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Combined use of mild heat treatment and refrigeration to extend the postharvest life of organic pepper sticks, as affected by fruit maturity stage

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ABSTRACT

Given the proscription of using chemicals from synthesis, the alternatives for postharvest management of organic produce are limited. Consequently, great interest is being devoted to develop and optimize alternative postharvest approaches. In this work we tested mild heat treatments for green and red fresh-cut peppers and evaluated their effect on quality maintenance under normal distribution and retail temperatures (4 °C). Pepper sticks (1 × 5 cm) at red and green ripening stages were heat-treated (HT) by immersion in water at 45, 50 or 55 °C (1, 3 or 5 min) and quality maintenance during storage was evaluated. Green peppers were more tolerant to HT than red fruit. Both green and ripe peppers subjected to hot water dips at 45 °C for 3 min showed lower spoilage than the control. The treatments markedly reduced soft rots (2 and 4 fold for red and green fruit respectively). Hot water dips also prevented shriveling, weight loss, color changes and contributed to maintain lower fruit respiration during storage. The treatments did not alter sugar content, acidity or antioxidant capacity. Despite of the effective control of soft rots only a slight reduction of microbial counts (<1 log CFU g^{-1}) was found. This suggests that other responses besides biocide effects or microbial wash-off are involved. The treatments delayed pectin solubilization and softening and prevented membrane leakage. Short mild heat treatments (45 °C, 3 min) may be a simple and appealing non-chemical approach to supplement the benefits of low temperature management, extending the shelf life of organic fresh-cut green and red peppers.

1. Introduction

Interests in eco-friendly food production systems and changing lifestyle have contributed to the rapid growth of organic vegetables in the last decade (James and Ngarmsak, 2011). Once limited to a few products, the organic fresh-cut vegetables segment currently includes a large variety of commodities (Goodburn and Wallace, 2013). Quality maintenance in these products is highly challenging, given their high perishability, and the limitation of using preserving agents which narrows the palette of postharvest alternatives. In addition, some studies have suggested that organic vegetables may be more perishable products than conventionally grown commodities (Amodio et al., 2007). In this scenario, the reassessment of physical treatments has gained great interest (Ölmez and Kretzschmar, 2009).

Postharvest heat treatments (HT) group a very diverse set of treatments in terms of their temperature range (35–60 °C), exposure time (few s to days) and heating conditions (microwave, infrared, hot vapor, hot air, hot water) (Fallik et al., 1996; Lurie, 1998; Inkha and Boonyakiat, 2010; Kusajima et al., 2012). They have been used for time in whole fruit and vegetables mainly to control insect pests (Schirra et al., 2000). HT have been reported to effectively control decay, reduce chilling injury (Sivakumar and Fallik, 2013), delay ripening (Lurie et al., 1996), senescence (Martínez and Civello, 2008) and browning (Kim et al., 1993; Saltveit, 2000). However, the outcome of postharvest HT is highly variable depending on treatment schedule, on the commodity and even on the cultivar considered (Lurie, 1998). Fruit ripening stage

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and degree of processing may have great impact on the benefits obtained (Koukounaras et al., 2008).

Peppers are, together with potato, tomato and eggplant among the most popular fruit crops in the Solanaceae family (Martínez et al., 2007). They have been traditionally marketed as whole fruits, but more recently fresh-cut forms have started to be distributed as well (Tadesse et al., 2002). Chilling injury, a common disorder in whole peppers held at temperatures below 7 °C, is not a main limiting factor in fresh-cut fruit (González-Aguilar et al., 2004). Instead, the main factors reducing the postharvest life of fresh-cut pepper include soft rots, shriveling of cut areas and extensive softening (Rodoni et al., 2015a). Field practices such as fertilization and the maturity at harvest may have a high impact in the quality and postharvest behavior of pepper strips (Piazzolla et al., 2012). Even under proper temperature management fresh-cut peppers have a relatively short postharvest life (7-10 days). Modified atmospheres (MAP) with 5 kPa CO_2 and 5 kPa O_2 were reported to provide moderate benefits on quality maintenance of fresh-cut peppers (Rodoni et al., 2015a). Postharvest HT has shown promising effects in whole peppers (Fallik et al., 1996). Hot water rinses (55 °C for 15 s) over brushes reduced decay and chilling injury incidence (Fallik et al., 1999). Sgroppo and Pereyra (2009) reported that hot water treatments (55 and 60 °C, 3 min.), before processing, improved antioxidant retention in fresh-cut green peppers. Post-cutting treatments may be more easily adaptable to current processing lines (Artés and Allende, 2005) and in some cases could be exploited to limit microbial loads in the washing water (Wulfkuehler et al., 2014). Although post-cutting hot water dips may be a relatively simple postharvest approach to extend the shelf life of organically grown fruits, they have not been tested to date. Whether or not the optimal treatment conditions and/or the induced responses differ depending on the ripening stage has not been established either.

In this work, we selected a proper post-cutting HT for fresh-cut organic green and red peppers. We also characterized the effect of combining HT and low temperature storage on quality maintenance.

2. Materials and methods

2.1. Plant material

Bell peppers (*Capsicum annuum* L.) cv. Jaen organically grown in La Plata, Argentina were harvested at both green and red stages and immediately transported to the laboratory. Fruit was washed with water, the peduncles, placenta and seeds were removed, and the pericarp was cut into 5 × 1 cm sticks and rapidly cooled to 4 °C.

