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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* $a_{initial}$