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Antioxidant capacity and total phenolic content of hydrothermally-treated 'Fuerte' avocado

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Abstract: Avocados possess high nutritional value with proven effectiveness in preventing cardiovascular diseases, attributed primarily to their unsaturated fatty acids content. This fruit is also rich in carotenoids and vitamins, particularly vitamin E. This work evaluates the antioxidant capacity and total phenolic content of hydrothermally-treated Fuerte avocado. Fruits were selected and hydrothermally treated at 45ºC for 5, 10, 15 and 20 min. They were then stored in a refrigerator (10 ± 1ºC and 90±5% relative humidity) and evaluated over a 15-day period. The total phenolic content increased up to the sixth day of storage, and decreased thereafter, without differences between the treatments. The percentage of antioxidant capacity of the control and the hydrothermally-treated samples for 5 and 10 min increased during storage. Untreated fruits showed the highest percentage of antioxidant capacity. However, the antioxidant capacity of avocado fruits subjected to these treatments declined starting on the twelfth day of storage, possibly due to the fruits' senescence. Hydrothermal treatments for 15 and 20 min delayed fruit senescence while the antioxidant capacity continued to increase up to the fifteenth day of storage. No significant correlation was found between antioxidant capacity and total phenolic content. The antioxidant capacity of ripe Fuerte avocado was higher than that of unripe or overripe avocado.

1. Introduction

Avocado (*Persea americana* Mill.) has considerable nutritional quality, with a high content of fibers, proteins and mineral salts, particularly potassium and vitamins, especially vitamin E (USDA, 2007). It also contains significant amounts of unsaturated fatty acids, which are beneficial for the prevention of cardiovascular diseases (Tango *et al*., 2004). Previous studies have also shown that this fruit contains anticarcinogenic lipophilic compounds such as carotenoids (Ding *et al*., 2007).

Wang *et al*. (2010) pointed out the scantiness of studies on the phytochemical composition of avocados and the lack of knowledge about the total phenolic content and antioxidant capacity of different avocado varieties, or cultivars. The aforementioned authors conducted studies to determine the antioxidant capacity of the pulp, seed and peel of different avocado varieties, but not the Fuerte variety. 'Fuerte' avocados are small and are highly valued in European and American markets.

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Antioxidants, which are compounds that inhibit and/or reduce the effects of free radicals (Soares *et al*., 2005), can be defined as compounds that protect the cells against the harmful effects of oxygen and nitrogen free radicals that are formed in oxidative processes. High free radical levels generate an imbalance, triggering oxidative stress, the metabolic process responsible for the onset of several types of chronic degenerative diseases. Antioxidants can be obtained by eating food containing vitamins E and C, carotenoids, phenolic compounds, and other compounds (Ali *et al*., 2008).

Phenolic compounds are responsible for most of the antioxidant activity in fruits, making them a natural source of antioxidants (Heim *et al*., 2002). The phenolic content in food and plants depends on a number of intrinsic factors such as the genus, species and cultivar, and on extrinsic factors such as agronomic and environmental factors, handling and storage (Thomas-Barberán and Espín, 2001).

Avocado is a climacteric fruit which ripens a few days after harvest (Hardenburg *et al*., 1986; Seymour and Tucker, 1993) and whose postharvest behavior can be influenced by temperature and storage time. The literature contains several studies about the increase in

the conservation period of avocado, involving the evaluation of storage temperature, the use of modified atmosphere with the application of wax, gamma irradiation and thermal treatment to prevent chilling injury (Zauberman *et al*., 1973; Castro and Bleinroth, 1982; Seymour and Tucker, 1993; Germano *et al*., 1996; De Oliveira *et al*., 2000; Sanches, 2006; Morgado, 2007; Donadon, 2009).

