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Effect of radiation intensity on the outcome of postharvest UV-C treatments

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ABSTRACT

Studies on the use of UV-C radiation of fresh produce have focused on the selection of appropriate doses (energy per unit area) for different commodities, but little attention has been placed on the effect of radiation intensity (dose per unit time). In this study, tomatoes (*Solanum lycopersicum* cv. Elpida) and strawberries (*Fragaria* × *ananassa* cv. Camarosa), were harvested (breaker and 100% of surface red color respectively) and treated with 4 kJ m^{-2} of UV-C, at low (3 W m^{-2}) or high (33 W m^{-2}) radiation intensities. Untreated fruits were used as controls. After the treatments and at different storage times the incidence of postharvest rots and the changes in fruit physical and chemical properties were determined. UV-C treatments reduced decay, with the effects being were more marked in fruit exposed to high intensities. Mold counts were unaffected by the treatments, suggesting that improved disease control did not result from greater germicide effect. In both fruit species exposure to UV-C radiation delayed ripening, evidenced as lower color development, pigment accumulation and softening. UV-C-treated fruit maintained better quality than the control. In strawberry, high intensity treatments were more effective to prevent deterioration than in tomato where the differences between UV-C treatments were subtler. Soluble solids, titratable acidity and ethanol soluble antioxidants were not affected regardless of the UV-C intensity. Consumer tests showed higher preference of fruit treated at high UV-C intensity. Results show that in addition to the applied dose, radiation intensity is a main factor determining the effectiveness of UV-C treatments and should not be over-sighted. For a given dose, increasing radiation intensity may in some cases maximize the benefits of UV-C on fruit quality, while significantly reducing the treatments time.

1. Introduction

UV-C treatments have been used to maintain quality and reduce postharvest losses in fruits and vegetables (Civello et al., 2006; Shama, 2007). UV-C exposure delayed ripening (Liu et al., 1993), senescence (Costa et al., 2006; Allende et al., 2006) and reduced spoilage (Stevens et al., 1996; Baka et al., 1999). Elicitation of plant tissues with UV-C irradiation improved fruit antioxidant capacity (Adrian et al., 2000). In tomato and strawberry, UV-C treatments reduced deterioration (Stevens et al., 1998; Barka et al., 2000; Pan et al., 2004; Allende et al., 2007) and promoted the accumulation of antioxidants (Erkan et al., 2008).

Total dosage (energy per unit area) is a main factor determining fruit responses to UV-C (Civello et al., 2006), but the intensity of the radiation (dose per unit time) may also determine treatment outcome. Liu and Zhang (2006) showed that raising UV intensity may increase the control of *E. coli*, *Staphylococcus aureus* and *Candida albicans*. In the case of UV-B radiation, it is known that exposure to high fluencies induces genes associated with wound, defense and stress responses (A-H-Mackerness et al., 2001; Kilian et al., 2007), while lower intensities activate pathways involved in damage protection and acclimation against excessive radiation (Brown and Jenkins, 2008). Remarkably, most studies analyzing postharvest UV-C treatments on harvested commodities have focused on selecting appropriate doses, but the role of radiation intensity for a given dose has received almost no attention. In this work, we evaluated the influence of radiation intensity on the efficacy of UV-C treatments in tomato and strawberry as model systems for climacteric and non-climacteric fruits.

2. Materials and methods

2.1. Plant material

Tomatoes (*Solanum lycopersicum* L. cv. Elpida) and strawberries (*Fragaria* × *ananassa* Duch., cv. Camarosa) grown in greenhouses in

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La Plata, Argentina at the breaker stage or having 100% surface color red, respectively, were harvested and immediately transported to the laboratory. Fruits having uniform size and color and free of external blemishes or infections were selected.

2.2. UV-C treatments and storage

Fruit was irradiated under a bank of 12 UV-C lamps (UV-C peak emission at 254 nm, TUV G30T8, 30 W, Philips, Argentina). A dose of 4 kJ m^{-2} was applied at two different radiation intensities (low: 3 W m^{-2}; high: 33 W m^{-2}). The treatment times for high and low intensity irradiations were 120 and 1200 s, respectively. UV-C dosage and radiation intensity were monitored with a radiometer (Cole-Palmer Instrument Company, Vernon Hills, IL, USA). Fruit was rotated to ensure uniform surface exposure to UV radiation. When the treatments were finished, fruit was packed in groups of 16 in plastic trays, covered with perforated polyvinyl chloride (PVC) and stored (10 or 20 °C for strawberry and tomato, respectively). Untreated fruit, packed and stored as previously described, was used as a control. At each sampling day fruit was immediately analyzed or frozen in liquid nitrogen and stored at −80 °C until use. Sixty four fruits were evaluated for each treatment and storage time. The complete experiment was repeated twice in two independent harvests.

