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Role of UV-B irradiation dose and intensity on color retention and antioxidant elicitation in broccoli florets (*Brassica oleracea* var. *Italica*)

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ABSTRACT

Postharvest UV exposure has been useful to i) delay senescence and ii) induce the accumulation of bioactive compounds in some vegetable species. However, no studies have been conducted to determine the treatment conditions (radiation dose and intensity) required to maximize these two diverse responses. In this work, we evaluated the effect of UV-B irradiation intensity (*Control*: 0, *Low*: 3.2, *Medium*: 4.0 and *High*: 5.0 W/m^2) and dose (0, 2, 4, 8, 12 kJ/m^2) on quality retention and antioxidant capacity of fresh broccoli florets during storage (4 °C for 17 days). Exposure to *Low* UV-B radiation and dose (2, 4 kJ/m^2) reduced broccoli weight loss, delayed yellowing and improved chlorophyll and chlorophyllide retention. After long term storage, no marked improvement on the antioxidant capacity was found regardless of the irradiation condition. Evaluations at short time after UV-B exposure (0, 2, 6, 18 h) indicated that the treatments elicited antioxidant accumulation. Greatest antioxidant capacity was found in broccoli subjected to *High* intensity UV-B. Increased levels of aliphatic glucosinolates were found 18 h after the UV-B irradiation, whereas phenolic antioxidants peaked 6 h after the treatment. Results showed that *Low* UV-B doses and intensities delayed chlorophyll degradation and may be useful to complement refrigeration in fresh broccoli. Instead, *High* intensity UV-B exposure may be better suited as a pre-treatment to increase the antioxidant capacity prior to further processing such us freezing.

1. Introduction

In the last decade, there has been increased interest in the evaluation of postharvest physical methods that could maintain or even improve vegetable quality, while circumventing the use of chemical additives (Vicente and Lurie, 2014). UV irradiation has been used for several years in the food industry mainly for disinfection purposes (packaging materials and water) (Civello et al., 2007). More recently, the direct use of UV treatments on foods surfaces has been started to be evaluated (Vicente and Lurie, 2014). For these applications, UV-C irradiation has been usually chosen, given its highest germicide effect (Civello et al., 2007). However, other regions within the UV spectrum are known to induce physiological responses in vegetables (Venditti and D'hallewin, 2014).

UV-B treatments have been reported to delay ripening and senescence. UV-B exposure delayed yellowing in broccoli stored at 15 °C (Aiamla-or et al., 2009, 2010). Whether the treatments are also beneficial in refrigerated inflorescences has not been tested. UV irradiation has been also shown to elicit antioxidant accumulation in some commodities (Jansen et al., 2010). Sun-exposed pears receiving higher UV-B light usually show higher anthocyanin level than shaded fruit (Sun et al., 2014). Increased contents of phenolic acids and flavonoids have been reported in tomatoes subjected to UV-B irradiation after harvest (Castagna et al., 2014). The induction of defensive responses against UV-B radiation has been shown to depend also on the geographic origin of the genotypes (Jansen et al., 2010). Research understanding the effects that the irradiation conditions exert on the outcome of UV treatments is still ongoing. A number of studies have analyzed the

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effect of UV-B radiation prior to harvest growing crops and characterized its role on plant physiology and fitness. However, this may markedly differ from the responses occurring in harvested organs. Most studies conducted so far have characterized the effects of UV irradiation dose treatments on different commodities (Lemoine et al., 2007, 2008; Aiamla-or et al., 2009; Avena-Bustillos et al., 2012). However, irradiation intensity as a major factor determining the outcome of the UV treatments on quality retention has been less studied. Cote et al. (2013) reported that for a fixed dose (4.1 kJ/m^2) high intensity UV-C treatments were more effective to prevent postharvest decay and softening in strawberry than those of low intensity exposure. The influence of UV-B irradiation intensity on postharvest senescence and antioxidant *status* of harvested broccoli has not been determined. The aim of this study was to determine the influence of different UV-B treatment conditions (dose and intensity) on visual quality retention and antioxidant capacity elicitation in fresh broccoli florets.

2. Materials and methods

2.1. Plant material

Field grown broccoli (*Brassica oleracea* var. *Italica*, cv. Legacy) heads were harvested at commercial maturity (when the individual flowers were still closed and had dark green color, and the inflorescences were compact) in La Plata, Argentina, and immediately transported to the laboratory. The heads presenting defects were eliminated and the remaining inflorescences were separated into individual florets. Samples were subsequently packaged in plastic trays weighing *ca.* 180 g each.

