



## Changes in color, vitamin C, carotenoids and tocopherols during ripening and senescence of tomato fruit

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### ABSTRACT

**Purpose:** Changes in color, vitamin C,  $\beta$ -carotene, lycopene,  $\alpha$ - and  $\delta$ -tocopherol were followed during ripening and senescence of mature-green tomatoes (*Lycopersicon esculentum* Mill cv. Rhapsody) maintained at 22 °C and 85% RH for up to 5 weeks.

**Research method:** Tomatoes were harvested at the mature-green stage and valued for color and the content of selected bioactive compounds ( $\beta$ -carotene, lycopene,  $\alpha$ - and  $\delta$ - tocopherols and vitamin C) during simulated retail market conditions (22 °C and 85% RH for 5 weeks). **Findings:** The tested tomato cultivar had a long postharvest life under the tested conditions, as the fruit maintained in edible conditions during the whole storage period. Vitamin C, lycopene,  $\alpha$ - and  $\delta$ -tocopherol presented their highest values after about 14 to 18 days after harvest, and  $\beta$ -carotene maintained a maximum content (0.84 mg/100 g) 8 days after harvest. The maximum content of vitamin C, lycopene,  $\alpha$ - and  $\delta$ -tocopherol were 0.036, 30, 0.27 and 0.0045 mg 100 g<sup>-1</sup> of fresh tissue, respectively. Our results indicate that 'Rhapsody' tomatoes harvested at the mature-green stage have shown important levels of vitamin E (tocopherols), C, and carotenoids (lycopene and  $\beta$ -carotene) after 14 to 17 days from harvest. **Research limitations:** There were no limitations. **Originality/Value:** This study evaluated the changes in the content of bioactive compounds in long shelf life tomatoes, of great importance for human health, for up to 5 weeks to determine the ideal moment for their consumption.

## INTRODUCTION

Tomato is an essential vegetable in the diet of people around the world and is the main vegetable produced worldwide; in 2022, up to 186 million tons were produced (FAOSTAT, 2022). Mexico is among the 10 biggest world producers of tomatoes (FAOSTAT, 2022), and is the main exporter to United States and Canada markets (SAGARPA, 2017). Tomato has become one of the main sources of nutritional and bioactive compounds for human health, fundamentally antioxidant vitamins, such as C, E and B12, with content of 4–22 mg/100 g fw, 0.9–3.8 mg/100 g fw, and 4–60 µg/100 g fw, respectively (Bianchi et al., 2023; Raiola et al., 2015; Figueira et al., 2017; Vats et al., 2022). Additionally, tomato fruit has high content of carotenoids, mainly lycopene (2.2–41.8 mg/100 g fw),  $\alpha$ - and  $\beta$ -carotenes (0.1–0.5 mg/100 g fw), and lutein (0.1–19.7 mg/100 g fw) (Flores et al., 2017; Loayza et al., 2021a,b; Vats et al., 2022; Bianchi et al., 2023). The intake of tomato fruit and its lycopene has been related with a lower risk of cancer mortality and prevention of cardiovascular diseases (Mazidi et al., 2020a,b). The consumption of bioactive components found in tomato fruit was reported to increase the natural antioxidants, such as plasma total antioxidant capacity, erythrocyte superoxide dismutase and glutathione peroxidase, and reduce the oxidative stress, serum malondialdehyde, and the inflammatory markers such as TNF- $\alpha$  (Ghavipour et al., 2015; Widjaja et al., 2022).

