



Color and Pigment Development of Mature-green Tomatoes Treated with Hot Water

FRANCISCO E. LOAYZA¹, JEFFREY K. BRECHT*¹, AND AMARAT H. SIMONNE²

¹Horticultural Sciences Department, University of Florida, Fifield Hall, Gainesville, FL 32611-0690

²Food Science & Human Nutrition Department, University of Florida, 359 FSHN Building, Newell Drive, Gainesville, FL 32611-0370

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Reversible (i.e., non-injurious) stress can induce up-regulation of the antioxidant system in plants. Therefore heat stress in the form of a hot water treatment can promote the synthesis of beneficial antioxidant compounds such as lycopene in tomato fruit. However, it has also been shown that hot-water treated mature-green 'Florida 47' tomatoes did not develop higher carotenoid content when fully ripe, even though they visually showed more rapid color development. Our objective was to demonstrate that hot-water treatment induces higher carotenoid accumulation in early ripening stages, but total carotenoid accumulation is limited by a cultivar's genetic potential. Mature-green 'Florida 47' tomatoes were immersed in water at 25 or 52 °C for 5 minutes, followed by treatment with 100 ppm ethylene for 48 hours at 20 °C, then stored at 20 °C. We found that the hot-water treatment significantly increased total carotenoids ($P = 0.002$) but moderately increased lycopene ($P = 0.116$). Hot water resulted in faster accumulation of lycopene and total carotenoids during ripening, with the largest significant differences occurring 7 days after treatment ($P = 0.009$ and $P = 0.032$, respectively). In conclusion, hot-water treatment had a highly significant effect on the synthesis of carotenoids, and a moderately significant effect on lycopene, but their final accumulation was apparently limited by the cultivar's genetic potential.

External stress can induce the production of radical oxygen species in plants. In response, plants generally defend themselves by increasing the capacity of their antioxidant system (Bénard et al., 2009, Grune, 2005; Mazhoudi et al., 1997; Raffo et al., 2006). Therefore the general hypothesis is that a controlled application of a stress can induce up-regulation of the antioxidant system without damaging the plant (Yahia et al., 2007). In the work reported here, the application of this stress was in the form of immersion in hot-water, which has been shown to promote the synthesis of antioxidant compounds in tomatoes (Brecht et al., 1999) and other fruits (Kim et al., 2007). However, in previous experiments, the application of the hot-water treatment to mature-green 'Florida 47' tomatoes did not result in higher carotenoid concentrations when the fruit reached their fully ripe stage. This lack of response might have been because 'Florida 47' tomatoes have a limited genetic potential for the synthesis of carotenoids. Hence the main objective of this work was to compare the synthesis of carotenoids and the development of red color between hot-water treated tomatoes and non-treated fruit during the ripening process.

Material and Methods

Tomatoes of the cultivar 'Florida 47' were harvested at the mature-green developmental stage in Ruskin, FL. The tomatoes' size was Large (6×6) and they were kept at 20 °C after packing.

In preparation for the hot-water treatment, the tomatoes were grouped in batches of 60 fruit. Every batch underwent either a hot-water treatment at 52 °C for 5 min or at 25 °C for 5 min as control treatment and they were then surface air dried at room temperature. All batches were then exposed to an ethylene treatment of 100 ppm for 48 h at 20 °C and the end of this treatment was designated as day 0. Tomatoes that did not show any sign of red color after 48 h ethylene exposure were discarded reducing the batch size to 30 fruit. The tomatoes were kept at 20 °C and batches of both treatments were sampled on days 3, 5, 7, 9, 11, and 13.

The visual color was related to the transition from green to red color which can be directly determined by a* value (CIE L* a* b* scale) using a Minolta Colorimeter CR-400. The a* value was measured at four different locations on each tomato fruit. One measurement was made externally on the epidermis at the blossom end, avoiding the blossom end scar if present, and the other three measurements were made internally on the peripheral, middle and core pericarp. The measurement of the peripheral pericarp was made beneath the epidermis at the blossom end; then the fruit was cut through the equatorial section and the core pericarp measurement was made at the center of the tomato and the middle pericarp measurement was made midway between the center of the tomato and the periphery.