2.2. UV-B treatments and storage

2.2.1. Effect of UV-B irradiation intensity and dose on quality retention of refrigerated broccoli florets

Trays containing broccoli florets were placed into the UV-B irradiation chamber equipped with 4 lamps (QFS-40, Philips, 290–340 nm). Samples were treated with UV doses of 2, 4, 8 and 12 kJ/m^2 at three different intensities: *Low*: 3.2 W/m^2, *Medium* 4.0 W/m^2 and *High*: 5.0 W/m^2. The treatment conditions were achieved by modifying the distance from the irradiation source to the samples as well as the number of lamps and irradiation time (Table 1). UV-B dose and intensity were determined with a digital radiometer (UVITEC, RX-003, France). Broccoli trays without UV-B treatment were used as controls. Samples were subsequently covered with perforated PVC film and stored in darkness at 4 °C for 17 days. Four trays containing at least 16 florets each were visually evaluated for color, weight loss, chlorophyll (*Chl*) and *Chl*-derivatives and antioxidant capacity as described in Section 2.3. Samples were immediately evaluated or otherwise frozen in liquid N_2 and stored at −80 °C until use. The experiment was repeated three times.

Table 1
Exposure times (min) for the UV-B treatment dose and combination evaluated.

Intensity		Lamps	Distance	UV-B doses (kJ/m^2)			
	(W/m^2)		(cm)	2	4	8	12
Low	3.2	2	30	10	20	45	65
Medium	4.0	4	30	7	15	32	48
High	5.0	4	15	6	12	25	39

2.2.2. Effect of UV-B intensity and dose on antioxidant elicitation and stability of the induced compounds after storage at −18 °C

Broccoli florets were selected and prepared as described in Section 2.1 and subjected to the following UV-B treatments:

a) *Low* intensity (3.2 W m^{-2}) and low dose (2.0 kJ m^{-2}), L2;
b) *Low* intensity (3.2 W m^{-2}) and high dose (12.0 kJ m^{-2}), L12;
c) *High* intensity (5.0 W m^{-2}) and low dose (2.0 kJ m^{-2}), H2;
d) *High* intensity (5.0 W m^{-2}) and low dose (12.0 kJ m^{-2}), H12.

Broccoli florets without UV-B treatment were used as controls. The trays were subsequently covered with perforated PVC film and held in darkness at 20 °C to simulate a delay (for 0, 2, 6 or 18 h) until further processing. After 0, 2, 6 or 18 h samples were taken and used for color, phenolics, antioxidant capacity and glucosinolate content. Samples were immediately evaluated or otherwise frozen in liquid N_2 and stored at −80 °C until use. Four trays were analyzed for each treatment and time. The experiment was repeated three times.

To determine the stability of the induced compounds after frozen storage, samples taken at the incubation time that showed the highest eliciting effect on antioxidant capacity (6 h) were stored in a commercial freezer at −18 °C for 30 d and subsequently thawed and assessed for antioxidant capacity.

2.3. Weight loss

Weight loss was determined by weighing the trays at harvest time and after 17 d of storage at 4 °C. Results were calculated as percentage of weight loss relative to the initial value. Four replicates were evaluated for each treatment and storage time.

2.4. Color

Color was evaluated with a chromameter (Minolta CR-400, Japan). The CIE L*, a* and b* values were determined and the Hue angle was calculated as tg^{-1} b*/a*. Twenty measurements were performed per tray of each treatment and storage time.

2.5. Chlorophyll and derivatives

Chlorophyll (*Chl*) and Chl-derivatives were extracted and determined according to Yang et al. (1998). Frozen broccoli tissue was ground in a mill and 1 g of the resultant powder was added to 5 mL acetone:water (80:20), vortexed, centrifuged at 3000 × *g* for 5 min at 4 °C. The supernatant was saved and the pellet was re-extracted as described above and the supernatants were pooled. Five milliliters of hexane were added to 5 mL of sample extract. After vortexing samples were centrifuged at 3000 × *g* for 5 min at 4 °C.

The absorbance of the lower acetone:water phase was measured at 665 nm to determine the content of Chlorophyllide a (*Chlide a*) according to Eq. (I) (Yang et al., 1998). This phase was acidified with 0.02 mL 25% v/v HCl, and the absorbance was measured at 665 nm and 653 nm to determine the content of total Pheophorbide (*Pheo* a_{total}) according to Eq. (II) (Lorenzen and Jeffrey, 1980; Marker et al., 1980). *Pheo* $a_{initial}$ content was calculated according to Eq. (III).

The upper phase (hexane) containing *Chl* and less polar *Chl* derivatives was splitted in two aliquots (Varela and Massa, 1981): a) Na_2SO_4 was added to one of the aliquots as a desiccant and the absorbance was measured at 663,6 nm and 646,6 nm to determined the content of *Chl a* according to Eq. (IV); b) 0.02 mL 25% v/v HCl and Na_2SO_4 was added and the absorbance was measured at 667 nm and 653 nm to determine the content of total Pheophytin a (*Phy* a_{total}) according to Eq. (V) (Lichtenthaler, 1987). The *Phy* $a_{initial}$

content was calculated according to Eq. (VI) (Lorenzen and Jeffrey, 1980; Marker et al., 1980). Two extracts were prepared and measured in triplicate for each treatment and storage time. Results were expressed as mg/kg fresh weight.