2.2. Treatment selection

Red and green peppers sticks were immersed in a stirred hot water stainless steel tank at 45, 50 or 55 °C for 1, 3 or 5 min. The water temperature was carefully monitored. The ratio of water to product was 10:1 to prevent temperature variations. After the treatments, the sticks were rapidly transferred to a cool water tank (2 °C) for 5 min and subsequently drained. One group of pepper sticks, without heat treatment, processed and cooled as indicated above was used as a control. The sticks (∼150 g) were subsequently packed in polyethylene terephthalate (PET) trays, covered with perforated PVC and stored at 4 °C for 3 and 12 days. Eight trays per treatment, storage time and maturity stage were evaluated. Two independent experiments from different harvests were conducted. The percentage of sticks showing heat damage symptoms (loss of tissue turgor, exacerbated softening and decay) for each experiment was registered. Fruit deterioration was also visually assessed based on a hedonic four level intensity scale (0 = excellent; 1 = good; 2 = acceptable and 3 = poor). Sticks decayed or having extensive softening or shriveling, were classified as poor. Samples with moderate softening or shriveling, but without decay, were categorized as acceptable. Sticks showing no marked softening and slight shriveling were considered good. Excellent sticks showed no visual symptoms of decay, dehydration or softening. A deterioration index (DI) was subsequently calculated as:

$$\mathrm{DI} = \frac{\sum(\text{Injury level} \times \text{Number of sticks in this level})}{\text{Total number of sticks}}$$

2.3. Quality retention of heat-treated green and red pepper sticks during refrigerated storage

Fresh-cut red and green organic pepper sticks were immersed in water at 45 °C for 3 min and cooled as described in Section 2.2. Thirty PET trays containing pepper sticks (∼150 g) and covered with perforated PVC were prepared for each ripening stage. Corresponding peppers without treatments, cooled and packed as previously mentioned, were used as controls. Samples were stored for 0, 7 or 12 days at 4 °C. At each sampling date, 10 trays were taken and used for quality assessment. When required representative

Table 1
Heat damage (%), deterioration index (DI), (0—excellent to 3—poor) and analyses of variance (ANOVA) probabilities values in control and heat-treated (45, 50 or 55 °C for 1, 3 or 5 min) red and green organic pepper sticks stored at 4 °C and 90–95% RH for12 days.[d]

			Heat damage (%)[a]		DI[b,c]	
	Temperature (° C)	Time (min)	Red	Green	Red	Green
Control	–	–	–	–	2.2 ± 0.2ab	1.8 ± 0.3c
	45	1	0c	0b	1.7 ± 0.3bc	1.2 ± 0.6d
	45	3	0c	0b	1.4 ± 0.6c	0.8 ± 0.2e
	45	5	0c	0b	1.7 ± 0.3bc	1.4 ± 0.3de
	50	1	0c	0b	1.9 ± 0.6bc	1.0 ± 0.3de
	50	3	0c	0b	2.6 ± 0.3a	0.9 ± 0.4de
	50	5	4 ± 3c	0b	2.6 ± 0.4a	2.0 ± 0.3c
	55	1	0c	0b	2.0 ± 0.6b	2.0 ± 0.3c
	55	3	74 ± 19b	0b	2.7 ± 0.4a	2.8 ± 0.4b
	55	5	96 ± 5a	20 ± 19a	3 ± 0a	3 ± 0a
	Temperature (*T*)		<0.0001	0.0002	<0.0001	<0.0001
	Time (*t*)		<0.0001	0.001	0.0344	<0.0001
	T × *t*		<0.0001	<0.0001	0.0829	<0.0001

[a] Registered after 3 days of storage ($n = 16$). Heat damaged sticks showed excessive juice exudate and exacerbated softening.
[b] Registered after 12 days of storage.
[c] Means ± standard deviation ($n = 16$).
[d] Values followed by different letters indicate significant differences within a column based on a Fisher test at a level of significance of $P < 0.05$.

subsamples taken and the values obtained from each harvest were averaged. Measurements were done immediately after sampling or otherwise fruit was frozen in liquid N_2 and stored at −80 °C until analysis. Three independent experiments form different harvests were conducted and were considered as biological replicates.

2.3.1. Soft rots incidence, shriveling and weight loss

The percentage of sticks showing soft rots or shriveling on each tray was recorded. Weight loss was determined by weighing the pepper sticks individually. For each stick weight loss was calculated as: $WL = 100(W_i - W_f)/W_i$, being W_i the initial sample weight and W_f the final weight. Results were expressed in percentage.

2.3.2. Extractable juice

For extractable juice determination, the filter paper press method described by Kauffman et al. (1986) was used. For each harvest a subsample of five pepper sticks was taken and was placed upside up in a weighed filter paper and compressed by a normal force of 2 kg for 30 s. The filter papers were removed and weighed again and fruit juice loss was calculated. Results were expressed as grams of extracted juice per kilogram of fresh weight (FW).

2.3.3. Electrolyte leakage

Samples of pepper sticks for each harvest, weighing approximately 15 g, were taken and put in plastic tubes containing 20 mL of mannitol 0.6 M for 5 min with gentle stirring. The tissue was removed and the conductivity of the solution was measured at 20 °C with a conductimeter (Oakton Model 510, IL USA). To evaluate the total amount of electrolytes in the tissue, the pepper sticks were placed back into the mannitol solution and ground in an Omnimixer (Sorvall Inc., CT, USA). The suspension was then centrifuged at 10,000 × *g* for 10 min and the conductivity of the supernatant was measured as described above. Results were expressed as the percentage of electrolytes that leaked out from the tissues during the incubation period.

2.3.4. Respiration rate

Approximately 150 g of pepper sticks from stored packages were put into a glass flask (3 L). The flask was sealed and incubated for 20 min at 4 °C and CO_2 production was monitored with an infrared sensor (Alnor Compu-flow, Model 8650, Alnor, USA). Results were expressed in milligrams of CO_2 produced per kilogram of FW in an hour.

