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Pre and post-harvest management affects functional quality of peach (*Prunus persica* L.) cv. Flavorcrest

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Abstract: The effect of rootstock, fertilization and post-harvest heat treatments on the antioxidant capacity and total phenolic content in fruits of peach cultivar Flavorcrest was studied. `Flavorcrest´ grafted on `MrS. 2/5´ and `Flordaguard´ rootstocks produced fruits with the highest antioxidant capacity while the activity in the fruit skin was around ten times higher than in the flesh. Treatment without fertilization produced the highest antioxidant capacity in fruit flesh while the fruit skin showed no significant differences between treatments. A moderate heat shock (34 and 42°C), evaluated at 24 h post-harvest, improved the antioxidant capacity of fruits but after keep them for 72 h at 20°C, the values were similar to those observed in untreated fruit. Pre-harvest (rootstocks and fertilization) and post-harvest (heat shock) treatments influenced the functional quality of 'Flavorcrest' peach cultivar fruits.

1. Introduction

In recent years, consumers have paid increasing attention to the health and nutritional aspects (vitamins contents, mineral elements and antioxidants) of horticultural products (Scalzo *et al.*, 2005). And fruits are generally beneficial to human health, conferring not only nutritive value but also physiological and biochemical benefits. They are also excellent functional foods, contributing to the prevention of degenerative diseases. These beneficial properties have been associated to the presence of bioactive compounds such as phenolics, carotenoids, tocopherols and ascorbic acid (Soobrattee *et al*., 2005).

Fruit phenolic compounds are relevant in terms of quality, as they have a role in visual appearance (pigmentation and browning), taste (astringency), and health-promoting properties (free-radical scavengers) (Tomás-Barberán and Robins, 1997). The flavonoids are a large group of phenolic compounds ubiquitously distributed in the plant kingdom, and they exhibit diverse biological activities (Erlund, 2004; Spencer *et al.*, 2004). Many of these biological functions have been attributed to their radical scavenging and antioxidant activity (Soobrattee *et al*., 2005) because they are highly reactive as hydrogen or electron donors (Amić *et al.*, 2003).

The phenolic content in plants varies among genotypes (Tomás-Barberán *et al.*, 2001), environmental conditions, nutrient availability, agricultural practices, and postharvest conditions (Giorgi *et al*., 2005; Chludil *et al*., 2008). In fruits, the phenolic composition varies greatly among cultivars and, generally, skin fruit tissues contain larger amounts of phenolics, anthocyanins and flavonols than flesh tissue (Wang *et al*., 1996). Due to chemical structure, these compounds are able to react with many active substances in the human body, showing high antioxidant activity (Amić *et al.*, 2003).

Peach is one of the most popular fruits in the world due to its high nutrient level and pleasant flavor. In addition to vitamins and carotenoids (Gil *et al*., 2002) peach contains important phytonutrients such as phenolic acids and flavonoids (Prior and Cao, 2000; Tomás-Barberán *et al.*, 2001; Remorini *et al*., 2008).

It is known that the antioxidant activity of peach fruit is dependent on rootstock/genotype combination, ripening time and post-harvest preservation (Di Vaio *et al*., 2001; Scalzo *et al.*, 2005). Worldwide, peaches are still principally produced by grafting selected varieties onto rootstocks. In addition to conferring resistance to diseases and tolerance to stressed soil conditions, rootstocks differentially influence tree physiology resulting in differences in growth and vigor (Layne, 1994). Moreover, the effects of rootstock type on the mineral composition and sugar and organic acid content of the fruit have been reported (Di Vaio *et al.*, 2001). Nevertheless, present knowledge of rootstock effects on peach fruit quality, and particularly

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on nutritional attributes of the fruit, is generally limited (Giorgi *et al*., 2005).

Stresses such as drought, extreme temperatures, low soil quality, nutrient levels and/or the presence of herbicides and pathogens have direct consequences in the proportion of secondary metabolites produced by plants. Often, plants growing in poor nutrient habitats or under stressful soil conditions contain a greater proportion of secondary metabolites (Tang *et al*., 1995) but there is scarce information about the effects of fertilizers on the production of phenolic compounds by plants.

