



UV-C treatments extend the shelf life of fresh-cut peppers by delaying pectin solubilization and inducing local accumulation of phenolics



L.M. Rodoni ^{a,*}, M.J. Zaro ^a, J.H. Hasperué ^a, A. Concellón ^{a,b,1}, A.R. Vicente ^{a,c,1}

^a CIDCA (Centro de Investigación y Desarrollo en Criotecología de Alimentos) (CCT La Plata CONICET-UNLP), 47 esq. 116, CP 1900, La Plata, Argentina

^b Comisión de Investigaciones Científicas Pcia de Buenos Aires (CIC-PBA), Argentina

^c LIPA (Laboratorio de Investigación en Productos Agroindustriales), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina, Calle 60 y 119, CP 1900, La Plata, Argentina

ARTICLE INFO

Article history:

Received 21 September 2014

Received in revised form

9 March 2015

Accepted 11 March 2015

Available online 19 March 2015

Keywords:

Quality

Postharvest

Fresh-cut

Irradiation

Minimally-processed

ABSTRACT

In recent years there has been increased interest in the search of environmentally-friendly treatments that could complement the benefits of proper temperature management in vegetables. In this work we evaluated the effect of UV-C treatments on quality retention of fresh-cut red peppers. To select the most suitable treatment condition red peppers sticks (5×1 cm) were UV-C irradiated (1.5; 3; 5; 6; 10 and 20 kJ m^{-2}) in the inner (I), outer (O) or both fruit surfaces (I + O). UV-C treatments with 10 kJ m^{-2} I + O were the most effective to reduce spoilage and were used for further evaluations. The selected treatment caused no alterations in sugars, color, acidity or antioxidant capacity and markedly reduced decay, weight loss, softening and pectin solubilization. UV-treated fruit also showed lower respiration rate and electrolyte leakage, indicating improved quality maintenance. UV-C exposure did not exert large direct germicide effects. Instead, histochemical analyses showed that the treatments elicited the superficial accumulation of hydroxycinnamic acid-derivatives (OH-CinAD). Overall, results show that UV-C treatments (10 kJ m^{-2} I + O) delay pectin solubilization and induce surface accumulation of phenolics and could be useful to complement low temperature storage and extend the shelf life of fresh-cut red peppers.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Fresh-cut vegetables have gained popularity in recent years in association with an increased consumer's interest in healthy and ready to use foods (James & Ngarmasak, 2011). However, the removal of natural barriers during processing markedly increases produce perishability (Singh & Alam, 2012; Mukhopadhyay & Ramaswamy, 2012).

Short UV-C irradiation has been suggested as a simple and environmental-friendly strategy to extend the postharvest life of fresh vegetables (Civello, Vicente, & Martínez, 2006). In addition to its germicide effect (Sinha & Häder, 2002), UV-C radiation induced beneficial physiological effects in plant tissues, delaying some

ripening and senescence-associated changes (Bu, Yu, Aisikaer, & Ying, 2013), activating defense genes (Pombo, Rosli, Martínez, & Civello, 2011) and inducing the accumulation phytoalexins (D'hallewin, Schirra, Manueddu, Piga, & Ben-Yehoshua, 1999). However, the responses of vegetables to UV-C are largely dependent on the radiation dose and fluence and on the commodity considered (Cote et al., 2013).

Bell peppers (*Capsicum annuum* L.) are, together with tomato, the most popular *Solanaceous* fruits worldwide. Peppers have been traditionally marketed at both green and red ripening stages as whole fruit. More recently they have been started to be offered as a fresh-cut vegetable snack (Tadesse, Hewett, Nichols, & Fisher, 2002). Even under proper refrigeration fresh-cut peppers have a relatively short postharvest life. Finding complementary post-harvest strategies could be useful to prevent quality deterioration. UV-C treatments decreased spoilage of fresh-cut green peppers (Rodoni, Concellón, Chaves, & Vicente, 2012). However, the responses of plants to UV radiation have been shown to depend on the developmental stage at which the tissues are irradiated (Civello et al., 2006). In order to improve the feasibility of UV-C treatments

* Corresponding author. CIDCA (Centro de Investigación y Desarrollo en Criotecología de Alimentos, Facultad de Ciencias Exactas-UNLP), Calle 47 y 116, CP 1900, La Plata, Argentina. Tel.: +54 2214249287; fax: +54 2214254853.

E-mail address: luisrodoni@gmail.com (L.M. Rodoni).

¹ Research Member of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

under commercial settings it is necessary to establish the proper treatment conditions for all the ripening conditions at which the fruit is marketed. In continuing our studies on UV-C responses of fresh-cut vegetables, the aim of this research was to select a suitable UV-C treatment for fresh-cut red peppers and to characterize the responses induced by the irradiation treatment in fruit quality and ripening physiology.

2. Materials and methods

2.1. Plant material and UV-C selection

Bell peppers (*C. annuum* L. cv. Jaen) grown in greenhouses in La Plata, Buenos Aires, Argentina, were harvested at the red stage and immediately transported to laboratory. Fruit was carefully selected for absence of decay and damage, and size uniformity. After that, fruit peduncles and seeds were removed and the pericarp was cut into 5×1 cm sticks, rapidly cooled to 10°C and then irradiated under a bank of 4 UV-C lamps (UV-C peak emission at 254 nm, TUV G30T8, 30W, Philips, Argentina) at a distance of 30 cm (fluence rate: 17 W m^{-2}) as follows:

- i) 3, 6, 10 or 20 kJ m^{-2} in the inner side of the pericarp (I)
- ii) 3, 6, 10 or 20 kJ m^{-2} in the outer side of the pericarp (O)
- iii) 1.5, 3, 5, 10 kJ m^{-2} in both the inner and outer sides (I + O).