Tomato is a climacteric fruit, and therefore it is usually harvested unripen, including in the green-mature stage, and continues its ripening after harvest (Quinet et al., 2019; Islam et al., 2023). Tomato fruit maturation and ripening involve a series of biochemical, physiological and structural changes, triggered by the production and action of ethylene (Tilahun et al., 2017). These changes can influence the content of nutrients and functional phytochemicals (Yahia et al., 2001; Tilahun et al., 2017). Some of the fruit maturation and ripening changes are used as maturation and ripening indices as well as harvesting indices, and as quality standards and attributes. These include color (due to pigments) changes, structural (softening) changes, changes in the contents of sugars, acids, and volatile compounds (Quinet et al., 2019). The main indicator of fruit ripening processes is associated with the degradation of chlorophylls and the synthesis of carotenoids with the consequent dedifferentiation of chloroplasts to chromoplasts (Tilahun et al., 2019), and therefore the maturation and ripening stages of tomatoes are commonly classified according to the color change of the fruit, mainly the intensity of the red color caused primarily by the carotenoid lycopene (USDA, 2005). However, there has been an increased interest in objective and integral measurements for a better selection of cultivars, maturation and ripening stages, harvesting indices, quality standards and attributes, and therefore for effective pre- and post-harvest practices to optimize the content of bioactive components and to extend the storage life of the fruit.

Tomatoes are highly perishable after harvest affected by several important factors that affect fruit quality, such as temperature, relative humidity, atmosphere, and other treatments (Kefas et al., 2024). Fruit of traditional tomato cultivars have a postharvest life of about 15–20 days at ambient temperature, but some cultivars have an extended postharvest life, and these are becoming more popular in the international market (Bal, 2021). Changes in bioactive compounds, of great importance for human health, in the long shelf life cultivars have received little attention. Therefore, the objective of this work was to follow the changes of some of the important bioactive compounds, during the ripening and senescence of 'Rhapsody' tomato fruit after harvest, over a long period extending from the mature-green stage to over ripening.

## MATERIALS AND METHODS

### Tomato samples

Forty tomatoes (*Lycopersicon esculentum* Mill cv. Rhapsody) free of blemishes, with uniform color and size, with an average weight of 210 g, was harvested at the mature-green stage, from a commercial hydroponic greenhouse in Querétaro, Mexico. After harvest, the tomatoes were placed to ripen naturally at 22 °C and 85 % RH. Fruit evaluation was done over a period of 35 days, and consisted of a sample of four fruits each time.

### Color measurement

Fruit external color was determined on two readings from each fruit in longitudinal adjustment (one near of peduncle and the other in the apex), using a CM-2002 Minolta colorimeter (model CM-2002, Minolta Co. Ltd., Osaka, Japan). The colorimeter was calibrated with a white pattern and zeroed during each evaluation. The variables  $a^*$ ,  $b^*$ ,  $L^*$ ,  $C^*$ , and  $h^\circ$  were recorded.

### Extraction and HPLC analysis of vitamin C

Five grams of fresh fruit pulp were homogenized with 10 mL of 0.1 M citric acid solution containing 0.05% EDTA and 5% methanol, and pH was adjusted to 2.35-2.40. The mixture was centrifuged at 11, 960 x g for 10 min at 2 °C, and 2.4 mL of the supernatant was filtered through a number 3 Whatman paper, and at least one hour before the analysis 1 mL of 1,2-benzenediamine in water (83.2 mg/100 mL) was added to it. Of this mixture, 40  $\mu$ L were injected automatically into an HP 1100 series high pressure liquid chromatography (HPLC) system (Agilent Technologies Co., Palo alto, CA, USA) equipped with an inline degasser, a thermostatic auto-sampler, 100  $\mu$ L loop and a diode array detector (DAD). The mobile phase was water/methanol (95:5 % v/v) containing 5 mM of hexadecyltrimethyl-ammonium bromide and 50 mM of  $\text{KH}_2\text{PO}_4$ , and it was pumped at 1.5 mL  $\text{min}^{-1}$  through a Waters  $\mu$ Bondapak  $\text{C}_{18}$  analytical column (3.9 x 300 mm, 10  $\mu$ m) kept at 25 °C. The ascorbic acid (AA) and the isoascorbic acid (IAA) were monitored at  $\lambda = 261$  nm, and the dehydroascorbic acid (DHAA) at  $\lambda = 348$  nm. The identification and quantification of AA, IAA, and DHAA were achieved by comparing the retention time values and the integrated peak areas with those of known amounts of the standards of these compounds purchased from Sigma (Sigma-Aldrich, St. Louis, MO). The peaks obtained were processed using the HP Chem Station program, and reported as total vitamin C.

