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Changes in quality parameters, antioxidant compounds and enzymes of phenolic metabolism of hot water-treated organic 'cherry' peppers

[1*]Avalos-Llano, K. R., [1]Sgroppo, S. C. and [2]Chaves, A. R.

[1]Laboratorio de Tecnología Química y Bromatología, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste (UNNE), Avenida Libertad 5450, 3400, Corrientes, Argentina
[2]Centro de Investigación y Desarrollo en Criotecnología de Alimentos, Centro Científico Tecnológico Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-La Plata, Calle 47 esq. 116, 1900, La Plata, Argentina

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Abstract

The present study aimed to evaluate the effect of hot water treatment on the quality, content of antioxidant compounds and activity of enzymes of the phenolic metabolism of organic 'cherry' peppers stored at 10°C for 14 days. The selected hot water treatment (55°C for 60 s) delayed the decay and kept the quality of 'cherry' peppers. Immediately after treatment, the concentrations of hydroxycinnamic acid derivatives were unchanged, whereas on day 14 they were decreased. No noticeable adverse effects on the content of carotenoids, ascorbic acid, total phenols, and glycosylated flavonoids or anti-radical activity were observed after 14 days. Immediately after the hot water treatment, the activities of phenylalanine-ammonia-lyase (PAL) and polyphenoloxidase (PPO) decreased significantly, whereas that of peroxidase (POD) increased. Throughout storage, PAL activity in treated peppers remained unchanged, whereas PPO and POD activities increased. Therefore, the treatment at 55°C for 60 s allowed maintaining the quality, inhibited PAL activity and did not markedly affect the content of antioxidant compounds of 'cherry' peppers during storage. Our results suggest that this treatment may be used as a nonpolluting and non-chemical technology to extend the shelf life of organic 'cherry' peppers.

Introduction

'Cherry' peppers (*Capsicum annuum* L. cv. Cherry) are attractive fruits due to their small size, sweet taste, red color at the ripe stage and content of bioactive compounds. *C. annuum* owe the red color to carotenoids, such as capsanthin, curcubixanthin A, zeaxanthin, β-carotene, β-cryptoxanthin, and α-carotene (Hornero-Méndez *et al.*, 2000; Marín *et al.*, 2004; Raffo *et al.*, 2007). Moreover, they are a good source of vitamin C and phenolic compounds, such as flavonoids, capsaicinoids, and phenolic acids (Shahidi and Naczk, 2004).

Although 'cherry' peppers are rich in antioxidant compounds, and are grown in several world regions, their consumption and commercial cultivation are not widespread. They are commercially produced in the United States, Mexico, Europe, and Asia, and are a potential non-traditional export crop in developing countries. In fact, they are grown in South Africa as a new high value commodity (ICCO, 2015).

To consider 'cherry' peppers as a commodity for export, it is necessary to control the main factors that reduce their postharvest quality. In peppers, these factors are chilling injury susceptibility and shriveling (which can be controlled at temperatures above 7°C and relative humidity above 90%), decay development, and changes in their antioxidant properties.

Quality maintenance in organic products is highly challenging, given their high perishability and the proscription of using chemical agents. Thus, the assessment of physical treatments has regained great interest (Rodoni *et al.*, 2016). Among them, mild heat treatments have been used to prevent physiological changes that lead to quality loss, which is usually related to phenolic metabolism involving the enzymes phenylalanine-ammonia-lyase (PAL), polyphenoloxidase (PPO) and peroxidase (POD) (Tomás-Barberán and Espín, 2001). These treatments are a nonpolluting and relatively simple technology that may be applied on non-traditional export crops, such as 'cherry' peppers.

