The effect of degreening on antioxidants of tangerine cv. Batu-55

S I Kailaku1,2, R Nurjanah1, I B Jamal1 and W Broto1

1Indonesian Center for Agricultural Postharvest Research and Development, Jl. Tentara Pelajar 12, Bogor, West Java, Indonesia
2Corresponding author, E-mail: sari.kailaku@gmail.com

Abstract. Indonesian Ministry of Agriculture has selected several superior citrus cultivars to be widely cultivated, one of which is tangerine cv. Batu-55 (Citrus reticulata). The shortcoming of this cultivar is its green colour when harvested, causing low attraction and selling price. Degreening is a common method practiced by farmers and traders to promote colour development in citrus. However, concerns on the effect of degreening methods on the internal quality of the fruit had arisen. The objective of this research was to study the effect of degreening treatment on the antioxidants of tangerine cv. Batu-55. Completely Randomized Design was applied with ethylene concentration (0, 1, 3, 5 ppm) and the temperature of storage rooms (ambient, air-conditioned) as factors. Ethylene exposure was applied for 24 hours. The parameters observed were vitamin C, flavonoid and phenol contents, total acid, and total soluble solid. The results showed that storage temperature gave more influence on vitamin C, flavonoid contents, total acid, and total soluble solid compared to the concentration of ethylene gas. There were no significant differences in the total soluble solid, total acid, vitamin C, and flavonoids content between samples with and without degreening treatment. This result suggested that degreening treatment would not negatively affect the internal quality of this particular cultivar of tangerine.

1. Introduction

Indonesian Ministry of Agriculture has selected several superior citrus cultivars to be widely cultivated, one of which is tangerine cv. Batu-55 (Citrus reticulata). This cultivar has competitive quality compared to imported citrus [1], which demand had increased significantly over the last few years [2]. The shortcoming of this cultivar is its green peel colour even when harvested after internal maturity achieved, causing low attraction and selling price. Degreening is a common method practiced by farmers and traders to promote colour development on the citrus peel. In this method, ethylene exposure is applied in order to induce attractive yellow or orange colour [3–7].

Citrus is mostly eaten fresh, and famous for its health-promoting benefits. These benefits are provided by the high concentrations of bioactive compounds with antioxidant properties, i.e., vitamin C and phenolic compounds. Vitamin C has been known as one of the most important micronutrients in citrus, especially as a water-soluble antioxidant and reducing agent. Flavonoids are also found abundantly in citrus and are a widely distributed group of polyphenolics, which also acts as good antioxidants [5].

Although the effects of degreening treatment on fruit qualities had been well documented in numerous studies [7–10], concerns on the negative effects on the internal quality of the fruit had been arisen [4, 6]. More studies showed that treatment conditions, e.g., ethylene concentration, duration of exposure, storage temperature, and citrus varieties, influenced the results of ethylene treatment with different significance between studies [5]. Therefore, the application of degreening treatment on
tangerine cv. Batu-55 needed to be preceded by a study to determine specific treatment conditions that result in the desired yellow colour while preserving the internal quality of the fruit.

A wide range of ethylene concentrations used in the degreening process had been reported, and 1-5 ppm of ethylene exposure was recommended in several studies [3, 4, 11–13], while other studies suggested higher concentration, from 40 ppm to 2,000 ppm [5, 6]. A length of exposure duration for 24 to 72 hours was recommended for citrus harvested in time with internal commercial quality achieved before harvest [11], while a longer duration of 5 days was suggested for early-harvested citrus [3]. Although these different conditions of degreening practice were reported to have no side effects on the antioxidants of citrus, a significant reduction of vitamin C was observed at shelf-life [8]. Moreover, these studies were conducted on different varieties and cultivars of citrus; therefore, they are not readily applicable to other varieties.

It was safe to conclude that degreening treatment must be designed carefully in order to enhance the result and prevent negative effects. The objective of the present research was to study the effect of degreening treatment on the internal quality, especially the antioxidants component of tangerine cv. Batu-55.

2. Materials and methods

**Materials**

Tangerine cv. Batu-55 was obtained from Experimental Plantation of Indonesian Citrus and Subtropical Fruits Research Institute at Malang District, East Java. The tangerines were harvested at the physiological age of 32-34 weeks after flowering. Ethylene gas from PT. Gas Depo Industry, Indonesia, was of 99.99% purity. Treated-samples were put in specially designed wooden boxes fully covered with a tarp, and the control sample was put on a display shelf.

**Degreening treatment**

The study applied Completely Randomized Factorial Design with three repetitions. Treatment factors included ethylene concentration (0, 1, 3, 5 ppm) with the predetermined duration of ethylene exposure of 24 hours [3, 4, 9, 10, 12, 14], and two different environmental conditions for storage, i.e., ambient temperature and air-conditioned room. After 24 hours of ethylene exposure, boxes were open to clear out the gas from the boxes. The samples were subsequently stored in their respective room according to treatment design. The temperature in each room was recorded every morning, noon, and afternoon.

