

ANTIOXIDANT PROPERTY OF “SWEET GRAPE” TOMATOES TREATED WITH HEAT SHOCK

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ABSTRACT - Postharvest conservation methods such as heat shock can increase the nutritional profile of fruits, inducing greater accumulation of antioxidants that participate in the physiological maintenance of fruits. The objective of this work was to evaluate the antioxidant properties of “Sweet Grape” tomatoes treated with heat shock in pre-storage. “Sweet Grape” tomatoes were heat shocked in hot water (45°C) for 10 min. and stored at room temperature for 9 days. β -carotene, ascorbic acid, total phenolic compounds, and antioxidant activity (DPPH, ABTS, and FRAP) were evaluated every 3 days. The pulp and skin of the treated tomatoes had higher β -carotene content than the control during storage, according to the findings. The content of total phenolic compounds in the pulp reduced, regardless of the treatment. There was a reduction of ascorbic acid, but with significantly higher values in the fruits treated on the third and sixth days of storage. The DPPH antioxidant activity of the pulp decreased, regardless of the treatment. In the skin there was a significant effect of the treatment until the sixth day. There was no variation in the ABTS antioxidant activity of the pulp of the treated tomatoes. There was no significant effect of heat shock on the FRAP antioxidant activity of the pulp until the sixth day. Heat shock promoted an increase in β -carotene in tomato skin, which was related to increases in its DPPH antioxidant activity. The heat shock treatment promoted greater retention of ascorbic acid in the fruit, but it was not able to avoid its reduction during storage. The tomatoes’ antioxidant biochemical response to heat shock is most expressed in their skin.

Keywords: *Solanum lycopersicum* L., bioactive compounds, post-harvest conservation, oxidative stress.

PROPRIEDADE ANTIOXIDANTE DE TOMATES “SWEET GRAPE” TRATADOS COM CHOQUE TÉRMICO

RESUMO - Métodos de conservação pós-colheita como choque térmico podem elevar o perfil nutricional de frutos, induzindo maior acúmulo de antioxidantes que participam da manutenção fisiológica dos frutos. O objetivo deste trabalho foi avaliar as propriedades antioxidantes do tomate “Sweet Grape” tratado com choque térmico em pré-armazenamento. Tomates “Sweet Grape” foram tratados com choque térmico em água quente (45°C) por 10 min., e armazenados em temperatura ambiente por 9 dias. β -caroteno, ácido ascórbico, compostos fenólicos totais e atividade antioxidante (DPPH, ABTS e FRAP) foram avaliados a cada 3 dias. Os resultados mostraram que polpa e casca dos tomates tratados apresentaram conteúdo de β -caroteno superiores ao controle durante o armazenamento. Os teores de compostos fenólicos totais da polpa reduziram, independente do tratamento. Houve redução de ácido ascórbico, mas com valores significativamente maiores nos frutos tratados no terceiro e sexto dia de armazenamento. A atividade antioxidante DPPH da polpa sofreu queda, independente do tratamento. Na casca houve efeito significativo do tratamento até o sexto dia. Não houve variação da atividade antioxidante ABTS da polpa dos tomates tratados. Não houve efeito significativo do choque térmico na atividade antioxidante FRAP da polpa até o sexto dia. O choque térmico promoveu aumento de β -caroteno da casca do tomate, estando relacionado aos aumentos da sua atividade antioxidante DPPH. O tratamento com choque térmico promoveu maior retenção de ácido ascórbico do fruto, mas não foi capaz de evitar sua redução durante o armazenamento. A resposta bioquímica antioxidante do tomate ao choque térmico tem maior expressão na sua casca.

Palavras-chave: *Solanum lycopersicum* L., compostos bioativos, conservação pós-colheita, estresse oxidativo.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most produced and consumed produce in Brazil and in the world, considered an excellent source of antioxidant compounds such as ascorbic acid, β -carotene, lycopene, phenolic compounds, among others, and its consumption is associated with the prevention of diseases such as cancer, heart diseases, and other chronic diseases (STAJCIC et al., 2015; ELBADRAWY; SELLO, 2016). Like other produce,

tomatoes also need special attention regarding the post-harvest conservation techniques used due to the acceleration of deteriorative processes during storage, leading to the loss of nutritional and commercial value (LUVIELMO; LAMAS, 2012).

