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Postharvest Losses of NOR Tomato Fruit Line MA 131-6-3 Treated by Ethephon and Calcium Carbide

Kerugian Pascapanen Buah Tomat NOR Galur MA 131-6-3 yang Diperlakukan dengan Ethephon dan Kalsium Karbida

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ABSTRACT

Ethephon and calcium carbide are artificial ripening compounds that are often used to faster fruit ripening during postharvest. These two compounds were induced in non-ripening (NOR) tomato fruit line MA 131-6-3 because it produced endogenous ethylene and lower respiration. This study aimed to determine the effect of ethephon and calcium carbide on postharvest losses of NOR tomatoes for 14 and 28 days after treatment. Exogenous application of 1,500 ppm ethephon and 15 g kg^{-1} was given to NOR tomato fruit of line MA 131-6-3 and ambient conditions at temperature 28.08±1.80 °C and relative humidity 75.67±2.09%. This experiment was carried out in Sidoarum village, Godean district, Sleman regency, Special Region of Yogyakarta province, Indonesia, in 125 m above sea level. As a result, ethephon can increase fruit rotting. Furthermore, calcium carbide can accelerate metabolism which has an impact on physiological loss in weight, changing color to yellowish (b), increasing vivid color (chroma), but the color of tomato fruit cannot turn red.*

Keywords: fruit ripening, fruit quality, postharvest, total soluble solids, total titratable acidity

ABSTRAK

Ethephon dan kalsium karbida adalah senyawa pematangan buatan yang sering digunakan untuk mempercepat pematangan buah selama pascapanen. Kedua senyawa ini diinduksi pada buah tomat galur non-ripening (NOR) MA 131-6-3 karena menghasilkan etilen endogen dan respirasi yang lebih rendah. Penelitian ini bertujuan untuk mengetahui pengaruh ethephon dan kalsium karbida terhadap kerugian pascapanen tomat NOR selama 14 dan 28 hari setelah perlakuan. Aplikasi eksogen dari 1,500 ppm ethephon dan 15 g kg^{-1} kalsium karbida diberikan pada buah tomat NOR galur MA 131-6-3 dan kondisi lingkungan pada suhu 28.08±1.80 °C dan kelembaban relatif 75.67±2.09%. Penelitian ini dilaksanakan di Desa Sidoarum, Kecamatan Godean, Kabupaten Sleman, Provinsi Daerah Istimewa Yogyakarta, Indonesia, pada ketinggian 125 m di atas permukaan laut. Hasilnya, ethephon dapat meningkatkan pembusukan buah tomat. Selanjutnya, kalsium karbida dapat mempercepat metabolisme yang berdampak pada susut bobot, mengubah warna menjadi kekuningan (b), meningkatkan warna cerah (chroma), tetapi warna buah tomat tidak dapat menjadi merah.*

Kata kunci: kematangan buah, kualitas buah, pascapanen, total asam tertitrasi, total padatan terlarut

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) plant originates from American Southern and spread in several countries such as Chile, Ecuador, and Peru (Abiso *et al*., 2015). Tomato can grow and develop well in lowlands (0-200 m above sea level), medium (200-500 m above sea level), and highlands (500-700 m above sea level) (Romadhon *et al*., 2018). Tomato fruit is essential for making tomato sauce and paste, and can be consumed fresh. Tomato has a relatively high nutritional content such as 3.84 g carbohydrates, 17.8 mg vitamin C, 24 g vitamin A, 2860 g lycopene, and others (USDA, 2022).

Tomato breeding at Universitas Gadjah Mada results in plants with high yields but challenging to ripen, usually mentioned as NOR tomato (Siddiqui *et al.,* 2016). This tomato line is a result of conventional breeding (pedigree selection) and as a result of selection F6 which has been reported by Murti *et al*. (2022). The utilization of NOR tomato genetic

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is postharvest handling with the aim of the shelf life of fruit. NOR tomatoes are suspected to be low in ethylene. The problem in enzymatic activity of 1-aminocyclopropane-1-carboxylic synthase (ACS) and 1-aminocyclopropane-1-carboxylic oxidase (ACO) and gene expression causes the production of endogenous ethylene in NOR tomato fruit to be low (Wang *et al*., 2020b). The initial formation of ACS and ACO started from 1-aminocyclopropane-1-carboxylic which was synthesized from S-adenosyl-L-methionine by ACS. Furthermore, ACO oxidizes to ethylene (Polko *et al*., 2019).