### Extraction and HPLC analysis of tocopherols

Ten grams of fresh tomato pulp were homogenized with 20 mL of ethanol for 2 min and centrifuged at 5000 x g for 5 min at 2 °C. The pellet was eliminated and 3.5 mL of petroleum ether were added to the extract, shaken, and 5mL of water were added. The mixture was centrifuged again, under the same conditions, and the upper layer was then separated and evaporated in a rotavapor (Yahia et al., 2007). The obtained mixture was dissolved in 1.5 mL of methanol and filtered through a polyethylene membrane of 0.45  $\mu$ m of pore prior to the injection of 50  $\mu$ L to the HPLC system. The HPLC system described above was used, but using a fluorescence detector. The mobile phase was methanol/water (95:5 % v/v) and it was pumped at 1mL/min through a Waters Symmetry  $\text{C}_{18}$  analytical column (4.6 x 150mm, 3.5 mm) that was kept at 25°C. The tocopherols were monitored at  $\lambda_{\text{ex}} = 294$  nm and  $\lambda_{\text{em}} = 326$  nm. The identification and quantification of  $\alpha$ - and  $\sigma$ -tocopherols were achieved by comparing the retention time values and the integrated peak areas with those of the samples

obtained from solutions of known concentrations of standard tocopherols purchased from Sigma-Aldrich (Saint Luis, MO, USA).

### Extraction and HPLC analysis of carotenoids

Six grams of fresh tomato pulp were dehydrated with NaSO<sub>4</sub>, and 30 mL of hexane-acetone-toluene-ethanol solution (33.3:23.3:23.3:20 v/v) were added. The saponification of samples was done by adding 1 mL of 40% KOH in methanol. This mixture was placed in a water bath at 56 °C and shaken for 20 min, after which it was cooled, and 15 mL of hexane were added, sample was shaken again, and 15 mL of aqueous 10% NaSO<sub>4</sub> were added. The samples were then kept in the darkness for 15 min, and after filtration of the extracts through polyethylene membranes of 0.45 µm pore, 25 µL were taken from the upper layer and injected into the HPLC system. The determinations were made at λ= 471 nm using a diode array detector. A YMC Carotenoid C<sub>30</sub> (4.6x150 mm, 3 µm) analytical column was used. A gradient system of a mobile phase was employed, which begun with 4% water, 81% methanol and 15% *tert*-butyl methyl ether, and finalized after 75 minutes to 4%, 18% and 78%, respectively (Yahia et al., 2007). As it is the case in the other HPLC analyses, lycopene and β-carotene were identified and quantified using commercial standards obtained from Sigma-Aldrich (Saint Luis, MO, USA).

### Statistical analysis

The experiment was repeated 3 times, using a completely randomized design, and the collected data were analyzed by comparing the means using the Tukey-Kramer test and linear regressions, employing the JMP software (SAS Institute Inc., Cary, NC).

## RESULTS AND DISCUSSION

### Color development and carotenoids

Significant color changes were observed during the first 8 to 11 days after harvest, followed by very slight variations (Fig. 1). The development of the tomato pigmentation consisted in the disappearance of the green color, and the acquisition of red tonalities (increases in *a*\* value). These changes in color are also reflected in hue (*h*<sup>o</sup>) value. The quotient of the parameters *a*\* (redness) and *b*\* (yellowness) (*a*\*/*b*\*) was also useful in describing these changes, whose biochemical explanation is based on the degradation of chlorophyll (green pigment) and a rapid biosynthesis and accumulation of carotenoids (Yahia et al., 2007), principally lycopene, responsible for the red pigmentation characteristic of tomato fruit (Tilahun et al., 2017). The *a*\* and *a*\*/*b*\* values showed good correlation with lycopene content (Fig. 2), according to equations 1 and 2, whose correlation coefficients (*r*<sup>2</sup>) were 0.97 and 0.94, respectively, where lycopene values were in mg/100 g of fresh tissue.