Peaches ripen and deteriorate quickly at ambient temperature. Cold storage has always been used as the main method to slow these processes as well as the development of decay (Wang *et al*., 2006). On the other hand, heat treatments have been used in postharvest fruit technology for insect disinfestations, decay control, ripening delay and modification of fruit responses to other stresses (Lurie, 1998; Paull and Chen, 2000). High-temperature stress induces biosynthesis of phenolic compounds such as flavonoids and phenylpropanoids (Wahid *et al*., 2007).

'Flavorcrest' is a common yellow-flesh mid season peach variety, widely cultivated in Argentina. The aim of this study was to determine the effect of rootstock, fertilization and post-harvest heat treatments on the antioxidant capacity and total phenolic contents of fruits of this cultivar.

2. Materials and Methods

Chemicals

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu reactive and anhydrous sodium carbonate was obtained from Sigma-Aldrich (Argentina); chlorogenic acid from Fluka (Argentina).

Fruits

Samples (fruit) were obtained from plants grown in controlled experimental plots at the INTA San Pedro Agricultural Experimental Station (San Pedro, Buenos Aires, 33° 44´ 34.7´ S, 59° 47´ 34.4´´ W). The number of replicates is described in each different experiment. After harvest, fruits were immediately transported to the laboratory.

Pre-harvest assays

Cultivar/Rootstock assay. The influence of genotype/rootstock combination was evaluated on fruits of the 'Flavorcrest' cultivar grafted on a) 'Mr. S 2/5' (natural hybrid of *Prunus cerasifera*); b) 'Flordaguard' (a sixth generation descendant from the cross 'Chico 11' x *Prunus davidiana* (Carr.) Franch, C-26712. 'Chico 11' was a seedling of 'Shau Thai', PI 65821) (Sherman *et al*., 1991); and c) 'Cuaresmillo' (a selection of *Prunus persica* (L.) Batsch, from seedlings of a population grown in mountainous regions of western Argentina) (Valentini *et al*., 2003).

A randomized block design was used for the experiment, with five replications per treatment and three trees per replication.

Twenty fruits per cultivar/rootstock combination were harvested at commercial maturity stage. Ten fruits were selected for chemical evaluation.

Fertilization assay. Evaluation of the fertilization effects was carried out on fruit harvested from trees growing on soils belonging to the order of Mollisols, great group *Argiudoles*, sub-group *Vertico* (Ramallo series). Soils of this series are fertile, lightly acidic in the surface, with a good content of organic matter and silty clay loam texture. The transition to the B2t horizon is gradual. The study was carried out on trees planted in June of 2005.

The experiment was comprised of a randomized block design with 12 plants, three plants per block. The assay consisted of four treatments: N, NK, NP, and NPK. Peach trees without fertilizer were used as control (C). Phosphorus, as calcium triple superphosphate (46-48% P_2O_5), 60 g/plant, and potassium, as potassium chloride (60% K_2O), 100 g/plant were applied after planting. Nitrogen, as calcium nitrate (15.5% N), 20 g/plant, was applied after planting in four different moments: November and December 2005, and September and October 2006. The data regarding tree vigor (trunk diameter and cumulative weight of pruned wood) have been published previously by González and Del Pardo (2011).

Post-harvest assay

Heat shock assay. Heat treatments were applied inside an adapted walk-in cooler (Frutitec, Río Negro, Argentina) provided with refrigeration, heating and humidification systems. The fruit was heated to 20, 34, and 42°C (±1°C), 90% RH, and kept at these conditions for 24 h. Another batch of fruit was cooled to 0°C±0.5°C and kept in cold storage for 24 h. A pool of fruit without treatment was used as control (C). The fruit was evaluated after 24 h and then kept at 20°C for 72 h.