Fruits that cannot produce endogenous ethylene to ripen need exogenous ethylene. The availability of exogenous ethylene expected to trigger fruit development. The particular fruit storage room or ripening room to apply exogenous ethylene does not allow the gas to come out or outside air to enter the room, therefore the ripening process will not be affected except by the applied ethylene. Ethephon (ET) and calcium carbide (CaC_2) are appropriate compounds that can produce exogenous ethylene. ET or 2-chloroethylphosphonic acid is a chemical compound that has the molecular formula $C_2H_6ClO_3P$ and it is commonly used to ripen fruit. ET when added to water produces hydrogen phosphate and hydrogen chloride. Furthermore, CaC_2 or a common name that is often used, namely carbide, it is a chemical compound that is useful for ripening fruit during postharvest. Respiration of fruit during postharvest produces water molecules which react with CaC_2 to produce calcium hydroxide ($Ca(OH)_2$) and acetylene (C_2H_2). C_2H_2 can be converted to C_2H_4, consequently it can induce fruit ripeness. Dhall and Singh (2013) reported that applying 1,500 ppm ET of fruit dipping on hybrid tomatoes can increase fruit ripening and lycopene. Meanwhile, 20 g kg^{-1} CaC_2 of kept near fruit increased sweetness and aroma in banana based on study by Nura *et al*. (2018). The research aims to determine effect of ET and CaC_2 on the postharvest losses of NOR tomatoes for 14 and 28 days after treatment (DAT).

MATERIALS AND METODES

Experimental Procedure

The tomato was grown open field from January to May 2021 in Hargobinangun village, Pakem district, Sleman regency, Special Region of Yogyakarta province, Indonesia with 715 m above sea level (7°36'52.7"S 110°25'43.3"E). The environment had temperature 24.12±1.80 °C, relative humidity 82.17±1.50%, sunlight intensity 24,039.42± 1,140.07 lux, and wind speed 2.52±0.30 ms^{-1}. Nutrients and pH of soil such as nitrogen total 0.25±0.00%, phosphate availability 12.16±0.00 ppm, potassium exchange 0.180± 0.00 me%, calcium exchange 4.390±0.00 me%, magnesium exchange 0.67±0.00 me%, and pH 5.50±0.60. The postharvest handling and treatment application in Sidoarum village, Godean district, Sleman regency, Special Region of Yogyakarta province, Indonesia, in 125 m above sea level (7°46'50.6"S 110°19'01.9"E) with temperature 28.08±1.80 °C and relative humidity 75.67±2.09%.

Seeds of NOR tomato line MA 131-6-3 were obtained from Plant Breeding Laboratory, Department of Agronomy, Universitas Gadjah Mada. Tomato seeds transplanted 21 days after seedling (DAS), and these plants were individually moved into a 40 cm x 40 cm polybag. The growing media used were regosol soil, cow manure compost, and rice husk ash (3:1:1). Cow manure compost as much as 2 ton ha^{-1} was applied near plant root before planting. Meanwhile NPK Mutiara was given 15,000 ppm (10, 20, 30, 40, and 50 days after planting (DAP)) and calcium fertilizer (Kalsi gro 98) 15,000 ppm (60 DAP). Treatment application of NPK and Kalsi gro 98 by mixing water-soluble fertilizer to soil of 250 mL per plant. The irrigation is given for two to three days and pest and disease (Dithane M-45 80 WP, Curacron, and Furadan 3GR) control is carried out weekly with foliar spray.

The research treatment was carried out with the application ET and CaC_2 given when the fruit had been harvested at 80, 85, and 90 DAP. ET application is made by mixing 1,500 ppm and adding 1 L of water into a bucket. Then the fruit is dipped and left for 15 minutes (Dhall and Singh, 2013). Furthermore, the application of CaC_2 is placed in a jar with a dose of 20 g kg^{-1}. The jar used is 3 L which contains 27 fruit per jar with fruit dipping. The characteristics of the fruit used in this study were clean fruit, non-cracking fruit, not infected with pathogens, and fruit weight of 40±20 g per fruit. Observation variables are physiological loss in weight (PLW), fruit rotting, fruit firmness, TSS (total soluble solids), TTA (total titratable acidity), TSS:TTA ratio, vitamin C, and fruit color (L*, a*, b*, hue, chroma, and redness value).