Post-harvest conservation techniques should be used to delay normal physiological processes of fruits, such as ripening and microbiological deterioration, to extend their shelf life. However, scientific reports have shown that

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certain post-harvest conservation methods, such as heat shock, in addition to delaying fruit degradation processes, have also improved their antioxidant nutritional profile (BA et al., 2022).

Thermal shock is a heat treatment applied to fruits by means of heated water, hot air, or steam (USALL et al., 2016), and has been used mainly in the control of post-harvest diseases of fruits by eliminating microbial spores (FRANCESCO et al., 2018). In addition to the benefits associated with the inhibition of microorganisms, studies show that heat shock is also capable of causing moderate physiological stress in the plant organ, sufficient to alter its non-enzymatic antioxidant profile by stimulating the synthesis of antioxidant compounds, such as ascorbic acid and phenolic compounds (NG; KUPPUSAMY, 2019; ZHANG et al., 2019).

Moderately applied heat to fruits during heat shock generates cellular stress capable of affecting the metabolic activity of the treated plant, which consequently leads to the induction of defense systems mediated by processes of synthesis and degradation of secondary antioxidant metabolites (TAIZ et al., 2017). Therefore, the objective of this work was to evaluate the antioxidant properties of "Sweet Grape" tomato treated with heat shock in pre-storage.

MATERIAL AND METHODS

Tomato (*Solanum lycopersicum* L.) fruits, cv. "Sweet Grape", were acquired from rural producers and uniform experimental samples were selected in terms of size, maturation pattern (red ripe), absence of mechanical damage or disease symptoms. The fruits were, then, sanitized in sodium hypochlorite solution (1%) and dried at room temperature (25°C) for 1 h.

The heat shock treatment was applied to the tomatoes by immersing the fruits in a hot water bath (Novatecnica, NT 266) at 45°C (± 2) for 10 min. Untreated fruits were considered control. After treatment, the tomatoes were dried at room temperature (25°C) for 1 h, placed in expanded polystyrene trays and stored for 9 days under bench top conditions (uncontrolled environment) at 25°C (± 4). Analytical samplings were performed every 3 days.

The experimental design used was completely randomized in a split-plot scheme with 5 replications for each sampling period. The levels of β -carotene, total phenolic compounds, ascorbic acid, and antioxidant activity (DPPH, ABTS and FRAP) were evaluated.

Initially, a lipophilic extraction of the tomato sample was carried out, according to the extraction methodology proposed by Pan et al. (2008). Dried samples of tomato pulp and skin were dried in an oven (50°C) for 24 h, then 0.5 g of the material was weighed and extracted in an automatic grinder homogenizer (FastPrep-24™ 5G) using 0.75 mL of isopropyl alcohol/ultra-pure water/hydrochloric acid solution (2:1:0.002, v/v/v) as extraction solvent for 40 s. The extract was homogenized again in an ultrasonic bath (Unique, USC-2850 a) for 30 min., and centrifuged at 14.462.g in a microcentrifuge

(Sigma 1-14k) for 10 min. Subsequently, 0.75 mL of n-hexane analytical standard was added in each sample and again taken to the ultrasonic bath for 30 min. The extract supernatant containing n-hexane was collected with the aid of a syringe, filtered through a syringe filter (Chromafil® Xtra PES-45 $\mu\text{m}/25$ mm) and transferred to 2 mL vials (Agilent Technologies).

The analysis of β -carotene content was performed in HPLC (High Performance Liquid Chromatography; Waters, Alliance), equipped with a Diode Array-DAD detector (Waters, 995) and a C18 column (250 x 4.6 mm, 3 μm). The chromatographic separation technique followed the methodology of García-Valverde et al. (2013). Isocratic method was used with 100% methanol in the mobile phase, flow of 1 mL min^{-1} and injection volume of 10 μL . The results were compared with β -carotene standard and expressed in mg β -carotene g^{-1} dry sample. The compound retention time was 23 min., and the wavelength of greatest absorption was 457.2 nm.