The lycopene and total carotenoid contents were determined by HPLC (Waters 2695 Separation Module) and photodiode array detector (Waters 996). For this procedure, a homogenate of 10 fruit was prepared and then carotenoids were extracted with methylene chloride (Ishida et al., 2001a, 2001b). Tomato lycopene (Sigma-Aldrich) was used as an external standard for quantification. The total carotenoids were calculated as the sum of the peak areas. The results were expressed as µg/g of fruit in dry weight.

*Corresponding author; phone: (352) 273-4778; email: jkbrecht@ufl.edu

In order to evaluate the global effect of the hot-water treatment, an analysis of variance was performed in which sampling time was considered as block and the water temperature as main factor; and LSD was used for mean comparison. In addition, the hot-water treatment and control were compared at each day using one-factor ANOVA with the water temperature as the only factor. SAS 9.2 was used for statistical analysis.

Results and Discussion

Comparing the development of red color between hot-water treated 'Florida 47' tomatoes and their control, we observed that hot-water treated tomatoes had a noticeably higher a^* value than

the control fruit on the epidermis almost until the end of the ripening process (Fig. 1). Similarly hot-water treated tomatoes had greater a^* values in the peripheral pericarp but only until day 7. The a^* value of hot-water treated tomatoes was also higher than the control in the middle and core pericarp, being greater in the former until day 7 and in the latter until day 9 (Fig. 2). The three pericarp sections and the epidermis followed a normal pattern of color development (Tijksens and Evelo, 1994). Visually, the epidermis of hot-water treated tomatoes had a more intense color than the control at the blossom end. Internally hot-water treated tomatoes had a more even red color and they no longer showed signs of green and yellow colors in comparison with the control, when fruits reached the pink developmental stage.

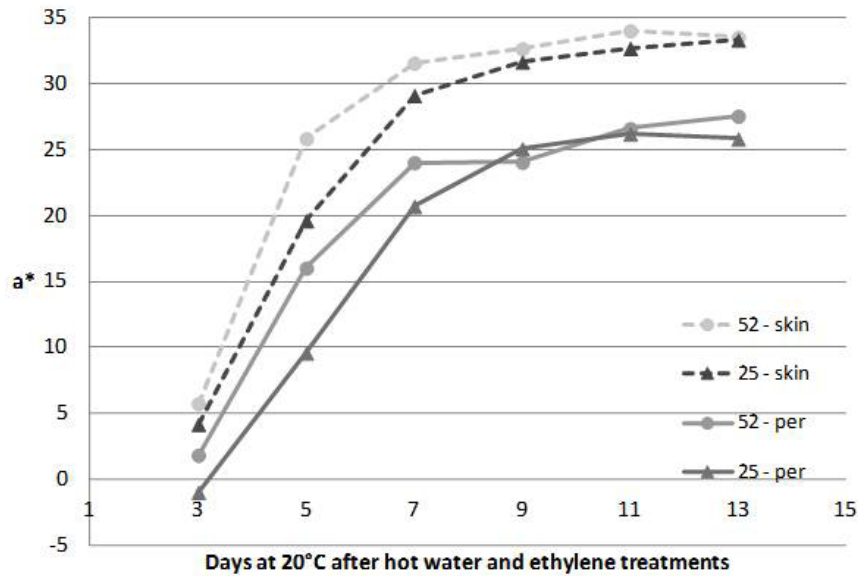


Fig. 1. The a^* value at the epidermis and in the peripheral pericarp during ripening of initially mature-green 'Florida 47' tomatoes. '52-per' was the a^* value average of 30 fruit measured in the peripheral pericarp of tomatoes treated with 52 °C water for 5 min; '25-per' was the control treated with 25 °C water for 5 min. '52-skin' was the a^* value average of 30 fruit measured on the epidermis of tomatoes treated with 52 °C water for 5 min; '25-skin' was the control treated with 25 °C water for 5 min.