Experimental Design

The research was conducted in two stages are trial and main research. A trial study was conducted to determine whether tomato fruit of line MA 131-6-3 could turn red if of ET concentrations (0, 1,600, and 5,000 ppm) were induced on the farm. Tomato plants were foliar spray treated at 65 DAP and observation variables were worked at 85 DAP (after harvested). The research design was carried out with Completely Randomized Block Design (CRBD) with five replications. The next step is to carry out the main research which is the fruit treated before storage. The research design was carried out with completely CRBD with four replications. Treatments were control, 1,500 ppm ET, and 20 g kg^{-1} CaC_2. The fruits are stored at ambient temperature.

Physicochemical Analyses

The instrument used to measure fruit firmness is the Bareiss Prüfgerätebau GmbH penetrometer type BS 61 II. Meanwhile, PLW and rotting are expressed in percentages. The formula for calculating these variables was as follows (Dhall and Singh, 2013):

$$\text{PLW (\%)} = \frac{A-B}{A} \text{ x } 100, \text{ Rotting (\%)} = \frac{R}{T} \text{ x } 100$$

Where A is the fruit weight at 0 days after treatment, B is the fruit weight at each sampling time, R is the number of rotting, and T is the number of fruit.

TSS:TTA ratio calculated with the formula is TSS/TTA (Khan *et al*., 2016). TSS was analyzed with a hand refractometer Atago series of ATC and TTA was analyzed by titration from AOAC (1990). The samples were cut and mashed then 10 g was taken. Furthermore, the mixtures were filtered with a tea filter and filter paper (Whatman No. 42). Take 25 mL of pulp, three drops of phenolphthalein 1% were added and titrated with 0.1 N NaOH. The formula for calculating TTA was as follows:

$$\text{TTA (\%)} = \frac{\text{mL NaOH x N NaOH x total material volume x equivalent weight}}{\text{titration material volume x g x 100}}$$

Where equivalent weight is 64 and g is the weight of the sample used. The vitamin C analysis method follows the modified Vitara (2021). Tomato fruit was taken and weighed as much as 2.5 g and added 50 mL of distilled water. The filtrate 10 mL was taken and put into a 100 mL volumetric flask, and distilled water was added to the mark and mixed with a vortex mixer. Furthermore, it was measured at an absorbance of 266 nm with a UV-Vis spectrophotometer. Then the numbers that appear on the spectrophotometer screen are regressed with vitamin C using Y = 0.106X + 0.0778. The value determination of vitamin C, flavonoid value is entered into the following formula:

$$\text{Vitamin C (mg 100 g}^{-1}) = \left(\frac{(C \times V) \times DF}{g}\right)$$

Where Y is spectrophotometer value, X is concentration of flavonoid, C is sample concentration (X value), V = volume of solution used, DF is dilution factor, and g is the weight of the sample used.

Fruit color measured using chromameter CR-400 and the color coordinates L*, a*, and b* were measured on all five sides of the fruit surface. The measurements are also carried out at the fruit surface's top, middle, and bottom. Fruit color is expressed as a color value by the Commission International de L'Eclairage. L* is the brightness level, with a larger range of 0 to 100 indicating a brighter level. a* is range -128 to 127, where the value of a*(-) indicates sample is getting greener, and a value a*(+) indicates the sample is getting redder. b* is range -128 to 127, where the value of b*(-) indicates sample is getting bluer, and the value of b*(+) indicates the sample is getting yellow. Hue values are categorized as 0◦ = red to purple, 90◦ = yellow, 180◦ = bluish to green, and 270◦ = blue. Chroma is defined as the intensity of color saturation from dull to vivid color (low to high values, respectively) (Khan *et al.,* 2016). Classification of color and mature stages of tomato fruit according to redness values such as -0.59 to -0.47 (green), -0.47 to -0.27 (breaker), -0.27 to 0.08 (turning), 0.08 to 0.60 (pink) 0.60 to 0.95 (light-red), and 0.95 to 1.21 (red) (Salas-Mendéz *et al.,* 2019). The formula for calculating these variables was as follows:

$$\text{Hue} = \tan^{-1}\frac{b^*}{a^*} + 180,\ \text{Chroma} = (a^{*2} + b^{*2})^{1/2},\ \text{Redness value} = \frac{a^*}{b^*}$$

Statistical Analysis

The statistical test of this research is Analysis of Variance (ANOVA). Before ANOVA, testing was carried out assuming a normal distribution and homogeneity. If there is a significant difference, then a post-hoc test with Tukey's Honestly Significant Difference (HSD) α = 0.05. The software is used for data analysis in SAS® OnDemand for Academics via a web browser (https://welcome.oda.sas.com/login). The data in the tables are mean±standard deviation (SD).