Total phenolic compounds were analyzed by the Folin-Ciocalteu spectrophotometric method according to Cheng et al. (2013). Dry samples of tomato pulp and skin were used. The results were expressed in mg EAG g^{-1} dry sample, gallic acid equivalent (GAE), through the calibration curve for gallic acid, at concentrations ranging from 0.071 to 0.689 mg EAG mL^{-1} . Ascorbic acid was determined according to Coelho et al. (2019), with adaptations. A 5 mL extract was prepared containing the tomato sample, to which 50 mL of oxalic acid (2%) was added. Titration was performed with 2,6-dichlorophenolindophenol sodium salt solution (0.01%). The value was calculated and expressed in mg ascorbic acid g^{-1} dry sample.

The antioxidant activity of the tomato pulp and skin was determined using the DPPH, ABTS, and FRAP methods. The determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging was performed according to Ballen et al. (2019), with results expressed in mg TE g^{-1} dry sample, Trolox equivalent (TE), using the calibration curve for Trolox at concentrations ranging from 0.077 to 0.413 mg TE mL^{-1} . The determination of ABTS⁺ [2,2'azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] free radical scavenging was performed according to Silva et al. (2018), with results expressed in mg TE g^{-1} dry sample, using the calibration curve for Trolox, at concentrations ranging from 0.121 to 0.655 mg TE mL^{-1} .

The determination of the reduction of iron ions through the FRAP method was performed according to Silva et al. (2018), with the results expressed in mg FSE g^{-1} of dry sample, ferrous sulfate equivalent (FSE), through the calibration curve for ferrous sulfate at concentrations ranging from 0.051 to 0.773 mg FSE mL^{-1} . Total phenolic compounds, ascorbic acid, and antioxidant activity were analyzed in triplicate.

The β -carotene content data were presented by means of the average values, and it was not possible to present statistical data due to the adopted analytical method. Data on ascorbic acid, antioxidant activity, and total

phenolic compounds were submitted to analysis of variance; and to the parameters, whose F test was significant, Tukey tests ($P < 0.05$) were applied. The statistical software Sisvar was used (FERREIRA, 2011).

RESULTS AND DISCUSSION

Figure 1 shows the results of β -carotene in the pulp and skin of tomatoes treated with heat shock. Between days

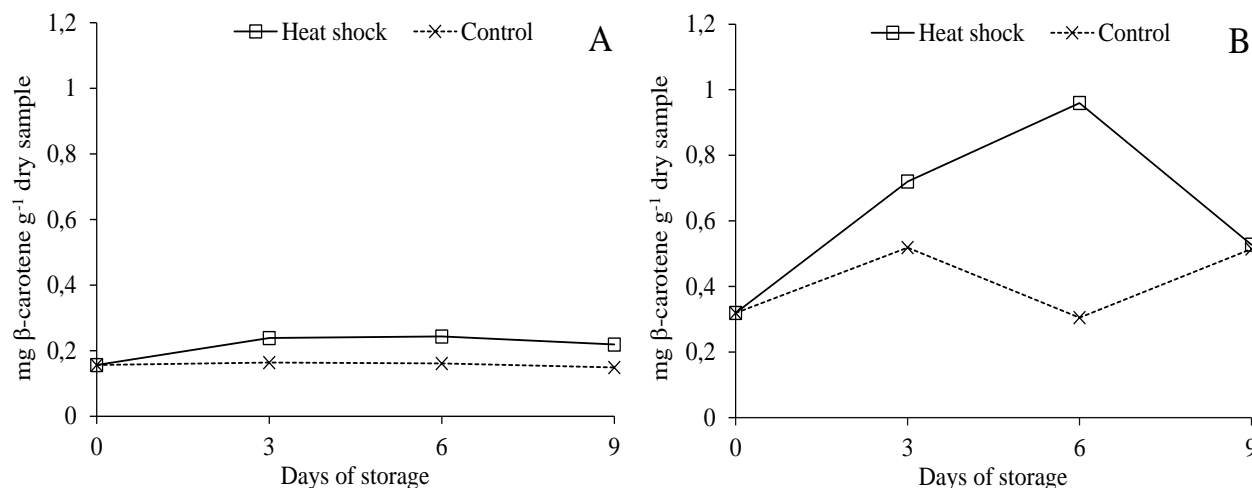


FIGURE 1 - Content of β -carotene in the pulp (A) and skin (B) of "Sweet Grape" tomatoes treated with heat shock and untreated (control) during storage ($25 \pm 4^\circ\text{C}$).