The effective treatment dose was measured with a radiometer (Cole-Palmer Instrument Company, Vernon Hills, IL, USA). Untreated pepper sticks were used as controls. After the treatments the fruit was packed into plastic trays ($12\text{ cm} \times 10\text{ cm} \times 4\text{ cm}$) covered with perforated PVC film and stored at 10°C for 5 days. Ten independent trays containing 20 sticks and weighing approximately 100 g were prepared for each treatment. At the end of storage period, the sticks were visually inspected, and the number of sticks with soft rots was registered. The percentage of soft rot per tray was calculated and used to select the best treatment.

2.2. Quality of UV-C treated bell pepper sticks during low temperature storage

Red pepper sticks were prepared as described in Section 2.1. Samples were treated with 10 kJ m^{-2} of UV-C radiation (fluence rate: 17 W m^{-2}) in the inner and outer sides of the pericarp (I + O). After the treatment, the pepper sticks were put into plastic trays covered with perforated PVC film and stored at 4°C for 0, 7 or 12 days. A control group without UV treatment was directly packed and stored as mentioned above. Twelve trays containing 20 pepper sticks were prepared for each treatment and storage time. Measurements were done immediately after sampling or otherwise fruit tissue was frozen in liquid N_2 and stored at -80°C until use. The whole experiment was repeated twice.

2.3. Weight loss

Weight loss was determined by weighing groups of five pepper sticks during storage. Sixteen groups were evaluated for each treatment and storage time. Weight loss (WL) was calculated as: $\text{WL} = 100 \times (W_i - W_f)/W_i$, being W_i the initial sample weight and W_f the final weight.

2.4. Decay and extractable juice

The percentage of sticks showing soft rots or fungal decay was visually assessed. Twelve trays containing 20 sticks each were used for each treatment and storage time. For extractable juice

determination five pepper sticks were placed outer side up in a weighed filter paper and compressed by a normal force of 19.6 N for 30 s. The filter paper weight gain was determined. Measurements were done in triplicate and results were expressed as grams of extracted juice per kilogram of fresh weight (fw).

2.5. Electrolyte leakage

Two red pepper sticks were weighed and suspended in plastic tubes with 20 mL of water for 5 min. The conductivity of the incubating solution was measured at 20°C with a conductivity meter (Oakton Model 510, IL USA). To evaluate the total amount of electrolytes in the tissue, the pepper sticks were placed in plastic tubes and ground with an Omni mixer (Sorvall Inc., Norwalk, CT, USA). The obtained suspension was centrifuged at $10\,000 \times g$ for 10 min and the conductivity of the supernatant was measured as described above. Results were expressed as percentage of electrolytes leaked out from the tissues in 5 min. Three replicates were prepared for each treatment and storage time.

2.6. Texture

The texture of the bell pepper sticks was determined by two different assays in a Texture Analyzer (TA.XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY, USA). For flexion assays bell pepper sticks $5 \times 1\text{ cm} \times 4\text{ mm}$ thick were placed in a platform standing at 2 points 3 cm far from each other. A dented probe with rectangular section ($10 \times 1\text{ mm}$) 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 outer side was determined. The slope of the force/time was calculated and expressed in N s^{-1} (Flex Resistance, FR). Sixty measurements were done for each treatment and storage time.

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 (MF) developed during the test was recorded. Sixty measurements were done for each treatment and storage time. Results were expressed in N.

2.7. Sugars and acidity

Frozen fruit tissue was ground in a mill (Model A11, IKA Works Inc., SP Brazil) and 1 g of the resulting powder was vortexed for 1 min in 5 mL of cold ethanol and centrifuged at $15\,000 \times g$ for 10 min at 4°C . The supernatant was collected and the pellet re-extracted with 5 mL of cold ethanol. Supernatants were pooled and brought to 100 mL with water. Sugars were measured with anthrone reagent (Andersson, Westerlund, & Aman, 2006). Glucose was used as a standard and results were expressed as grams of glucose per kilogram of fw. Four measurements were done for each treatment and storage time.

For acidity, frozen tissue (10 g) was pulverized and added to 100 mL of water. Then samples were titrated with 0.1 mol L^{-1} NaOH until pH 8.2 (AOAC, 1980). Four independent measurements were done for each treatment and storage time. Results were expressed as $\text{H}^+ \text{ mmol kg}^{-1} \text{ fw}$.

2.8. Surface color

Surface color was measured with a colorimeter (Minolta, Model CR-400, Osaka, Japan) to obtain the L^* , a^* and b^* values. The hue angle was calculated as $\text{tg}^{-1} b^*/a^*$. Sixty measurements were done on the outer side of the sticks for each treatment and storage time.

2.9. Respiration rate

Approximately 150 g of pepper sticks from stored packages were incubated in a flask (3 L) for 20 min at 4 °C. CO₂ was measured with an infrared sensor (Alnor Compu-flow, Model 8650, Alnor, USA). Measurements were done in triplicate for each treatment and storage time and results were expressed in milligrams of CO₂ produced per kilogram of fw in an hour.

2.10. Antioxidant capacity against DPPH• and ABTS•⁺ radicals

Aliquots of the concentrated ethanolic extracts, prepared as described in Section 2.7, were used for antioxidant capacity evaluations. The DPPH• assay was done according to Brand-Williams, Cuvelier, and Berset (1995). The antioxidant capacity was defined as EC₅₀^d (kg⁻¹).

The ABTS•⁺ assay was performed according to Arnao, Cano, and Acosta (2001). Three measurements were done for each treatment and storage time. Results were expressed as mmol of Trolox equivalents (TE) per kg⁻¹ of fw.