$$Chlide\ a\ (\text{ug/mL}) = A_{665}\ (614/74.9) \quad \text{(I)}$$

$$Pheo\ a_{total}\ (\text{ug/mL}) = 22.42\ A_{665} - 6.81\ A_{653} \quad \text{(II)}$$

$$Pheo\ a_{initial}\ (\text{ug/mL}) = Pheo\ a_{total} - Pheo\ a_{chlidea} = Pheo\ a_{total} - Chlide\ a\ (591/614) \quad \text{(III)}$$

$$Chl\ a\ (\text{ug/mL}) = 12.25\ A_{663.6} - 2.55\ A_{646.6} \quad \text{(IV)}$$

$$Phy\ a_{total}\ (\text{ug/mL}) = 22.42\ A_{667} - 6.81\ A_{653} \quad \text{(V)}$$

$$Phy\ a_{initial}\ (\text{ug/mL}) = Phy\ a_{total} - Phy\ a_{chla} = Phy\ a_{total} - Chl\ a\ (871/892) \quad \text{(VI)}$$

2.6. Phenolic compounds

Frozen broccoli was ground in a mill and 1.2 g of the resultant powder was added to 10 mL of cold ethanol (96% v/v) and vortexed for 30 s. After centrifuging at 13,000 × *g* for 10 min at 4 °C the supernatant was collected. Total phenolic content was determined according to Singleton et al. (1999) using the Folin-Ciocalteu reagent. Test tubes containing aliquots of 0.10 mL ethanol extract and 0.75 mL distilled water were mixed with 50 mL Folin-Ciocalteu (1:1 v/v in water). After 3 min, 0.10 mL 20% (m/v) Na_2CO_3 in 0.1 mol/L NaOH was added and completed with distilled water to a final volume of 2 mL. The reaction mixture was incubated for 90 min at room temperature. The absorbance at 760 nm was measured in a spectrophotometer (Hitachi U-1900, Japan). Total phenolics content was calculated using chlorogenic acid (CGA) as a standard. Three extracts were prepared for each treatment and storage time. Results were expressed as CGA equivalents in mg/kg fresh weight.

2.7. Antioxidant capacity

Ethanolic extracts were prepared as described in *section 2.6* and used for antioxidant capacity assay. Measurements were done according to Arnao et al. (2001) using the ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) method. The stock solution of stable radical-cation $ABTS^{\bullet+}$ was prepared using 7 mmol/L of the ammonium salt of ABTS in 2.45 mmol/L $K_2S_2O_8$ and left overnight at room temperature before use. An ethanolic dilution was performed to reach an absorbance of 0.700 ± 0,05 at 734 nm. Aliquots of 0.01 mL ethanol extract were added to 0.04 mL distilled water. Then, 1 mL $ABTS^{\bullet}$+ diluted solution was added, vortexed and after 6 min the absorbance at 734 nm was measured in a spectrophotometer. Trolox® was used as a standard and results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in mg/kg fresh weight. Measurements were conducted in triplicate.

2.8. Glucosinolates (GSs)

GSs were extracted and quantified as previously described (Cargnel et al., 2014) using freeze-dried tissue and sinigrin (2-propenyl-glucosinolate, Sigma-Aldrich, USA) as internal standard. Desulphoglucosinolates were analyzed by HPLC (Knauer Euroline) on a Restek Pinnacle II C18 (5.0 μm, 4.6 × 150 mm) column with solvents A (water) and B (20% acetonitrile), eluted with a gradient of 1% B at 0 min, 10% B at 10 min, 75% B at 22–24 min, with an equilibration time of 10 min and a flow rate of 1 mL/min. The injection volume was 20 μL, and elution was monitored with a diode array detector at 229 nm. Several GSs were identified on the basis of their relative retention times and UV spectra: a) Aliphatics: glucoraphanin and others (in minor concentrations: glucoiberin, progoitrin, glucoalyssin, gluconapin, glucoerucin); b) Indolyls: glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin. To calculate molar concentrations of individual GS, relative response factors were used to correct for absorbance difference between the internal standard and other compounds. Finally, results were expressed as mmol of sinigrin equivalent/kg of fresh weight.

2.9. Statistical analysis

Experiments were performed in a factorial design being the factors: UV-B dose, UV-B intensity and storage time. Data were analyzed by ANOVA and means were compared by a Fisher test at a significance level of 0.05 (Lemoine et al., 2008).

3. Results and discussion

3.1. Effect of UV-B irradiation intensity and dose on quality retention of refrigerated broccoli florets

We initially evaluated the changes in broccoli florets treated at three different UV-B intensities (*Low*: 3.2, *Medium*: 4.0, *High*: 5.0 W/m^2) and with five distinct doses 0, 2, 4, 8, 12 kJ/m^2 after refrigerated storage (4 °C) for 17 days. UV-B treated broccoli florets showed lower weight loss than the control after storage (Fig. 1). The greatest reduction in weight loss was found in samples treated with doses of 2 and 4 kJ/m^2 for the *Low* or *Medium* UV-B intensities, respectively. Reduced dehydration has been reported in broccoli treated with UV-C (Lemoine et al., 2008) as well as in other commodities. Manzocco and Nicoli (2015) suggested that UV-C irradiation may reduce water loss through a formation of a thin dried layer on the commodity surface which may in turn restrict water vapor flux. An improved maintenance of tissue integrity in UV irradiated commodities in which senescence is delayed may also contribute to explain the reduced water loss (Lemoine et al., 2008).