$$\text{Ln (lycopene)} = -0.87767667 + 0.07675095 (a^*) + 0.00691 (a^*)^2 \quad (1)$$

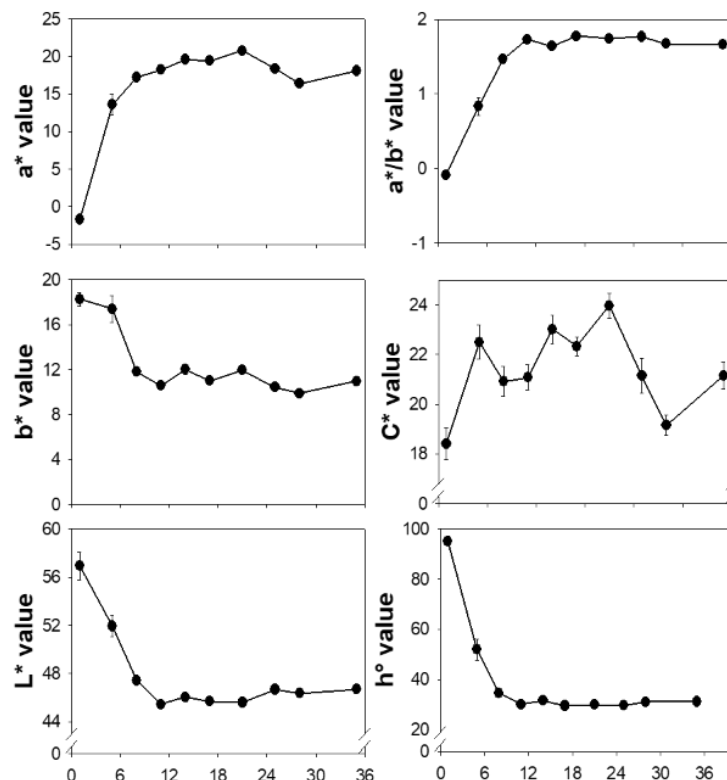
$$\text{Ln (Lycopene)} = -0.73015483 + 2.19329353 (a^*/b^*) \quad (2)$$

In contrast, Loayza et al. (2021a) did not find significant correlations between the color changes and lycopene content in ‘BHN-602’ tomatoes, while other studies have reported associations between color changes in tomatoes with carotenoids such as β-carotene (Flores et al., 2017).

The luminosity (*L*\*) value decreased almost 21% after 36 days of storage, and the major decrease was observed during the first 12 days. The chroma (*C*\*) values slightly increased

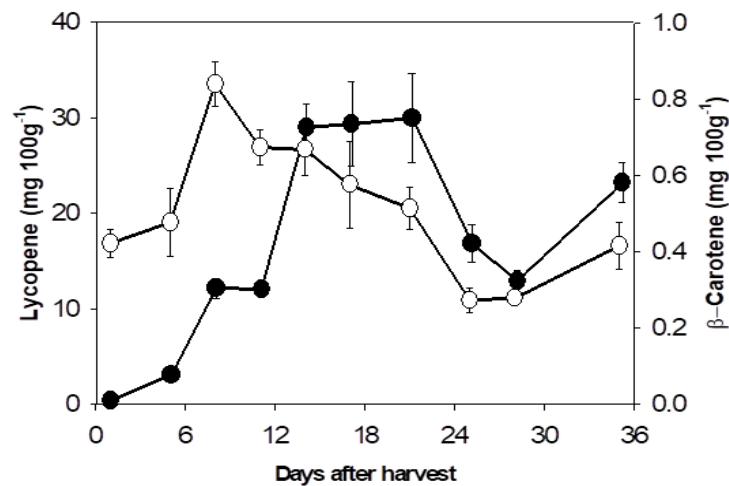
(14%) during ripening. Soto-Zamora et al. (2005) also found similar reductions (20%) in tomatoes stored at 20 °C. Changes in color during ripening have been reported in shorter postharvest periods in other cultivars. Mazón-Abarca et al. (2022) found higher reductions in the hue value after 9 days of storage in fruit of the cultivar TA234 in comparison with our study, where major changes were found after 12 days of storage of ‘Rhapsody’ tomatoes.