In Israel and other countries, a treatment with a hot water rinse and brushing is used in commercial packing lines of fresh commodities for export, such as peppers, melons, mangoes and sweet corn (Fallik

*Corresponding author.
Email: *kavalosllano@exa.unne.edu.ar*

et al., 1999; Fallik, 2004). The treatment requires low water consumption, the water can be recycled, and the heat is applied mainly as gas combustion (Sivakumar and Fallik, 2013). In addition, previous works have reported the effects of hot water treatments on the quality and certain antioxidant properties of pepper fruit during storage (González-Aguilar *et al*., 1999, 2000; Fallik *et al.*, 1999, 2009; Raffo *et al.* 2007; Ilić *et al*., 2012). Thus far, only one of these studies has assessed the effect of a hot water treatment on the content of ascorbic acid (AA) and phenols in peppers (Raffo *et al*., 2007). However, to our knowledge, there are no studies on the effect of this kind of treatment on dehydroascorbic acid (DHA) or the enzymes related to phenolic metabolism. Therefore, the aim of the present work was to study the effect of a hot water treatment on quality parameters, contents of carotenoids, AA and DHA, and phenolic compounds, anti-radical activity, and PAL, PPO and POD activities in organic 'cherry' peppers stored at 10°C for 14 days.

Materials and Methods

Plant material and selection of the hot water treatment

C. annuum L. cv. Cherry peppers organically grown in Corrientes, Argentina (27º 27' S, 58º 49' W), were harvested at the physiological ripeness stage having 90% surface red color. Peppers with uniform size (10.10 ± 2.43 g) and free of damage were selected, washed with 100 µL L^{-1} chlorinated water solution for 20 s, and randomly divided into six groups. To select the most suitable hot water treatment five groups were subjected to different treatments (50ºC for 60 s, 55ºC for 60 s, 55ºC for 120 s, 55ºC for 180 s, and 60ºC for 60 s) in a thermostatic water bath with agitation system, and the other group was subjected to no treatment as a control. Afterwards, 70 g of control peppers and 70 g of peppers from each treated group were randomly selected and packed in PET crystalline trays (7.5 cm x 6.5 cm x 4 cm). Packages were covered loosely with PVC film (thickness, 10 µm; O_2 permeability, 10.78 cm^3 m^{-2} atm^{-1} day^{-1}; CO_2 permeability, 51.32 cm^3 m^{-2} atm^{-1} day^{-1}; water vapor permeability, 38 g m^{-2} day^{-1}) to minimize dehydration and stored at 10 ± 2ºC and 90 ± 5% relative humidity for 14 days (as in commercial storage). Three trays of control peppers and three trays of peppers from each treated group were analyzed at 10 and 14 days. The effects of the different treatments were evaluated by monitoring the general appearance index (I), and the soft rot and fungal decay. The general appearance index was evaluated taking into account color, brightness, softness, shriveling, and presence of macroscopic soft rot and fungal growth, based on a score from 1 to 4 (1: very good or fresh, 2: good, 3: fair, and 4: poor) as follows: $I = (1n + 2n + 3n + 4n)/N$, where: n was the number of peppers corresponding to each score, and N was the total number of peppers analyzed. Scores above 2 were considered as an indicator that peppers had lost marketable quality. Decay was expressed as the percentage of fruit affected by soft rot and macroscopic fungal growth with respect to the total number of peppers evaluated.

Effects of the selected hot water treatment (55ºC for 60 s) on physic-chemical quality during storage

The peppers treated at 55°C for 60 s and the corresponding controls were packed as described above, and stored at 10ºC for 14 days. Three trays of control peppers and three of treated peppers were taken for analysis at 0, 2, 5, 10 and 14 days. Samples were analyzed at the sampling time or frozen at -20°C for further analysis.

Weight loss

Weight loss was determined by weighing three replicate packages containing the peppers from each treatment and storage time. Results were calculated as percentage of weight loss.

Color

L^*, a^*, and b^* values were determined with a colorimeter (Minolta, Model CR-300, Osaka, Japan) and the hue angle was calculated as $\tan^{-1}$ (b^*/a^*). Twenty peppers were analyzed for each treatment and storage time by measuring in three zones of each pepper.