Yellowing is the main factor contributing to broccoli deterioration during postharvest storage (Büchert et al., 2011). Previous

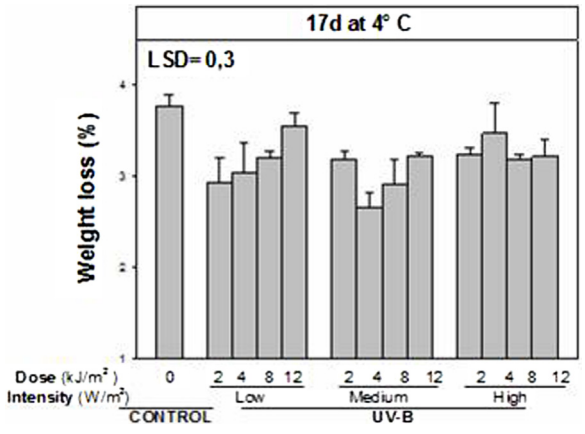

Fig. 1. Weight loss in control and treated broccoli florets subjected to different combinations of UV-B irradiation intensity (*Low*: 3.2 W/m^2,*Medium* 4.0 W/m^2 and *High*: 5.0 W/m^2) and dose (2, 4, 8, 12 kJ/m^2) and stored at 4 °C for 17 days. The standard deviation and LSD ($P < 0.05$) are shown.

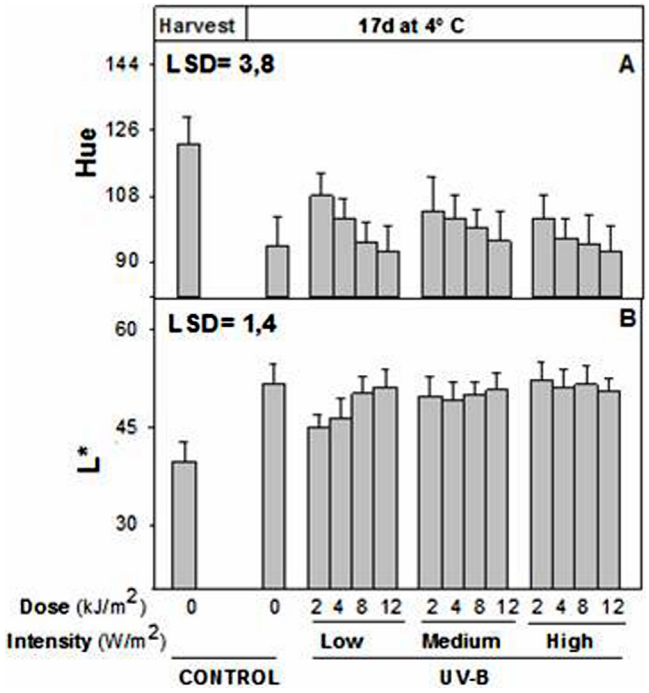

Fig. 2. A) Hue angle, B) lightness (L*) in control and treated broccoli florets subjected to different combinations of UV-B irradiation intensity (*Low*: 3.2 W/m^2, *Medium* 4.0 W/m^2 and *High*: 5.0 W/m^2) and dose (2, 4, 8 and 12 kJ/m^2) at harvest and after 17 days of storage at 4 °C in darkness. The standard deviation and LSD ($P < 0.05$) are shown.

work in UV-B and UV-C irradiated broccoli showed that the outcome on color retention is markedly dependant on the irradiation dose (Aiamla-or et al., 2009; Lemoine et al., 2007). However, whether or not the irradiation intensity has a significant role on the observed effects has not been determined. The florets showed significant yellowing after storage as depicted from the drop in the Hue values and increased florets lightness (Fig. 2A and B). Results showed that the effect of UV-B exposure on color retention is also highly dependent on the irradiation intensity used. Noteworthy, at *Medium* and *High* UV-B intensities the effect on color retention was negligible regardless of the dose applied. These results differ from those found by Aiamla-or et al. (2010) who found that exposure to 4.4 kJ/m^2 UV-B was less effective than treatments with doses of both 8.8 and 13.1 kJ/m^2, though in this case the intensities were 50% higher than those tested in our study. For *Low* intensity UV-B treatments the dose had a marked effect on color retention. The best results were observed in the florets treated with low doses (2 or 4 kJ/m^2).

To gain further insight regarding the role of UV treatment conditions on broccoli color we determined the changes on the levels of chlorophyll a and its derivatives chlorophyllide a (*Chlide a*), pheophytin a (*Phy a*) and pheophorbide a (*Pheo a*). During storage, a marked loss of chlorophyll a (30–70%) occurred regardless of the treatment applied (Fig. 3A). Treatments at *High* UV-B intensity induced higher *Chl a* loss than the control. The similar color observed in this case between control and UV irradiated samples may be then likely due to the accumulation of *Chlide a* (Fig. 3B), which despite of its higher water solubility induced by phytol removal shows similar optical properties than *Chl a*.