2.3.5. Color

Surface color was measured on the outer side of the sticks with a colorimeter (Model CR-400, Minolta, Osaka, Japan) to obtain L^*, a^* and b^* values. The *hue* angle was calculated as $180 - tg^{-1}(b^*/a^*)$ and $tg^{-1}(b^*/a^*)$ for green and red sticks respectively. Twenty measurements were conducted for each harvest replicate and averaged.

2.3.6. Sugars, acidity and pH and antioxidant capacity against DPPH• and ABTS•+ radicals

For each harvest replicate a subsample of 10 pepper sticks was used for sugar, acidity and pH analyses. For sugar measurements fruit tissue was ground in a mill (Model A11, IKA Works Inc., SP, Brazil) and approximately 1 g of the resulting powder was vortexed for 1 min in 5 mL of cold ethanol and centrifuged at 15,000 × *g* for 10 min at 4 °C. The supernatant was collected and the pellet was re-extracted with 5 mL of cold ethanol. The supernatants from both extractions were pooled and brought to 100 mL with distilled water. Sugars were measured with the anthrone reagent (Andersson et al., 2006). Glucose was used as a standard and results were expressed as grams of glucose per kg of FW. The DPPH• assay was done according to Brand-Williams et al. (1995), with minor modifications. Two milliliters of aliquots of the ethanolic extracts, prepared for sugar analysis were brought to 10 mL with ethanol and used for DPPH• determinations. Test tubes containing 0, 50, 75, 125, 175 and 225 mL of sample and ethanol to a final volume of 500 μL were prepared. After that 800 μL of a 60 mg L^{-1} solution of the radical DPPH• in ethanol were added. Samples were vortexed and incubated at 20 °C for 60 min. The absorbance at 515 nm was measured and the equivalent mass of fruit tissue required to consume 50% of the initial DPPH• was calculated (EC_{50}). The antioxidant capacity was defined as EC_{50}^{-1} (kg^{-1}). One measurement was done for each tray.

The ABTS•+ assay was performed according to Arnao et al. (2001), with minor modifications. The ABTS•+ stock solution was prepared by weighing 7 mmol of ABTS•+ ammonium salt and 2.45 mmol of $K_2S_2O_8$, which were added to water to make 1 L and allowed to react overnight at 20 °C in darkness. The ABTS•+ working solutions were prepared by diluting the stock solution to an absorbance of 0.700 ± 0.03 at 734 nm. Ten microliters of ethanolic fruit extracts, prepared for sugar analysis, where added to 1 mL of ABTS•+ working solution, vortexed, and incubated for 6 min. The absorbance at 734 nm was measured. Corresponding blanks without fruit extract were used to determine the stability of the ABTS•+. Results were expressed as millimols of Trolox equivalents per kg^{-1} of FW.

For pH and acidity evaluations fruit pulp was frozen in liquid nitrogen, ground in a mill and 10 g of the resulting powder were added to 100 mL of water. Samples were titrated with 0.1 mol L^{-1} NaOH until pH 8.2 (AOAC, 1980). Results were expressed as H^+ mmol L^{-1} of FW.

2.3.7. Bacteria, molds and yeasts

Approximately 25 g of pepper sticks were stirred in 225 mL of 0.1% w/v peptone for 15 min. From the resulting suspensions, dilutions from 10^{-2} to 10^{-5} were prepared and 1 mL of each dilution was poured into the appropriate medium (Petrifilm™ Plates 6400 and 6407, 3 M, St. Paul, MN USA) in three technical triplicates. Plates for aerobic mesophilic bacteria counts were incubated at 30 °C for 3 days and for yeast and molds at 20 °C for 5 days. Results were expressed as log of colony forming units (CFUs) per gram of FW. Fruit from two independent harvest replicates were evaluated.

Table 2
Analyses of variance (ANOVA) probabilities values for ripening stage (R), heat treatment (HT, 43 °C, 3 min) and their interaction (R × HT).

Parameter	Ripening stage (R)	Treatment (HT)	R × HT
Soft rot	0.0743	<0.0001	0.4313
Shriveling	0.6281	<0.0001	0.2885
Weight loss	0.7201	0.0053	0.828
Extractable juice	<0.0001	<0.0001	<0.0001
Electrol. leakage	<0.0001	<0.0001	<0.0001
Respiration rate	<0.0001	0.0001	0.219
L^*	0.001	0.0543	0.6153
Hue	<0.0001	0.4159	0.8391
Acidity	<0.0001	0.3129	0.8960
pH	<0.0001	0.8525	0.5253
Sugars	<0.0001	0.1679	0.9070
DPPH•	<0.0001	0.6113	0.8657
ABTS•+	<0.0001	0.3300	0.9166
Firmness	<0.0001	0.0005	0.6524
BR[a]	<0.0001	0.0103	0.1400
WSP[b]	<0.0001	0.0022	0.0023
Bacteria	0.0042	0.0008	0.4699
Molds and yeast	<0.0001	0.0001	0.3180

[a] Bending resistance.
[b] Water soluble pectin.