Fruit quality parameters

Flesh firmness (FF) was measured on two opposite sides in the equatorial zone of individual fruits with an Effegi 327 Fruit Pressure Tester (Milano, Italy) and expressed as kg/cm^2. Total soluble solids (TSS) were determined in juice from the longitudinal side opposite the suture, with an N1 Atago hand refractometer (Osaka, Japan) and reported as °Brix. Color determination was performed with a Minolta Chroma Meter CR-300 (Osaka, Japan). Results were expressed as L^*, C^* $[(a^2 + b^2)^{1/2}]$ and h^o $(\tan^{-1} b/a)$ color units calculated from a^* (green chromaticity) and b^* (yellow chromaticity).

Sample extractions

Extractions were carried out using 3 g of fresh fruit skin or flesh homogenized in 15 ml of 7% acetic acid in methanol. Tubes were stored for 24 h at 4°C. They were then centrifuged (10 min at 2000 g), filtered and stored at 4°C in darkness until use. Ten fruits per treatment were processed for chemical analyses.

Fruit functional quality

Assay of DPPH radical scavenging activity. The DPPH method was adapted from Brand-Williams *et al*. (1995). A total of 20 µl of peel extract or 200 µl of flesh extract were diluted to 1 ml with methanol. The diluted sample reacted with 2 ml of $DPPH^{\bullet+}$ (150 µM in methanol) at 30°C. Decrease in absorbance was measured at 517 nm after 30 min. Results were expressed as µmols of ascorbic acid equivalents/g of fresh weight (µmol AEAC/g FW).

Total phenolic content. Total phenolic content (TPC) was determined by the Folin-Ciocalteu method (Swain and Hillis, 1959) using chlorogenic acid as standard for the calibration curve. Results were expressed as µmols of chlorogenic acid equivalent/g of fresh weight (CAE/g FW). The sample (20 µl of peel extract or 200 µl of flesh extract) or standard (0, 50, 100, 200, 300, 400, 500 µl of 0.4 g/l chlorogenic acid) were diluted with water to a final volume of 4.45 ml. 50 µl of Folin-Ciocalteu reagent (2 N) were added. After 3 min sodium carbonate (0.1 N) was added. Results were read at 725 nm after 1 h.

Statistical analyses

Data were submitted to analysis of variance and Duncan tests were conducted to identify differences among means. Pearson Correlation test was used to determine the correlations among means. Statistical significance was declared at $p<0.05$.

3. Results and Discussion

Fruit quality parameters

Cultivar/Rootstock assay. Rootstock influence was found to not be significant for firmness (6.8-7.5 kg/cm^2) and soluble solid content (10.9-11.3°Brix). While fruit skin lightness (L*) and hue angle (h^o) values were significantly higher in 'Flavorcrest' fruits grafted on 'Mr.S 2/5' and 'Flordaguard' rootstock, while chroma values (C*) were not significantly affected by rootstock (Table 1).

Fertilization assay. Fruit from NP and NPK treatments presented the greatest firmness with no significant differences in comparison to control. Although fertilizer affected soluble solid content, it did not have pronounced effects. Fruit skin color characteristics, lightness (L*), hue angle (h^o) and chroma (C*) values showed no significant differences with fertilizer treatments (Table 1).

Heat shock assay. In general, fruit firmness decreased after storage at 20°C for 72 h. Flesh lightness (L*) and hue angle (h^o) were not modified by treatments. Chroma (C*) values decreased with 34 °C treatments (24 h) with respect to control (Table 1).

Table 1 - Firmness (FF), total soluble solids (TSS) and color (LCH system) of 'Flavorcrest' peach fruit grafted on 'Mr.S 2/5', 'Flordaguard' and 'Cuaresmillo' rootstocks included in fertilization and heat shock assays