There was an increase in the β -carotene content of the skin of tomatoes treated with heat shock on the third ($0.72 \text{ mg } g^{-1}$) and sixth days ($0.96 \text{ mg } g^{-1}$) of storage (Figure 1B), which were superior to the initial period ($0.32 \text{ mg } g^{-1}$) and to the control in the same periods (0.52 and $0.30 \text{ mg } g^{-1}$, respectively). The higher accumulations of β -carotene in the skin and pulp of tomatoes treated with heat shock, compared to controls, indicate that there was an induction of synthesis mechanisms of this carotenoid. That is, heat shock can improve this nutritional attribute of the tomato. The physiological stress caused by heat from heat shock can induce metabolic pathways of synthesis of antioxidant compounds, such as β -carotene, responsible for cell balance and maintenance (TAIZ et al., 2017; ZHANG et al., 2019). According to Couso et al. (2012), the synthesis of carotenoids is directly related to the greater generation of reactive oxygen species, which, according to Imahori (2014), is a consequence of oxidative stress.

It has also been shown that the content of β -carotene from tomato skin was higher in relation to pulp, both relative to their respective dry matter (Figure 1A), and that the response to its accumulation in the skin, due to heat shock, was greater than in the pulp. As it is a characteristic pigment of the tomato that undergoes great variation in its content, especially during ripening, variations in the levels of carotenoids in tomatoes are more present in the skin due to their high content in relation to pulp (D'AQUINO et al., 2016).

3 and 9 of storage, tomatoes treated with heat shock showed pulp β -carotene content (0.22 to $0.24 \text{ mg } g^{-1}$, respectively) higher than the control (0.15 to $0.16 \text{ mg } g^{-1}$, respectively, for the same period). In addition, the heat shock treatment promoted an increase in β -carotene in the tomatoes during storage, when the content at the initial time ($0.16 \text{ mg } g^{-1}$) was lower than those found in the other periods.

The results of the total phenolic compounds of the tomatoes treated with heat shock are shown in Figure 2. Regardless of the treatment, there was a reduction of total phenolic compounds in the tomato pulp during storage (Figure 2A). However, on the sixth and ninth days of storage, tomatoes treated with heat shock showed increasing (2.18 and $3.17 \text{ mg } g^{-1}$, respectively) and significantly ($P < 0.01$) higher content of total pulp phenolic compounds than controls (1.87 and $1.19 \text{ mg } g^{-1}$, respectively), suggesting that there was an induction of accumulation of total phenolic compounds in the pulp due to the heat shock treatment.

In the tomato skin, there was an increase in total phenolic compounds on the third day of storage in the heat shocked and untreated fruits (Figure 2B), but in the treated fruits the content of total phenolic compounds ($7.40 \text{ mg } g^{-1}$) was significantly lower than in the control ($9.02 \text{ mg } g^{-1}$). On the sixth and ninth days of storage, there was a reduction of total phenolic compounds and there were no significant differences between treated and control fruits, suggesting that the heat shock treatment did not influence the content of total phenolic compounds in the tomato skin.

Phenolic compounds are secondary metabolites with antioxidant properties that accumulate more frequently in the epidermis of fruits due to their protective role against ultraviolet radiation and defense against pathogens (VUOLO et al., 2019) - a fact that explains their higher content in the skin.