Respiratory activity

Peppers (100 g) were put into a hermetically sealed jar, and the CO_2 produced was measured every 5 min for 1 h by using an IR sensor (Alnor Compu Flow® Model 8650). The CO_2 production rate was calculated from the straight line obtained. Results were expressed as µL CO_2 g^{-1} h^{-1}.

Firmness

The maximum force (N) required for a 3-mm-diameter flat probe to penetrate a sample to a depth of 3 mm at a rate of 0.5 mm s^{-1} was recorded by using a Texture Analyzer (TA-XT2i, Stable Micro Systems Texture Technologies, Scarsdale NY, USA). Twenty peppers were used for each treatment and storage time by measuring eight times on the equatorial zone of each pepper.

Sugars and acidity

Frozen peppers were ground in a refrigerated mill, and 10 g of ground tissue was homogenized with 30 mL of ethanol for 15 min at 0°C in the dark. The homogenate was centrifuged at 5000×g for 10 min, and total sugars were determined using anthrone reagent (Southgate, 1976). The absorbance was measured at 620 nm in a spectrophotometer (Metrolab 1700, Buenos Aires, Argentina), and glucose was used as standard in the 0-26 mg L^{-1} concentration range. Results were expressed as g of glucose kg^{-1} of fresh tissue. Titratable acidity was measured according to the AOAC (1990) procedure.

Total carotenoids

Five grams of ground tissue was homogenized with 35 mL of acetone at 0°C for 20 min in the dark. The carotenoids were extracted with petroleum ether 35-60 bp, and the absorbance was measured at 450 nm. The extinction coefficient was 2.5 x 107 L kg^{-1} m^{-1} (Davies *et al*., 1970). Results were expressed as mg of β-carotene kg^{-1} of fresh tissue.

Ascorbic and dehydroascorbic acids

Four grams of ground tissue was homogenized with 10 mL of 60 g L^{-1} trichloroacetic acid solution at 0°C for 30 min in the dark. The homogenate was centrifuged at 2,000×g for 15 min, and the supernatant was used to determine the contents of AA and DHA according to Kampfenkel *et al*. (1995). The assay was based on the reduction of Fe^{3+} by AA and the spectrophotometric detection of Fe^{2+} complexed with 2,2'-dipyridyl. DHA was reduced to AA by preincubation of the sample with dithiothreitol (DTT). Subsequently, total AA was determined by the 2,2'-dipyridyl method. Then, the DHA concentration was calculated from the difference of total AA (with pretreatment with DTT) and AA (without pretreatment with DTT). To calculate the total AA and AA concentrations, AA (Sigma Chemical, St. Louis, MO, USA) was used as standard in the 0-12 mg L^{-1} concentration range. Results were expressed as mg kg^{-1} of fresh tissue.

Phenolic compounds

The ethanolic extract was prepared as described previously for sugars, and total phenol content was determined using Folin-Ciocalteau reagent (Singleton *et al*., 1999). The absorbance was measured at 760 nm, and chlorogenic acid (Sigma Chemical, St. Louis, MO, USA) was used as standard in the 0-14 mg L^{-1} concentration range. Results were expressed as g of chlorogenic acid $kg^{-1}$of fresh tissue.

The phenolic compounds of peppers were separated and quantified by HPLC using a Shimadzu LC-10AT (Shimadzu Corp., Tokyo, Japan) with a Shimadzu SPD-10A UV-visible detector. Ground tissue (5 g) was added to 30 mg of butylated hydroxyl-toluene (Sigma Chemical, St. Louis, MO, USA) and homogenized in 20 mL of methanol for 10 min at 0°C. The homogenate was centrifuged at 5,000×g for 10 min. The supernatant was evaporated to dryness in a rotary evaporator at 40ºC and then dissolved in methanol:water 70:30. The extract was filtered through a 0.45 µm nylon membrane and injected onto the HPLC (20 µL). The chromatographic column was ZORBAX SB-C8 (Agilent Technologies, USA) (5 µm, 4.6 mm x 150 mm). The mobile phases were 50 g L^{-1} formic acid (A) and methanol (B) (Marín *et al*., 2004). A linear gradient was used starting with 20% B in A and increasing to 70% B in A at 55 min, then decreasing back to 20% B at 60 min. The flow rate was 0.8 mL min^{-1} and the detection was recorded at 340 nm. Results were expressed as g hydroxycinnamic acid derivatives (HA) and glycosylated flavonoids (GF) kg^{-1} fresh tissue.