2.3. Quality evaluation

2.3.1. Decay and mold counts

The external appearance of each fruit and the presence of macroscopic fungal growth were visually evaluated. Results were expressed as percentage of decayed fruit.

For mold counts 25 g of fruit from control, low and high intensity UV-C-treated fruits before storage were stirred in 250 mL of sterile water for 15 min. From the resulting suspension, two series of dilutions from 10^{-1} to 10^{-4} were prepared and 1 mL of each dilution was seeded in an appropriate medium (Petrifilm™ Plates 6407, respectively; 3 M, Argentina) in triplicate. Plates were incubated at 20 °C for 5 days. Results were expressed as log of colony forming units (CFU) per gram of fresh fruit.

2.3.2. Weight loss

Fruit was weighed before packing and during storage. Weight loss (WL) was calculated from initial (IW) and final weights (FW) as described below:

$$\mathrm{WL}(\%) = \frac{\mathrm{IW} - \mathrm{FW}}{\mathrm{IW}} \times 100$$

2.3.3. Color

Surface color was evaluated with a colorimeter (Minolta, Model CR-400, Osaka, Japan) by measuring the L^*, a^* and b^* values. The hue angle and chroma were calculated as $t\,g^{-1}$ b^*/a^* and $(a^{*2} + b^{*2})^{1/2}$, respectively. Sixty measurements were done in both strawberry and tomato for each treatment and storage time.

2.3.4. Anthocyanins

Frozen strawberry fruit was ground in a mill and 0.1 g of the resulting powder was poured into 5 mL of methanol containing 1% v/v HCl. The slurry was centrifuged at 9000 × *g* for 10 min at 4 °C, the supernatant was saved and its absorbance at 515 nm was measured. Antocyanin content was calculated by using the extinction coefficient of pelargonidin-3-glucoside the most abundant anthocyanin in strawberry (ε = 36,000 L mol^{-1} cm^{-1}) (Woodward, 1972) Measurements were performed in triplicate and results were expressed as micromol per kilogram of fresh fruit.

2.3.5. Lycopene

Frozen tomato was ground in a mill and 0.1 g of the resulting powder was extracted with 5 mL of hexane:acetone:ethanol (2:1:1). The sample was vortexed and 1 mL of water was added. The upper phase was carefully extracted and its absorbance at 503 nm was determined. Measurements were performed in triplicate. Results were calculated by using ε = 172,000 L mol^{-1} cm^{-1}(Taber et al., 2008) and expressed as milligrams of lycopene per kilogram of fresh weight.

2.3.6. Firmness

Firmness was determined in a texture analyzer (model TA.XT2; Stable Micro Systems Texture Technologies, Scarsdale, NY, USA) by compressing 3.5 or 10 mm the strawberry or tomato fruit tissue in the equatorial zone, respectively, at a rate of 1 mm s^{-1} with a 3 mm-diameter probe. The force during the test was recorded as a function of distance. Thirty fruit having similar size were used for each treatment and storage time and measured twice on opposite sides. Results were expressed as the slope of the curve in N mm^{-1}.

2.3.7. Respiration

The respiration rate was measured with a CO_2 an IR sensor (Alnor Compu-flow, Model 8650, Alnor USA). Fruit trays were enclosed in tightly-sealed hermetic flasks and gases were allowed to accumulate for 20 min and incubated. Oxygen levels never dropped below 18% and CO_2 levels remained below 0.2% in all treatments and perfect linearity was observed during 1 h confinement. The sensor was introduced in the flask to perform CO_2 measurements and the respiration rate was calculated. Results were expressed in mmols of CO_2 kg^{-1} s^{-1}. Three replicates were evaluated for each treatment and storage time.

2.3.8. Soluble solids

Fruit was ground in a mill and the obtained slurry was filtered through a cloth. Soluble solid content was measured in a calibrated digital refractometer (Atago, WA, USA). Four measurements were done for each treatment and storage time. Results were expressed as g kg^{-1} of fresh fruit.