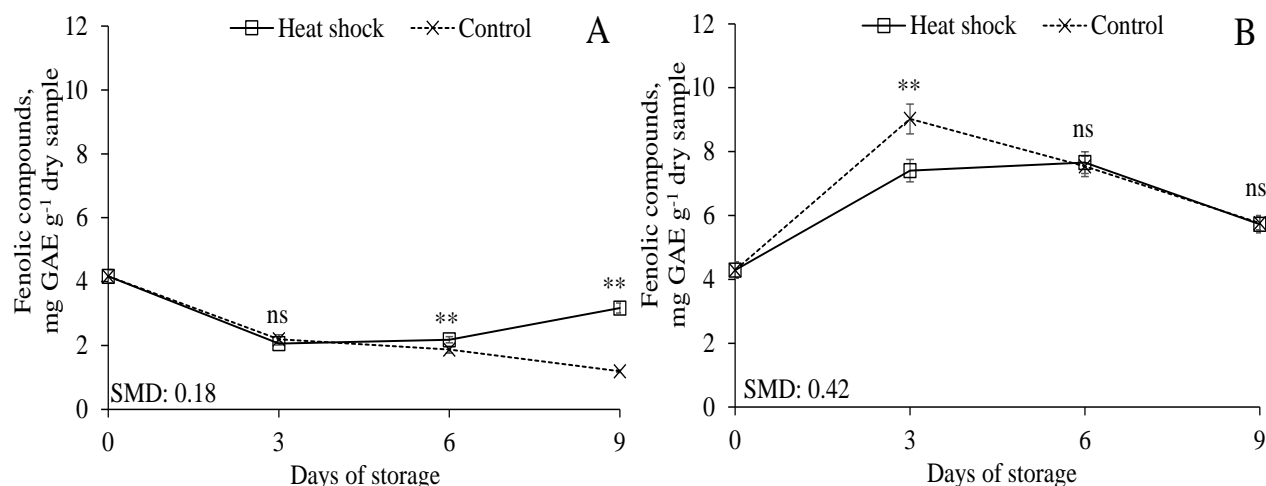


FIGURE 2 - Total phenolic compounds in the pulp (A) and skin (B) of ‘Sweet Grape’ tomatoes treated with heat shock and untreated (control) during storage ($25 \pm 4^\circ\text{C}$). *Significant with a 5% chance of error, **Significant with a 1% chance of error. SMD = significant minimum difference, ns = not significant.

The ascorbic acid content of both heat shock and control tomato groups reduced during storage (Figure 3). Nonetheless, on the third and sixth days of storage, the reduction of ascorbic acid in the treated tomatoes was significantly lower compared to the control, which represents a positive response to the heat shock treatment. In percentage terms, on the third day of storage, the ascorbic

acid content of the treated fruits (6.67 mg g^{-1}) was reduced by 41.4%, while in the control (3.96 mg g^{-1}) this reduction was greater, at 65.2%. In addition, on the sixth day, the ascorbic acid content of the treated tomatoes continued to decrease, but it was still significantly higher compared to the control (5.26 and 4.01 mg g^{-1} , respectively).

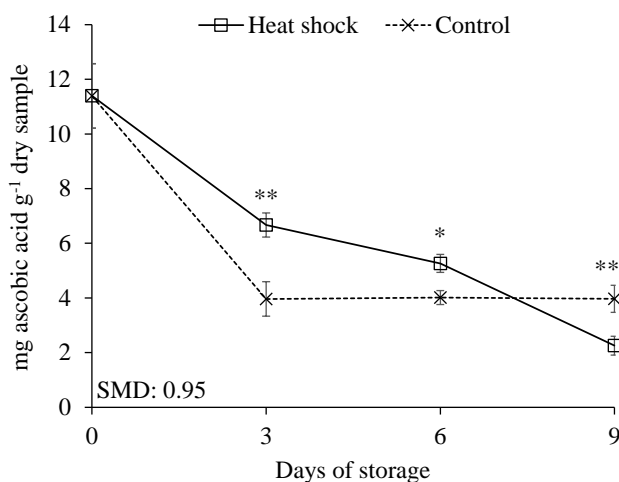


FIGURE 3 - Ascorbic acid content in the pulp of heat shocked and untreated ‘Sweet Grape’ tomatoes (control) during storage ($25 \pm 4^\circ\text{C}$). *Significant with a 5% chance of error, **Significant with a 1% chance of error. SMD = significant minimum difference, ns = not significant.

Despite the high instability of ascorbic acid against oxidative processes and heat (KELEBEK et al., 2017), these results suggest that heat shock influenced higher ascorbic acid content of tomato. However, contrary results were found by Loayza et al. (2020), who observed lower ascorbic acid content in tomatoes heat treated with hot water at 54°C .