Anti-radical activity

The ethanolic extract was prepared as described previously for sugars, and different aliquots of the extract were added to test tubes containing 3.4 mL of 0.030 g L^{-1} 2,2-diphenyl-1-picrylhydrazyl (DPPH.) (Sigma Chemical, St. Louis, MO, USA) in methanol (total volume: 4 mL). The absorbance at 517 nm was measured when the reaction reached a plateau (35 min). Results were expressed as EC_{50}^{-1} (Vicente *et al*., 2005) in kg^{-1} fresh tissue.

Enzyme activity assays

PAL activity was measured according to Lemoine *et al.* (2010) with modifications. Ground tissue (3 g) was homogenized with 20 mL of extraction buffer (0.1 mol L^{-1} $Na_2B_4O_7.10H_2O$, 5 mmol L^{-1} 2-mercaptoethanol, 2 mmol L^{-1} EDTA, and 6 g L^{-1} polyvinylpolypyrrolidone (PVPP, pH 8.8) for 10 min at 4°C, and the homogenate was centrifuged at 10,000×g for 20 min at 4ºC. PAL activity was determined at 37°C in a mixture containing 1,100 µL of 0.03 mol L^{-1} pH 8.8 sodium borate buffer, 250 µL of enzyme extract, and 150 µL of 0.01 mol L^{-1} L-phenylalanine. At predetermined times (0-30 h), 400 µL of the reaction mixture was removed, and the reaction was immediately stopped by adding 400 µL of 5 mol L^{-1} HCl, cooled and centrifuged. The absorbance was measured at 290 nm. Results were expressed as the change in optical density s^{-1} kg^{-1} of protein.

To prepare the enzyme extract of PPO and POD

assays, 3 g of ground tissue was homogenized with 20 mL of phosphate buffer (0.1 mol L^{-1} KH_2PO_4, 0.1 mol L^{-1} Na_2HPO_4, pH 7.0, 1 mmol L^{-1} PMSF, 0.1 mmol L^{-1} EDTA, 1 mL L^{-1} Triton X-100 and 6 g L^{-1} PVPP) for 10 min at 4°C. Then, the homogenate was centrifuged at 10,000×g for 20 min at 4ºC. PPO activity was determined at 30°C in a mixture containing 100 mmol L^{-1} phosphate buffer, pH 6.0, 20 mol L^{-1} catechol, and 300 µL of enzyme extract in a total volume of 3 mL, and the absorbance was measured at 410 nm. POD activity was determined by measuring the increase in absorbance at 470 nm according to Lemoine *et al*. (2010) with modifications. The reaction mixture contained 1,280 µL of buffer (0.1 mol L^{-1} KH2PO4 and 0.1 mol L^{-1} Na_2HPO_4, pH 7.0), 60 µL of enzyme extract, 500 µL of 2.5 mL L^{-1} guaiacol, and 160 µL of 8 mmol L^{-1} H_2O_2. PPO and POD activities were expressed as the change in optical density s^{-1} kg^{-1} of protein.

The protein content was determined according to Bradford (1976), using bovine serum albumin as the standard protein.

Statistical analysis

Analysis of variance and the Fisher's test at $P<0.05$ for comparison of means were performed using the InfoStat 2015 Version (Di Rienzo *et al.*, 2015) software package. Three independent experiments were performed according to a factorial design. The factors were the treatment (with or without) and the storage time.