The most effective treatment in terms of color retention (*Low* intensity and dose of 4 kJ/m^2), retained higher level of native *Chl a* (Fig. 3A) at the end of the storage period. Interestingly, these samples also accumulated higher levels of *Chlide a* and *Pheo a* (Fig. 3B and D) than the control. Early work suggested that phytol removal was the first committed step in chlorophyll catabolism, and that subsequent Mg^{+2} removals would yield olive green *Pheo a* (Amir-Shapira et al., 1987; Matile et al., 1999). Chlorophyllase (Chlase) and magnesium dechelatase (MDS) were reported to be involved in these steps during normal chloroplast disassembly (Langmeier et al., 1993; Kaewsuksaeng et al., 2007). Most recently the identification of plant pheophytinases (PPH) suggested that direct removal of the central Mg-atom from native *Chl* may be an important *in vivo* route for *Chl* degradation as well. This is supported by the stay green phenotype of PPH knock-out mutants in *Arabidopsis* (Schelbert et al., 2009). The relative importance of the different *Chl* catabolic pathways in most commercially important products in which loss of green color is a major quality index remains to be established. Aiamla-or et al. (2010) suggested that UV-B irradiation may modulate the activity of *Chl* degrading enzymes. Results from our work suggest that high intensity UV-B treatments at doses ranging between 2 and 12 kJ/m^2 may favor chlorophyll a dephytilation and increase *Pheo a*. In contrast, low

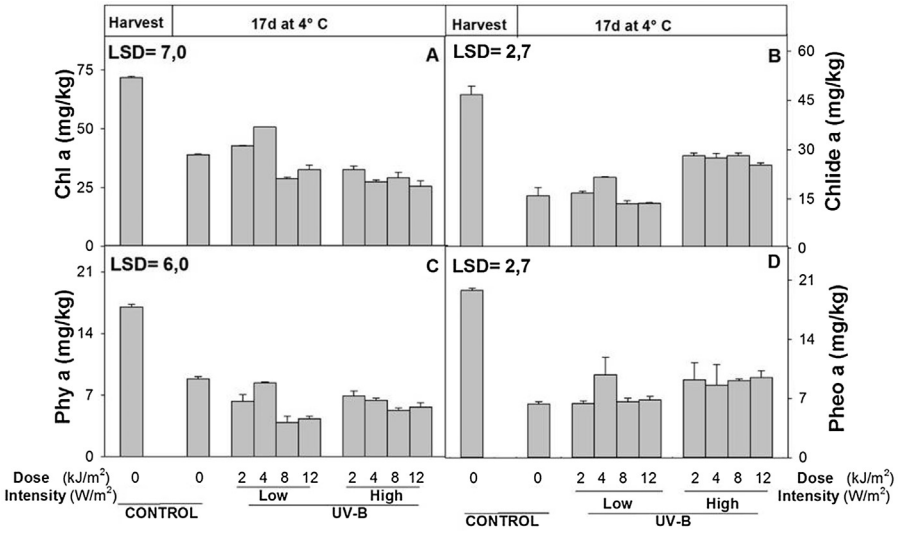

Fig. 3. A) Chlorophyll a (*Chl a*), B) Chlorophyllide a (*Chlide a*), C) Pheophytin a (*Phy a*), D) Pheophorbide a (*Pheo a*) in control and treated broccoli florets subjected to different combinations of UV-B irradiation intensity (*Low*: 3.2 W/m^2, *High*: 5.0 W/m^2) and dose (2, 4, 8, 12 kJ/m^2) at harvest and after 17 days of storage at 4 °C. The standard deviation and LSD ($P < 0.05$) are shown.

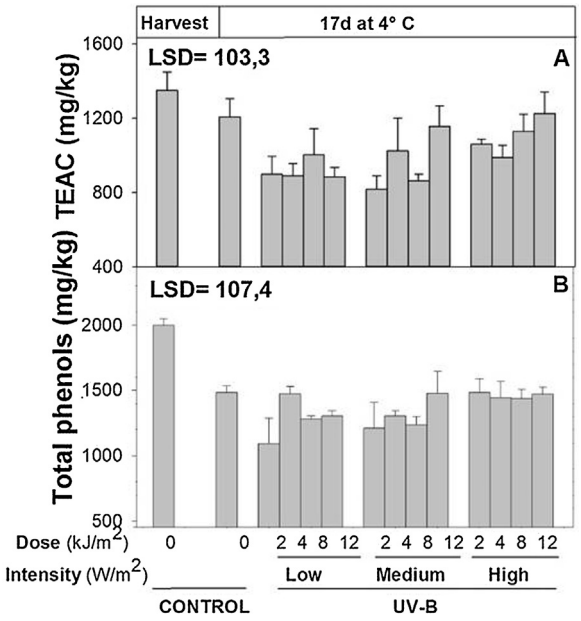

Fig. 4. A) Trolox Equivalent Antioxidant Capacity (TEAC) and B) Total Phenols in control and treated broccoli florets subjected to different combinations of UV-B irradiation intensity (*Low*: 3.2 W/m^2, *Medium* 4.0 W/m^2 and *High*: 5.0 W/m^2) and dose (2, 4, 8 and 12 kJ/m^2) at harvest and after 17 days of storage at 4 °C in darkness. The standard deviation and LSD ($P < 0.05$) are shown.