Figure 4 shows the results of antioxidant activity of tomatoes treated with heat shock. The DPPH antioxidant activity of tomato pulp decreased during storage, regardless of treatment (Figure 4A), but, especially on the third day,

fruits treated with heat shock showed significantly lower DPPH antioxidant activity (2.16 mg g^{-1}) than control (4.44 mg g^{-1}), and this was not associated with β -carotene results, total phenolic compounds, and ascorbic acid from tomato pulp. On the other hand, the antioxidant activity of the skin of the treated fruits (Figure 4B) increased during storage (third and sixth days), unlike the control that showed DPPH antioxidant activity (6.01 and 5.98 mg g^{-1}) significantly lower than treated tomatoes (13.13 and 11.40 mg g^{-1}) in the same periods.

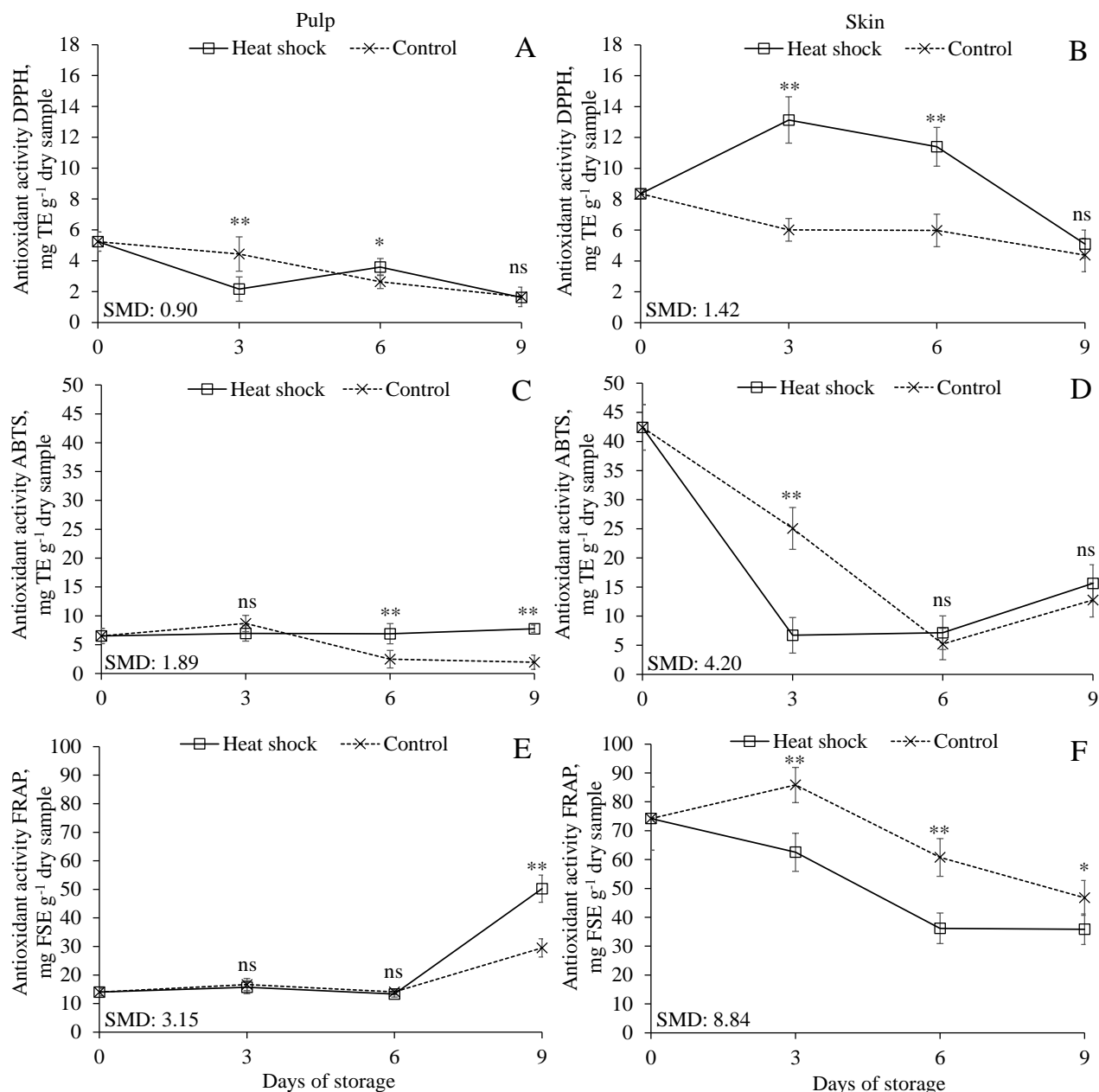


FIGURE 4 - Methods of analysis of DPPH, ABTS, and FRAP antioxidant activity in the pulp (A, C, E) and skin (B, D, F) of heat-shocked and untreated (control) "Sweet Grape" tomatoes during storage ($25 \pm 4^\circ\text{C}$). *Significant with a 5% chance of error, **Significant with a 1% chance of error. SMD = significant minimum difference, ns = not significant.