2.3.9. Antioxidant capacity

The free radical scavenging capacity of tomato and strawberry fruit was tested according to the procedure described by Brand-Williams et al. (1995). Samples (0.8 g for strawberry and 1.2 g for tomato) were ground in 5 mL of ethanol and the mixture was centrifuged at 9000 × *g* for 10 min at 4 °C. Aliquots of this ethanolic extracts (0, 150, 300, 450 and 600 μL) and ethanol to a final volume of 1 mL were added to test tubes containing 3 mL of 0.040 g L^{-1} 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) in methanol prepared daily. The absorbance at 515 nm was measured at different times with a spectrophotometer (DU650 Model, Beckman Instruments Inc., Berkeley, CA, USA) until the reaction reached a plateau (90 min). The percentage of remaining $DPPH^{\bullet}$ against the extract volume was then plotted to obtain the amount of sample necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50%, which was defined as EC_{50}. The antioxidant capacity was expressed as $EC_{50}{}^{-1}$ in kg^{-1}. Two independent extracts were prepared for each fruit, treatment and time point evaluated and each measurement was done in triplicate

2.3.10. Titratable acidity

Frozen fruit was ground in a mill and 10 g of the obtained powder were suspended in 100 mL of H_2O. Acidity was determined by titration with 0.1 mol L^{-1} NaOH until pH 8.2 (AOAC, 1980). Results were expressed as mmol of H^+ L^{-1}. Four extracts were prepared for each treatment and storage time.

2.3.11. Sensory analysis

A sensory analysis based on a preference test with 40 non-trained consumers was performed (Hough et al., 2006; Ryffel et al., 2008). Control and UV-C-treated (4 kJ m^{-2}, at both high and low intensity) fruit stored for 2 days were presented to consumers. The samples were exhibited in a random position and coded with a three digit number. The panelist evaluated and scored the samples between like and dislike based on visual and manual inspection similar to those performed at purchasing centers. The results were analyzed assigning the score: 3 = most, 2 = moderate, 1 = least preferred; and finally adding the scores of each sample.

2.4. Statistical analysis

Experiments were performed according to a factorial design. Data were analyzed by ANOVA and the means were compared by a Tukey test at a significance level of 0.05.

3. Results

3.1. Decay and mold counts

Control strawberries showed higher decay than UV-C-treated fruit, independently of the intensity used (Fig. 1A). The main cause of decay was fungal attack by *Botrytis*. Changing UV radiation fluency resulted in clear differences in the incidence of postharvest rots. After 5 days at 10 °C, only 12% of the strawberries irradiated with the highest intensity showed fungal growth, compared to 46% and 68% in low intensity-treated and control fruit respectively. The berries irradiated with an intensity of 33 W m^{-2} also presented less exudate and calyx wilting than untreated controls and fruit exposed low fluency UV-C (data not shown). A reduced incidence of postharvest rots as a result of UV-C exposure was also found in tomato. After 9 d of storage at 20 °C, 23% of the control fruit were decayed while only 8% and 5% of low and high UV intensity presented disease symptoms, respectively (Fig. 1B). Interestingly no differences in the counts of yeast and molds were found among treatments immediately after irradiation (data not shown).

3.2. Weight loss and respiration rate

Non-irradiated strawberry fruit lost ~6 and 11% of fresh weight after 3 and 5 days at 10 °C and similar values were found in fruit irradiated with low intensity UV-C. The treatment performed at the highest intensity significantly reduced weight loss (Fig. 1C). In tomato, after 4 days of storage at 20 °C no differences were found between control and treated fruit regardless of the UV intensity. In contrast, after 9 days at 20 °C, fruit irradiated with high UV-C fluency showed lower weight loss than the control (Fig. 1D).

Strawberry respiration rate increased during storage. After 5 days control fruit presented higher values than UV-C irradiated fruit (Fig. 2C). In tomato the respiration rate showed an increasing tendency during the shelf-life period, but no differences were detected among treatments (Fig. 2D).

Fig. 2A and B shows the results of a consumer preference test for stored strawberries and tomatoes either untreated or exposed to low or high UV-C intensity. Fruit evaluation was performed after 2 days storage. This time point was selected because we did not want consumers to discriminate between healthy and decayed fruit. The selection was made for each treatment based on visual and manual inspection. In strawberry, UV treated fruit at both low and high fluency showed higher preference than the control. The highest preference corresponded to high UV-C intensity-treated fruit. In tomato, the consumers preferred fruit treated with high UV-C intensity and no differences were detected between fruit subjected to low fluency irradiation and the control.