2.11. Phenolic compounds

Aliquots of the concentrated ethanolic extracts, prepared as described in Section 2.7, were used to determine phenolic compounds according to Singleton, Orthofer, and Lamuela-Raventos (1999). Phenolic compounds were calculated by using gallic acid (GA) as standard. Samples were measured by triplicate and results were expressed mg of GA per kg of fw.

2.12. Histochemical localization of phenolic compounds

Phenolics *in situ* localization was done with Neu's reagent as described by Mondolot et al. (2006) with slight modifications. Phenolics related compounds were visualized by a light green fluorescence when excited under UV light. To start the staining, longitudinal pepper sticks sections (2 mm thick), were dipped in 10 mL of 10 g L⁻¹ 2-amino-ethyl-diphenylborinate in absolute methanol for 30 s. Samples were immediately examined in a light stereomicroscope (Modular Stereomicroscope Leica MZ10 F, Leica Microsystems Ltd., Germany). Samples were excited at 425 nm and emission at 480 nm was evaluated. Images were obtained with a digital color camera Leica DFC490 (Leica Microsystems Ltd., Germany, 8 megapixels). Negative controls were obtained by analyzing slices dipped in absolute methanol.

2.13. Yeast, molds and bacteria

Pepper sticks weighing 25 g were stirred in 225 mL of 1 g L⁻¹ peptone for 15 min in duplicate. Serial dilutions were made and poured into the appropriate media for aerobic bacteria (Petri-film™ plates 6400, 3 M, St. Paul, MN USA) and molds and yeasts (Petri-film™ 6407) in triplicate. Plates for aerobic mesophilic bacteria evaluations were incubated at 30 °C. For yeast and mold evaluations the plates were incubated at 20 °C for 5 days. Results were expressed as log of colony forming units (CFUs) per gram of fw.

2.14. Water soluble pectin (WSP)

Approximately 7 g of pepper sticks were ground in 20 mL of water with an Omnimixer. The suspension was vortexed and centrifuged at 10 000 × g for 10 min at 4 °C. The supernatant was precipitated by adding 3 volumes of cold ethanol. Samples were then centrifuged at 10 000 × g for 10 min at 4 °C, the pellet

dissolved in HAC/NaAc buffer (pH 5.0; 50 mmol L⁻¹) to obtain the water soluble pectins (WSP). Three independent extractions were done for each treatment, and storage time. Uronic acids in the WSP were determined as previously reported by Blumenkrantz and Asboe-Hansen (1973). Results are expressed as grams of galacturonic acid equivalents per kg of fw.

2.15. Statistical analysis

Experiments were performed according to a factorial design. Results were analyzed with the PC-SAS software package (SAS Institute Inc., Cary, NC, USA). The model assumptions of homogeneity of variance and normality were tested by means of Levene's and Shapiro–Wilk's tests, respectively. Means were compared by a Tukey test at a significance level of $\alpha \leq 0.05$.

3. Results and discussion

3.1. UV-C treatment selection

The initial screening experiments were conducted at 10 °C in order to select the most appropriate condition among 6 different UV doses and 3 exposure conditions (inner, outer and both surfaces) more rapidly. Although, fruit metabolism could result in different responses depending on the temperature such differences in this case (~5 °C) would not alter the relative outcome of the different treatments. After 5 days at 10 °C, 56% of control sticks showed incipient soft rots. UV-C treatments with a dose of 1.5 kJ m⁻² (I + O) or 3 kJ m⁻² on a single surface did not reduce soft rots (Table 1). Lower reduction of soft rots was found in fruit treated with 3 kJ m⁻² I + O and 6 kJ m⁻² I. The treatments with 5 kJ m⁻² I + O and 10 kJ m⁻² in one side reduced soft rot incidences by 30–41%. The highest reduction of soft rots was achieved when a UV-C dose of 10 kJ m⁻² was applied on both fruit sides. In this case only 7% of sticks presented soft rots after 5 days (Table 1). This shows that red fresh-cut peppers can tolerate a higher dose than that previously reported for whole fruits (Vicente et al., 2005). The treatments with 20 kJ m⁻² applied on one surface resulted in lower reduction of soft rots. This differs from green pepper, in which exposure to 20 kJ m⁻² UV-C in the O side was equivalent to a 10 kJ m⁻² I + O treatment (Rodoni et al., 2012). Based on these results, the treatment with a dose of 10 kJ m⁻² I + O was selected for further experiments combining UV-C exposure and low temperature storage.

Table 1

Incidence of soft rots in red bell pepper sticks treated with 0 (control); 1.5; 3; 5; 6; 10 or 20 kJ m⁻² of UV-C radiation in the inner, outer or both inner and outer, and stored at 10 °C for 5 days^{a,b}.

Treatment	Inner side (kJ m ⁻²)	Outer side (kJ m ⁻²)	Soft rots (%)
Control	0	0	56 ± 10ab
1.5 I + O	1.5	1.5	41 ± 9bcd
3 I	3	0	40 ± 8bcd
3 O	0	3	60 ± 8ab
3 I + O	3	3	40 ± 9bcd
6 I	6	0	47 ± 10abcd
6 O	0	6	53 ± 14abc
5 I + O	5	5	41 ± 3bcd
10 I	10	0	30 ± 11d
10 O	0	10	35 ± 10bcd
10 I + O	10	10	7 ± 5e
20 I	20	0	31 ± 18cd
20 O	0	20	36 ± 6bcd

^a The means ± the standard deviation are shown ($n = 10$).

^b Different letters indicate significant differences between treatments based on a Tukey's test ($\alpha \leq 0.05$).