intensity UV-B exposure at doses of 4 kJ/m^2 reduced broccoli yellowing by increasing the levels of *Chl a, Chlide a* and *Pheo a.*

A number of works have suggested that mild stress conditions induced by UV-B irradiation could be a valuable technological strategy to increase the antioxidant capacity of fresh produce. Higher contents of phenolic antioxidants have been reported in UV-B treated fresh-cut carrots (Avena-Bustillos et al., 2012), grape (Martínez-Lüscher et al., 2014) and tomato (Castagna et al., 2014). However, the responses reported in the literature are highly variable depending on the commodity considered and treatment applied (Ribeiro et al., 2012; Jansen et al., 2010). In the case of broccoli, UV-C treatments improved the antioxidant capacity (Costa et al., 2006; Lemoine et al., 2007). The effect of UV-B on broccoli florets antioxidant status has not been established. Increased flavonoid accumulation was reported in broccoli sprouts (Mewis et al., 2012; Topcu et al., 2015), but the responses to UV exposure are known to be dependent on the ontogenic stage of the vegetable (Schreiner et al., 2009). The TEAC and the level of phenolic compounds of control broccoli decreased (12% and 26%, respectively) during storage (Fig. 4A and B). In contrast to what has been reported in other commodities, none of the 12 different UV-B treatments evaluated in this work improved broccoli antioxidant capacity after postharvest storage and rather decreased it. Treatments with 12 kJ/m^2 and *Mid* or *High* irradiation intensity maintained after 17 days of refrigerated storage similar levels of TEAC than the control.

3.2. Effect of UV-B irradiation dose and intensity on short term antioxidant elicitation

Several studies have reported that mild stress could induce a rapid burst of reactive oxygen species (ROS), which may in turn trigger the accumulation of antioxidants or other protecting molecules (Agrawal et al., 2009; A-H-Mackerness, 2000). However, the physiological understanding of plant responses to abiotic stresses has been seldom exploited in the food industry In some cases this response has been transient, with tissues recovering the basal levels of the elicited metabolites shortly after removal of the stressor (Mittler, 2002). For instance, heat shock proteins with chaperonin function are rapidly up regulated (upon 3 h) in plant tissues chilled and/or exposed to heat and decrease when samples are transferred to normal temperatures (Lurie, 1998). In order to evaluate if such response was occurring in UV-B treated broccoli we evaluated the changes in AOX at short times upon irradiation (0, 2, 6 and 18 h). None of the treatments evaluated were visually distinguishable during the 18 h period at 20 °C nor showed differences in color (Hue and L*) (Fig. 5A and B).

Glucosinolates are one of the most relevant phytochemicals in broccoli and other *Brassicaceae* species. The levels and types of GSs have been reported to depend on the cultivar considered (Bhandari and Kwak, 2015). In the present work, the aliphatic GSs were 3-fold higher than indolyl GSs at harvest (Fig. 6A). Some studies have reported increased GS accumulation in response to abiotic stresses (Variyar et al., 2014). The responses to UV are markedly dependent on the irradiations conditions. In broccoli flower bud, Rybarczyk-Plonska et al. (2016) found that both total aliphatic and indolyl GSs content were unchanged during storage at 0 or 4 °C, and even when florets were transferred to 10 or 18 °C for 3 days with a combination of visible light (19 mmol/m^{2}s) and UV-B irradiation (20 kJ/m^{2}d) treatment during 12 h per day. Wang et al. (2011) found higher GS contents in *Arabidopsis thaliana* leaves after 1 h of UV-B exposure, though a significant decline, particularly indolyl GS, was recorded after 12 h of UV-B exposure. Here *High* intensity and low dose irradiation (H2) decreased total GSs content after 2 h, though a subsequent increase was observed. The highest levels of GSs were achieved after 18 h of the UV-B treatment. Aliphatic glucoraphanin showed the highest induction in response to UV-B exposure (Fig. 6B and C). Mewis et al. (2012) also found a preferential increase of aliphatic GSs in UV-B irradiated (0.3 kJ/m^2) broccoli sprouts. They proposed a marked induction of genes involved in later steps of GS biosynthesis and especially in those related with the aliphatic GS. Further work is necessary to determine the metabolic switches induced by UV-B irradiation.