3.3. Color, pigments and sensory analysis

Strawberry fruit lightness (L^*) decreased during storage (Fig. 3A). UV-C treatment delayed darkening, being the effect similar for both intensities. Surface color saturation (chroma)

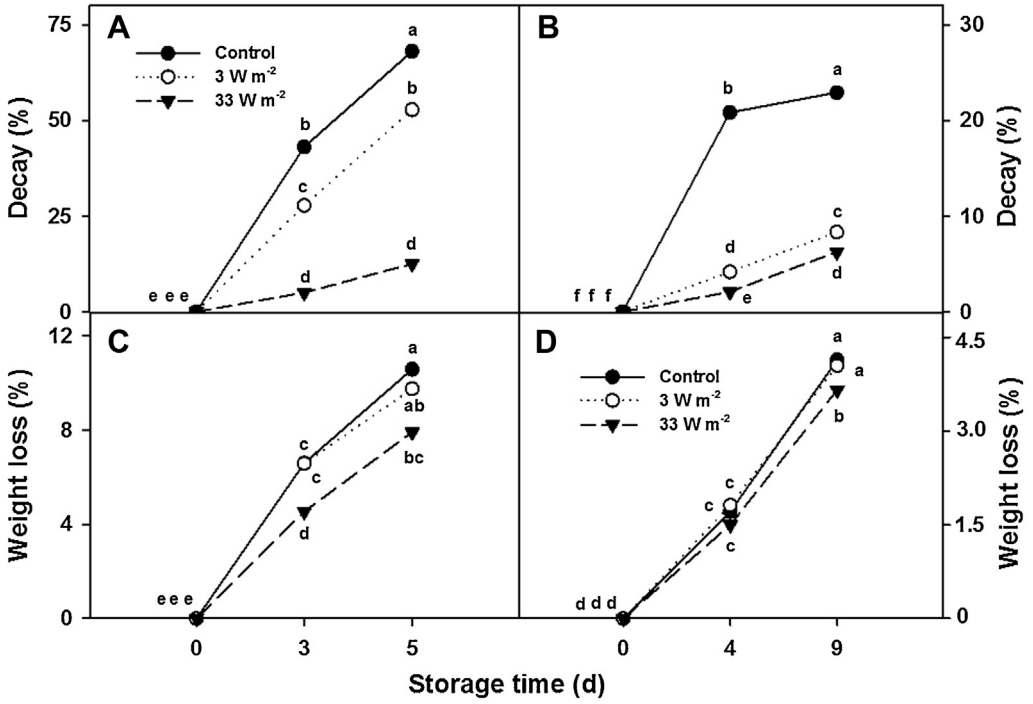

Fig. 1. Decay (**A**, **B**) and weight loss (**C**, **D**) in control and UV-C-treated (4 kJ m^{-2}) strawberry (**A**, **C**) and tomato fruit (**B**, **D**) with different intensities of radiation (3 or 33 W m^{-2}) during storage. Different letters indicate significant differences on a Tukey test at a level of significance of $P \leq 0.05$.