Results and Discussion

Selection of the hot water treatment

The hot water treatments at 55°C were the most suitable ones to maintain a good general appearance and avoid the macroscopic soft rot and fungal growth in 'cherry' peppers for 14 days at 10°C (Table 1). The treatment at 55°C for 60 s was selected for further studies because the exposure time was the lowest to obtain the same benefits. Since the effect of each hot water treatment depends on the cultivar, size, maturity and growing season (Fallik, 2004), in other pepper cultivars, other authors have selected a different temperature-time combination as the most effective one to maintain the quality (Fallik *et al.,* 1999; González-Aguilar *et al.*, 1999; Raffo *et al*., 2007; Ilić *et al*., 2012).

In 'cherry' peppers, the treatment at 55°C for 60 s maintained a good general appearance (I = 2), and controlled soft rot and fungal decay (Table 1). However, in control peppers, the fungal decay limited the storage life. Similar results have been found in other peppers (Fallik *et al.*, 1999, 2009; Ilić *et al.*, 2012). The treatment allowed controlling the postharvest decay probably because the treatment removes dirt, spores and latent infections from the surface or the first few layers under the fruit epidermis, and limits the sites of fungal penetration into the fruit by sealing open stomata (Fallik *et al.,* 1999; Ban *et al*., 2015).The heat treatment may also inhibit spore germination and mycelial growth, cause ultrastructural changes in fungal cell components, or induce defense responses such as the accumulation of phytoalexins (Sivakumar and Fallik, 2013).

Table 1. Changes in the general appearance index (I) and decay of 'cherry' peppers at 10 and 14 days of storage at 10°C.

Treatment*	I		Decay (%)	
	10 days	14 days	10 days	14 days
C	3.11	3.55	44	75
T 50°C/60 s	1.60	2.30	5	6
T 55°C/60 s	1.28	2.00	0	0
T 55°C/120 s	1.30	2.06	0	0
T 55°C/180 s	1.33	2.06	0	0
T 60°C/60 s	1.73	2.35	0	6

*C: control peppers, T: treated peppers. (LSDI = 0.06; LSD_{Decay} = 3.54).

The soft rot and macroscopic fungal growth were the principal deterioration factors in cherry peppers during storage. In contrast, shriveling, softening and visual color changes had no incidence on the appearance of peppers, which is in accordance with the results of weight loss, firmness and color parameters.

Effect of the selected hot water treatment on physico-chemical quality during storage

Quality parameters

In green peppers, González and Tiznado (1993) observed initial signs of shriveling when the weight loss was 5%. In contrast, in the present study, 'cherry' peppers treated at 55°C for 60 s lost less than 1.40% of their weight on day 14 (Table 2) because the film used to cover the peppers served as a barrier to water evaporation.

Hot water treatments can either delay or disrupt the fruit ripening, thus inhibition of color development may be indirectly observed (Fallik, 2004), and the respiration rate, firmness, and content of pigments may be affected (Paul and Chen, 2000). In the present study ('cherry' peppers treated at 55°C for 60 s), no

Table 2. Changes in weight loss (%), L^* and hue angle values, and respiratory activity (μL CO_2 h^{-1} g^{-1}) of control (C) and treated (T) 'cherry' peppers stored for 14 days at 10°C.

Treatments	Days at 10°C				
	0	2	5	10	14
Weight loss					
C	0.00	0.13	0.33	0.57	1.37
T	0.00	0.09	0.37	0.70	1.28
*L**					
C	35.64	34.96	35.53	35.50	35.11
T	35.64	35.33	36.43	36.42	35.87
Hue					
C	24.75	24.92	23.81	23.52	23.83
T	24.75	24.43	25.52*	25.32*	25.28*
Respiratory activity					
C	32.26	35.77	32.24	26.88	48.84*
T	12.92*	33.28	35.83	34.62	38.53

*Significantly different values from corresponding control peppers at P<0.05 according to a least significant difference (LSD) test. ($LSD_{Weight\ loss}$ = 0.32; LSD_{L^*} = 0.45; LSD_{Hue} = 0.56; $LSD_{Respiratory\ activity}$ = 10.37).

changes in lightness and a slight increase in the hue angle were found during storage (Table 2). Although, this increase may indicate a slight additional red color development, no changes in visual color were observed when the general appearance was evaluated.