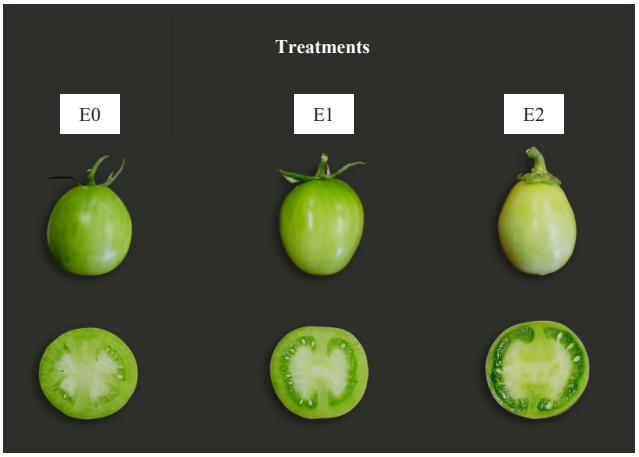
RESULTS AND DISCUSSIONS

The result by trial study is an increase in color brightness (L*) and an increase in turning (b*) (Table 1), but still unable to make this tomato red (Figure 1). The trial results form the basis fore advanced research on postharvest treatment. PLW and rotting are shown in Table 2. CaC_2 treatment (5.09%) was significant with control (1.62%) in 14 DAT. The effect of CaC_2 with ambient conditions can accelerate PLW therefore that the transpiration process in fruit takes place quickly. Increased transpiration rate and carbohydrates are the main causes of high PLW in tomatoes stored at room temperature (Pinheiro *et al*., 2013; Rab *et al*., 2013). Hybrid tomato fruit is at the ambient room (12.36%) significance with cold room (5.07%) of 7 to 28 days of storage for a mean of all treatments (Okolie *et al*., 2012). High temperature causes an increase in respiration, ethylene production, and transpiration rate (Ayomide *et al*., 2019). Transpiration and PLW are usually positively correlated with fruit ripening during storage. Tomato fruit is susceptible to shrinkage or weight loss caused by water

Table 1. Effect of different levels of ethephon on L*, a*, b*, hue, chroma, and redness value on farm (mean ± SD)

Treatments	L*	a*	b*	Hue	Chroma	Redness value
Control	52.31±1.00b	-11.70±0.92	17.50±0.31b	120.74±1.12	22.46±1.01	-0.67±0.05b
1,600 ppm Ethephon	57.08±2.61ab	-11.25±0.76	18.46±0.39b	118.92±2.00	22.78±0.18	-0.61±0.03ab
5,000 ppm Ethephon	62.18±1.98a	-10.27±0.95	20.37±0.36a	117.22±1.73	21.93±0.76	-0.50±0.04a

Note: Numbers followed by the same letter in the same column were not significantly different based on Tukey's HSD test at α = 0.05 (n = 5)



Note: E0 = control; E1 = 1,600 ppm ethephon; E2 = 5,000 ppm ethephon

Figure 1. Effect of different levels of ethephon at fruit color ripening on farm treatment

loss. The optimal relative humidity for storing tomatoes on mature green is 85-95%. Increased evapotranspiration in fruit can occur because the relative humidity is below the optimal value, causing the fruit to shrink (Arah *et al*., 2015). Meanwhile, rotting is shown in Table 2. ET (58.34%) was significant with CaC_2 (0.00%) at 28 DAT. ET causes NOR tomato fruit to accelerate rotting caused by direct contact of the fruit with water. This problem worsen if the fruit has several wounds and bruises that are not visible therefore it becomes an entry point for pathogenic infection by *Botrytis cinerea* during treatment (Akter *et al*., 2019). Hybrid tomato fruit with the treatment of sage essential oil 0.1% compared to control decreased rotting symptoms at 7 and 14 days of storage by 1.00% and 1.08% (Tzortzakis *et al*., 2019). The author's study results are lower than Tzortzakis *et al*. (2019) at 14 days of storage.