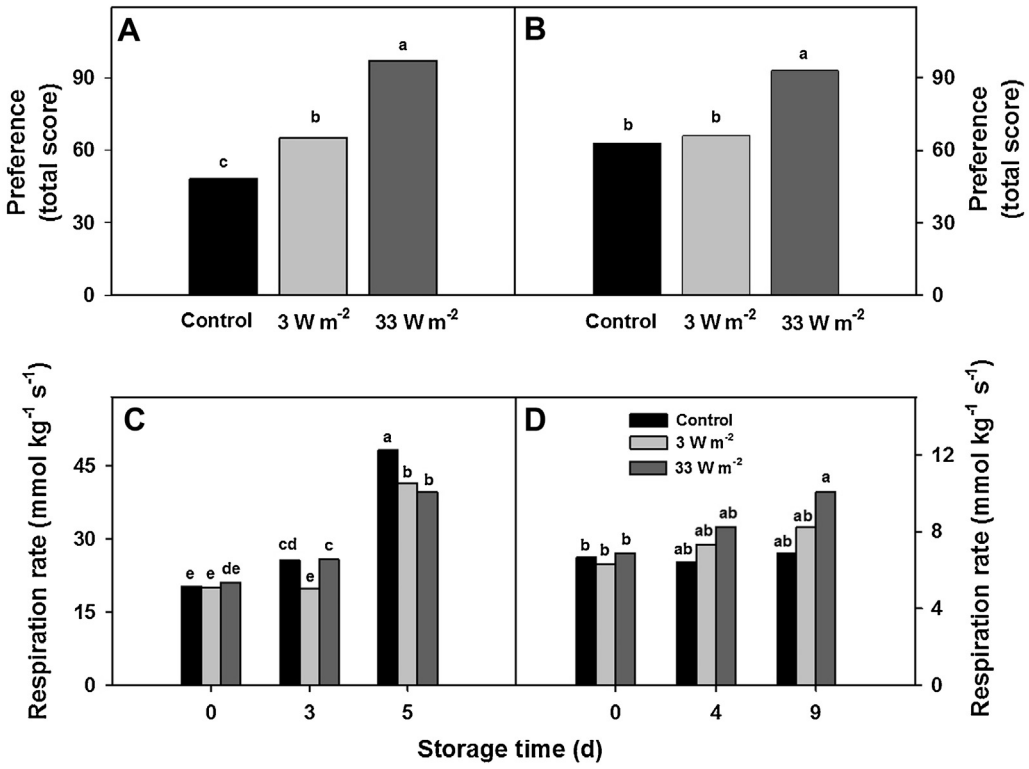

Fig. 2. Consumer preference (**A**, **B**) and respiration rate (**C**, **D**) in control and UV-C-treated (4 kJ m^{-2}) strawberry (**A**, **C**) and tomato fruit (**B**, **D**) with different intensities of radiation (3 or 33 W m^{-2}) during storage. Different letters indicate significant differences on a Tukey test at a level of significance of $P \leq 0.05$.

also decreased as storage time progressed, but this change being slowed-down by exposure to high UV-C intensity. Instead, no difference was observed between control and low UV-C intensity-irradiated fruit (Fig. 3C). The hue angle showed a continuous reduction during storage accompanying red color development (Fig. 3E). After 3 days of storage, fruit treated with a fluency of 33 W m^{-2} maintained higher hue values than the control. The differences were also observed after 5 days at 10 °C. The delay in color development was associated with a lower accumulation of anthocyanins. After 3 days, the anthocyanin content of high fluency treated fruit was already below of that found in the controls (Fig. 4C). Subsequently, anthocyanin concentration increased in both control and low intensity treated strawberries. After 5 days, high intensity UV-C-treated fruit presented 540 μmol kg^{-1} of pelargonidin-3-glucoside. At this time point, anthocyanin concentration was 25% higher in control and low intensity UV-C-treated fruit. Similarly to strawberry, tomato fruit the lightness and hue angle decreased in during storage, while color saturation increased (Fig. 3B, D and F). The effect of UV-C treatment on tomato color was lower than in strawberry (Fig. 3B); no effect on chroma or hue angle was detected in response to irradiation at both fluencies assayed (Fig. 3D and F). Lycopene showed a gradual increase during storage in all fruit groups and no differences among them were found either (Fig. 4B).

3.4. Firmness

Firmness decreased almost 50% in control strawberries after 3 days of storage at 10 °C (Fig. 4C). Softening was significantly reduced in UV-C-treated fruit. High UV radiation intensity was more efficient to prevent softening. In fact, during the first 3 days of storage no changes in firmness were detected in high UV-C intensity-treated fruit. After 5 days the UV-C-treated fruit still remained firmer than the control. In tomato, there was a slight reduction of softening by UV-C treatments but no differences resulted from changing the radiation intensity (Fig. 4D).

3.5. Soluble solids, titratable acidity and antioxidant capacity

UV-C exposure did not alter the levels of soluble solids neither in tomato nor in strawberry. Similarly, fruit antioxidant capacity did not change significantly during storage and was not affected by the UV-C treatments (Tables 1 and 2). Titratable acidity was slightly reduced in tomato fruit exposed to a UV-C intensity of 33 W m^{-2}. In strawberry it increased during storage (Table 1). Interestingly, fruit irradiated at high intensity showed lower values than the control at the end of the storage period. In contrast tomato, high intensity irradiation resulted in lower acidity at the end of the storage period.

Table 1
Soluble solids, titratable acidity and antioxidant capacity in control and UV-C treated (dose 4 kJ m^{-2}) strawberry fruit with low (3 W m^{-2}) or high (33 W m^{-2}) intensity during storage at 10 °C for 0, 3 or 5 days. Different letters indicate significant differences on a Tukey test at a level of significance of $P \leq 0.05$.