**Analysis**

Total soluble solid content was measured using a refractometer with results in °Brix, while the percentages of total acid and vitamin C content (mg/100 ml) were determined by titration method [4]. Total phenolic content was determined by using the Folin-Ciocalteu method for sample preparation and spectrophotometer for measurement [4]. Total flavonoids content was determined following the method of [15]. Statistical analysis was conducted with Analysis of Variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT) at a 95% confidence interval.

3. Results and discussion

**Degreening process**

Data record showed that the temperature in the storage room with the ambient condition was 25-29°C, and that of the air-conditioned room was 18-22°C. This temperature was applied from ethylene exposure to storage period for each sample according to their respective treatments.

The condition of the degreening process used in this present study resulted in fully yellow colour at the fifth day in an air-conditioned room and at the seventh day in ambient temperature for the samples with 5 ppm ethylene exposure. In contrast, a lower concentration of ethylene resulted in later peel colour change (6-7 days in ambient temperature and 8-10 days in an air-conditioned room) [16].

External appearance, especially colour, is the first determining factor in consumers’ decision to buy citrus. Unfortunately, internal maturity and quality of citrus do not always correspond with external
quality [3, 10, 14]. Fully yellow tangerines are more attractive for consumers than the green ones, even if mature. Naturally, large differences between day and night temperatures may help stimulate the colour development in citrus. In a tropical climate such as Indonesia, where day temperature is a considerably high and only small difference between day/night temperature, the colour of citrus peel is not enhanced optimally, and fruit often retains their greenish colour even after maturity achieved [17]. Hence the degreening treatment using ethylene, which is carried out by farmers and traders to achieve external commercial quality [11].

Ethylene induces the activity of the enzyme that catalyses the chlorophyll degradation, chlorophyllase (chlase) [7]. As the chlorophyll was degraded, carotenoid content was increased. Up to 14% increase of carotenoids was observed after 35 days of storage following ethylene treatment in a previous study [18]. This was proven in [16], where yellow colour significantly more developed by higher ethylene concentration, indicating the degradation of chlorophyll and the formation of carotenoids.

**Total soluble solids and total acid**

Data on total soluble solids (TSS) showed that there was no significant difference resulted from the concentration of ethylene gas (p=0.488) and the combination of ethylene concentration and storage temperature (p=0.523). However, a statistically significant difference was shown on the effect of different room temperature (p=0.034), where the mean TSS of tangerine cv. Batu-55 stored in ambient temperature was 11.03±0.60 °Brix, and that of stored in the air-conditioned room was 10.45±0.74 °Brix.

The previous study found that ethylene exposure on tangerine did not result in noticeable differences in total soluble solids [18].

Similar to the data of TSS, only different storage temperatures had a significant influence on total acid (p=0.029). Ethylene concentration and combination of treatments did not show a statistically significant effect on total acid (p=0.840 and p=0.722, respectively). The tangerines stored in the air-conditioned room had higher total acid content (0.37±0.07%) compared to those stored in ambient temperature (0.31±0.06).

The level of TSS and total acid and their ratio strongly determine the taste of citrus. In the present study, both parameters were not affected by ethylene exposure, which corresponds with the results of previous studies [4, 19]. The study reported by [4] was also supported by flavour evaluations. A trained panel confirmed that the flavour of citrus was not altered with or without ethylene exposure, nor a sense of off-flavour was detected after exposure of ethylene.

Moreover, it was observed in the present study that there was only a slight reduction of TSS and total acid from the initial sample to after degreening process, where full yellow colour had been achieved (figure 1). However, after storage, the ratio of TSS and total acid of tangerines cv. Batu-55 exposed with ethylene was found similar to that of initial and before storage (degreened). Meanwhile, the ratio of TSS and total acid of samples stored in ambient temperature was considerably higher. This confirms the observation that in the degreening process, the temperature was more influential on the internal quality of fruit, compared to ethylene concentration [6].
Figure 1. Total soluble solids (above) and total acid (below) of tangerine cv. Batu-55 before degreening (initial), after the achievement of fully yellow colour (degreened) and after storage, as affected by different ethylene concentration and storage temperature (ambient=25-29°C; air-conditioned=18-22°C).

Vitamin C

As shown in figure 2, higher vitamin C content was observed in most samples stored in ambient temperature (39.72±5.94 mg/100ml) compared to those stored in the air-conditioned room (35.51±4.36 mg/100ml). Statistical analysis showed that only storage temperature significantly affected vitamin C content (p=0.036), while ethylene exposure and combination of both treatments did not (p=0.334 and p=0.434, respectively).