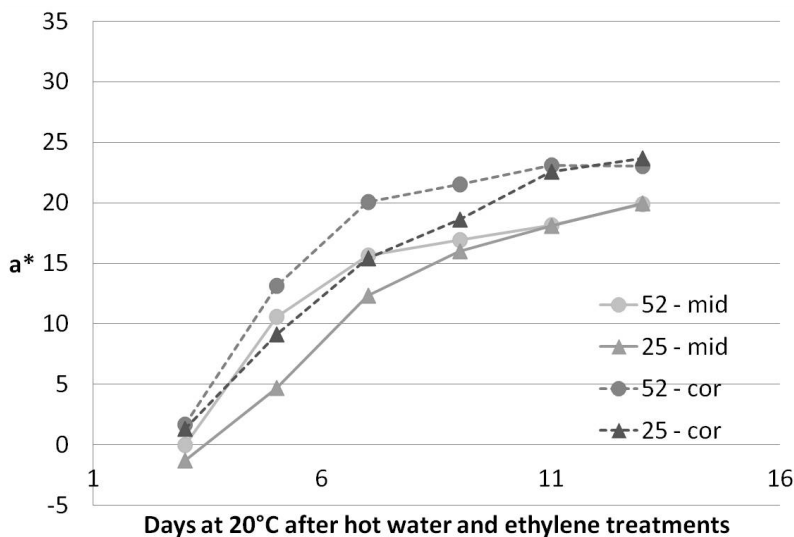


Fig. 2. The a^* value in the middle and core pericarp of initially mature-green 'Florida 47' tomatoes during ripening. '52-cor' was the a^* value average of 30 fruit measured in the core pericarp of tomatoes treated with 52 °C water for 5 min; '25-cor' was the control treated with 25 °C water for 5 min. '52-mid' was the a^* value average of 30 fruit measured in the middle pericarp of tomatoes treated with 52 °C water for 5 min; '25-mid' was the control treated with 25 °C water for 5 min.

In tomatoes, the red color is due to the presence of carotenoids, particularly lycopene. As expected, the hot-water treatment significantly increased the content of total carotenoids over the course of ripening ($P = 0.002$). On average, the hot-water treated tomatoes had over 50 $\mu\text{g/g}$ of fruit DW higher content of total carotenoids than the control (Fig. 3). Furthermore, the hot-water treatment had an overall moderate effect on the lycopene content ($P = 0.116$). Even though, on average, hot-water treated tomatoes had higher lycopene content, this difference was not significant at 95% level of confidence; usually heat stress reduces lycopene content (Raffo et al., 2006) although a controlled, non-injurious heat exposure induces its synthesis.

Observing the evolution of the carotenoid synthesis during ripening, hot-water treated tomatoes had higher carotenoid content during the ripening process, being significantly higher on days 7 and 9 (Fig. 4). Similarly, hot-water treated tomatoes had higher lycopene content, but there was no significant differences on any day (Fig. 5). Therefore, the comparison showed that hot-water treated tomatoes synthesized greater amounts of carotenoids early in the ripening process; however, when the tomatoes were full-ripe on day 13, there was no longer a difference in carotenoids content between hot-water treated tomatoes and the control. Both treatments produced a carotenoids profile consistent with earlier reports (Alquezar et al., 2008).

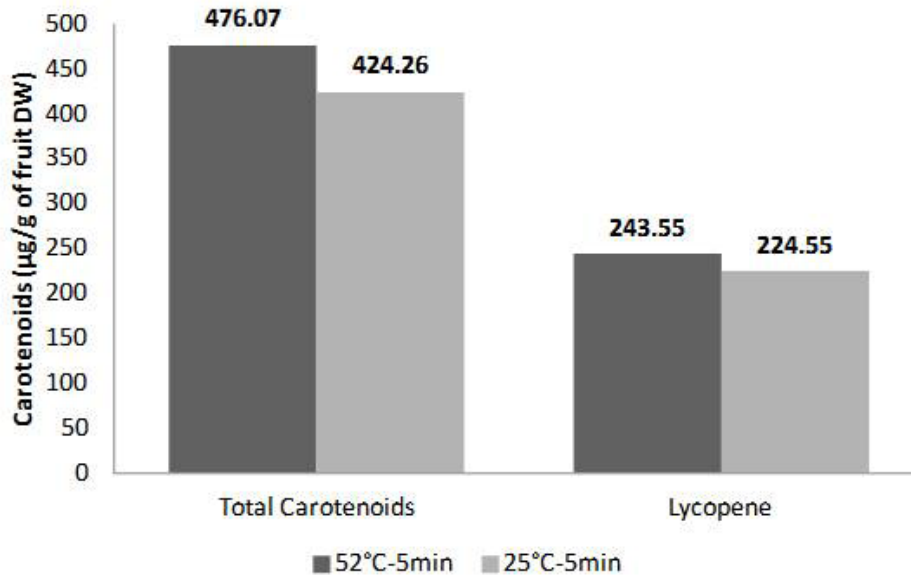


Fig. 3. Average total carotenoid and lycopene content during ripening of ‘Florida 47’ tomatoes subjected to hot-water treatment. ‘52 °C–5min’ represents the average content of total carotenoids and lycopene of during the ripening process of tomatoes treated with 52 °C water for 5min whereas ‘25 °C–5min’ was the control treated with 25 °C water for 5 min.