		Time at 10 °C (days)		
		0	3	5
Soluble solids (g kg^{-1})	Control	101 a	104 a	92 a
	3 W m^{-2}	95 a	102 a	100 a
	33 W m^{-2}	95 a	93 a	94 a
Titratable acidity ([H$^+$] mmol L^{-1})	Control	169.8 de	160.3 ef	259.9 a
	3 W m^{-2}	174.9 cd	155.7 f	246.0 b
	33 W m^{-2}	170.9 de	182.1 c	248.1 b
Antioxidant capacity (kg^{-1})	Control	515.4 a	503.2 a	553.9 a
	3 W m^{-2}	458.2 a	453.9 a	641.6 a
	33 W m^{-2}	364.7 a	465.9 a	591.2 a

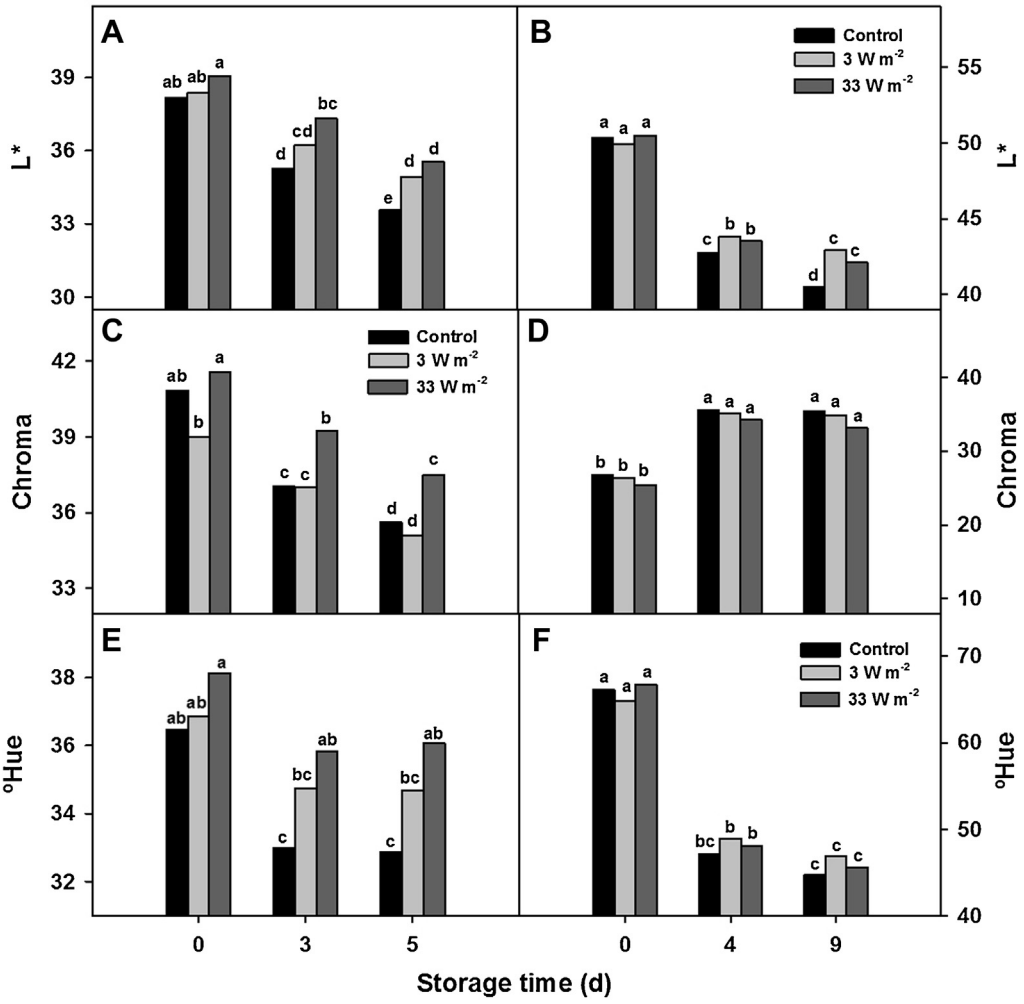

Fig. 3. Lightness (**A**, **B**), color saturation (chroma) (**C**, **D**) and tone (hue) (**E**, **F**) in control and UV-C-treated (4 kJ m^{-2}) strawberry (**A**, **C**, **E**) and tomato fruit (**B**, **D**, **F**) with different intensities of radiation (3 or 33 W m^{-2}) during storage. Different letters indicate significant differences on a Tukey test at a level of significance of $P \leq 0.05$.