3.2. Combination of UV-C treatment and low temperature storage

3.2.1. Mass loss, soft rots, fungal decay, extractable juice and electrolyte leakage

As reported for green peppers (Rodoni et al., 2012) no symptoms of chilling injury were observed in red pepper sticks throughout the storage period at 4 °C. Fruit shelf life was rather limited by soft rot incidence, excessive softening and dehydration. The photochemical

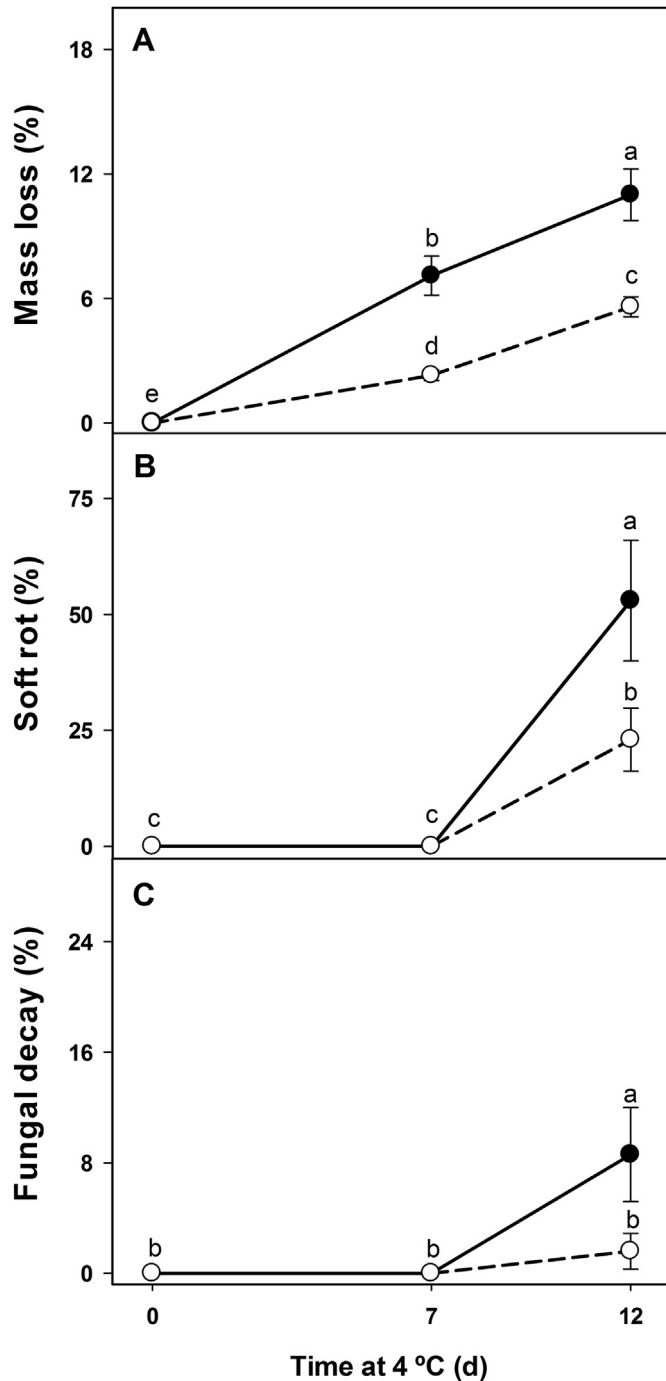


Fig. 1. A. Mass loss, B. soft rots and C. fungal decay in control (●) and UV-C treated (10 kJ m^{-2} , ○) red pepper sticks (—, △) stored for 0, 7 or 12 days at 4 °C. Different letters indicate significant differences between treatments based on a Tukey's test ($\alpha \leq 0.05$). The error bars represent the confidence interval ($n = 60$ and 12 for mass loss and soft rots and fungal decay respectively).

treatments have proven effectivity to reduce dehydration. Indeed, weight loss was, after 5 days of storage, two-fold higher in the controls than in UV-C treated sticks (Fig. 1). Manzocco et al. (2011) also found lower weight loss during refrigerated storage of UV-C treated apple cubes. The authors speculated that this could have resulted from the formation of a thin dried film in surface increasing the resistance to water flux. Treated peppers showed, at the end of the storage period, lower incidence of soft rots and fungal decay than the control (Fig. 1B and C). Untreated peppers also showed higher levels of the extractable juice (EJ) and electrolyte leakage (EL) during storage, indicating higher tissue and membrane disruption than UV-C treated sticks (Fig. 2A and B).

3.2.2. Texture

Control and UV-C treated sticks showed before storage similar firmness values (ca. 6.0 N) (Fig. 3A). After 12 days the firmness of control peppers decreased to 50%. In contrast no significant change in tissue firmness was found in UV-C treated fruit. The flex resistance (FR) of control sticks decreased from 0.15 to 0.10 N s^{-1} during the 12-d storage period while remaining unchanged in UV-C treated sticks (Fig. 3B). Vicente et al. (2005) showed that UV-C treatments retarded softening in whole red peppers. Water loss has been suggested as a major factor contributing to pepper softening (Díaz-Pérez, Muy-Rangel, & Mascorro, 2007). However, UV radiation has been shown to affect the activity of some cell wall