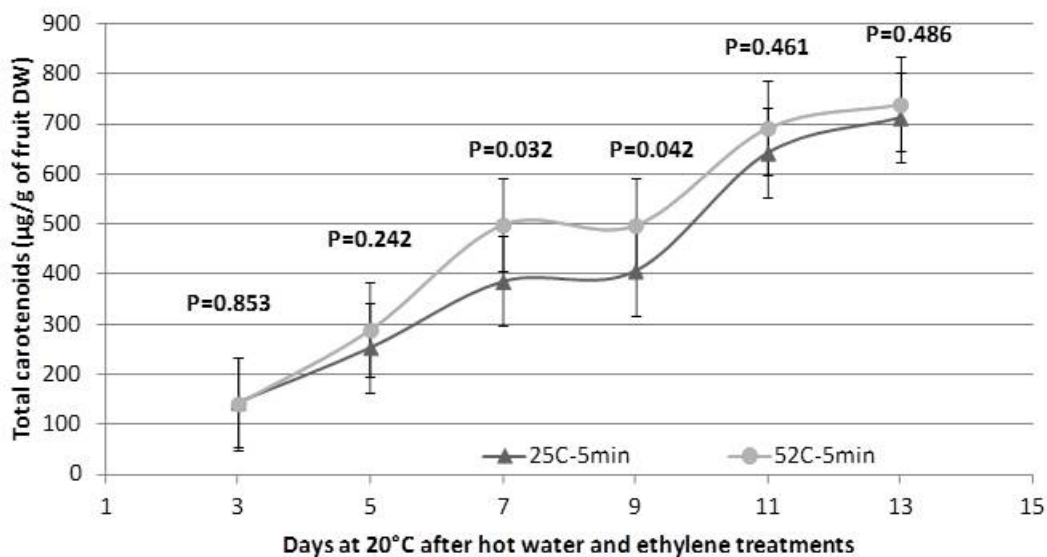


Fig. 4. Total carotenoids accumulation during ripening of initially mature-green ‘Florida 47’ tomatoes subjected to hot-water treatment. ‘52C–5min’ represents the average content of total carotenoids of three homogenates during the ripening process of tomatoes treated with 52 °C water for 5 min whereas ‘25C–5min’ was the control treated with 25 °C water for 5 min. The P -value of the simple ANOVA is noted for each day.