Lycopene content increased continuously after 21 days of storage to up to 30 mg/100 g fw and then slightly decreased until day 36 to 23 mg/ 100 g fw (Fig. 2). These lycopene levels are within the range reported for tomatoes of other cultivars (2.2–54.9 mg/100 g fw) (Flores et al., 2017; Loayza et al., 2021a; Vats et al., 2022; Bianchi et al., 2023). Once the tomatoes have developed their red color (after 8 to 11 days, see Fig. 1), the maximum  $\beta$ -carotene content (0.84 mg 100 g<sup>-1</sup>) has been reached (Fig. 2), which surpassed the content (0.51  $\pm$  0.1 mg/ 100 g fw) reported in 21 red tomato cultivars (Flores et al., 2017). In our study, the  $\beta$ -carotene content after 35 days was similar with that at day 0 (0.41 mg 100 g<sup>-1</sup>). It has been suggested that lycopene content increases during tomato ripening due to an increase in the activity of enzymes such as 6-methyl-5-hepten-2-one, promoted by ethylene action (Pu et al., 2020). Few studies have followed the changes in the content of carotenoids during the ripening and senescence periods of tomatoes (only 10–20 d postharvest) (Tilahun et al., 2019; Pu et al., 2020). Yahia et al. (2007, 2005) reported major variations related to storage temperatures, where chilling temperatures (4°C) delayed the carotenoid accumulation in tomato fruit.



**Fig. 1.** Changes of color parameters during the ripening and senescence of ‘Rhapsody’ tomatoes harvested at the mature-green stage and maintained at 22 °C and 85% RH. Vertical bars indicate standard error of the mean.





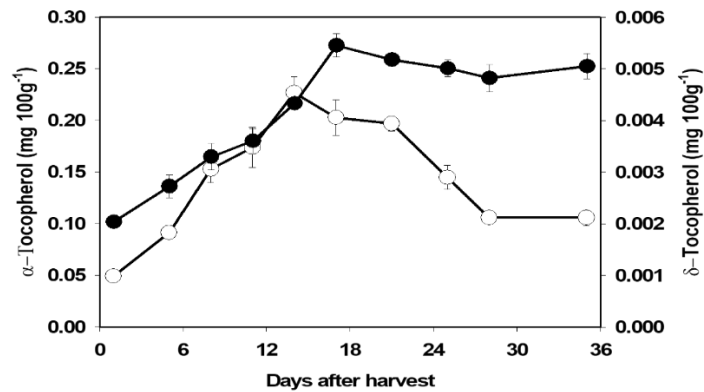
**Fig. 2.** Changes in lycopene (—●—) and β-Carotene (—○—) content during the ripening and senescence of ‘Rhapsody’ tomatoes harvested at the mature-green stage and maintained at 22 °C and 85% RH. Vertical bars indicate standard error of the mean.