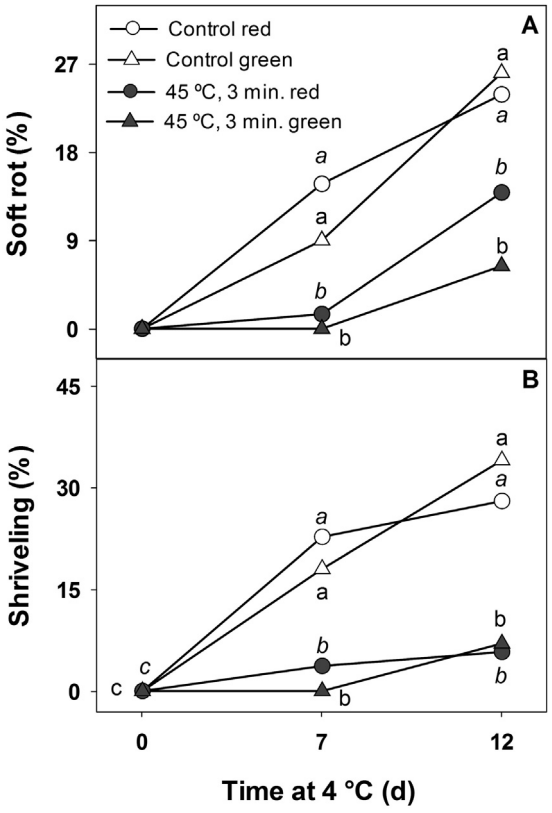

Fig. 1. Soft rot (A) and shriveling (B) in control and heat-treated (45 °C, 3 min) red and green organic pepper sticks stored for 0, 7 or 12 days at 4 °C and 90–95% HR. Different letters for a given ripening stage and storage time indicate significant differences based on a Fisher test at a level of significance of $P < 0.05$.

2.3.8. Texture

The texture of the bell pepper sticks was determined by two different (puncture and bending) assays in a Texture Analyzer (TA. XT2, Stable Micro Systems Texture Technologies, NY, USA). Firmness was determined in the inner side of the pepper sticks by compressing the fruit tissue 2 mm in equatorial zone at a rate of 0.5 mm s^{-1} with a 3 mm diameter flat probe. The maximum force developed during the test was recorded. Twenty measurements were done for each harvest.

For bending resistance assays, the bell pepper sticks with 5 × 1 cm and 4 mm thick were placed in a platform standing at 2 points 3 cm far from each other. A dented probe with rectangular section (10 × 1 mm) was used to displace the center of the pepper sticks at a rate of 0.5 mm s^{-1} and the force required to bend the sticks 6 mm, by applying a normal force on the cuticle side, was determined. Results were calculated as the slope of the force/time curve and expressed in N s^{-1}. Twenty measurements were done for each harvest replicate.

2.3.9. Water-soluble pectin (WSP)

Approximately 7.5 g of pepper sticks were ground in 20 mL of distilled water in an Omnimixer. The suspension was vortexed and centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant was saved and precipitated by adding 3 volumes of cold ethanol. Samples were then centrifuged at 10,000 × *g* for 10 min at 4 °C, the pellet was saved and dissolved in HAc/NaAc buffer (pH 5.0; 50 mmol L^{-1}) to obtain the water soluble pectins (WSP). Three extractions were done for each treatment, ripening stage and storage time. The concentration of uronic acids in the WSP was determined as previously reported (Blumenkrantz and Asboe-Hansen, 1973). Results are expressed as grams of galacturonic acid per kilogram of FW.

2.3.10. Statistical analysis

Results were analyzed by ANOVA with the InfoStat software package (Di Rienzo et al., 2010). The model assumptions of homogeneity of variance and normality were probed by means of Levene's and Shapiro–Wilk's tests, respectively. A two factorial (temperature, time) design was performed for treatment selection. For the experiment assessing quality changes in UV-treated peppers means and pooled standard deviation (Pooled sd) for all physicochemical determination was calculated. Means were analyzed by a factorial test and compared by a Fisher test at $^{*}P < 0.05$.

3. Results and discussion

3.1. Treatment selection

Although peppers are known to be chilling sensitive (Cantwell, 2015) the incidence and severity of the disorder depends both storage temperature and duration (Liu et al., 2015). In this case, probably due to the short storage period of fresh-cut peppers relative to intact fruit, manifestations of chilling injury (surface depressions, pitting and discoloration) were minimal, regardless of the ripening stage. This has been reported in previous studies (González-Aguilar et al., 2004), though some works have found chilling injury in fresh-cut peppers (Kang and Lee, 1997). The main factors contributing to fruit deterioration were, at both maturity stages, soft rots, shriveling and softening. Results showed that for both ripening stages the effect of treatment temperature and duration was significant (Table 1). The interactions between treatment temperature and duration were significant in green peppers for both deterioration index and heat damage but only for heat damage in red ripe fruit. Immediately after the heat treatments, no symptoms of heat damage were observed. However, exposure of red pepper sticks to water at 50 °C for over 1 min or 55 °C for 1, 3 and 5 min favored tissue softening, juice exudate and rapid decay (Table 1). The optimal treatment temperatures for fresh-cut peppers are much lower than those reported for unprocessed fruit. Previous work in whole red fruit showed that immersion at 55 and 60 °C (Fallik et al., 1999) prevented spoilage without causing heat damage. In green peppers, González-Aguilar et al. (2000) reported that exposure to 53 °C for 4 min reduced chilling injury with no adverse effects on the fruit. However, the influence of pepper ripening stage on heat tolerance has not been determined in a single study. In the present work, green fresh-cut peppers proved more tolerant to HT, as they showed slight heat damage symptoms only after the most intense condition tested (55 °C, 5 min) (Table 1). Unripe peppers have been shown to be more tolerant than ripe fruit to other postharvest treatments, including UV irradiation, CO_2 enrichment and O_2 reduction (Rodoni et al., 2015a,b).

Treatments at 45 °C for 1, 3 or 5 min and at 50 °C for 1 or 3 min markedly reduced deterioration in green pepper sticks when compared to the control (Table 1). These treatments did not damage the fruit and were equally effective to prevent pepper visual quality loss. In red fruit, the effective HT window was narrower; deterioration of ripe fruit was reduced by hot water treatments at 45 °C for 3 min (Table 1). The distinct heat susceptibility between green and red peppers may be due to differences in tissue physiological tolerance to high temperatures. However, this is not a general response and, in mango, heat tolerance has been shown to increase as fruit ripen (Jacobi et al., 2001). Green pepper showed higher resistance to blanching treatments than red fruit (Castro et al., 2008).