These results of DPPH antioxidant activity of tomato skins during storage had a positive association with the results of β -carotene and total phenolic compounds. However, the effect of heat shock on the DPPH antioxidant activity of tomato skins, verified in Figure 4B, was associated only with β -carotene, which also increased its content in the skin of tomatoes treated with heat shock, i.e., β -carotene was determinant for DPPH antioxidant activity.

In fact, the heat shock was crucial to improving the antioxidant activity of the tomato skin, but not of its pulp, especially because the pulp concentrates water-soluble antioxidant substances, such as ascorbic acid, which were degraded during storage.

The results of the ABTS antioxidant activity of the pulp and skin of tomatoes are shown in Figure 4C and 4D. Tomatoes treated with heat shock did not reduce ABTS antioxidant activity during storage, with a total average of 7.03 mg g^{-1} (Figure 4C), while in the control there were reductions ($P < 0.01$) of the ABTS antioxidant activity on the sixth and ninth days of storage (2.49 and 1.96 mg g^{-1} , respectively), when compared to pulp from treated tomatoes (6.90 and 7.77 mg g^{-1}).

On the other hand, in the tomato skin (Figure 4D) there was a reduction in the ABTS antioxidant activity, especially on the third day for tomatoes treated with heat shock (6.72 mg g^{-1}), which was significantly lower than the control (25.07 mg g^{-1}). These results indicate that the ABTS

antioxidant activity was not able to express the antioxidant activity of the skin at the level of association corresponding to the increases in β -carotene (Figure 1B) and total phenolic compounds (Figure 2B) found in the skin, which are jointly important antioxidant compounds concentrated in tomato skins.

Concerning FRAP antioxidant activity (Figure 4E and 4F), there was no significant antioxidant variation between the pulp of treated and control tomatoes up to 6 days of storage (Figure 4E), with mean values of 14.37 and 14.93 mg g⁻¹, respectively, showing that there was maintenance of antioxidant activity during this period. In the skin (Figure 4F), the FRAP antioxidant activity of tomatoes treated with heat shock suffered reductions during storage and was lower than the control in all periods.

This suggests that antioxidant compounds in the skin may have been degraded due to heat shock, such as total phenolic compounds. Loayza et al. (2020) working with tomatoes observed different patterns in the values referring to FRAP antioxidant activity among the varieties of tomatoes treated with hot water (54°C). That is, according to these authors, heat treatment triggers different antioxidant responses in different tomato cultivars.

In general, the heat shock treatment promoted an increase in β -carotene in the tomatoes during storage, which in turn was present in higher content in the tomato skin. On the other hand, the content of total phenolic compounds in the pulp of treated tomatoes reduced, while in the skin there was an initial increase, but with lower values than the control on the third day of storage.

Ascorbic acid had its content reduced in tomatoes, regardless of treatment, with significantly lower losses in treated tomatoes, indicating response to treatment. The DPPH antioxidant activity of the tomato pulp was reduced during storage, while the values increased in the skin until the sixth day in treated tomatoes. The ABTS antioxidant activity of the pulp of treated tomatoes did not change significantly during the period, but it was superior to the control, as well as in the skin.

Finally, the FRAP antioxidant activity also did not change in the pulp of the treated and control tomatoes. Conversely, the skin showed a reduction in values, suggesting that certain antioxidant compounds in the skin may have been degraded due to the thermal shock, as pointed out by the values for ABTS and FRAP antioxidant activity.

CONCLUSIONS

Heat shock promoted an increase in β -carotene in tomato skin, which was related to increases in its DPPH antioxidant activity.

The heat shock treatment promoted greater retention of ascorbic acid in the fruit, but it was not able to avoid its reduction during storage.

The tomatoes' antioxidant biochemical response to heat shock has greater expression in their skin.

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