### Changes in tocopherols and vitamin C

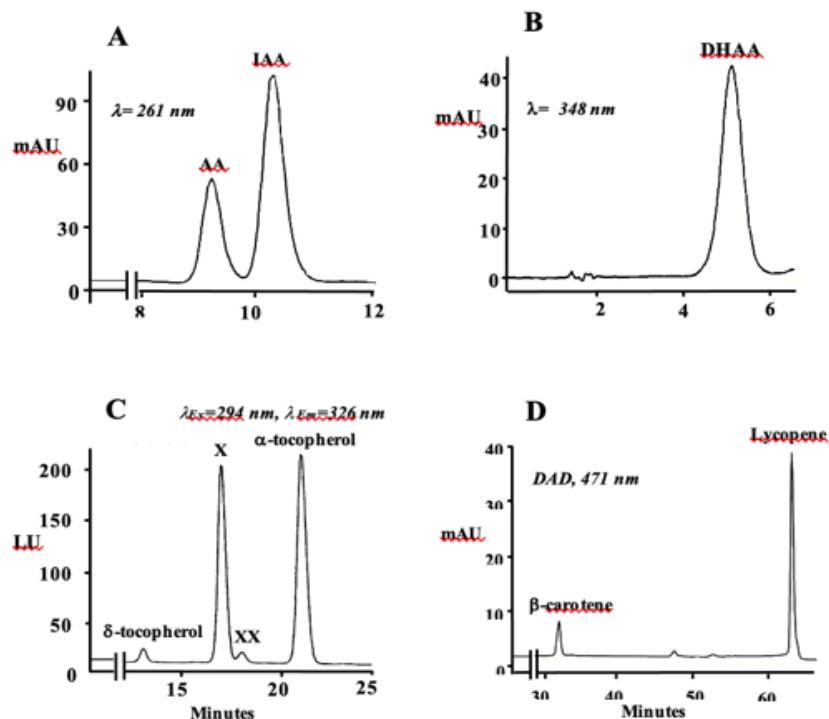
The maximum levels of δ- and α-tocopherols were reached after 14 and 17 days after harvest, respectively, and then started to decrease (Fig. 3). This tendency, especially the peak of these two vitamins of vitamin E agrees with the development of the red color of the fruit. Chlorophyll biodegradation produces phytol, which is a limiting factor in the synthesis of tocopherols by the 2-methyl-6-phytylquinol methyltransferase during tomato ripening (Vats et al., 2022). The limited amount of chlorophyll and its degradation during ripening, and thus the limited production of phytol groups might be the reason behind the reduction of the tocopherols after reaching their peak (Soto-Zamora et al., 2005). The reduction was slower for α-tocopherol (Fig. 3), probably because other tocopherols are good substrates for methyltransferase (Guo et al., 2022), which is responsible for the formation of this compound. In contrast, Figueira et al. (2017) found higher losses of α-tocopherol, up to 62.8% less α-tocopherol in ripe fruit than in full mature green ‘Gordal’ tomatoes, and slight increase in the content of δ- and γ-tocopherols. Yahia et al. (2007) reported a reduction in the content of α-tocopherol after 14 days and then increased until day 30 of storage at 20 °C, whereas in fruit stored at 4 °C, the content slightly increased during storage. The obtained tocopherol chromatograms (Fig. 4C) imply that analyzed tomatoes may contain at least another isomer of tocopherol (β or γ or both together), which is in agreement with results reported by Abushita et al. (2000) and Figueira et al. (2017). The chromatograms (Fig. 4) show two compounds (X and XX), eluted between α- and δ-tocopherol (α- as three-methylated and δ- as the mono-methylated vitamins, respectively), which exhibited fluorescence response at  $\lambda_{ex}$  = 294 nm and  $\lambda_{em}$  = 326 nm, a chemical property that characterizes tocopherols. The peaks X, XX, or both of them, could represent a mixture of bimethylated tocopherols as well as tocotrienols. The content of tocopherols in tomato fruit has been reported from 0.17 up to 3.83 mg/100 g fw, being α-tocopherol the most abundant (Raiola et al., 2015; Figueira et al., 2017).