Immediately after the treatment at 55°C for 60 s, the CO_2 production was reduced by 60%, whereas on day 14 it increased to values 21% lower than in controls (Table 2). On the other hand, firmness (initial value: 10.26 ± 2.03 N), total sugar content (initial value: 1.31 ± 0.05 g glucose 100 g^{-1}) and titratable acidity (initial value: 0.24 ± 0.01 g citric acid 100 g^{-1}) remained unchanged throughout storage, and no differences were found between control and treated peppers (data not shown). Other authors reported similar effects on the respiration rate and firmness in other pepper cultivars (Fallik *et al.*, 1999; González-Aguilar *et al.*, 1999; Raffo *et al*. 2007; Ilić *et al.*, 2012). In citrus fruit and peaches, Lurie and Tonutti (2014) found that the heat treatment evaluated caused an increase in sugars and a decrease in acids, likely due to a low glycolysis and an increased respiration during the treatment. In contrast, our results may indicate a moderate use of respiratory substrates by the peppers because of the moderate to low respiratory rate and metabolic activity during cold storage, as reported by González-Aguilar *et al.* (1999) and Raffo *et al*. (2007).

Table 3. Changes in the contents of total carotenoids (mg β-carotene kg^{-1}), total phenols (g chlorogenic acid kg^{-1}), ascorbic acid (mg kg^{-1}) and dehydroascorbic acid (mg kg^{-1}), and anti-radical activity (kg^{-1}) of control (C) and treated (T) 'cherry' peppers stored for 14 days at 10°C.

Treatments	Days at 10°C				
	0	2	5	10	14
Total carotenoids					
C	275.94	263.06	298.04	288.01	322.02
T	278.38	300.92*	305.92	291.75	306.01
Ascorbic acid (AA)					
C	227.82	289.92	225.57	266.77	228.00
T	210.82	293.44	211.03	235.35*	193.40*
Dehydroascorbic acid (DHA)					
C	30.40	28.51	34.99	34.61	28.48
T	21.10*	11.40*	21.94*	18.00*	23.32
Total phenols					
C	1.05	0.98	1.17	1.07	1.07
T	1.07	0.95	1.05*	1.07	0.99*
Anti-radical activity (x 10^{-4})					
C	1.40	1.13	1.42	1.47	1.58
T	1.41	1.12	1.29*	1.51	1.46*

*Significantly different values from corresponding control peppers at P<0.05 according to a least significant difference (LSD) test. ($LSD_{total\ carotenoids}$ = 17; $LSD_{total\ phenols}$ = 0.04; LSD_{AA} = 18.19; LSD_{DHA} = 8.14; $LSD_{anti\text{-}radical\ activity}$ = 487).

Antioxidant compounds

Pepper ripeness is associated with carotenoids accumulation, which can be ascribed to continuation of synthesis and metabolic interconversion during postharvest (Raffo *et al*., 2007). Although heat treatments can modulate the rate of ripening (Lurie and Tonutti, 2014), in the present study, the increase in carotenoids of control and treated peppers during storage was similar (P>0.05) (Table 3). Thus, the heat treatment did not prevent carotenoid biosynthesis as previously reported (Ilić *et al.*, 2008).

Since AA and DHA are biologically active forms of vitamin C, it is also important to measure both acids. AA content contributed 88-91%, whereas DHA contributed 12-9% of the total vitamin C at the beginning of storage (Table 3). Similar and higher AA contributions have been reported in other pepper cultivars (Jiménez *et al.,* 2003; Marín *et al*., 2004; Andrade Cuvi *et al.*, 2011).

After 2 days of storage, the content of AA increased (Table 3), suggesting that AA biosynthesis continued in both control and treated peppers during postharvest storage. This has also been observed by Martínez *et al.* (2005) and Andrade Cuvi *et al.* (2011). Subsequently, the AA content decreased and, in treated peppers, reached values slightly lower than in

controls at the end of storage. However, the hot water treatment limited AA degradation to less than 10%, similar to that reported by Raffo *et al*. (2007). Thus, after 14 days of storage, treated peppers retained 92% of vitamin C in the AA form.