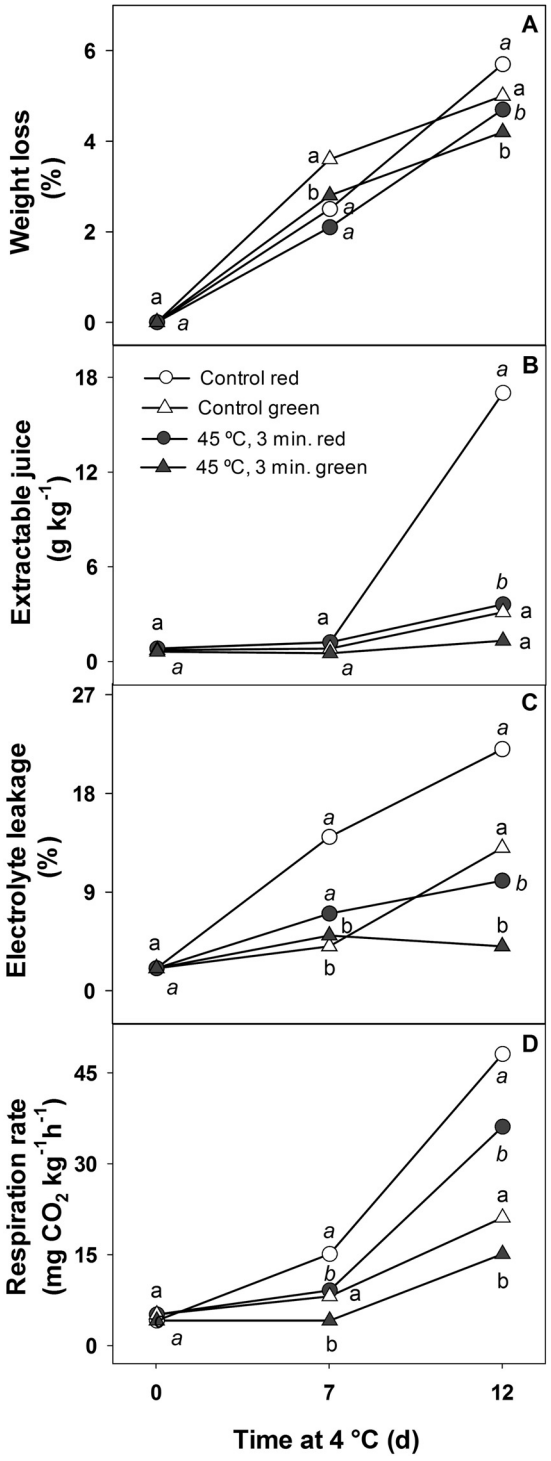

Fig. 2. Weight loss (A), extractable juice (B) electrolyte leakage (C) and respiration rate (D) in control and heat-treated (45 °C, 3 min) red and green organic pepper sticks stored for 0, 7 or 12 days at 4 °C and 90–95% HR. Different letters for a given ripening stage and storage time indicate significant differences based on a Fisher test at a level of significance of $P < 0.05$.

Previous work by Sgroppo and Pereyra (2009) reported that hot water pre-cutting dips at 55 °C for 3 min may be useful in fresh-cut green peppers. Results herein show that, when performed after cutting, pepper hot water treatments should not exceed 45 °C or 50 °C in red and green fruit respectively. Hot water treatments at 45 °C for 3 min were selected for further experiments to evaluate the effect of combining HT and low temperature storage on fruit physical, chemical and microbiological quality of organic red and green pepper sticks.

3.2. Quality retention of heat-treated green and red pepper sticks during refrigerated storage

The heat treatment did not have significant effect in fruit color, sugars, acidity or antioxidant capacity, but affected ion leakage, weight loss, decay and microbial populations (Table 2). Except for electrolyte treatment and extractable juice, the interaction between fruit ripening stage and HT was not significant.

3.2.1. Soft rot, shriveling and weight loss

In contrast to whole fruit in which *Botrytis* and *Alternaria* are the main causes of decay, spoilage of both green and red pepper sticks was mainly due to soft rots (SR). SR of fresh-cut vegetables has been usually associated with pectolitic microorganisms, including bacteria *Pseudomonas* spp., *Erwinia* spp. and several genera of fungi (Barth et al., 2010; Jacxsens et al., 2003). After 7 days of storage, the incidence of SR was significantly lower in heat-treated peppers regardless of the ripening stage. At the end of the storage period SR incidence was 2 and 4 fold lower in heat-treated (HT) red and green peppers than in the control (Fig. 1A).

Shriveling of cut surfaces was close to 20 and 4% in control and HT red pepper sticks after 1 week at 4 °C (Fig. 1B). Green treated pepper sticks showed shriveling only after 12 days of storage, but at much lower levels than the control. The reduction of shriveling of cut areas caused by the HT was associated with a decrease in weight loss (Fig. 2A). The HT green sticks showed lower weight loss than untreated fruit after both 7 and 12 d at 4 °C. Ripe peppers subjected to the HT also presented 20% lower weight loss than the control at the end of the storage period. These results differ from previous work in whole fruit, in which HT caused no variations or increased weight loss (Fallik et al., 1996; Ilić et al., 2012; González-Aguilar et al., 1998, 2000).

3.2.2. Extractable juice, electrolyte leakage and respiration rate

Fruit extractable juice showed no changes during the first week of storage. Subsequently, increased juice exudate was found in control peppers a both ripening stages, being higher in ripe fruit. At 12 days green and red sticks subjected to heat treatment presented 2 and 4 fold lower levels of extractable juice than the corresponding controls (Fig. 2B). Electrolyte leakage also increased with storage. At the last sampling date, both green and red sticks subjected to HT presented lower EL than the controls (Fig. 2C).