Treatment	FF	TSS	L	C	H
				Peel color characteristics	
Rootstock					
Mr. S 2/5	6.81 a	11.31 a	69.03 a	46.64 a	85.63 a
Flordaguard	7.41 a	11.20 a	66.86 a	45.85 a	79.44 a
Cuaresmillo	7.21 a	10.88 a	62.34 b	44.86 a	71.18 b
Fertilization					
C	5.95 a	12.16 a	55.67 a	43.55 a	61.48 a
N	4.77 b	11.13 b	59.38 a	45.44 a	63.95 a
NP	5.77 a	11.96 ab	57.81 a	45.58 a	61.14 a
NK	3.93 c	11.40 ab	56.84 a	45.17 a	59.95 a
NPK	5.16 ab	11.97 b	55.15 a	45.23 a	58.04 a
Heat Shock				*Flesh color characteristics*	
Control	7.87 a	11.02 ab	73.94 a	49.64 a	99.80 a
0°C	7.84 a	10.96 ab	74.16 a	46.88 ab	98.92 a
20°C	7.42 a	10.70 b	73.63 a	46.35 ab	98.43 a
34°C	7.70 a	11.20 ab	74.57 a	44.13 b	99.49 a
42°C	8.12 a	12.45 a	75.15 a	45.94 ab	97.55 a
Control + 3D	5.92 a	11.44 a	73.83 a	48.72 a	96.49 a
0°C + 3D	5.88 a	11.20 a	71.44 a	46.67 a	96.87 a
20°C + 3D	2.79 b	11.28 a	72.99 a	46.56 a	95.99 a
34°C + 3D	4.47 ab	11.58 a	72.33 a	45.25 a	94.86 a
42°C + 3D	2.01 b	12.05 a	70.96 a	47.45 a	92.72 a

Values are the mean of 30 replications. Means followed by the same letters are not significantly different (p= 0.05).

Fruit functional quality

Cultivar/Rootstock assay. Total antioxidant capacity was determined in fruit flesh and skin. The fruit flesh of 'Flavorcrest' grafted on 'Flordaguard' and 'Mr. S 2/5', both middle vigor rootstocks, presented the highest AEAC/g FW values, with 1.77 and 1.65 µmols of ascorbic acid equivalents/g of fresh weight, respectively (Fig. 1). Previous studies have shown that total antioxidant capacity changes as a function of the rootstock. Remorini *et al*. (2008) demonstrated that 'Mr.S 2/5' produced fruits with the highest total antioxidant capacity, attributing this to low-vigor properties. Despite these results, they did not find a link between rootstock vigor and total antioxidant capacity. On the other hand, Scalzo *et al*. (2005) observed higher antioxidant capacity values with vigorous rootstock. Light has been reported to be one of the major environmental factors that affect phenolic production (Par and Bolwell, 2000). Fruits of dense foliage trees receive less light and this could affect the phenolic content. Phenolics are the major antioxidant compounds in peach fruits (Tomás-Barberan *et al*., 2001).

Fruit skin total antioxidant capacity was approximately five times higher (8.8-10.6 µmols AEAC/g FW) than that of flesh and showed no significant differences between rootstocks (Fig. 1).

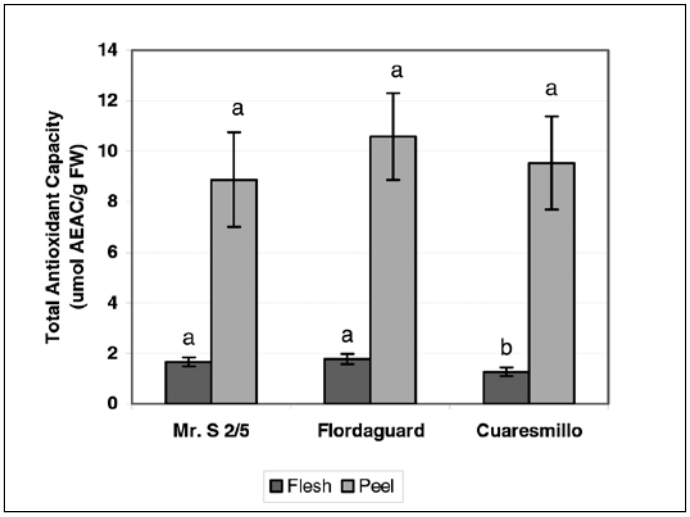

Fig. 1 - Total antioxidant capacity determined by DPPH assay in flesh and peel of fruits of Flavorcrest cultivar grafted on 'Mr.S 2/5', 'Flordaguard' and 'Cuaresmillo' rootstocks. Values are means (± S.E.) of 10 replicates. Inside each group (flesh or peel), means followed by the same letters are not significantly different (p= 0.05).