4. Discussion

In the last decade there has been an increased concern about the use of agrochemicals in fresh produce. Some pesticides have been banned and in many cases legislation has moved toward a reduction in residue limits allowance. Since then, more research efforts have focused on residue-free technologies to maintain the quality of fresh fruits and vegetables such as UV-C (Shama, 2007). Several studies have evaluated the effect of different UV-C doses, but strikingly the influence of the intensity of the radiation has received almost no attention. Various studies have shown that UV-C treatments applied at appropriate doses reduced decay in strawberry and tomato (Maharaj et al., 1999; Pan et al., 2004; Erkan et al., 2008). This effect is well documented, and has been associated with both germicide properties of the radiation as well as activation of defence mechanisms (Charles et al., 2009; Pombo et al., 2010).

Table 2
Soluble solids, titratable acidity and antioxidant capacity in control and UV-C-treated (dose 4 kJ m^{-2}) tomato fruit with low (3 W m^{-2}) or high (33 W m^{-2}) intensity during storage at 20 °C for 0, 4 or 9 days. Different letters indicate significant differences on a Tukey test at a level of significance of $P \leq 0.05$.

		Time at 20 °C (days)		
		0	4	9
Soluble solids (g kg^{-1})	Control	42 a	39 a	38 a
	3 W m^{-2}	39 a	38 a	41 a
	33 W m^{-2}	41 a	40 a	43 a
Titratable acidity ([H^+] mmol L^{-1})	Control	62.63 ab	67.56 a	58.51 bc
	3 W m^{-2}	66.28 a	59.84 bc	56.59 c
	33 W m^{-2}	58.34 bc	55.55 c	48.70 d
Antioxidant capacity (kg^{-1})	Control	46.6 a	43.9 a	43.6 a
	3 W m^{-2}	45.2 a	43.7 a	41.2 a
	33 W m^{-2}	42.8 a	48.1 a	43.7 a

The germicidal effect of UV radiation is affected by the dose applied and the microorganism considered. Marquenie et al. (2002) found that the viable conidia of *Botrytis cinerea* decreased linearly with the applied UV-C dose. Increasing the intensity of UV radiation resulted in greater inactivation of pathogens in water (Sommer et al., 1996a,b). In the present work, the treatments did affect mold viability, showing that greater decay control by high intensity UV-C irradiation, at this dose, is not due to risen germicide action. This, together with the large difference of decay among treatments, suggests that some indirect mechanism mediated by the fruit might be involved. Several studies have demonstrated that UV-C radiation induces defensive responses in fruits which my in turn affect the susceptibility of the hosts against rotting microorganisms (D'hallewin et al., 2000, Mercier et al., 1993 and Mercier et al., 2001; Douillet-Breuil et al., 1999). Pombo et al. (2010) found that UV-C treatment of strawberries increases the expression of genes and the activity of defensive enzymes. The activation of genes coding for β-1,3-glucanases and chitinases by UV-C has been