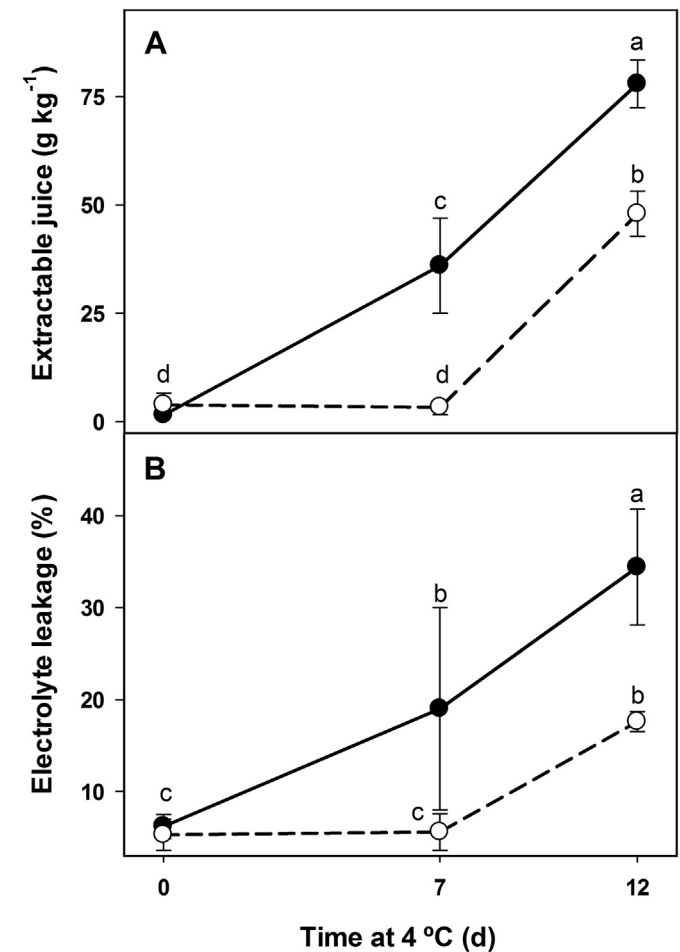


Fig. 2. A. Extractable juice and B. electrolyte leakage in control (■) and UV-C treated (10 kJ m^{-2} , ○) red pepper sticks (—, □) stored for 0, 7 or 12 days at 4 °C. Different letters indicate significant differences between treatments, based on a Tukey's test ($\alpha \leq 0.05$). The error bars represent the confidence interval ($n = 3$).

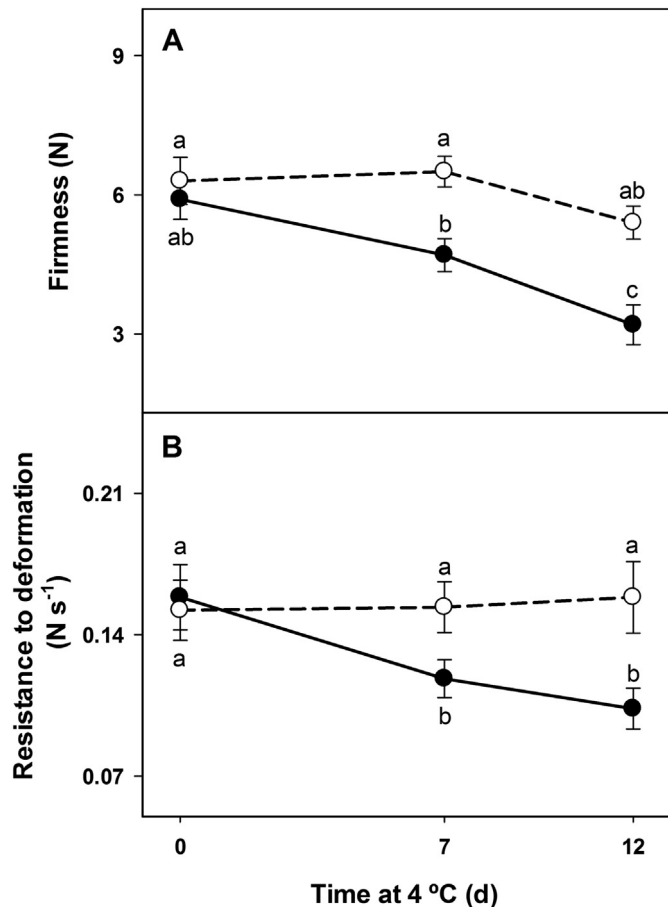


Fig. 3. A. Firmness and B. flex resistance in control (■) and UV-C treated (10 kJ m⁻², 1 + 0) red pepper sticks (□) during storage at 4 °C for 0, 7 or 12 days. Different letters indicate significant differences between treatments, based on a Tukey's test ($\alpha \leq 0.05$). The error bars represent the confidence interval ($n = 60$).

hydrolases (Civello et al., 2006) and their role on pepper softening is not fully understood.

3.2.3. Respiration rate, sugars, acidity, color, and antioxidants

The respiration rate (RR) of control pepper sticks was ca. 10 mg CO₂ kg⁻¹ h⁻¹ (Table 2) and increased three-fold during storage. In contrast to that, and in association with an improved maintenance

Table 2

Sugars, acidity, pH, color (hue and lightness), respiration rate and antioxidants capacity measured against the radicals DPPH• or ABTS^{•+} and total phenolics, in control and UV-C treated (10 kJ m⁻², 1 + 0) red pepper sticks during storage at 4 °C for 0, 7 and 12 d^{a,b}.

Time at 4 °C (d)		Sugars ^c	Acidity ^d	pH	Color		Respiration ^e	Antioxidants		
					Hue	Lightness		ABTS ^{•+} f	DPPH [•] g	Phenols ^h
0	Control	50 ± 2a	32 ± 3b	5.19 ± 0.01ab	45 ± 6a	32 ± 4bc	13 ± 2cd	5.5 ± 0.2bc	6.1 ± 0.3cd	995 ± 6c
	UV-C	48 ± 4a	30 ± 1b	5.34 ± 0.02a	43 ± 5ab	31 ± 3c	11 ± 1d	5.25 ± 0.1c	5.6 ± 0.1d	1018 ± 3c
7	Control	57 ± 1a	40 ± 2a	5.02 ± 0.01bc	42 ± 7ab	36 ± 3a	26 ± 1b	6.20 ± 0.1ab	7.6 ± 0.3ab	1185 ± 17a
	UV-C	49 ± 1a	38 ± 1a	4.92 ± 0.04c	39 ± 6b	36 ± 2a	17 ± 1bcd	5.7 ± 0.2abc	6.8 ± 0.2bc	1157 ± 22ab
12	Control	54 ± 4a	37 ± 1a	5.07 ± 0.09bc	43 ± 6ab	35 ± 3a	45 ± 2a	6.3 ± 0 a	7.4 ± 0.1ab	1029 ± 29c
	UV-C	44 ± 1a	40 ± 4a	4.94 ± 0c	41 ± 4ab	34 ± 2ab	25 ± 9bc	6.0 ± 0.3abc	7.8 ± 0.1a	1121 ± 12b