The effect of rootstock on flesh total phenolic content was significantly different. 'Flavorcrest' grafted on 'Flordaguard' (1.14 µmol CAE/g FW) and 'Mr.S 2/5' (1.02 CAE/g FW) showed the highest values. Fruit skin TPC was higher (ten times) than that of flesh and no differences were observed between rootstocks. Other authors also found a higher phenolic content in fruit skin compared to flesh (Tomás-Barberán *et al*., 2001; Remorini *et al*., 2008), reporting values two to four times higher.

Total antioxidant capacity and total phenolic content were positively correlated in flesh (r= 0.8052) and peel (r= 0.8190), which suggests that phenolic compounds greatly contribute to the total antioxidant capacity (Fig. 2).

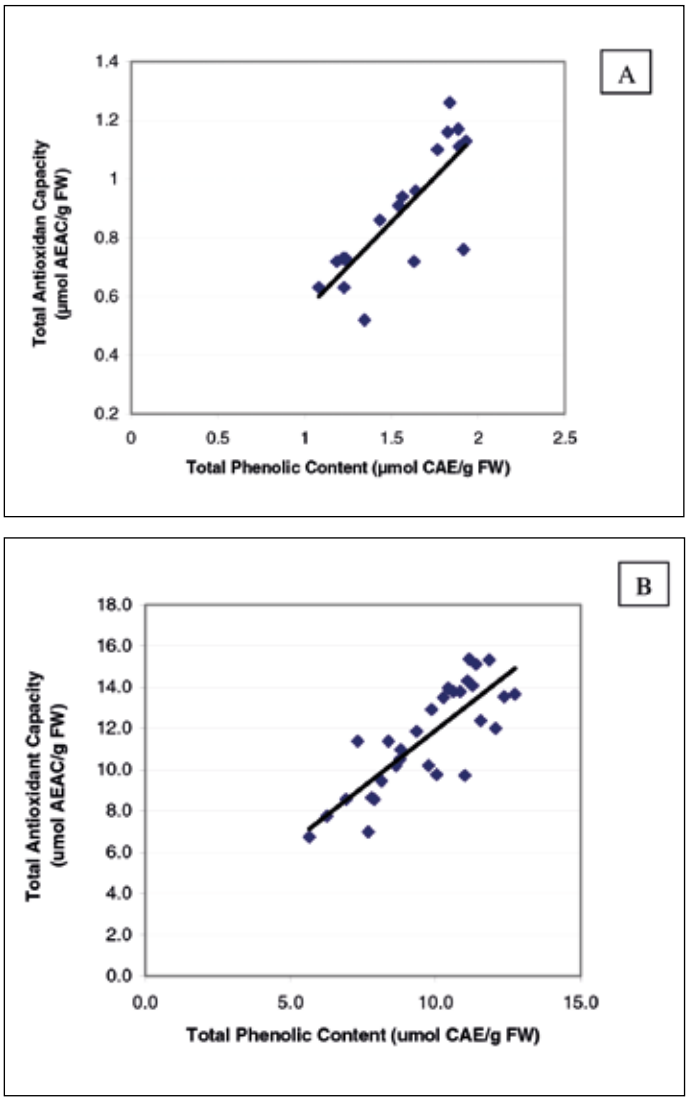

Fig. 2 - Correlation between total phenolic content (µmol CA/g FW) and total antioxidant capacity (µmol AEAC/g FW) of flesh A) and B) fruit skin of Flavorcrest cultivar, grafted on 'Mr.S 2/5', 'Flordaguard' and, 'Cuaresmillo' rootstocks.

Fertilization

Flesh from control fruits (without fertilization) had the highest antioxidant capacity (2.2 µmols AEAC/g FW), whereas the antioxidant capacity decreased (with respect to control) when N (1.55 µmols AEAC/g FW), NP (1.75 µmols AEAC/g FW) and NPK (1.79 µmols AEAC/g FW) treatments were applied, and even more so with NK (1.32 µmols AEAC/g FW). Fruit skin showed no significant differences (Fig. 3).