Thermal treatment has been applied postharvest to solve the problem of contamination by fungal diseases and insect infestation in fruit (Fawcett, 1922 *apud* Couey, 1989) or to reduce problems caused by low storage temperatures (Kluge *et al*., 2006). To this end, thermal treatments are performed prior to refrigeration, in the form of conditioning, or during refrigerated storage, in the form of intermittent warming. Thermal conditioning consists of exposing fruits briefly to moderate (15 to 25ºC) or high temperatures (37 to 53ºC) before putting them in refrigerated storage (Kluge *et al*., 2006).

Daiuto and Vieites (2008) conducted a study on Hass avocado to evaluate the polyphenol oxidase (PPO) and peroxidase (POD) content in unripe and ripe fruits hydrothermally treated at 45ºC for 10 min and stored at 9ºC (±1). The enzyme inactivation in ripe fruits subjected to the treatment was 78 to 94% compared to untreated fruits. Daiuto *et al*. (2010) evaluated the weight loss and respiratory rate of 'Hass' avocado by subjecting it to different physical treatments (thermal, UV and gamma radiation) and reported a decrease in the intensity of the fruit's respiratory peaks.

The evaluation of antioxidant capacity has become increasingly important to determine the effectiveness of natural antioxidants in protecting vegetable products against oxidative damage and loss of their commercial and nutritional value. Therefore, the present research focused on an evaluation of the antioxidant capacity and total phenolic content of 'Fuerte' avocado subjected to hydrothermal treatment.

2. Materials and Methods

'Fuerte' avocados were harvested carefully at the point of physiological maturation and according to their oil content. The fruits, which were selected with a view to uniform size, color and absence of injuries and defects, were hydrothermally treated in a water bath at 45°C for 5, 10, 15 and 20 min (four treatments), after which they were stored under refrigeration (10±1°C and 90±5% relative humidity). Fruits not subjected to the hydrothermal treatment were used as control. The fruits of these five treatments were evaluated at three-day intervals for two weeks.

Fruit extraction

The extraction process was performed with a solvent mixture of ethanol:water (80:20 v/v). Fruit extracts were obtained in triplicate. Aliquots of 3.0 g of pulp were weighed and placed in Falcon tubes, to which were added 30 ml of an ethanol:water mixture (80:20 v/v). The tubes containing pulp and solvent were then processed at room temperature in a Turrax crushing disperser for several minutes, and then centrifuged at 5000 X G for 15 min. The extracts were filtered and stored in dark vials at 8ºC for no longer than a week prior to analysis.

Total polyphenol analysis

The total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method, as described by Singleton *et al*. (1999), using gallic acid as standard. An aliquot of 0.5 ml of the resulting extracts was then transferred to a test tube and 2.5 ml Folin-Ciocalteu reagent diluted in water 1:10 was added. The mixture was allowed to rest for 5 min, after which 2 ml of sodium carbonate 4% was added and the tubes were left to stand for 2 hr in the dark. The absorbance was measured in a spectrophotometer operating at a wavelength of 740 nm. A blank sample was subjected to the same procedure and conditions. The results are expressed in μg GAE/100 g^{-1} of dry weight.

DPPH radical scavenging activity

The radical scavenging activity was determined by DPPH method (Mensor *et al*., 2001). Tocopherol and BHT at a concentration of 90 μg ml^{-1} were used as standards. The reaction mixture consisted of 500 μl of fruit extract, 3.0 ml of ethanol 99%, and 300 μl of the DPPH radical in a solution of ethanol 0.5 mM, which was incubated for 45 min at room temperature in the dark. The negative control was prepared by replacing the volume of extract for an equal volume of the extraction solvent. A processing time of 45 min was defined after determining the half maximal effective concentration, Ec50. To determine the stabilization time, readings of the antioxidant in five concentrations (1, 2, 3, 4 and 5 g) were taken at 15-min intervals (Sanches-Moreno *et al*., 1998). According to Do Rufino *et al*. (2007), in subsequent experiments with the same fruit, readings can be limited to the previously established time (Ec50 time), accompanied by the initial reading of the control. The blank was prepared by substituting the volume of the DPPH solution for an equal volume of solvent.