^a The means ± the standard deviation are shown ($n = 4$ for sugars, acidity and pH; $n = 60$ for color parameters; $n = 3$ for respiration; $n = 3$ for antioxidants).

^b Different letters indicate significant differences between treatments based on a Tukey's test ($\alpha \leq 0.05$).

^c Expressed as g kg⁻¹.

^d Expressed as mmol H⁺ kg⁻¹.

^e Expressed as mg CO₂ kg⁻¹ h⁻¹.

^f Expressed as mmol of TE kg⁻¹.

^g Expressed as kg⁻¹ × 10⁻⁵.

^h Expressed as mg of gallic acid kg⁻¹.

Table 3

Microbiological counts (yeast, mold and mesophilic aerobic bacteria), in control and UV-C treated (10 kJ m⁻², 1 + 0) red pepper sticks during storage at 4 °C for 0, 7 or 12 d^{a,b}.

		Time at 4 °C (days)		
		0	7	12
Yeasts and molds (log CFU g ⁻¹)	Control	3.3 ± 0.2c	5.6 ± 0.1b	7.4 ± 0.1a
	UV-C	3.2 ± 0.1c	5.6 ± 0.1b	7.3 ± 0.1a
Bacteria (log CFU g ⁻¹)	Control	3.0 ± 0.1de	5.2 ± 0.1c	8.0 ± 0.1a
	UV-C	2.6 ± 0.1e	3.4 ± 0.1d	7.3 ± 0.1b

^a The means ± the standard deviation are shown ($n = 3$).

^b Different letters indicate significant differences between treatments based on a Tukey's test ($\alpha \leq 0.05$).

of tissue integrity, UV-C treated peppers maintained lower RR than the controls.

Total sugar content was 50 g kg⁻¹ and no changes were found between control and treated fruit (Table 2). The antioxidant capacity (AC) measured against the ABTS^{•+} or DPPH• radicals increased during storage following the normal trend of ascorbic acid accompanying pepper ripening (Howard, Talcott, Brenes, & Villalon, 2000), without differences between treatments. Fruit acidity, surface redness and lightness (L*) increased slowly during storage, without differences between treatments (Table 2). Overall,

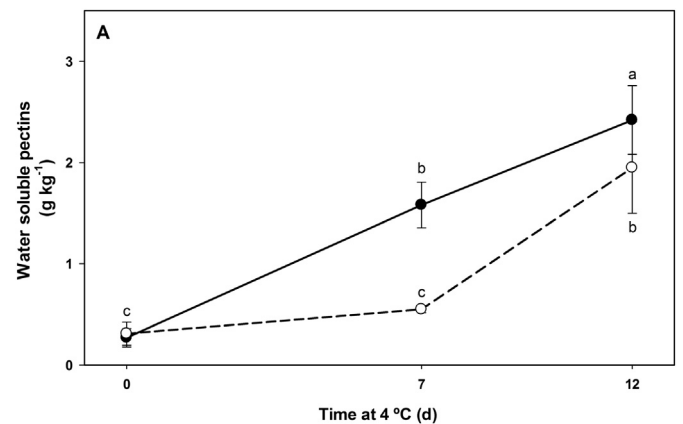


Fig. 4. Water soluble pectin in control (■) and UV-C treated (10 kJ m⁻², 1 + 0) red pepper sticks (□) during storage at 4 °C for 0, 7 or 12 days. Different letters indicate significant differences between treatments, based on a Tukey's test ($\alpha \leq 0.05$). The error bars represent the confidence interval ($n = 3$).

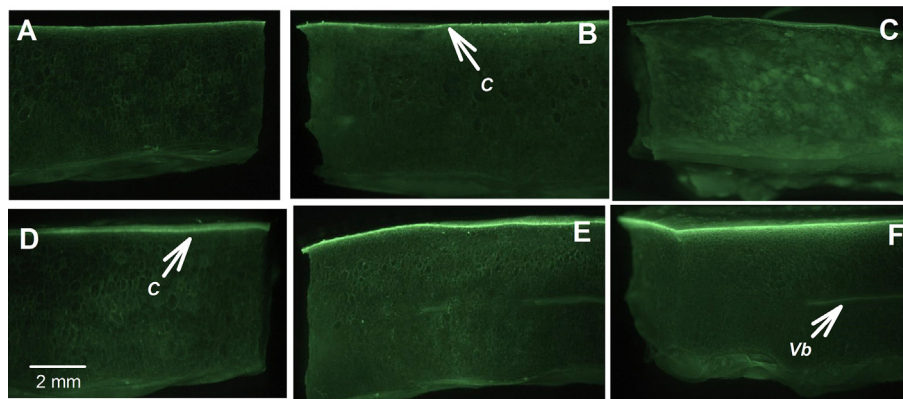


Fig. 5. Histolocalization of hydroxycinnamic acid phenolic-derivatives by fluorescence emission upon reaction with 2-amino-ethyl-diphenylborinate in control (A–C) and UV-C treated (10 kJ m^{-2} , I + O) red pepper sticks (D–F) during storage at 4°C for 0 (A, D); 7 (B, E) or 12 days (C, F). C: Cuticle, Vb: vascular bundles. Magnification: $12.5\times$.

the selected UV-C treatments reduced decay and prevented texture deterioration while not impairing other quality attributes.