This finding was in line with that of [4, 20] who compared vitamin C contents from various species of citrus and found no noticeable differences in vitamin C contents of samples during and degreening process. A review by [21] confirmed that numerous studies had concluded that ethylene degreening was an effective method to improve the colour of citrus, without negatively affecting the nutritional properties, especially vitamin C. Moreover, not only vitamin C is not affected by ethylene treatment, but also the antioxidant capacity of several types of clementines [20]. The temperature and humidity conditions are needed to be optimal in order to maintain the internal quality of citrus during long term storage. The effect of temperature on vitamin C was found different in different citrus fruit, and different storage duration. Several studies observed higher vitamin C with lower temperatures and lower vitamin C after a longer duration of storage. Meanwhile, other studies found that certain types of
citrus can maintain their vitamin C content in different storage temperatures and after long term storage [21].

Regarding the current study, the average vitamin C content of samples stored in ambient temperature was higher compared to those stored in the air-conditioned room. This may be one of the examples of certain citrus that can retain vitamin C content despite room temperature, as mentioned in [21].

Figure 2. Vitamin C content of tangerine cv. Batu-55 before degreening (initial), after the achievement of fully yellow colour (degreened) and after storage, as affected by different ethylene concentration and storage temperature (ambient=25-29°C; air-conditioned=18-22°C).

Flavonoids
Exogenous ethylene was found to affect several enzymes in plant metabolic pathways. It was hypothesized that ethylene could influence the biosynthesis of its metabolites, such as carotenoids and flavonoids [22].

In the present study, the concentration of ethylene had no significant difference in the flavonoids content of tangerine cv. Batu-55 after degreening process (p=0.166). However, the interaction of it with the storage temperature showed a significant effect (p=0.038), as well as the effect of storage temperature alone (p=0.000). As shown in figure 3, the flavonoid content after storage was higher in samples stored in an air-conditioned room (395.49±79.80 ppm) compared to the ones in ambient temperature (267.46±79.05 ppm). Meanwhile, further analysis of the effect of the interaction of ethylene concentration and temperature showed that significantly higher flavonoid content was observed in almost all samples exposed to 5 ppm ethylene and stored in the air-conditioned room (390.25 – 446.64 ppm). Lower flavonoid content was measured in tangerines stored in ambient temperature (294.02 – 318.60 ppm), with the ones exposed with 5 ppm ethylene and stored in an air-conditioned room (262.28 ppm). The lowest flavonoid content was found in samples exposed with 3 ppm ethylene and stored in ambient temperature (151.81 ppm).

[22] elaborated that grapefruits degreened with 10 ppm ethylene had an increased level of flavonoid content, compared to the non-degreened and fruits degreened with 5 ppm ethylene. However, after transfer to storage in low temperature (11°C) for seven days, the flavonoid content was significantly higher in non-degreened fruits compared to the degreened fruits. After 35 days of storage, the flavonoid levels of non-degreened and 5 ppm ethylene-exposed fruits were similar, and lower compared to those exposed with 10 ppm ethylene. This level was also similar to the level measured at harvest (day zero). In the end, it can be concluded that changes in flavonoid pathway genes occurred during the storage of citrus, causing different measurement results in citrus treated with and without ethylene.
Figure 3. Flavonoids content (above) and phenol content (below) of tangerine cv. Batu-55 before degreening (initial), after the achievement of fully yellow colour (degreened) and after storage, as affected by different ethylene concentration and storage temperature (ambient=25-29°C; air-conditioned=18-22 °C).

Phenol

Phenol is one of the compounds in citrus fruits responsible for its protective effects as an important source of antioxidants [5]. In the current research, an increase of phenol content in citrus stored in ambient temperature was observed with the higher level of ethylene exposure, while no similar tendency was shown in citrus stored in the air-conditioned room (figure 3).

Similar to vitamin C, different types of citrus fruits respond differently to degreening treatment and storage conditions. It was reported that fruits with higher total phenolic content also tended to have higher total ascorbic acid. Moreover, the changes in the level of total phenolic content, as well as vitamin C, were proven dependent not only on storage conditions but also on the cultivar [5].

4. Conclusions

Storage temperature was shown to have more influence on vitamin C, flavonoid contents, total acid, and total soluble solid compared to the concentration of ethylene gas. There were no significant differences between samples with and without ethylene treatment. This result suggested that
degreening treatment would not negatively affect the antioxidant components of this particular cultivar of tangerine.