Heat-treated fruits showed lower DHA content than the controls both immediately after the treatment and throughout the storage period (Table 3). The fact that this occurred without the accumulation of AA, its immediate precursor, suggests that the heat treatment accelerated the DHA turnover rather than its reduction through the Halliwell-Asada cycle. Previous works have shown that the heat treatment may induce the accumulation of reactive oxygen species (ROS) (Lurie and Pedreschi, 2014; Lurie and Tonutti, 2014), which may be in part responsible for the decreased pool of DHA and AA found in treated peppers. Besides, the potential physiological role of the decrease in the contents of AA and DHA was not very important from a nutritional perspective because the decrease in vitamin C content was not higher than 10%.

Regarding the content of total phenolic compounds, no significant differences (P>0.05) were observed between control and treated peppers immediately after the treatment (Table 3). After day 2, the total phenolic content of treated peppers decreased slightly and then increased and reached values approximately 8% lower than those of controls on day 14 (Table 3).

Phenolic compounds, such as coumaric, ferulic and sinapic acids, quercetin, luteolin, and other O-glycosylflavones and C-glycosylflavones, have been found in another pepper cultivar (Marín *et al*., 2004). In our work, the total GF contents were initially about 3.7 fold higher than the total HA contents (Table 4). Similarly, higher levels of flavonoids than hydroxycinnamic derivatives were found in red peppers, but the ratio was 2.5 (Marín *et al*., 2004; Raffo *et al.*, 2007). In the present study, at the beginning of storage, no differences (P>0.05) were detected in the total HA concentration between control and treated peppers (Table 4). After 14 days of storage, treated peppers displayed 46% lower HA content than controls. On the other hand, there were no changes (P>0.05) in the total GF content caused by the heat treatment throughout storage. In contrast, Raffo *et al.* (2007) reported that the heat treatment (53ºC for 4 min) did not affect HA levels, but decreased the GF content in another pepper cultivar.

Immediately after the treatment, no differences were found in anti-radical capacity between control and treated peppers (Table 3). Although the activity decreased by 20% in both control and treated peppers on day 2, it later increased and reached values slightly higher than the initial ones. Heat stress before storage and low temperature during storage may lead to higher phenolic metabolism and antioxidant capacity in peppers (Ilić *et al*., 2012).

Table 4. Content of phenolic compounds (g kg^{-1} fresh tissue) of control (C) and treated (T) 'cherry' peppers, expressed as total hydroxycinnamic acid derivatives (HA) and glycosylated flavonoids (GF), at 0 and 14 days of storage at 10°C.

Treatments	Days at 10°C	
	0	14
HA		
C	0.20	0.26
T	0.19	0.14*
GF		
C	0.76	0.79
T	0.76	0.80

*Significantly different values from corresponding control peppers at P<0.05 according to a least significant difference (LSD) test. (LSD_{HA} = 0.03; LSD_{GF} = 0.05).

Several compounds, such as flavonoids, phenolic acids, amino acids, AA, tocopherols and pigments may contribute to the total antioxidant capacity of fruits and vegetables. In our work, the evolution of the anti-radical capacity was mainly associated with the trend of total phenols. The decrease in anti-radical activity after 2 days may be explained in part by the slight decrease in total phenolic content, whereas the lower anti-radical capacity in treated peppers after 14 days may be related to lower levels of HA compounds. Alvarez-Parrilla *et al*. (2011) and Materska (2014) also reported a good correlation between total phenolic compounds and antioxidant capacity in other pepper cultivars.