3.2.4. Molds and bacterial counts

Despite the known germicidal effect of UV-C irradiation (Sinha & Häder, 2002), a very slight reduction in microbial populations was observed immediately after the UV-C treatment (Table 3). After 7 days of storage at 4°C , no differences in yeast and molds populations were found between control and treated sticks. In contrast, bacterial populations were lower in UV-C treated pepper after 7 days. At the end of storage, yeast and molds and bacterial counts reached comparably high levels in controls and UV-C treated peppers (Table 3). Interestingly irradiated peppers showed lower decay than the control (Fig. 1B and C), suggesting that the treatments may have modulated the host disease susceptibility.

3.2.5. Water soluble pectin

The level of water soluble pectin (WSP) before storage was ca. 0.3 g kg^{-1} (Fig. 4). WSP content rose to 1.6 g kg^{-1} in the controls during the first week of storage but showed no variation in irradiated peppers. After 12 days of storage UV-C treated fruit still presented lower level of WSP than the controls (Fig. 4). The selected treatment reduced the disassembly of cell wall polyuronides.

3.2.6. Analysis of phenolic compounds

The level of total phenolic compounds in control and UV treated were comparable when measured in whole sticks samples (Table 2). In order to further evaluate if UV-C treatments elicited the local accumulation of phenolics, we performed histochemical analyses of the pepper sticks by fluorescence microscopy after reaction of the fruit tissues with 2-amino-ethyl-diphenylborinate (DPB) (Mondolot et al., 2006). Phenolic compounds were present throughout the stick section but were predominantly accumulated in the cuticle region and central vascular bundles. The green fluorescence derived from the accumulation of hydroxycinnamic acid-derivatives increased after 7 days of storage in both control and UV-C treated sticks (Fig. 5A–F). Interestingly irradiated peppers showed higher accumulation of phenolics than control fruits. Towards the end of storage period, the signal intensity in the surface of control pepper sticks decreased. In turn, the mesophyll cells fluoresced suggesting a loss of cellular compartmentation (Fig. 5C). In contrast UV-C sticks still showed high fluorescence in the cuticle region (Fig. 5F). The induction of phenolics by UV radiation has been previously reported (Venditti & D'hallewin, 2014). However,

the plant responses to UV radiation are highly dependent on the fruit species, ontogenic stage, UV dose, and surface exposed. Studies with UV-B light (305–310 nm) on different organs of nasturtium (*Tropaeolum majus* L.) proved that plant response to short-term and moderate UV-B exposure is plant organ, tissue- and age-specific (Schreiner, Krumbein, Mewis, Ulrichs, & Huyskens-Keil, 2009) and in a number of studies, UV exposure did not elicit phytoalexin accumulation (Civello et al., 2006). UV-C irradiation with 10 kJ m^{-2} I + O was sufficient to induce the accumulation of hydroxycinnamic acid-derivatives in pepper fruit. In addition, the surface buildup of phenolics depicted from the microscopy images provided also interesting information regarding the local nature of the fruit response to the irradiation treatment. Phenolic compounds are known to inhibit microbial growth and development. Further work is needed to determine whether or not the increased levels of phenolics on the fruit surface contributed to the reduced decay found in UV-C treated pepper sticks. However, the lack of germicidal effects of the UV-C treatment applied, together with the known antimicrobial capacity of several phenolics (Lattanzio, Lattanzio, & Cardinali, 2006), provides support to this hypothesis (del Río et al., 2004; Charles, Goulet, & Arul, 2008). The potential defensive role of phenolics by cross-linking and reinforcing the cell walls (Schoch et al., 2001; Wang, Chantreau, Sibout, & Hawkins, 2013) cannot be ruled out and also deserves further investigation.

4. Conclusions

In this work we selected an appropriate UV-C dose (10 kJ m^{-2}) and mode of application (I + O) for fresh-cut ripe peppers. When combined with refrigerated storage the UV-C treatments markedly reduced soft rots, respiration and softening without impairing other quality attributes such as sugar, acids, color or antioxidant capacity. The UV-C treatment caused only minor germicide effects in molds and moderate reductions of mesophylic aerobic bacteria. Exposure to 10 kJ m^{-2} of UV-C radiation reduced pectin solubilization and induced the superficial accumulation of phenolic hydroxycinnamic acid-derivatives which may be modulating the host susceptibility to decay. UV-C treatments (10 kJ m^{-2} I + O) could be useful to complement low temperature storage and extend the shelf life of fresh-cut red peppers.

Acknowledgments

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (Argentina) Project PICT #2009-0059 and

Comisión Nacional de Investigaciones Científicas y Técnicas (CONICET) PIP-2009-0353.