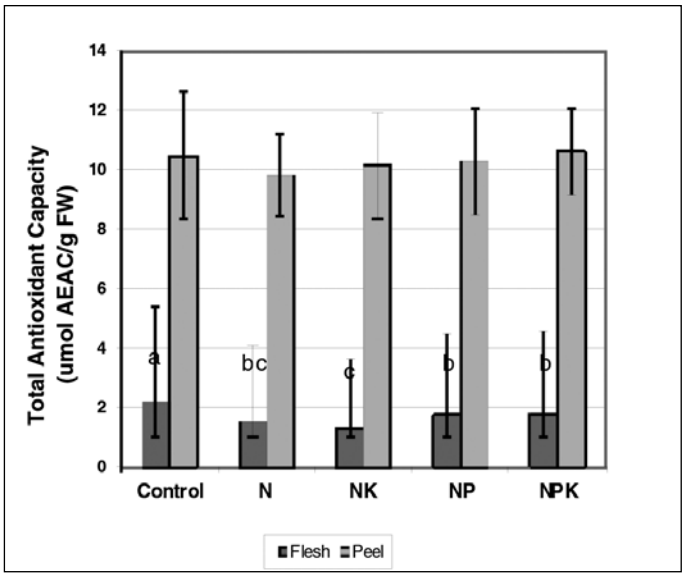

Fig. 3 - Total antioxidant capacity determined by DPPH assay in flesh and fruit skin of Flavorcrest cultivar under fertilizer N, NK, NP and NPK. Values are means (±S.E.) of 10 replicates. In bars corresponding to flesh values, means followed by the same letters are not significantly different (p= 0.05).

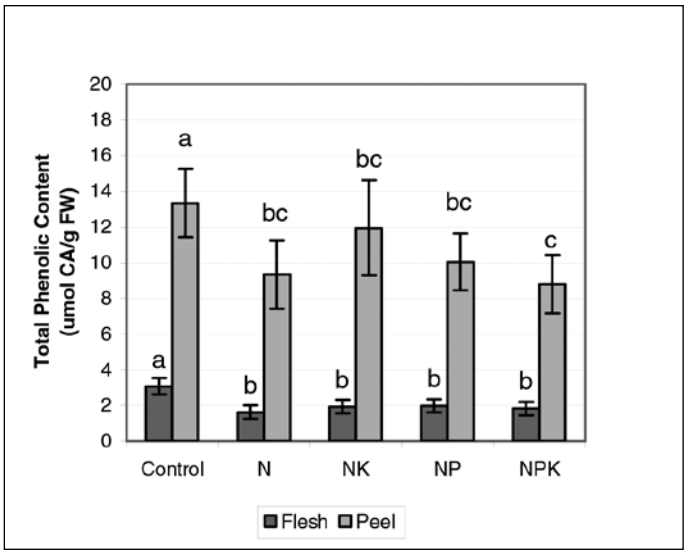

Fig. 4 - Total phenolic content determined by Folin-Ciocalteau assay in flesh and fruit skin of Flavorcrest cultivar under fertilizer N, NK, NP and NPK. Values are means (±S.E.) of 10 replicates.

According to the 'C/N balance theory', when N is readily available, plants will primarily synthesize compounds with high N content (e.g. protein to growth). Instead when N availability is limited, metabolism changes towards carbon-containing compounds such as starch, cellulose, and non N-containing secondary metabolites such as phenolics and terpenoides (Haukioja *et al*., 1998). In plants, it has been shown that competition between protein and phenolic synthesis exists for the common precursor L-phenylalanine (Riipi *et al*., 2002). The relative differences in the release of nutrients from various fertilizers could lead to different C/N ratios in plants and this in turn leads to a difference in the production of secondary metabolites (Brandt and Molgaard, 2001).

Fertilization treatments were found to not significantly affect the vegetative variables (trunk cross sectional area and pruned wood) (González and Del Pardo, 2011). Environmental stresses including nutrient deficiency are known to activate the biosynthesis of phenylpropanoid compounds (Dixon and Paiva, 1995), which could explain why the highest antioxidant activity was found without fertilizer treatment.