Table 3
Lightness (L^*), surface color (*hue*) and sugars in control and heat-treated (45 °C, 3 min) red and green organic pepper sticks stored for 0, 7 or 12 days at 4 °C and 90–95%.[a]

Time at 4 °C (d)		L^*		Hue		Sugars (g kg^{-1})	
		Red	Green	Red	Green	Red	Green
0	Control	31a	32a	50a	127a	43a	20a
	HT	31a	31a	50a	128a	44a	19a
7	Control	35a	32a	47b	128a	46a	20a
	HT	34a	30b	48b	127a	44a	19a
12	Control	33a	32a	46b	125b	45a	22a
	HT	33a	31a	51a	127a	41a	20a
Pooled sd		1		1		2	

[a] Values followed by different letters for a given ripening stage and storage time indicate significant differences based on a Fisher test at a level of significance of $P < 0.05$.

Table 4
Acidity, pH and antioxidant capacity against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals in control and heat-treated (45 °C, 3 min) red and green organic pepper sticks stored for 0, 7 or 12 days at 4 °C and 90–95%.[a]

Time at 4 °C (d)		Acidity (H^+ mmol L^{-1})		pH		DPPH$^{\bullet}$ ($kg^{-1} \times 10^{-5}$)		ABTS$^{\bullet+}$ (mmol of TE kg^{-1})	
		Red	Green	Red	Green	Red	Green	Red	Green
0	Control	28a	16a	5.0a	6.0a	4.0a	2.4a	8.2a	2.9a
	HT	27a	16a	5.0a	5.9a	4.3a	2.3a	8.0a	2.7a
7	Control	31a	13a	5.0a	6.2a	4.0a	2.7a	8.7a	2.5a
	HT	30a	12a	4.9a	6.4a	4.1a	2.6a	8.6a	2.5a
12	Control	34a	17a	4.7a	5.9a	3.8a	2.6a	7.6a	2.5a
	HT	32a	15a	4.7a	5.9a	3.6a	2.9a	7.6a	2.2a
Pooled sd		2		0.1		0.3		0.3	

[a] Values followed by different letters for a given ripening stage and storage time indicate significant differences based on a Fisher test at a level of significance of $P < 0.05$.

In contrast to Piazzolla et al. (2012) full-colored peppers showed in the current study similar respiration rate than mature-green peppers. Regarding the effects of HT on fruit respiration rate (RR) it has been reported that it could depend of the physiological age of the commodity and the type of treatment (Lurie 1998; Paull and Chen, 2000). Herein no differences were found between control and treated sticks prior to storage. This differs from previous reports by González-Aguilar et al. (1998, 2000) who found increased RR in heat treated peppers. This may have resulted from the milder conditions used in the present study. As fruit was hold at 4 °C the RR rose probably in response to tissue damage (Watada et al., 1996). The increase in RR was more marked in red sticks than in unripe fruit. However, at both ripening stages, the HT caused a lower increase in RR. At the last sampling date, the RR of HT sticks was 30% lower than that of control sticks (Fig. 2D). In whole red pepper a HT (55 °C for 12 s) also reduced respiration rate during storage (Fallik et al., 1999). Taken together, results indicated that mild water HT may be useful to reduce tissue disruption and organic fresh-cut pepper metabolic activity. Improved maintenance of tissue integrity may have also contributed to reduce the susceptibility of the host to SR. However, this should be confirmed by post-treatment inoculation studies.

3.2.3. Color, sugars, acidity and antioxidants

We further evaluated the changes in fruit physico-chemical quality during storage. Lightness (L^*) showed no variation during storage and was not affected by the treatments. The hue angle of red sticks was close to 50 at beginning of storage. The hue angle decrease in red control sticks at the end of storage. In contrast, HT peppers showed no variations in color throughout the storage period (Table 3). Carotenoid biosynthesis has been shown to slowly proceed in peppers even after harvest (Ilić et al., 2012). Some carotenoid biosynthetic enzymes are highly sensitive to heat. In tomato, exposure to temperatures as low as 38 °C inhibit the phytoene synthase, a key enzyme for biosynthesis of precursors of pepper ketocarotenoids (Lurie et al., 1996). The hue angle of control unripe sticks decreased during storage, indicating a slight loss of dark green surface color (Table 3). Previous works have been shown that HT can reduce chlorophyll degradation (Costa et al., 2005) by inhibiting the catabolic enzymes chlorophyllase and peroxidase (Funamoto et al., 2002). However, direct comparisons are not possible since the treatments used herein are less drastic than those reported in those studies. Even in case of lack of inhibition of these enzymes it is plausible that the HT prevented color changes by redirecting metabolism from senescence to heat acclimation responses (Spadoni et al., 2014). Regardless of the mechanism involved, results show that mild hot water treatment are effective to prevent color changes in both green and ripe fresh-cut peppers.

At harvest sugar content was higher ripe peppers than in green fruit (40 and 20 g kg^{-1} respectively) (Table 3). Similar results were reported by Piazzolla et al. (2012). Acidity was 100% higher in red peppers than in unripe fruit and, similarly to sugars, the changes during storage or in response to the treatments were negligible (Table 4). Antioxidant (AOX) capacity as measured by both the DPPH$^{\bullet}$ and ABTS$^{\bullet}$ assays was almost three fold higher in red fruit. This was likely due to a higher accumulation of ascorbic acid, which represents the most hydrophilic AOX in ripe pepper (Ornelas-Paz et al., 2013). Piazzolla et al. (2012) also reported that phenol content in full-colored peppers was higher than in mature-green fruit. Further studies to address the specific effect on each antioxidant group may be of useful. Even though a slight reduction in the AOX capacity was found during storage, but without differences derived from the HT, indicating that the nutritional value of the fruits was seldom affected (Table 4).

