A.R.B. (2018). Difference of storage temperature on the quality of "selayar" citrus. *Journal TABARO* **2**, 145-151.
- Arzam, T.S., Hidayati, I., Poerwanto, R., and Purwanto, Y.A. (2015). Precooling and ethylene concentration at degreening to generate orange color of tangerine peels. *Jurnal Hortikultura Indonesia* **25**, 257-265.
- Dhyan, C., Sumarlan, S.H., and Susilo, B. (2014). The Influence of Bee Wax Coating and Storage Temperature on Guava's Quality (*Psidium guajava* L.). *Jurnal Bioproses Komoditas Buah Tropika*. **2**, 79-90.
- Goutama, Y.A. (2018). Application of Ethepon and Bee Wax As a Degreening Effort and to Extend Shelf-life of "Garut" Keprok Fruit (*Citrus reticulata* L.). [THESIS]. Institut Pertanian Bogor.
- Jimenez, C.M., Cuquerella, J., and Martínez-Javega, J.. (1981). Determination of a color index for citrus fruit degreening. *Proceeding of the International Society of Citriculture* **2**, 750-753.
- Jomori, M.L.L., Sestari, Terra, F.A.M., Chiou, D.G., and Klauge, R.A. (2010). Degreening of "Murcott" tangor with ethepon treatments. Proc. IV International Postharvest Symposium. *Acta Hort* **877**, 815-820.
- Ladaniya, M.S. (2008). "*Citrus Fruit: Biology, Technology, and Evaluation*". pp 229-278. Academic Press.
- Manera, J., Brotons, J., Conesa, A., and Porras, I. (2012). Relationship between air temperature and degreening of lemon (*Citrus lemon* L. Burm. F) peel color during maturation. *Australian Journal of Crop Science* **6**, 1051-1058.
- Mayuoni, L., Tietel, Z., Patil, B.S., and Porat, R. (2011). Does ethylene degreening affect internal quality of citrus fruit?. *Postharvest Biology and Technology* **62**, 50-58.
- Monica, R.E. (2015). Consumer Attitudes and Sensitivity Prices on "Medan" Orange and "Mandarin" Orange in Yogya Bogor Junction. [THESIS]. Institut Pertanian Bogor.
- Musdalifah, N., Purwanto, Y.A., and Poerwanto, R. (2016). Effect of temperature and storage period on the change in color of "siam Pontianak" citrus after degreening. *Warta IHP* **33**, 39-48.
- Nacing, S. (2017). Application of Ethepon on Degreening "Garut" Tangerine (*Citrus reticulata* L.). [THESIS]. Institut Pertanian Bogor.
- Peng, G., Xie, X.L., Jiang, Q., Song, S., and Xu, C.J. (2013). Chlorophyll a/b binding protein plays a key role in natural and ethylene-induced degreening of Ponkam (*Citrus reticulata* Blanco). *Science Horticulture* **160**, 37- 43.
- Ramadhani, N., Purwanto, Y.A., and Poerwanto, R. (2015). Effect ethylene exposure duration and temperature at degreening to generate orange color of tangerine fruit from Banyuwangi. *Jurnal Hortikultura Indonesia* **25**, 277-286.
- Redrigo, M.J., Alquezar, M., Alos, E., Medina, V., Carmona, L., and Bruna, M. (2013). Novel carotenoid cleavage in the biosynthesis of fruit specific apocarotenoid pigment. *Journal of Experimental Botany* **64**, 4461-4478.
- Rodrigo, M.J., and Zacarias, L. (2007). Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (*Citrus sinensis* L. Osbeck) fruit. *Postharvest Biology and Technology* **43**, 12-22.
- Shahid, M.N., and Abbasi, N.A. (2011). Effect of beewax coatings on physiological changes in fruits of sweet orange cv. "blood red". *Sarhad Journal of Agriculture*. **27**, 385-394.
- Stewart, I., and Wheaton, T.A. (1971). Carotenoids in citrus: their accumulation induced by athylene. *Journal of Agriculture and Food Chemistry*. **20**, 448-449.
- Zhou, J., Sun, D., Zhang, L., Dai, X., Xu, X., and Chen, K.. (2010). Preferential accumulation of orange-colored carotenoids in Ponkan (*Citrus reticulata*) fruit peel following postharvest application of ethylene or ethephon. *Scientia Horticulturae* **126**, 229-235.