5. References
[1] Hanif Z, Zamzami L. 2015. Trend Jeruk Impor dan Posisi Indonesia sebagai Produsen Jeruk Dunia. 2015. Epub ahead of print 2015. DOI: 10.13140/RG.2.1.4207.7601.
[2] Sugiyatno A. 2015. Proses Inversi Menuju Inovasi Jeruk Keprok Batu 55. In: Inovasi Hortikultura Pengungkit Peningkatan Pendapatan Rakyat, pp. 91–99.
[3] Tietel Z, Weiss B, Lewinsohn E, et al. 2010. Improving taste and peel color of early-season Satsuma mandarins by combining high-temperature conditioning and degreening treatments. Postharvest Biol Technol; 57: 1–5.
[4] Mayuoni L, Tietel Z, Patil BS, et al. 2011. Does ethylene degreening affect internal quality of citrus fruit? Postharvest Biol Technol; 62: 50–58.
[5] Sdiri S, Navarro P, Monterde A, et al. 2012. Effect of postharvest degreening followed by a cold-quarantine treatment on vitamin C, phenolic compounds and antioxidant activity of early-season citrus fruit. Postharvest Biol Technol; 65: 13–21.
[6] Poole ND, Gray K. 2002. Quality in citrus fruit: to degreen or not degreen? Br Food J; 104: 492–505.
[7] Peng G, Xie X, Jiang Q, et al. 2013. Chlorophyll a/b binding protein plays a key role in natural and ethylene-induced degreening of Ponkan (Citrus reticulata Blanco). Sci Hortic (Amsterdam); 160: 37–43.
[8] Chaudhary P, Jayaprakasha GK, Porat R, et al. 2012. Degreening and postharvest storage influences ‘Star Ruby’ grapefruit (Citrus paradisi Macf.) bioactive compounds. Food Chem; 135: 1667–1675.
[9] Mayuoni L, Sharabi-schwager M, Feldmesser E, et al. 2011. Effects of ethylene degreening on the transcriptome of mandarin flesh. Postharvest Biol Technol; 60: 75–82.
[10] Moscoso-Ramírez PA, Palou L. 2014. Effect of ethylene degreening on the development of postharvest penicillium molds and fruit quality of early season citrus fruit. Postharvest Biol Technol; 91: 1–8.
[11] Lado J, Rodrigo MJ, Zacarias L. 2014. Maturity indicators and citrus fruit quality. Stewart Postharvest Rev; 2: 1–6.
[12] Cronje PJR, Barry GH, Huysamer M. 2011. Rind breakdown of ‘Nules Clementine’ mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol Technol; 60: 192–201.
[13] Arpaia ML. 2015. Research Review Report: Citrus Degreening. Postharvest Technology Center, University of California, Davis, CA, 2015.
[14] Conesa A, Brotons JM, Manera FJ, et al. 2014. The degreening of lemon and grapefruit in ethylene atmosphere: A cost analysis. Sci Hortic (Amsterdam); 179: 140–145.
[15] Shin Y, Liu RH, Nock JF, et al. 2007. Temperature and relative humidity effects on quality, total ascorbic acid, phenolics and flavonoid concentrations, and antioxidant activity of strawberry. Postharvest Biol Technol; 45: 349–357.
[16] Kailaku SI, Jamal IB, Nurjanah R, et al. 2019. The Influence of Ethylene Gas and Storage Temperature in The Degreening Process of Tangerine Cultivar Batu-55. In: Asian Food Conference. Denpasar.
[17] Barry GH, Wyk AA Van. 2006. Low-temperature cold shock may induce rind colour development of ‘Nules Clementine’ mandarin (Citrus reticulata Blanco) fruit. 40: 82–88.
[18] Zhou J, Sun C, Zhang L, et al. 2010. Preferential accumulation of orange-colored carotenoids in Ponkan (Citrus reticulata) fruit peel following postharvest application of ethylene or ethephon. Sci Hortic (Amsterdam); 126: 229–235.
[19] Chaudhary PR, Jayaprakasha GK, Patil BS. 2015. Ethylene degreening modulates health promoting phytochemicals in Rio Red grapefruit. Food Chem; 188: 77–83.
[20] Sdiri S, Navarro P, Abda J, et al. 2010. Antioxidant activity and vitamin C are not affected by degreening treatment of Clementine mandarins (abstract). In: XXVIII International
Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on Postharvest Technology in the Global Market, pp. 893–899.

[21] Mditchwa A, Magwaza LS, Tesfay SZ, et al. 2017. Postharvest factors affecting vitamin C content of citrus fruits: A review. Sci Hortic (Amsterdam); 218: 95–104.

[22] Chaudhary PR, Bang H, Jayaprakasha GK, et al. 2017. Effect of ethylene degreening on flavonoid pathway gene expression and phytochemicals in Rio Red grapefruit (Citrus paradisi Macf.). Phytochem Lett; 22: 270–279.