3.2.4. Bacteria, molds and yeast

Mold and yeast counts were close to 3.0 log CFU g^{-1} before storage. A slight decrease in counts was observed at day 7 in red stick, but at the end of storage period, mold and yeast counts were indistinguishable in controls and treated red sticks (Table 5).

Bacterial counts were before storage *ca.* 4.0 log CFU g^{-1} in red and green sticks. The HT did not cause an immediate reduction of the bacterial populations. An increase in bacterial CFU was found during storage in both control ant HT sticks, regardless of the ripening stage (Table 5). Noteworthy, only a slight reduction of microbial count (<1 log CFU g^{-1}) was found at 7 and 12 days despite of the effective control of soft rot, suggesting that other

Table 5
Aerobic mesophilic bacteria and molds and yeast in control and heat-treated (45 °C, 3 min) red and green organic pepper sticks stored for 0, 7 or 12 days at 4 °C and 90–95%.[a]

Time at 4 °C (d)		Bacteria (log CFU g^{-1})		Molds and yeast (log CFU g^{-1})	
		Red	Green	Red	Green
0	Control	3.7a	4.4a	2.9a	2.0a
	HT	3.6a	4.0a	2.4a	1.7a
7	Control	5.4a	5.8a	6.0a	4.6a
	HT	4.6b	5.6a	5.3b	4.1a
12	Control	7.6a	8.1a	7.5a	5.5a
	HT	7.6a	7.1b	7.6a	4.8b
Pooled sd		0.3		0.1	

[a] Values followed by different letters for a given ripening stage and storage time indicate significant differences based on a Fisher test at a level of significance of $P < 0.05$.

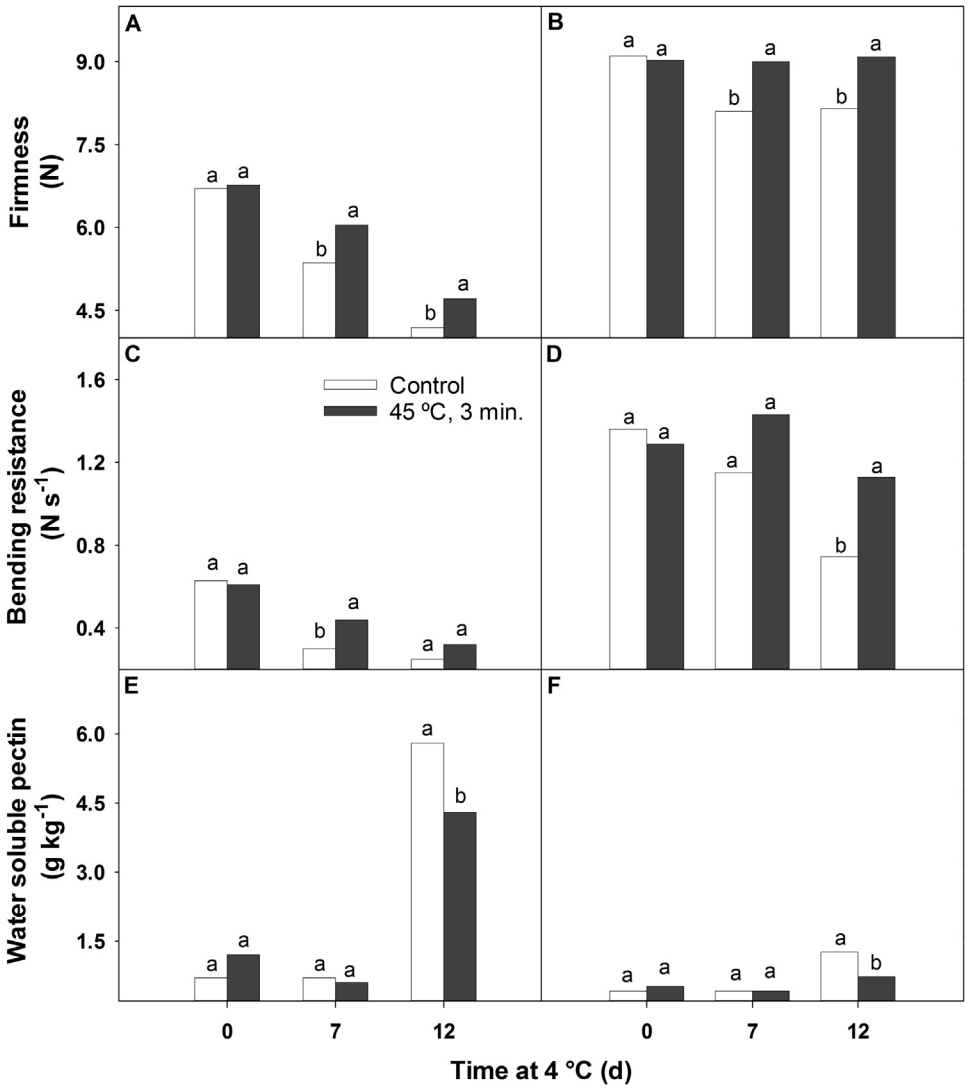

Fig. 3. Firmness (A and B), flex resistance (C and D) and water soluble pectin (E and F) in control and heat-treated (45 °C, 3 min) red (A, C and E) and green (B, D and F) organic pepper sticks stored for 0, 7 or 12 days at 4 °C and 90–95% HR. Different letters for a given ripening stage and storage time indicate significant differences based on a Fisher test at a level of significance of $P < 0.05$.

responses, besides biocide effects or microbial wash-off, were involved. *In vitro* studies has been found that germ tube elongation of *Botrytis cinerea* and *Alternaria alternata* spores were affected following 3 min at 45 °C (Fallik et al., 1996). Without being lethal a treatment of 40 °C for 5 or 10 min retarded *Monilinia fructicola* germ tube length and growth (Liu et al., 2012). In strawberry hot air treatments at 45 °C for 3 h did not affect *Rhizopus* and *Botrytis* conidia viability but reduced their germination rate (Pan et al., 2004). The effects of mild HT in postharvest spoiling bacteria have been less characterized and may deserve further studies.