The content of vitamin C (Fig. 5) was within the reported range for tomato fruit (4–23 mg/ 100 g fresh fruit) (Tilahun et al., 2017; Bianchi et al., 2023). It decreased slightly during the first 11 days after harvest and then increased during 11 and 17 days, and gradually decreased again, however, there were no significant differences between the initial and final amounts of this vitamin. Yahia et al. (2007 and 2005) reported similar contents of ascorbic acid, but an increase of isoascorbic acid during tomato ripening after 30 and 35 days of

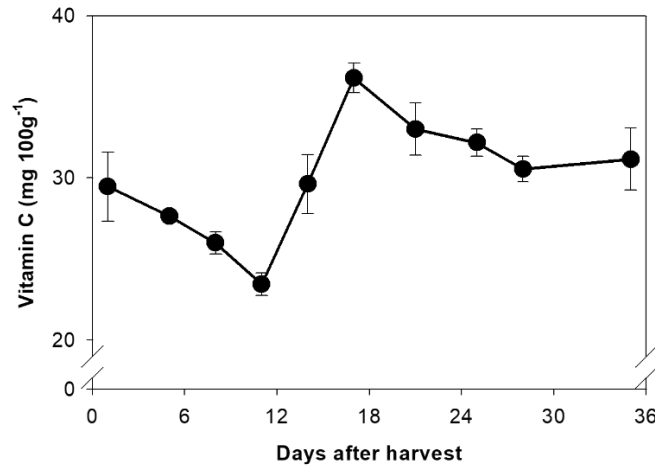
storage at 10 and 20 °C. To the contrary, Tilahun et al. (2017) reported a slight decrease (17.2–20.3%) in the content of ascorbic acid from tomatoes at breaker (initiation of green-red) stage and red fruits of cv. ‘TY Megaton’ and ‘Yureca’ at similar storage conditions ( $20 \pm 2$  °C). The increase of vitamin C during fruit ripening has been related to the production of the intermediary GDP-1-galactose promoted by the GDP-mannose 3’, 5’-epimerase family (Vats et al., 2022). However, the ripening metabolism varied in different cultivars.



**Fig. 3.** Changes in  $\alpha$ -tocopherol (●) and  $\delta$ -tocopherol (○) content during the ripening and senescence of ‘Rhapsody’ tomatoes harvested at the mature-green stage and maintained at 22 °C and 85% RH. Vertical bars indicate standard error of the mean.



**Fig. 4.** Typical chromatograms of ascorbic acid (AA). A: isoascorbic acid (IAA), B: dehydroascorbic acid (DHAA), C:  $\delta$ -tocopherol,  $\alpha$ -tocopherol, and D:  $\beta$ -carotene and lycopene. X and XX are thought to be  $\gamma$ - and  $\beta$ -tocopherol, respectively. The response of the photodiodes array detector (DAD) and the fluorescence detector (FLD) were in milli absorbance units (mAU) and luminescence units (LU), respectively.



**Fig. 5.** Changes in vitamin C content during ripening and senescence of 'Rhapsody' tomatoes harvested at the mature-green stage and maintained at 22 °C and 85% RH. Vertical bars indicate standard error of the mean.

## CONCLUSION

Our results indicated that 'Rhapsody' tomatoes harvested at the mature-green stage and ripened naturally had adequate levels of vitamin E (tocopherols), vitamin C, lycopene, and  $\beta$ -carotene for 14 to 17 days. The storage at 20 °C was suitable for the promotion of carotenoids accumulation in tomato fruit. The content of these bioactive antioxidant compounds was maintained during the ripening stage, and therefore the consumption of the fruit or its use for processing during this period would not cause major losses in these health-promoting phytochemicals. However, extended periods, beyond 11-17 days of ripening when the fruit are harvested at the mature-green stage, could result in significant losses in important bioactive components, and therefore decreased health benefits.

## Conflict of interest

The authors declare that there is no conflict of interest.

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