TSS, TTA, and TSS:TTA ratio are shown in Table 3 and the data showed no significance. TSS:TTA ratio, research by Khan *et al*. (2016) shows that fruit ripening inhibitors such as 1,000 nL L^{-1} 1-hexylcyclopropene (HCP) can reduce TSS:TTA ratio by 7.22 compared to a control of 8.80. A decrease in TSS was also found in Nasef (2018) study with cucumbers (*Cucumis sativus* L.) dipped in hot water at 25 °C (control), 45 °C, and 55 °C. The reduction in TSS could be due to the quality deterioration of fruit. NOR tomato decrease TTA because this response occurs in the citric acid content, which increases with maturity and stage of ripening (Ruelas-Chacon *et al*., 2017). In addition, Kahramanoğlu and Usanmaz (2019) reported a decrease in TSS during postharvest treatment of cucumber fruit with lemongrass oil and propolis extract (alone or in interaction) with modified atmosphere packaging. TTA decreased in the maturity stages in the percentage of titratable acidity. Total organic acids decreased during fruit ripening caused by increased respiration, while organic acids were the substrate in the process (Teka, 2013). The correlation data between TTA and vitamin C at 14 and 28 DAT shows that the relationship is solid, with a value of 96% dan 89% (data not shown).

Vitamin C and fruit firmness are shown in Table 4 and the data showed no significance. The variable of vitamin C, case study reported by Siddiqui *et al*. (2016) is ripening inhibitor (RIN) tomato fruit result vitamin C of 26.12 mg 100 g^{-1} from the genotype of BCT-11. The current experiment showed that vitamin C is higher than Siddiqui *et al*. (2016) found, with a value of 32.85 mg 100 g^{-1} in control at 14 days

Table 2. Effect of treatments on PLW and rotting (mean±SD)

Treatments	PLW (%)		Rotting (%)	
	14 DAT	28 DAT	14 DAT	28 DAT
Control	1.62±0.42b	3.59±1.49	0.00±0.00	16.67±33.34ab
1,500 ppm Ethephon	1.45±0.72b	5.39±2.75	0.00±0.00	58.34±41.94a
20 g kg^{-1} Calcium Carbide	5.09±1.25a	6.75±1.83	0.00±0.00	0.00±0.00b

Note: PLW = physiological loss in weight; numbers followed by the same letter in the same days after treatment (DAT) were not significantly different based on Tukey's HSD test at α = 0.05 (n = 4)

Table 3. Effect of treatments on TSS, TTA, and TSS: TTA ratio (mean±SD)

Treatments	TSS (%Brix)		TTA (%)		TSS:TTA ratio	
	14 DAT	28 DAT	14 DAT	28 DAT	14 DAT	28 DAT
Control	4.31±0.47	3.79±0.25	0.13±0.03	0.10±0.02	34.33±8.07	37.93±6.02
1,500 ppm Ethephon	4.44±0.43	3.98±0.05	0.12±0.03	0.07±0.04	41.18±16.03	85.56±76.58
20 g kg^{-1} Calcium Carbide	4.19±0.13	3.63±0.44	0.12±0.01	0.08±0.02	35.66±3.96	45.51±9.21

Note: TSS = total soluble solids; TTA = total titratable acidity; numbers followed by the same letter in the same days after treatment (DAT) were not significant different based on Tukey's HSD test at α = 0.05 (n = 4)

Table 4. Effect of treatments on vitamin C and fruit firmness (mean±SD)

Treatments	Vitamin C (mg 100 g^{-1})		Fruit firmness (Newton)	
	14 DAT	28 DAT	14 DAT	28 DAT
Control	32.85±12.56	23.21±4.93	19.58±5.69	12.21±6.08
1,500 ppm Ethephon	19.83±2.08	18.20±0.81	22.89±6.38	10.08±6.48
20 g kg^{-1} Calcium Carbide	25.64±2.41	17.99±1.88	18.95±2.74	13.21±3.54

