

## UV-C treatment delays postharvest senescence in broccoli florets

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### Abstract

Central broccoli heads (cv. de Cicco) were harvested and treated with UV-C light (4, 7, 10, or 14 kJ m<sup>-2</sup>). All treatments delayed yellowing and chlorophyll degradation at 20 °C but the irradiation dose of 10 kJ m<sup>-2</sup> allowed retaining the highest chlorophyll content yet had lower amounts of pheophytins than every treatment other than 7 kJ m<sup>-2</sup>. This dose was selected to analyze the effect of UV-C on postharvest broccoli senescence at 20 °C. The UV-C treatment delayed yellowing, chlorophyll *a* and *b* degradation, and also the increase in pheophytins during storage. The activity of chlorophyll peroxidase and chlorophyllase was lower in UV-C treated broccoli. Instead, Mg-dechelatase activity increased immediately after the treatment, but after 4 and 6 d this activity was lower in UV-C treated florets than in controls. Treated broccoli also displayed lower respiration rate, total phenols and flavonoids, along with higher antioxidant capacity. The results suggest that UV-C treatments could be a useful non-chemical method to delay chlorophyll degradation, reduce tissue damage and disruption, and maintain antioxidant capacity in broccoli.

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**Keywords:** UV-C; Chlorophyll degradation; Broccoli; Mg-dechelatase; Chlorophyllase; Peroxidase

### 1. Introduction

One of the main goals of postharvest technology in broccoli is to delay senescence symptoms (Page et al., 2001). Broccoli quality is highly reduced after harvesting because of the loss of green color and the yellowing of its sepals as a consequence of chlorophyll catabolism (Funamoto et al., 2002). Matile et al. (1999) proposed a pathway for chlorophyll (Chl) degradation in which chlorophyll *a* is transformed in chlorophyllide *a* by the action of chlorophyllase (Hörtnersteiner, 1999; Matile et al., 1999), and chlorophyllide *a* is then transformed in pheophorbide *a* by Mg-dechelatase. Tetrapyrrolic rings are broken producing non-colored Chl derivatives. Alternatively, Chl could be directly degraded by peroxidases, which produce other Chl derivatives in an oxidative reaction (Matile, 1980; Funamoto et al., 2002).

Other detrimental changes during broccoli postharvest senescence are tissue disruption, lipid peroxidation, protein degradation and the loss of antioxidant compounds, which decreases the nutritional value of the product (Page et al., 2001). Many different techniques have been examined to extend broccoli postharvest life, including the use of refrigerated storage (Toivonen, 1997), controlled and modified atmospheres (Jacobsson et al., 2004), heat treatments (Funamoto et al., 2002), application of 1-MCP (Ku and Wills, 1999; Able et al., 2002), cytokinins (Clarke et al., 1994), and ethanol (Suzuki et al., 2004).

Many studies have shown the deleterious effects of UV light on plant tissues, such as decreased protein synthesis, impaired chloroplast function, and DNA damage (Danon and Gallois, 1998; Brosché et al., 1999). However, the concept of hormesis establishes that it is possible to obtain a beneficial effect from application of a low or sublethal dose of an agent capable of inducing physical or chemical stress (Luckey, 1980). Specifically, UV radiation can induce the antioxidant

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system in sunflower cotyledons as a response to the stress generated by the applied radiation (Costa et al., 2002b). Recently, sub-lethal doses of UV-C have been assayed as a possible strategy in postharvest technology. Low doses of UV-C can reduce decay in grapefruit (D'hallewin et al., 2000), strawberries (Baka et al., 1999; Pan et al., 2004), and boysenberries (Vicente et al., 2004). In addition, UV-C irradiation has been useful to delay some ripening-associated processes (Barka et al., 2000) and to reduce chilling injury in pepper (Vicente et al., 2005). However, to our knowledge, the effect of short duration UV-C pulses on postharvest senescence has not been reported. Therefore, the objective of this work was to select a suitable UV-C treatment to delay postharvest senescence of broccoli and to evaluate the effect of these treatments on chlorophyll degradation and antioxidant levels during storage at 20 °C.