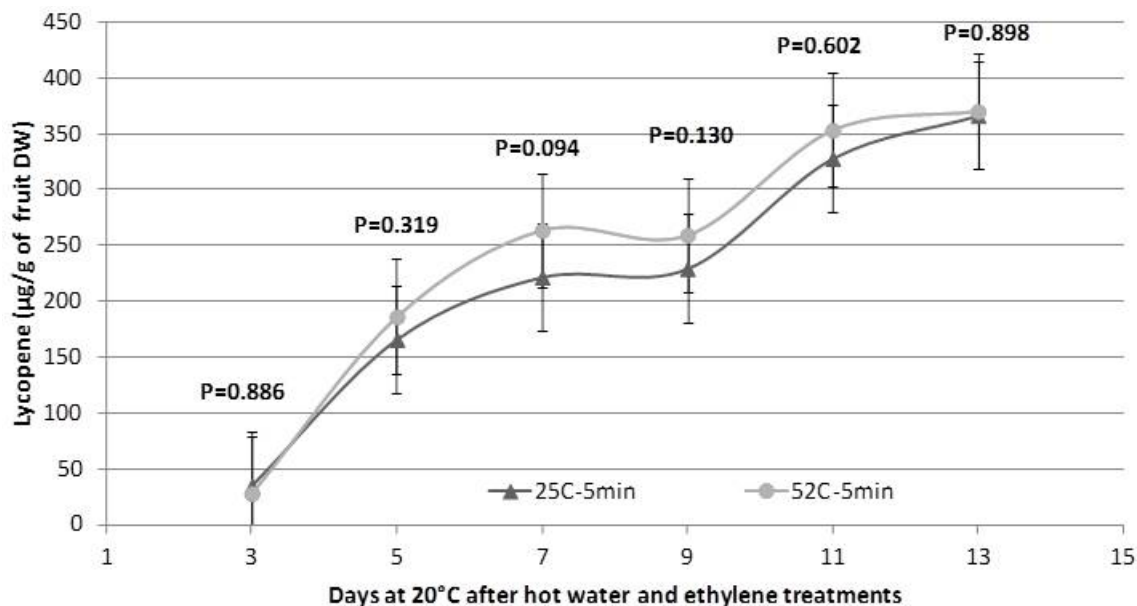


Fig. 5. Lycopene accumulation during ripening of initially mature-green 'Florida 47' tomatoes subjected to hot-water treatment. '52C-5min' represents the average content of lycopene of three homogenates during the ripening process of tomatoes treated with 52 °C water for 5 min whereas '25C-5min' was the control treated with 25 °C for 5 min. The *P*-value of the simple ANOVA is noted for each day.

Multiple regression was tested in order to predict the total carotenoid or lycopene contents using *a** value measured on several locations of the tomatoes. Those multiple regressions are shown below:

$$[\text{Lycopene}] = 0.081(a^*\text{cor}) + 0.291(a^*\text{mid}) + 0.071(a^*\text{per}) + 0.120(a^*\text{epi}) + 5.645$$

$$[\text{Total Carotenoids}] = 0.247(a^*\text{cor}) + 0.496(a^*\text{mid}) + 0.143(a^*\text{per}) + 0.450(a^*\text{epi}) + 2.203$$

Both multiple regression models were highly significant ($P < 0.0001$), but the R^2 was low in both cases. However, the multiple regression models showed a better correlation between carotenoid content and *a** color values than simple regressions among *a** values measured at different locations (Table 1). In general, both *a** value and carotenoid content in tomatoes had a poor correlations with lycopene content in comparison with predicted lycopene in tomato puree or products by other spectrophotometric methods (Davis et al., 2003; Watada et al., 1976). In addition, the *a** value measurements in our research were made on small areas which

might not have accurately represented the heterogeneous red color intensity in the tomato due to the uneven distribution of carotenoids in the whole fruit. Consequently, the linear correlation between real values and predicted values from the multiple regression models were poor with R^2 of 0.471 for lycopene and 0.504 for total carotenoids.

Conclusion

We conclude that the hot-water treatment had a significant effect in accelerating the accumulation of total carotenoids in 'Florida 47' tomatoes, but, in the case of lycopene, this effect was moderate. Similarly, the hot-water treatment produced higher *a** values on the epidermis and in the peripheral, middle and core pericarp regions, particularly early in the ripening process. However, those significant differences between treated and non-treated tomatoes did not persist through the fully ripe stage. Finally we found that while 'Florida 47' positively responded to the hot-water treatment, this cultivar apparently has a limited genetic capacity to synthesize carotenoids that did not allow a net increase in carotenoids content in the ripe fruit.

Table 1. Correlation matrix of R^2 values^z for lycopene and total carotenoids vs. *a** values at different locations and with multiple regression model.

	<i>a*</i> in core pericarp	<i>a*</i> in middle pericarp	<i>a*</i> in peripheral pericarp	<i>a*</i> on epidermis	Multiple regression model
Lycopene	0.4274 ($P < 0.0001$)	0.4993 ($P < 0.0001$)	0.3993 ($P < 0.0001$)	0.4218 ($P < 0.0001$)	0.5268 ($P < 0.0001$)
Total carotenoids	0.4396 ($P < 0.0001$)	0.4882 ($P < 0.0001$)	0.4085 ($P < 0.0001$)	0.4554 ($P < 0.0001$)	0.5352 ($P < 0.0001$)

^zThe R^2 was obtained as follows: each homogenate result was correlated with the *a** values of the corresponding 10 fruit. In other words, for every analytical measurement, there were 10 measurements of color. The *P*-value of each correlation is noted below its corresponding R^2 .

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