The highest total phenolic content in flesh (3.37 µmol CAE/g FW) and fruit skin (13.34 µmol CAE/g FW) was obtained in plants without fertilization (C), which differed significantly from the rest of the treatments (Fig. 4). Flesh total phenolic content showed a low correlation with antioxidant capacity (r= 0.48).

Heat shock treatments

In this assay, the effect of post-harvest temperature on the functional quality of fruit flesh was evaluated. Total antioxidant capacity was significantly different between fruit flesh evaluated at 24 h and fruit held at 20°C for 72 h. The moderate heat shock treatments (34°C and 42°C) at 24 h improved the antioxidant capacity (0.76 µmols and 0.84 µmols AEAC/g FW, respectively) in comparison to control (0.48 µmols AEAC/g FW), 0°C (0.49 µmols AEAC/g FW) and 20°C (0.52 µmols AEAC/g FW). AEAC (µmol/g FW) showed no significant differences between treatments after keeping fruits for 72 h at 20°C. Comparing fruits evaluated at 24 and 72 h, the total antioxidant capacity was significantly increased in control, 0°C and 20°C treatments and significantly decreased in 34°C and 42°C treatments (Fig. 5).

Total phenolic content in the flesh, evaluated 24 h after treatment applications, was significantly higher at 34°C (0.41 CAE/g FW) and 42°C (0.49 CAE/g FW) than control

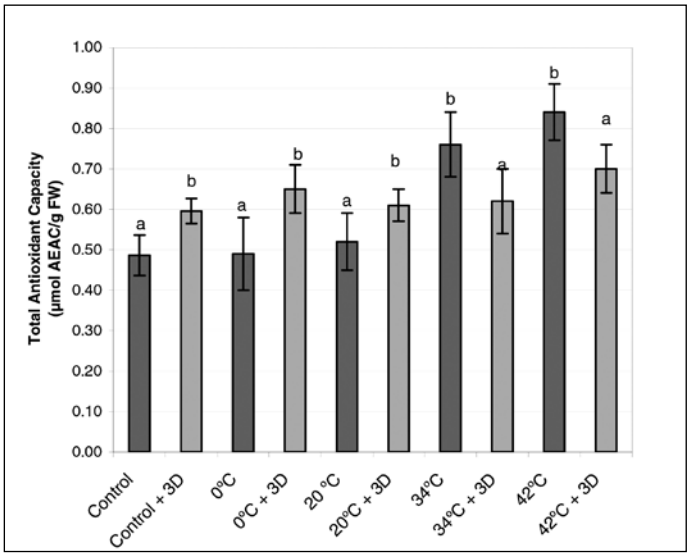

Fig. 5 Total antioxidant capacity determined by DPPH assay in flesh of Flavorcrest cultivar submitted to different temperature treatments: 0, 20, 34, and 42°C. Fruit was evaluated at 24 h (dark grey bars) and after 72 h (light grey bars) from treatment application. Each bar indicates the mean (±S.E.) of 5 replications.

(0.30 CAE/g FW), 0°C (0.30 CAE/g FW) and 20°C (0.34 CAE/g FW), following the same behavior as antioxidant capacity. After 72 h at 20°C, all treatments differenced of control. When treatments for the two evaluation periods (24 and 72 h) were compared, the only heat treatment that showed a significant difference was 42°C (Fig. 6). There was a positive correlation between total antioxidant capacity and total phenolic content (r=0.67) (Fig. 7).

Heat treatment affects several aspects of fruit ripening such as ethylene production and cell wall degradation (Lurie, 1998). Thermal stress enhances activities of oxidative stress enzymes and induces the accumulation of phenolic compounds like flavonoids and phenylpropanoids (Wahid *et al*., 2007). A previous study on peach cultivars showed that heat treatments promoted the development of red color in the fruit flesh (Budde *et al*., 2002) which could link these phenomena to an increased synthesis of phenolic compounds.