Note: Numbers followed by the same letter in the same days after treatment (DAT) were not significantly different based on Tukey's HSD test at α = 0.05 (n = 4)

of storage. The horticultural product that is considered an essential nutrient because of its potent antioxidant activity is vitamin C (Liu *et al*., 2018). Meanwhile for fruit firmness, Li *et al.* (2014) reported higher fruit firmness in RIN control with 222.54 g force (2.18 Newton) at 0 DAT. The results of the two observation variables are lower than the author's (Control at 14 DAT) results. Artificial ripening treatment resulted in fruit expression such as pectin degradation and softening caused by the downregulation of polygalacturonase and beta-galactosidase genes (Cheema *et al*., 2014). In addition, fruit softening causes cell wall composition, intracellular materials, and structure deterioration (Fagundes *et al*., 2015). Cell wall composition changes such as cellulose and pectin are directly related to fruit firmness. The activity of cell wall degradation enzymes during postharvest can cause fruit softening. Enzymes that play an active role in cell wall degradation are cellulase, pectin methylesterase, and polygalacturonase (Bu *et al*., 2013).

Fruit color with L*, a*, b*, hue, and chroma are shown in Table 5 and 6, and then the variables b* and chroma are significant. The increase in the turning color (b*) of NOR tomato can be treated with CaC_2. The correlation of lycopene with tomato red color is usually solid, but not in this NOR tomato. The color of tomato at the end of storage is turning, therefore it is suspected that flavonoid accumulation occurs (Orsi *et al*., 2021). Tomato fruit cannot turn red because the carotenoid biosynthetic pathway that produces lycopene is inhibited due to mutations in the PSY1 gene therefore lycopene synthesis is abnormal (Chattopadhyay *et al*., 2021). It is an accumulation of phytoene precursors which eventually turn color of tomato into turning, and not be red. Wang *et al*. (2020a) reported that the inhibiting action of the new protein on ethylene signal transduction is therefore intense that ET and CaC_2 cannot overcome it. As a result, a new protein acts as a repressor. In addition, the effect of CaC_2 was significant compared to the control at 14 and 28 DAT. The value of chroma indicates color saturation from dull to vivid color. Anthocyanin accumulates in the skin of NOR tomato control. The darker skin of tomatoes have high anthocyanin. It is evidenced by Bassolino *et al*. (2013) and Zhang *et al*. (2013), the fruit of Aft/Aft atv/atv and Del/Ros1 can be sufficient to reduce susceptibility to *B. cinerea* in fruit.

Table 5. Effect of treatments on L*, a*, and b* (mean±SD)

Treatments	L*		a*		b*	
	14 DAT	28 DAT	14 DAT	28 DAT	14 DAT	28 DAT
Control	62.20±4.21	67.50±1.42	-4.53±0.77	-3.14±1.02	23.06±2.75b	25.38±1.84b
1,500 ppm Ethephon	65.14±1.49	67.30±3.74	-5.21±1.22	-2.10±1.69	26.46±3.38ab	28.58±3.12ab
20 g kg^{-1} Calcium Carbide	64.40±0.86	66.23±1.65	-5.96±1.24	-2.41±1.11	29.27±1.03a	30.98±0.81a

Note: Numbers followed by the same letter in the same days after treatment (DAT) were not significantly different based on Tukey's HSD test at α = 0.05 (n = 4)

Table 6. Effect of treatments on hue, chroma, and redness value (mean±SD)

Treatments	Hue		Chroma		Redness value	
	14 DAT	28 DAT	14 DAT	28 DAT	14 DAT	28 DAT
Control	100.77±2.89	96.65±2.41	23.52±2.59b	25.59±1.78b	-0.20±0.05	-0.12±0.04
1,500 ppm Ethephon	99.63±2.94	94.11±3.40	27.00±3.26ab	28.70±3.03ab	-0.20±0.06	-0.08±0.06
20 g kg^{-1} Calcium Carbide	100.60±2.47	94.03±1.97	29.89±0.85a	31.09±0.74a	-0.20±0.05	-0.08±0.04

Note: Numbers followed by the same letter in the same days after treatment (DAT) were not significantly different based on Tukey's HSD test at α = 0.05 (n = 4)

CONCLUSION

All treatments did not support the maturity of NOR tomato on farm and postharvest. CaC_2 can only increase PLW, b*, and chroma. The NOR tomato applied with CaC_2 had a lighter and more turning color than other treatments. The effect of CaC_2 on the increase in PLW results in decreased fruit quality when marketed.

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