The free radical scavenging activity was determined in the form of Antioxidant Activity (AA), using the equation: AA (%) = 100- [(Aa – Ab) x 100] / Ac, where: Aa = absorbance of the sample; Ab = absorbance of the blank; and Ac = absorbance of the negative control. All the analyses were performed in triplicate and accompanied by a control.

A variance analysis was performed using Tukey's test for multiple comparisons of the averages, at a significance level of 5%. The data were then subjected to a regression analysis and to Pearson's correlation for the two parameters evaluated, using the SAS version

9.2 software program.

3. Results and Discussion

Table 1 presents the average and standard deviation of total polyphenols identified in 'Fuerte' avocado. Although the four treatments produced similar results, a difference was detected as a function of storage time (p=0.007).

Total polyphenol content was higher in the control treatment. Mean values of 45.7, 47.0, 47.1 and 47.2 μg GAE/100 g^{-1}, respectively, were obtained in 5, 10, 15 and 20 min hydrothermal treatment, while the control showed 49.8 GAE/100g^{-1}. The lowest value obtained was 42.7 μg on the first day of analysis and the highest was 62.1 μg for the control treatment on the sixth day of analysis. The composition of phenolic compounds in fruit may be modified as a function of the environment and postharvest factors, including processing and storage. Processing and storage can induce prolonged enzymatic and chemical oxidation of phenolic compounds, contributing to their reduction (Kaur and Kapoor, 2002). Many studies have shown that phenolic compounds generally decrease in climacteric fruit such as tomatoes, bananas, mangos and guavas during ripening (Haard and Chism, 1996; Lakshimnarayana *et al*., 1970; Mitra and Baldwin, 1997; Selvaraj and Kumar, 1989).

The total phenolic content increased up to the sixth day of storage, decreasing thereafter due to the onset of senescence (Fig. 1). Daiuto *et al*. (2010) found that the average respiratory peak of 'Hass' avocado subjected to different physical treatments occurred on the ninth day of storage, after which senescence set in. This decrease can be attributed to a series of chemical and enzyme amendments that occur during the accelerated process of maturation of this fruit. These changes may include glycoside hydrolysis by glycosidases, phenol oxidation by phenoloxidases, and polymerization of free phenolic content (Robards *et al*., 1999).

The average antioxidant capacity measured in the treatments varied from 21.1% (20 min treatment) to 28.5% (control). The lowest value obtained was 17.6% on the first day of analysis and the highest was 67.6% on the twelfth day of analysis in the control treatment (Table 2). The overall average, taking into account the days of storage time, indicated an increase in antioxidant capacity.

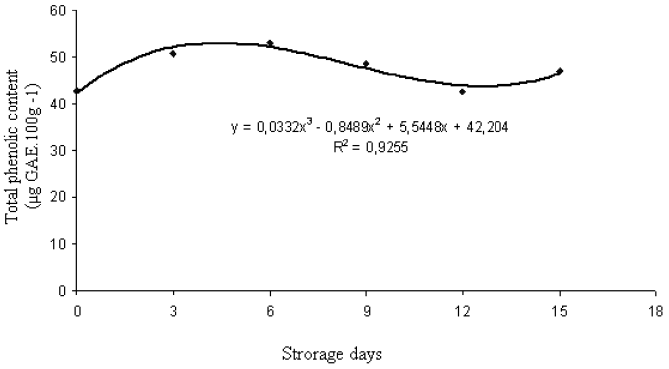


Fig. 1 - Total phenolic content (μg GAE/100g^{-1}) of hydrothermally-treated 'Fuerte' avocado (overall average of storage days).