References

- Andersson, R., Westerlund, E., & Aman, P. (2006). Cell-wall polysaccharides: structural, chemical and analytical aspects. In A. C. Eliasson (Ed.), *Carbohydrates in food* (p. 151). New York: CRC Press, Taylor & Francis Group.
- AOAC. (1980). *Official methods of analysis* (13th ed.). (p. 359). Washington, DC: Association of Official Analytical Chemists.
- Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry*, 73, 239–244.
- Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, 54, 484–489.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28, 25–30.
- Bu, J., Yu, Y., Aisikaer, G., & Ying, T. (2013). Postharvest UV-C irradiation inhibits the production of ethylene and the activity of cell wall-degrading enzymes during softening of tomato (*Lycopersicon esculentum* L.) fruit. *Postharvest Biology and Technology*, 86, 337–345.
- Charles, M. T., Goulet, A., & Arul, J. (2008). Physiological basis of UV-C induced resistance to *Botrytis cinerea* in tomato fruit IV. Biochemical modification of structural barriers. *Postharvest Biology and Technology*, 47, 41–53.
- Civello, P. M., Vicente, A. R., & Martínez, G. A. (2006). UV-C technology to control postharvest diseases of fruits and vegetables. In R. Troncoso-Rojas, M. E. Tiznado-Hernández, & A. González-León (Eds.), *Recent advances in alternative postharvest technologies to control fungal diseases in fruits and vegetables* (pp. 31–102). Kerakla, India: Transworld Research Network.
- Cote, S., Rodoni, L. R., Miceli, E., Concellón, A., Civello, P. M., & Vicente, A. R. (2013). Effect of radiation intensity on the outcome of postharvest UV-C treatments. *Postharvest Biology and Technology*, 83, 83–89.
- Díaz-Pérez, J. C., Muy-Rangel, M. D., & Mascorro, A. G. (2007). Fruit size and stage of ripeness affect postharvest water loss in bell pepper fruit (*Capsicum annuum* L.). *Journal of the Science of Food and Agriculture*, 87, 68–73.
- D'hallewin, G., Schirra, M., Manueddu, E., Piga, A., & Ben-Yehoshua, S. (1999). Scoparone and scopoletin accumulation and ultraviolet-C-induced resistance to postharvest decay in oranges as influenced by harvest date. *Journal of the American Society for Horticultural Science*, 124, 702–707.
- Howard, L. R., Talcott, S. T., Brenes, C. H., & Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum species*) as influenced by maturity. *Journal of Agriculture and Food Chemistry*, 48, 1713–1720.
- James, J. B., & Ngarmasak, T. (2011). *Processing of fresh-cut tropical fruits and vegetables: a technical guide*. Food and Agriculture Organization of the United Nations (FAO) (Accessed 2014). In: <http://www.fao.org/docrep/014/i1909e/i1909e00.htm>.
- Lattanzio, V., Lattanzio, V. M. T., & Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In F. Imperato (Ed.), *Phytochemistry: Advances in research* (pp. 23–27). Italy: Università degli Studi di Foggia.
- Manzocco, L., Da Pieve, S., Bertolini, A., Bartolomeoli, I., Maifrenia, M., Vianello, A., et al. (2011). Surface decontamination of fresh-cut apple by UV-C light exposure: effects on structure, colour and sensory properties. *Postharvest Biology and Technology*, 61, 165–171.
- Mondolot, L., La-Fisca, P., Buatois, B., Talansier, E., De-Kochko, A., & Campa, C. (2006). Evolution in caffeoylquinic acid content and histolocalization during *Coffea canephora* leaf development. *Annals of Botany*, 98, 33–40.
- Mukhopadhyay, S., & Ramaswamy, R. (2012). Application of emerging technologies to control Salmonella in foods: a review. *Food Research International*, 45, 666–677.
- Pombo, M. A., Rosli, H. G., Martínez, G. A., & Civello, P. M. (2011). UV-C treatment affects the expression and activity of defense genes in strawberry fruit (*Fragaria × ananassa*, Duch.). *Postharvest Biology and Technology*, 59, 94–102.
- del Río, J. E., Gómez, P., Báidez, A., Fuster, M. D., Ortuño, A., & Frías, V. (2004). Phenolic compounds have a role in the defense mechanism protecting grapevine against the fungi involved in Petri disease. *Phytopathologia Mediterranea*, 43, 87–94.
- Rodoni, L. M., Concellón, A., Chaves, A. R., & Vicente, A. R. (2012). Use of UV-C treatments to maintain quality and extend the shelf life of green fresh-cut bell pepper (*Capsicum annuum* L.). *Journal of Food Science*, 77, 632–639.
- Schoch, G., Goepfert, S., Morant, M., Hehn, A., Meyer, D., Ullmann, P., et al. (2001). CYP98A3 from *Arabidopsis thaliana* is a 3'-hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway. *Journal of Biological Chemistry*, 276, 36566–36574.
- Schreiner, M., Krumbein, A., Mewis, I., Ulrichs, C., & Huyskens-Keil, S. (2009). Short-term and moderate UV-B radiation effects on secondary plant metabolism in different organs of nasturtium (*Tropaeolum majus* L.). *Innovative Food Science and Emerging Technologies*, 10, 93–96.
- Singh, S., & Alam, M. S. (2012). Preservation of fresh-cut fruits and vegetables: current status and emerging technologies. *Stewart Postharvest Reviews*, 8, 1–10.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178.
- Sinha, R. P., & Häder, D. P. (2002). UV-induced DNA damage and repair: a review. *Photochemical & Photobiological Science*, 1, 225–236.
- Tadesse, T., Hewett, E. W., Nichols, M. A., & Fisher, K. J. (2002). Changes in physicochemical attributes of sweet pepper cv. Domino during fruit growth and development. *Scientia Horticulturae*, 93, 91–103.
- Venditti, T., & D'hallewin, G. (2014). Use of ultraviolet radiation to increase the health-promoting properties of fruits and vegetables. *Stewart Postharvest Reviews*, 10, 3.
- Vicente, A. R., Pineda, C., Lemoine, L., Civello, P. M., Martínez, G. A., & Chaves, A. R. (2005). UV-C treatments reduce decay, retain quality and alleviate chilling injury in pepper. *Postharvest Biology and Technology*, 35, 69–78.
- Wang, Y., Chantreau, M., Sibout, R., & Hawkins, S. (2013). Plant cell wall lignification and monolignol metabolism. *Frontiers in Plant Science*, 4, 1–14.