Enzymes of phenolic metabolism

Immediately after treatment, PAL activity of 'cherry' peppers decreased 52% relative to controls (Table 5). Throughout storage at 10ºC, the treatment had no effect on the PAL activity of 'cherry' peppers, which may explain the trend of total phenols (Table 3). Shao & Tu (2014) found that a hot air treatment reduced PAL and total phenolic content in loquat fruits during storage at 4ºC. In contrast, Jin *et al.* (2009) and Zong *et al*. (2010) respectively found that a heat treatment increased PAL activity of tomato fruits stored at 20ºC and peaches stored at 0ºC. In the peaches, total phenolic content was also increased, which may be associated with chilling

Table 5. Evolution of the specific activity of PAL (ΔOD s^{-1} kg^{-1} protein), PPO (ΔOD s^{-1} kg^{-1} protein), and POD (ΔOD s^{-1} kg^{-1} protein) of control (C) and treated (T) 'cherry' peppers stored for 14 days at 10°C.

Treatments	Days at 10°C			
	0	5	10	14
PAL				
C	31.94	16.80	17.47	31.70
T	15.25*	17.57	17.86	15.29*
PPO				
C	430.33	417.00	588.33	390.50
T	203.33*	332.17	562.00	460.17
POD (x 10^{-4})				
C	4.31	5.29	9.29	6.58
T	6.31*	4.79	9.13	7.66*

*Significantly different values from corresponding control peppers at $P<0.05$ according to a least significant difference (LSD) test. (LSD_{PAL} = 5.6; LSD_{PPO} = 100.83; LSD_{POD} = 5580).

tolerance. The effect on PAL, the key enzyme of the polyphenol biosynthesis pathway, was influenced by the temperature and time of treatment, the storage temperature, and the type of fruit or vegetable.

In the present study, 'cherry' peppers showed a decrease of 53% in PPO activity immediately after treatment (Table 5). This is because PPO is not a highly heat-stable enzyme and its activity decreases very quickly when the temperature increases beyond 25°C (Maghoumi *et al*., 2014). In contrast, Ciou *et al.* (2011) reported that PPO activity increased in water caltrop pericarp treated at 50-60ºC. Different sources of enzymes may possess different heat resistances. During storage at 10°C, the PPO activity of the treated peppers increased, and no differences were observed between control and treated peppers. As shown in Tables 3 and 5, the heat treatment reduced the PPO activity at the beginning of storage, although it did not prevent the loss of phenols during longer storage times.

The heat treatment induced an increase in POD activity of 47% (Table 5) immediately after the treatment. In contrast, Castro et al. (2008) found that pepper POD subjected to blanching temperatures (70-98ºC) was inactivated, suggesting that pepper POD has a low stability at high temperature. During storage, POD increased and no significant differences ($P>0.05$) between the activity of control and treated 'cherry' peppers were detected up to day 10 when a maximum value was reached (Table 5). After 14 days, the treated samples showed POD activity 16% higher than the controls, which may explain the low level of phenols detected. In water caltrop pericarp treated at 50-60ºC, high PPO and POD activities resulted in degradation of phenolic compounds (Ciou *et al.*, 2011).

Since an accumulation of heat shock transcription factors may persist during storage and allow a diversion of protein synthesis associated with the heat response to occur over extended times, the heat treatment may also affect phenolic metabolism during storage (Ferguson *et al.*, 2000). Moreover, the changes induced by high temperature persist for a long time when the product is stored at a low temperature after the heat stress (Lurie and Pedreschi, 2014). This may explain the short-term effect of the treatment on POD and PPO and the long-term effect on PAL and phenolic compounds.

Conclusion

The hot water treatment at 55ºC for 60 s maintained a good general appearance and controlled the soft rot and fungal decay in 'cherry' peppers stored at 10°C. Moreover, the treatment had no negative effects on the quality or antioxidant properties. On the other hand, the hot water treatment did not affect the contents of HA and GF compounds immediately after its application, despite the initial changes in PAL, PPO, and POD activities. However, the slight changes in the concentrations of HA after 14 days may be associated with the enzyme trend. The results show that the treatment at 55°C for 60 s may be used as a nonpolluting technology to extend the shelf-life of organic 'cherry' peppers at 10°C without dramatically affecting the antioxidant properties.

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