## 2. Materials and methods

### 2.1. Selection of UV-C optimal dose

Broccoli (*Brassica oleracea* L. var. Italica, cv Cicco) heads were obtained from producers in La Plata, Buenos Aires Province, Argentina, and immediately transported to the laboratory. To select the most suitable experimental conditions, different UV-C light (peak emission at 254 nm) doses were applied. Each dose was assayed on 30 broccoli heads placed in plastic trays (2 heads per tray). Heads were placed vertically in order to assure a homogeneous irradiation on florets and put under a bank of 4 UV-C lamps (TUV G30T8, 30W, Philips). The heads were irradiated at a distance of 30 cm to obtain doses of 0 (control), 4, 7, 10 and 14 kJ m<sup>-2</sup>. The flux intensity of lamps was measured with a digital radiometer (Cole-Parmer Instrument Company, Vernon Hills, IL, USA). After treatment, the broccoli heads were loosely covered with PVC film to diminish water loss and stored at 20 °C for 5 d in darkness. The heads were weighed every day and the weight loss was determined. Fifteen heads were sampled immediately after the treatment and after 5 d of storage. Superficial color was measured using intact broccoli heads. Then, individual florets were removed from the 15 broccoli heads, taking care to remove as much of the floret pedicel as possible. Florets were randomly grouped into two replicate sets, frozen in liquid nitrogen, and stored at -80 °C until analysis. Total chlorophyll and pheophytin contents were evaluated as described in Section 2.5. The whole experiment was repeated three times, and since the same trend was found only results from the first experiment are shown.

### 2.2. Experiments using 10 kJ m<sup>-2</sup> UV-C treatments

Eighty broccoli heads were irradiated as described in Section 2.1 in order to obtain a dose of 10 kJ m<sup>-2</sup>. After treatment the heads were loosely covered with PVC and stored at 20 °C

for 6 d in darkness. Eighty heads without UV-C treatment were directly brought to 20 °C and used as controls. Twenty heads were sampled immediately after the treatment and after 2, 4 and 6 d of storage. Superficial color and respiration rate were measured using intact broccoli heads. Then, individual florets were removed, grouped, and stored as described in Section 2.1. The entire experiment was repeated twice and since the same trend was found only results from the first experiment are shown.

### 2.3. Color measurement

Superficial color was determined by measuring parameters  $L^*$ ,  $a^*$ , and  $b^*$  with a chromameter (Minolta CR300, Osaka, Japan). The hue angle ( $h^\circ$ ) was calculated as  $h^\circ = \tan^{-1}(b/a)$ , when  $a > 0$  and  $b > 0$ , or as  $h^\circ = 180^\circ - \tan^{-1}(b/a)$  when  $a < 0$  and  $b > 0$ . Nine positions on each of 20 heads were measured for each treatment and storage time.

### 2.4. Respiration rate

Broccoli heads weighing approximately 120 g were placed in 1000 mL jars, sealed and incubated at 20 °C for 1 h. Gas samples were withdrawn with a 1 mL syringe through a septum fitted in the jar lid. The CO<sub>2</sub> content in the jars was determined using a gas chromatograph (Varian, CX 3400, CA, USA) equipped with an Alltech CTRI column and a thermal conductivity detector. Temperatures in the injector, column and detector were set at 120, 30 and 120 °C respectively. Helium was used as carrier and the flow rate was set at 0.33 mL s<sup>-1</sup>. Results were expressed as mg kg<sup>-1</sup> s<sup>-1</sup>. Three jars were prepared per condition analyzed, and each jar was measured twice.

### 2.5. Analytical and enzyme assays

In each of the two complete experiments performed, two extracts were done for each treatment and storage time analyzed, and measurements were done at least in duplicate.

**Chlorophylls and pheophytins:** Approximately 60 g of frozen broccoli florets was crushed in a mill and 0.5 g of the obtained powder was poured into 5 mL of acetone:water (80:20), stirred and then centrifuged at 3000 × g for 15 min. The supernatant was used to determine the content of chlorophyll and pheophytin according to Lichtenthaler (1987) with slight modifications (Costa et al., 2005a).

**Antioxidant capacity:** The free radical scavenging capacity of broccoli florets was adapted from Brand-Williams et al. (1995). Approximately 60 g of frozen broccoli florets was crushed in a refrigerated mill and 2 g of the powder obtained was homogenized in 30 mL of ethanol. The mixture was centrifuged at 9000 × g for 10 min at 4 °C and the supernatant was utilized to evaluate antioxidant capacity according to Costa et al. (2005b). The antioxidant capacity was expressed as EC<sub>50</sub><sup>-1</sup>.

**Phenols and flavonoids:** Approximately 60 g of frozen broccoli florets was crushed in a refrigerated mill and samples of 0.5 g were homogenized in 6 mL of ethanol. The mixture was centrifuged at  $9000 \times g$  for 10 min at  $4^\circ\text{C}$ . Three milliliters of the resultant supernatant were brought to 50 mL with water. The extracts were used to determine total phenols according to Singleton et al. (1999) with modifications. Two hundred microliters of crude extract was added to 1110  $\mu\text{L}$  of water and 100  $\mu\text{L}$  of Folin–Ciocalteau reagent. After 3 min at  $25^\circ\text{C}$ , 1.5 mL of saturated solution of  $\text{Na}_2\text{CO}_3$  was added, and the reaction mixture was incubated for 1 h at the same temperature. The absorbance was measured at 760 nm and total phenols were calculated by using phenol as standard. Results were expressed as g of phenol per kg of tissue. Total flavonoids were measured by a colorimetric assay developed by Zhishen et al. (1999). Aliquots of appropriately diluted samples obtained as above described were used. Total flavonoids were expressed as g of catechin per kg of fresh tissue.