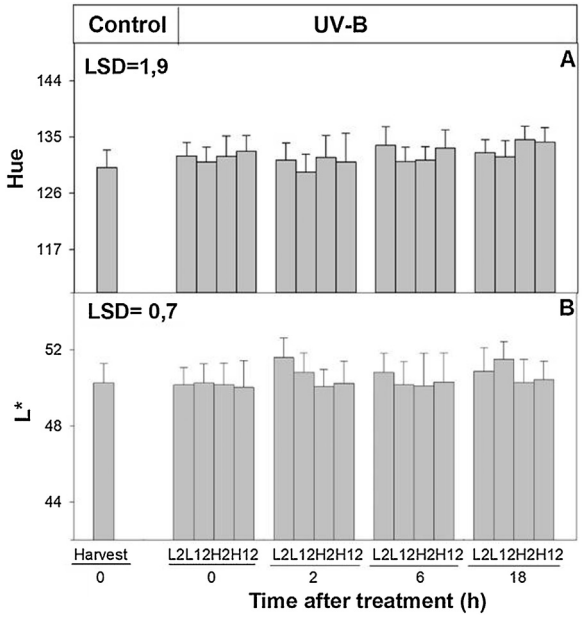

Fig. 5. A) Hue angle, B) Lightness (L*) in control and treated broccoli florets subjected to low UV-B intensity and low dose, L2 (3.0 W/m^2, 2 kJ/m^2); low UV-B intensity high dose, L12 (3.0 W/m^2, 12 kJ/m^2); high UV-B intensity and low dose, H2 (5.0 W/m^2, 2 kJ/m^2); and high UV-B intensity high dose, H12 (5.0 W/m^2, 12 kJ/m^2); treatments and held for 0, 2, 6 or 18 h at 20 °C in darkness. The standard deviation and LSD ($P < 0.05$) are shown.

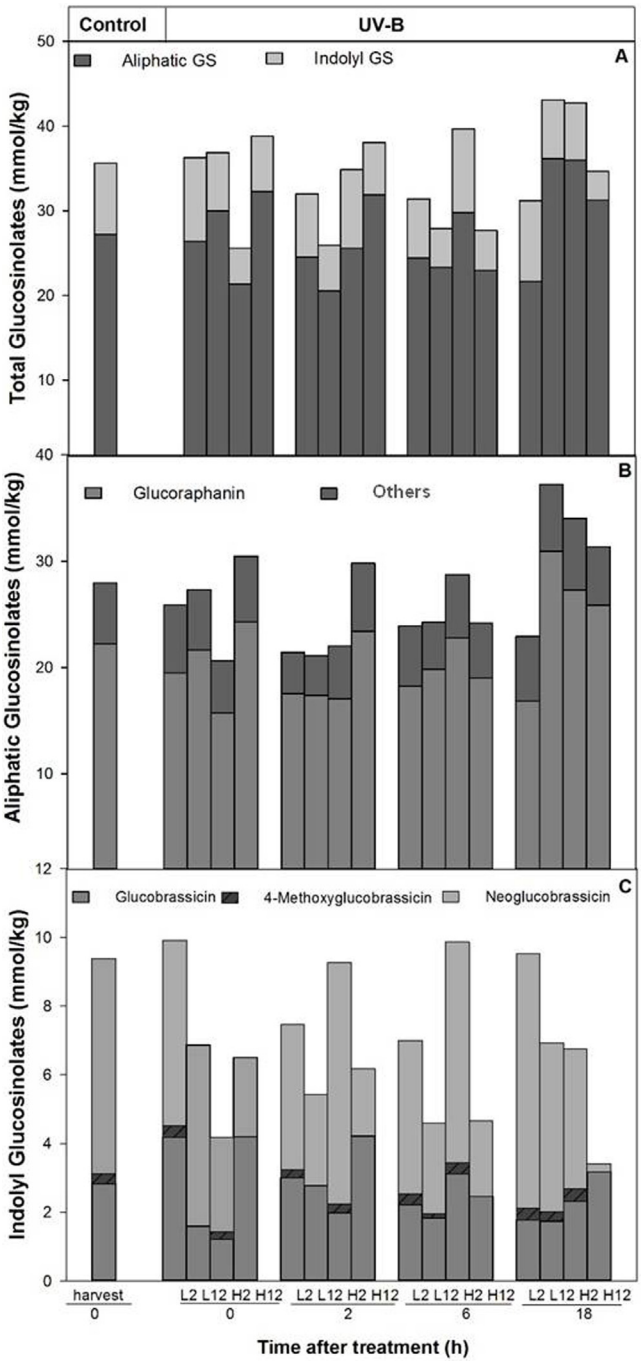

Fig. 6. A) Total, B) Aliphatic and C) Indolyl glucosinolates in control and treated broccoli florets subjected to low UV-B intensity and low dose, L2 (3.0 W/m^2, 2 kJ/m^2); low UV-B intensity high dose, L12 (3.0 W/m^2, 12 kJ/m^2); high UV-B intensity and low dose, H2 (5.0 W/m^2, 2 kJ/m^2); and high UV-B intensity high dose, H12 (5.0 W/m^2, 12 kJ/m^2); treatments and held for 0, 2, 6 or 18 h at 20 °C in darkness.

For all treatments besides those at the lowest intensity and dose the antioxidant capacity rapidly dropped after UV-B irradiation (Fig. 7A). Interestingly, the antioxidant capacity began to increase afterwards in all four treatments tested. TEAC values peaked 2 and 6 h after the treatment for *Low* and *High* intensity irradiation, respectively. At longer times, the samples tended to recover initial antioxidant capacity values. The broccoli samples exposed to *High* UV-B intensity showed a significant increase in antioxidant capacity after 6 and 18 h relative to the initial values at harvest.