Table 1 - Average and standard deviation of the total polyphenol content in hydrothermally-treated 'Fuerte' avocado as a function of treatment and storage day

	Storage days						
Treatments	0	3	6	9	12	15	Overall average per treatment
Control	42.7±2.7	41.8±2.7	62.1±20.5	54.2±1.3	36.5±9.3	61.7±3.5	49.85±13.2
5 min	42.7±2.7	51.8±12.8	42.3±13.5	52.9±15.4	42.7±3.7	42.2±6.0	45.75±9.9
10 min	42.7±2.7	58.5±2.7	48.0±4.6	48.8±6.5	44.1±7.5	39.8±15.1	47.05±9.5
15 min	42.7±2.7	51.6±5.1	58.4±9.1	41.4±15.8	42.8±4.5	45.6±6.0	47.15±9.4
20 min	42.7±2.7	49.3±1.7	54.6±10.1	45.3±6.8	45.6±2.9	45.4±7.9	47.25±6.5
Overall average per storage day	42.7B±2.7	50.6AB±8.8	53.1A±13.0	48.5AB±10.3	42.4B±6.1	46.9AB±10.8	

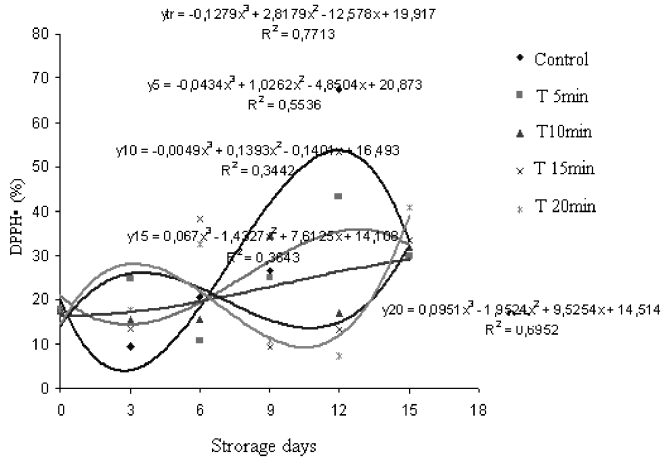
Upper case letters compare overall averages on each storage day

Table 2 - Average and standard deviation of antioxidant capacity of hydrothermally-treated 'Fuerte' avocado as a function of hydrothermal treatment and storage day

	Storage days						
Treatments	0	3	6	9	12	15	Overall average per treatment
Control	17.6aB±1.1	9.3aB±2.0	20.6abB±7.2	26.6aB±6.8	67.6aA±1.4	29.4aAB±3.6	28.5±19.5
5 min	17.6aB±1.1	24.7aAB±1.0	10.8bB±6.4	25.1aAB±9.7	43.2aA±33.6	29.6aAB±12.6	25.1±16.6
10 min	17.6aB±1.1	15.4abA±4.0	15.7abA±7.4	34.3aA±16.9	16.9bA±0.7	31.7aA±12.2	21.9±11.2
15 min	17.6aB±1.1	13.2aAB±4.3	38.4aA±5.4	9.3aB±4.5	13.2bAB±0.7	33.4aAB±8.4	20.9±13.4
20 min	17.6aB±1.1	17.7aAB±2.5	32.6AB±13.3	10.81B±3.8	7.2bB±4.8	40.9aA±34.6	21.1±17.8
Overall average per storage day	17.6±1.1	16.1±5.9	23.6±12.9	21.2±12.9	29.6±17.5	33.0±15.7	

Lower case letters compare averages per treatment per day.
Upper case letters compare averages of each treatment on each storage day.

Considering all the treatments, the highest antioxidant capacity was found in the control treatment, followed by the 5-min thermal treatment. Figure 2 shows increasing values of antioxidant capacity over storage time in the control and the 5- and 10-min hydrothermal treatments.



Legend: T = thermal treatment

Fig. 2 - DPPH antioxidant activity of hydrothermally-treated Fuerte avocado.

The highest percentages of antioxidant capacity were obtained in the fruit without hydrothermal treatment. The percentage of antioxidant capacity declined in thermally-treated fruits starting on the twelfth day of storage, possibly due to senescence.