**Enzyme activities:** Approximately 60 g of frozen broccoli florets was crushed in a mill, and 3 g of the obtained powder was poured into 30 mL of the following extraction buffer: 0.1 M  $\text{Na}_2\text{HPO}_4$ , 0.1 M  $\text{NaH}_2\text{PO}_4$ , 0.1% (v/v) Triton X-100, 30 g  $\text{L}^{-1}$  polyvinylpolypyrrolidone (PVPP), 1 mM phenyl methyl sulfonyl fluoride (PMSF), pH 6.5. The mixture was stirred for 2 h at  $4^\circ\text{C}$  and centrifuged at  $9000 \times g$  for 20 min at  $4^\circ\text{C}$ . The supernatant was separated, vacuum-filtered and used to determine enzyme activities (Chlorophyllase, Mg-dechelatase and Chl-peroxidase) according to Costa et al. (2005a).

## 2.6. Statistical analysis

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

## 3. Results

### 3.1. Selection of optimal UV-C dose

Broccoli heads were irradiated with different doses of UV-C light (4, 7, 10 or  $14 \text{ kJ m}^{-2}$ ) and then stored at  $20^\circ\text{C}$  for 5 d to accelerate senescence. During storage at  $20^\circ\text{C}$ , all control and treated samples had a weight loss around 1.5% per day (data not shown). Initial hue values were approximately 126 and decreased in all samples during storage at  $20^\circ\text{C}$ , while initial  $L^*$  values were approximately 40 and increased along the storage (Table 1). After 5 d at  $20^\circ\text{C}$ , the lightness increased less in irradiated than in control broccoli, independently of the UV-C dose applied. Also, the reduction of hue angle was affected, indicating that UV-C treatment delayed the broccoli yellowing. The lowest change in hue angle was found after application of a dose of  $7 \text{ kJ m}^{-2}$ . According to

Table 1

Change of lightness ( $L^*$ ), hue, chlorophylls, and pheophytins in control and UV-C treated (0, 4, 7, 10 and  $14 \text{ kJ m}^{-2}$ ) broccoli during stay at  $20^\circ\text{C}$

|  | Days at $20^\circ\text{C}$ |       |
|--|----------------------------|-------|
|  | 0                          | 5     |
| $L^*$ (LSD = 3.8)                                |                            |       |
| Control  | 39.2                       | 53.8  |
| $4 \text{ kJ m}^{-2}$                            | 41.8                       | 48.1  |
| $7 \text{ kJ m}^{-2}$                            | 41.2                       | 45.8  |
| $10 \text{ kJ m}^{-2}$                           | 40.7                       | 44.2  |
| $14 \text{ kJ m}^{-2}$                           | 40.7                       | 45.7  |
| Hue (LSD = 3.2)                                  |                            |       |
| Control  | 127.1                      | 112.5 |
| $4 \text{ kJ m}^{-2}$                            | 126.8                      | 116.6 |
| $7 \text{ kJ m}^{-2}$                            | 127.4                      | 120.3 |
| $10 \text{ kJ m}^{-2}$                           | 126.3                      | 116.2 |
| $14 \text{ kJ m}^{-2}$                           | 126.8                      | 113.7 |
| Chlorophylls ( $\text{mg kg}^{-1}$ ) (LSD = 2.9) |                            |       |
| Control  | 236.2                      | 32.0  |
| $4 \text{ kJ m}^{-2}$                            | 235.1                      | 51.3  |
| $7 \text{ kJ m}^{-2}$                            | 234.9                      | 58.0  |
| $10 \text{ kJ m}^{-2}$                           | 236.0                      | 74.6  |
| $14 \text{ kJ m}^{-2}$                           | 236.5                      | 73.0  |
| Pheophytins ( $\text{mg kg}^{-1}$ ) (LSD = 3.2)  |                            |       |
| Control  | 13.6                       | 29.4  |
| $4 \text{ kJ m}^{-2}$                            | 14.3                       | 15.6  |
| $7 \text{ kJ m}^{-2}$                            | 13.9                       | 5.5   |
| $10 \text{ kJ m}^{-2}$                           | 14.4                       | 11.8  |
| $14 \text{ kJ m}^{-2}$                           | 13.7                       | 26.7  |

In each case, the least significant difference (LSD) at  $P < 0.05$  is indicated.

chlorophyll amount data, chlorophyll degradation with 10 and  $14 \text{ kJ m}^{-2}$  doses causing the greatest response (Table 1). It is worthy to note that although chlorophyll degradation was highly delayed after the treatment at  $14 \text{ kJ m}^{-2}$ , no difference in the hue angle value was found. The accumulation of pheophytin (Pheo) was detected during broccoli senescence (Table 1). Broccoli heads treated with 4, 7 and  $10 \text{ kJ m}^{-2}$  accumulated less Pheo than the untreated samples, while those broccoli heads treated with  $14 \text{ kJ m}^{-2}$  showed similar Pheo amounts than the control. Based on superficial color and the chlorophyll and pheophytin amounts, the dose of  $10 \text{ kJ m}^{-2}$  was selected to analyze the effect of UV-C treatments on senescence and chlorophyll degradation.