The reduction in soft-rots at the end of the storage period may have also resulted from changes in the host susceptibility induced by the HT (Lurie 1998). A number of works have found that HT can induce defense responses including the reinforcement of ultra-structural barriers (Schirra et al., 2000) and the accumulation of antimicrobial compounds (Inkha and Boonyakiat 2010). Other stresses such as UV exposure have been recently shown to induce the accumulation of hydroxycinammic acid-derivatives in the surface of fresh-cut pepper (Rodoni et al., 2015b). Heat treatments have been also shown to induce wound healing responses (Shao et al., 2010), which may also reduce tissue susceptibility to pathogens. Passive responses such higher maintenance of tissue integrity, delayed ripening and senescence may also contribute to reduce tissue colonization by opportunistic pathogens.

3.2.5. Texture and water soluble pectin

Before storage, the firmness was *ca.* 6.7 and 9.0 N for red and green peppers respectively (Fig. 3A and B). Piazzolla et al. (2012) also reported lower firmness in mature yellow peppers compared with immature fruit. The HT was highly effective to prevent softening during storage, especially in green fruit. Control green sticks softened during the first week, as opposed to HT sticks, which maintained similar firmness values than at harvest. At the last sampling date, treated green sticks remained firmer than the control. The softening rate of red pepper sticks was also reduced by the HT. At 7th day the firmness dropped more rapidly in control red sticks. At the end of storage period the HT red sticks still remained firmer (Fig. 3A).

Prior to storage the bending resistance (BR) was almost 100% higher in green than in red sticks (Fig. 3C and D). During the initial 7 days of storage, the BR of control red sticks decreased by 50% and only half as much in HT sticks. As for firmness, the effect of HT on

stick rigidity was also more marked in green sticks. Treated green sticks hold, at the end of the storage period, similar BR values than at harvest as opposed to controls fruit which showed a clear drop.

We further determined the changes in water soluble pectin of control and treated sticks during storage. Pectin solubility increased markedly after 12 days of storage, especially in red ripe fruit. The HT delayed polyuronide solubilization in both green and red sticks (Fig. 3E and F). Although cell-wall degradation is limited in pepper compared to other fruit species softening to a melting texture (Jen and Robinson, 1984; Ogasawara et al., 2007; Cheng et al., 2008), firmness loss proceeds during postharvest storage even without substantial dehydration. The lower softening rate of HT sticks could result in part from the inhibition of the activity of cell-wall degrading enzymes. In tomato, a reversible polygalacturonase (PG) inhibition was observed during treatments at 38 °C (Lurie et al., 1996). In strawberry, warm air (45 °C, 3 h) caused a transient reduction in the expression of PG and other cell-wall degrading enzymes (Martínez and Civello, 2008). Though the treatments used in this work are milder than those usually reported to affect wall turnover, a superficial effect may be sufficient to delay cell-wall disassembly and improve tissue tolerance against pathogens. Another effect that has been observed in plant tissues subjected to mild HT is a stimulation of pectin methylesterases (Vicente et al., 2005). In this scenario, unesterified galacturonic acids residues would be readily available for ionic bridges stabilizing the pectic matrix (Grant et al., 1973). In addition, polygalacturonase would have lower affinity against Ca^{2+} pectates (Vicente et al., 2007). Low temperature blanching treatments have been used to improve firmness in some vegetables (Anthon and Barrett, 2006). In green pepper, a treatment at 70 °C for 1 min, applied prior to freezing, prevented softening (Castro et al., 2008). This effect has been found in heat treated apple slices (Kim et al., 1993), fresh-cut peach (Koukounaras et al., 2008), fresh-cut melon (Aguayo et al., 2008) and kiwifruit (Beirão-da-Costa et al., 2006). Whether or not this is the case in HT peppers sticks would require direct measurements. It is worth noting however, that the association between tissue firmness, pectin solubility and soft rot (SR) incidence was not high at day 7. At this sampling date, control peppers showed higher SR incidence and lower firmness, but the levels of water soluble pectin showed no differences between control and HT sticks. This suggests that other responses besides improved cell wall are contributing to reduce decay in HT fresh-cut organic peppers.

4. Conclusion

Results show that green fresh-cut peppers are more tolerant to hot water treatments than red ripe fruit. The window for effectively in heat treating unripe pepper sticks ranges from 45 °C 1 min to 50 °C for 3 min. In the case of red ticks the optimal temperature is 45 °C, without differences in the treatment outcome between duration of 1, 3 or 5 min. In a second part of this study, we determined the effect of combining hot water dips at 45 °C for 3 min with low temperature management on fruit physical, chemical and microbiological quality. This treatment markedly controlled soft rots, shriveling, weight loss and color changes during storage. In addition, it delayed softening and prevented the rise in electrolyte leakage, pectin solubility, extractable juice and respiration detected in untreated fruit. Moreover, the treatments did not cause negative changes in sugars, pH, acidity or antioxidant capacity. Overall, results indicate that short mild heat treatments (45 °C, 3 min) may be a simple and appealing non-chemical approach to supplement the benefits of low temperature management and extend the shelf-life of organic fresh-cut green and red peppers.

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