The fruits thermally treated for 15 and 20 min showed values of 33.4 and 40.9%, respectively, after 15 days of storage. However, the values declined between the sixth and twelfth day of storage. This tendency for the percentage of antioxidant capacity to decrease may be a result of the thermal treatment, but the profile presented here with low values at nine and 12 days may be a consequence of the heterogeneity of fruit samples. This may explain the results of this research, which indicated that the antioxidant capacity of pulp thermally treated for 15 and 20 min increased up to the fifteenth day of storage. With a less intense respiratory peak, the degradation reactions were also diminished.

Arancibia-Avila *et al*. (2008) reported that total polyphenols, flavonoids and anthocyanins were significantly higher ($p < 0.05$) in ripe durian fruit than in unripe or overripe fruit (*Durio zibethinus* Murr., cv. Mon Thong).

The overall average antioxidant capacity during the storage period was 17.6, 16.1, 23.6, 21.2, 29.6, and 33.0% for the different treatments, indicating the tendency for antioxidant capacity to increase as the fruits ripened. The total phenolic content is not necessarily involved in the quantification of antioxidant activity (Jacóbo-Velasquéz and Cisneros-Zevallos, 2009). The correlation analysis of antioxidant capacity and phenolic compounds in Fuerte avocado did not reveal significant results ($p=0.992$ and $r=0.001$). Arancibia-Avila *et al*. (2008) found a correlation of 0.98 between the total phenolic content and antioxidant capacity of durian fruit. These authors concluded that the high polyphenol content was the main factor responsible for the fruit's antioxidant capacity. Wang *et al*. (2010) found a significant correlation between the total phenolic content and antioxidant capacity ($\geq = 0.79$) of avocados of different cultivars. The two parameters evaluated by these authors showed no correlation with the chlorophyll and carotenoids content ($r<0.1$). Furthermore, for these authors the high correlation found between procyanidins and the polyphenol content and antioxidant capacity suggests that this compound is the main polyphenol contributing to the antioxidant capacity of avocado. In the present research, the low correlation found for the evaluated parameters may indicate that another food metabolite is responsible for the antioxidant activity of avocado. It should be noted that vitamin E is a powerful antioxidant which may also contribute to the antioxidant capacity of avocado fruits.

In a study of the effect of heat treatment on the antioxidant capacity of vegetables, Melo *et al*. (2009) found that several events that occur during this treatment explain changes in the antioxidant activity of foods, which may be increased, reduced or unaltered. In situations in which the antioxidant activity of food increases, heat treatments favor the partial oxidation of the bioactive compound with the highest ability to donate a hydrogen atom to a radical starting from the hydroxyl group, and/or the aromatic structure of the polyphenol is more able to withstand the displacement of the unpaired electron around the ring. Moreover, heat treatments may favor the formation of new compounds such as Maillard reaction products (reductones), which exhibit antioxidant activity (Nicoli *et al*., 1999). Because refrigeration is the most efficient method for controlling fruit maturation, it may have contributed to maintaining the antioxidant capacity of the fruits during the storage period. The heat treatment had a negative effect on the maintenance of the fruit's antioxidant capacity compared to that of the control. The longer the fruit is exposed to a hydrothermal treatment, the higher the loss of its antioxidant capacity.

4. Conclusions

The total phenolic content increased up to the sixth day of storage, decreasing thereafter, without differences between the treatments. The antioxidant capacity of the control fruit and the fruit hydrothermally treated for 5 and 10 min increased throughout the storage period. The highest percentages of antioxidant capacity were obtained for the fruit without heat treatment. The percentage of antioxidant capacity of these treatments declined starting on day 12 of storage, possibly due to senescence. Hydrothermal treatments of 15 and 20 min delayed senescence, with antioxidant capacity continu-

ing to increase up to the fifth day of storage. No significant correlation was found between the antioxidant capacity and the content of phenolic compounds. The antioxidant capacity of ripe 'Fuerte' avocado was higher than that of unripe or senescent fruit.

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