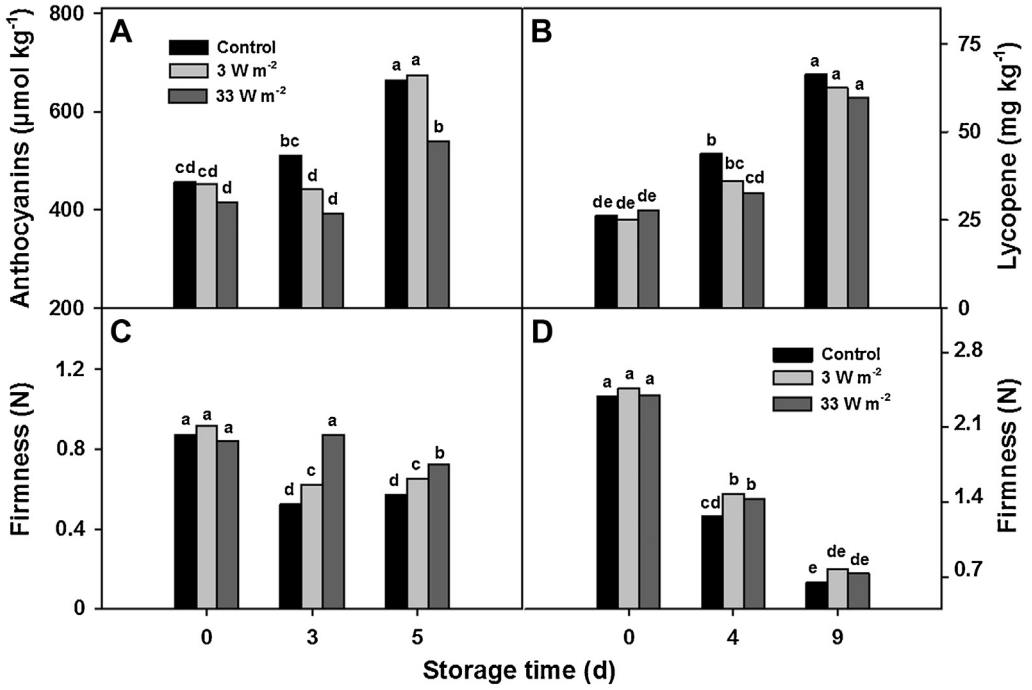

Fig. 4. Anthocyanins (**A**), lycopene (**B**) and firmness (**C**, **D**) in control and UV-C-treated (4 kJ m^{-2}) strawberry (**A**, **C**) and tomato fruit (**B**, **D**) with different intensities of radiation (3 or 33 W m^{-2}) during storage. Different letters indicate significant differences on a Tukey test at a level of significance of $P \leq 0.05$.

reported (El Ghaouth et al., 2003), but the effect of UV-C intensity on the activation of these defense responses is not well established. Schreiner et al. (2009) suggested that UV radiation fluency can significantly affect the accumulation of phenolics with antimicrobial properties. It would be of great interest to determine the intensity-dose response on the activation of defense mechanisms. Moreover, the reinforcement of cell wall by cross-linkages of phenolic compounds, lignification and suberization in response to UV exposure has also been reported (Charles et al., 2008).

UV-C treatments may also affect the microorganisms' virulence. Pan et al. (2004) reported that UV-C irradiation, delayed the germination of *Botrytis* and *Rhizopus* conidia. A general delay of ripening may result in fruit with reduced susceptibility against spoilage-causing organisms (Barka et al., 2000).

Irradiated fruit showed lower weight loss and delayed color development and softening than the control. It is important to note that both strawberries and tomatoes treated at the highest intensity reached full ripening for consumption. The reduction of fruit weight loss was more marked in fruit treated at high UV intensity. This could be associated with improved maintenance of tissue integrity, but some studies have also reported that exposure to UV-C could induce the biosynthesis of hydrophobic healing polymers such as suberin (Charles et al., 2008). Moreover, fresh-cut apples treated with UV-C showed lower dehydration that the control. This effect was associated with the formation of a thin film on the product surface hindering water evaporation (Manzocco et al., 2011).

Exposure to high intensity UV-C in strawberry increased the efficacy of the treatments, reducing softening, one of the main changes limiting its postharvest life. Pigment biosynthesis and surface darkening were also decreased by UV-C treatment. Also for these attributes the effect of the radiation fluency was less accentuated in tomato. It may be hypothesized that low intensity UV-C might have been sufficient to reduce ethylene biosynthesis and have a similar impact than high intensity treatment, but this needs to be further evaluations. Previous studies have indicated that some antioxidant compounds could be affected by UV-C irradiation. However the results are highly variable. Allende et al. (2007) found that UV-C exposure decreased procyanidin content and antioxidant compounds in strawberry. In contrast, Erkan et al. (2008) found increased ORAC in UV-C-treated strawberries. In the present study, the content of hydrophilic antioxidant contents was not affected by the UV-C treatments. Results show that the elicitation or degradation of bioactive compounds is greatly dependent on the variety tested. Finally the treatments did not caused dramatic changes in soluble solids or acidity. The increase in titratable acidity at the end of the storage period in strawberry could be related to the fact that fruits already harvested 100% red are reaching over-ripeness and initiating senescence. Although the fruit is still aerobically respiring some cells and zoned may have under these conditions fermentative reactions may be favored. In addition to that, accumulation of TCA intermediates may occur.

Overall, the study suggests that besides the total dose, the intensity of the radiation is a key factor affecting the efficacy of the treatments and that the effects are distinct in different fruits. High UV-intensity may in some cases increase the outcome of the treatments and also reduce treatment times. Excessively high intensities could result in oxidative reactions and alterations of nutritional and/or organoleptic fruit quality; it would be of great interest to determine the maximum intensities tolerated by different commodities, which is currently unknown.

5. Conclusions

Results show that the intensity of UV-C radiation is a key factor determining the effectiveness of UV-C treatments in fruits. In strawberry, for a dose of 4 kJ m^{-2} raising UV-C radiation intensity from 3 to 33 W m^{-2} reducing treatment times tenfold improved quality maintenance. In tomato, the benefits from increasing UV-C intensity are subtler. Increasing UV-C radiation intensity might be of interest from a technological perspective since it could increase the effectiveness while reducing the exposure time to perform the treatments, increasing their feasibility for industrial applications.

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