The changes in broccoli TEAC followed a similar pattern to that observed in phenolic compounds (Fig. 7B), suggesting that this was

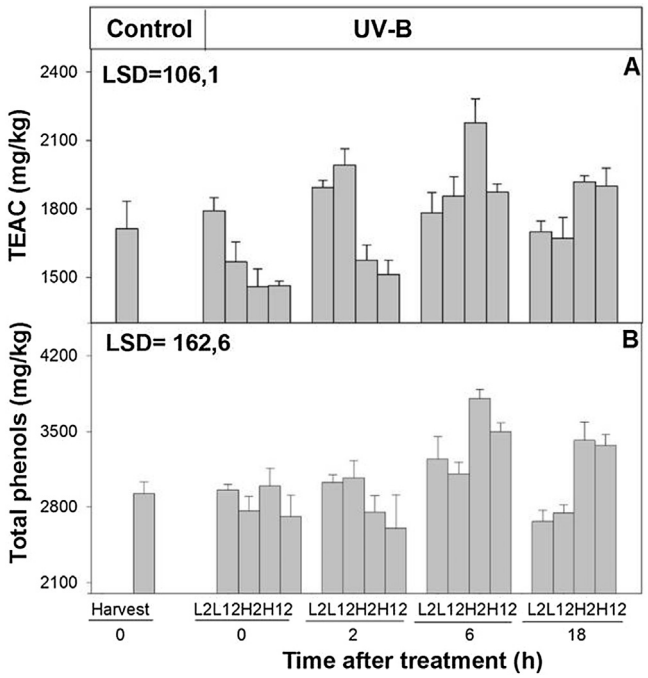

Fig. 7. A) Trolox Equivalent Antioxidant Capacity (TEAC) and B) Total Phenols in control and treated broccoli florets subjected to low UV-B intensity and low dose, L2 (3.0 W/m^2, 2 kJ/m^2); low UV-B intensity high dose, L12 (3.0 W/m^2, 12 kJ/m^2); high UV-B intensity and low dose, H2 (5.0 W/m^2, 2 kJ/m^2); and high UV-B intensity high dose, H12 (5.0 W/m^2, 12 kJ/m^2); treatments and held for 0, 2, 6 or 18 h at 20 °C in darkness. The standard deviation and LSD ($P < 0.05$) are shown.

the main group of antioxidants elicited by the treatments. Previous works have reported that UV irradiation can induce key enzymes in the phenyl-propanoid pathway such as phenylalanine ammonia lyase and chalcone synthase (Nigro et al., 2000 Tomás-Barberán and Espín, 2001; Pombo et al., 2011). UV-B treatments of asparagus spears increased PAL within 2 h (Eichholz et al., 2012). The lack of complete correlation between phenolics and TEAC indicate that UV-B may be modulating other antioxidant groups as well. Besides that, results from this work indicate that improved color retention and phenolic antioxidants elicitation in broccoli by UV-B treatments are feasible, though the time frame for these effects and the irradiation conditions required are distinctly different. UV-B treatments at low doses and intensities could be useful to delay chlorophyll degradation and senescence after long storage. Instead, *High* intensity UV-B exposure may be envisioned as a treatment to induce antioxidant capacity prior other intense processing treatments detaining vegetable metabolism. The stability of the UV-B induced antioxidants after freezing, storage and thawing provides some support for this approach (Table 2).

Table 2
Trolox Equivalent Antioxidant Capacity (TEAC, mg Trolox equivalents/kg fresh weight) in control and treated broccoli florets subjected to low UV-B intensity and low dose, L2 (3.0 W/m^2, 2 kJ/m^2), low UV-B intensity high dose, L12 (3.0 W/m^2, 12 kJ/m^2), high UV-B intensity and low dose, H2 (5.0 W/m^2, 2 kJ/m^2) and high UV-B intensity high dose, H12 (5.0 W/m^2, 12 kJ/m^2) irradiation held for 6 h at 20 °C, frozen and stored at −18 °C for 30 days. The standard deviation and least significant differences (LSD) at $P < 0.05$ are shown.

Time at −18 °C (d)	Control	UV-B			
		L2	L12	H2	H12
0	1678 ± 26	1783 ± 89	1857 ± 83	2178 ± 105	1833 ± 51
30	1681 ± 75	1718 ± 88	1833 ± 243	2103 ± 133	1878 ± 79
LSD	102				

4. Conclusion

Low UV-B irradiation intensity and doses (2 and 4 kJ/m^2) reduced broccoli weight loss, delayed chlorophyll degradation and senescence. UV-B treatments did not improve broccoli TEAC levels and phenolic compounds after long term storage, regardless of the irradiation conditions tested. In contrast, *High* intensity UV-B irradiation transiently increased broccoli TEAC levels 6 h after the treatments. Antioxidant capacity buildup resulted mainly from the accumulation of phenolic compounds. *High* intensity UV-B also induced aliphatic glucosinolates 18 h after the treatment. Overall, results show that *Low* UV-B doses and intensities delay chlorophyll degradation and may be then useful to complement refrigeration. Instead, *High* intensity UV-B exposure may be better suited for the freezing industry as a pre-treatment to increase the antioxidant capacity prior to further processing.

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