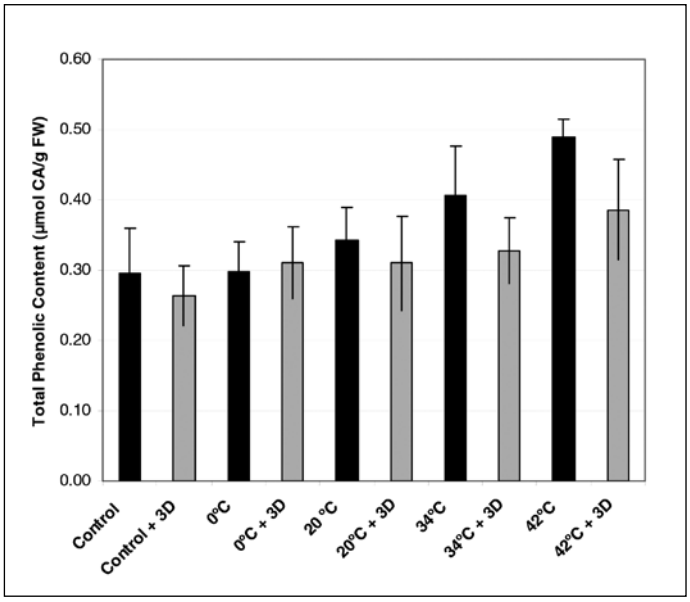

Fig. 6 - Total phenolic content determined by Folin-Ciocalteau assay in flesh of Flavorcrest cultivar subjected to different temperature treatments: 0, 20, 34, and 42°C (± 1°C). Fruit was evaluated at 24 h (Black bars) and after 72 h (gray bars) from treatment application. Each bar indicates the mean (± S.E.) of 5 replications.

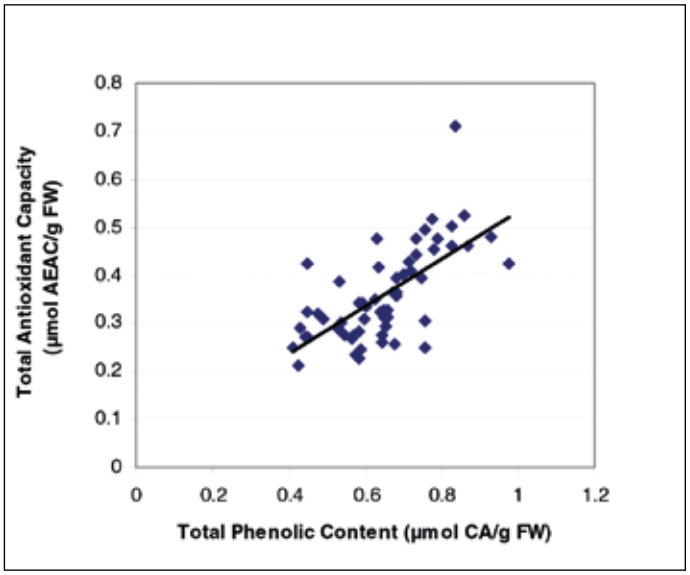

Fig. 7 - Correlation between total phenolic content (µmol CA/g FW) and total antioxidant capacity (µmol AEAC/g FW) of fruit flesh in the heat treatment assay.

4. Conclusions

The results of this study show that pre-harvest (rootstocks and fertilization) and post-harvest (heat shock) treatments influence the functional quality of 'Flavorcrest' peach fruits. 'MrS. 2/5' and 'Flordaguard' rootstocks produced fruits with the highest antioxidant capacity and phenolic content, whereas 'Cuaresmillo', the most commonly used peach rootstock in our peach growing area, showed the lowest. Although these results could be attributed to vigor it is not possible to determine a general behavior; assays with other rootstocks could be useful.

It has been reported that soluble phenolics are the principal contributors to the total antioxidant capacity. The accelerated plant growth induced by fertilization may cause a reduction in concentrations of phenylpropanoids (Haukioja *et al*., 1998), resulting in the lowest antioxidant capacity observed in fertilized treatments.

Heat stress causes accumulation of secondary metabolites of a multifarious nature in plants (Wahid *et al.*, 2007). While higher antioxidant capacity was observed in heat-treated fruit at 24 h, the total antioxidant capacity values were similar to those observed in non heat-treated fruit after they were held for 72 h at 20°C.

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