### 3.2. Effect of UV-C treatment on postharvest senescence of broccoli

#### 3.2.1. Superficial color and pigments content

Broccoli heads were UV-C treated ( $10 \text{ kJ m}^{-2}$ ) and superficial color parameters and pigment content were evaluated through the senescence period. Hue value decreased in all samples during storage at  $20^\circ\text{C}$ , but lower changes were observed in UV-C treated samples, in agreement with the visible yellowing delay (Table 2). On the contrary,  $L^*$  value increased during storage but treated samples presented a lower increase in lightness than the controls (Table 2).

Table 2

Change of lightness, hue, chlorophyll *a* and *b* in control and UV-C ( $10 \text{ kJ m}^{-2}$ ) treated broccoli during storage at  $20^\circ\text{C}$

|  | Days at $20^\circ\text{C}$ |        |        |        |
|--|----------------------------|--------|--------|--------|
|  | 0                          | 2      | 4      | 6      |
| <i>L*</i>                                    |                            |        |        |        |
| Control                                      | 40.55                      | 40.97  | 46.37  | 51.20  |
| UV-C   | 39.27                      | 38.75  | 39.97* | 41.26* |
| Hue  |                            |        |        |        |
| Control                                      | 123.6                      | 126.0  | 119.1  | 113.4  |
| UV-C   | 122.6                      | 125.5  | 124.6* | 122.3* |
| Chlorophyll <i>a</i> ( $\text{mg kg}^{-1}$ ) |                            |        |        |        |
| Control                                      | 151.2                      | 123.1  | 83.2   | 57.4   |
| UV-C   | 154.3                      | 167.9* | 129.1* | 92.2*  |
| Chlorophyll <i>b</i> ( $\text{mg kg}^{-1}$ ) |                            |        |        |        |
| Control                                      | 109.1                      | 80.7   | 49.7   | 29.8   |
| UV-C   | 102.3                      | 103.4* | 72.8*  | 47.4*  |

The asterisk indicates that the value is significantly different from the corresponding control at  $P < 0.05$ .

Total chlorophyll decreased during storage at  $20^\circ\text{C}$ , and chlorophyll degradation rate was slowed by the UV-C treatment (Fig. 1A). After 4 d at  $20^\circ\text{C}$ , UV-C treated florets had approximately 53% more chlorophyll than control florets. The levels of chlorophyll *a* and *b* decreased during storage at  $20^\circ\text{C}$  and the UV-C treatments delayed both chlorophyll *a* and *b* degradation (Table 2). In the case of Pheo, an accumulation was observed after 2 and 4 d of incubation at  $20^\circ\text{C}$ , followed by a reduction after 6 d at  $20^\circ\text{C}$  (Fig. 1B). No differences in Pheo content were found either immediately after the UV-C treatment or after 2 d at  $20^\circ\text{C}$ , but afterwards the UV-C treated broccoli accumulated less Pheo than control broccoli.

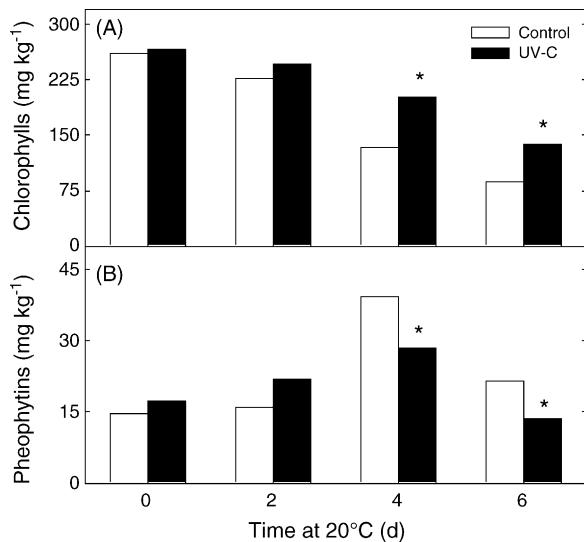


Fig. 1. Total chlorophyll (A) and pheophytin (B) content in control and UV-C treated broccoli ( $10 \text{ kJ m}^{-2}$ ) during storage at  $20^\circ\text{C}$ . The asterisk indicates that the value is significantly different from the corresponding control at  $P < 0.05$ .

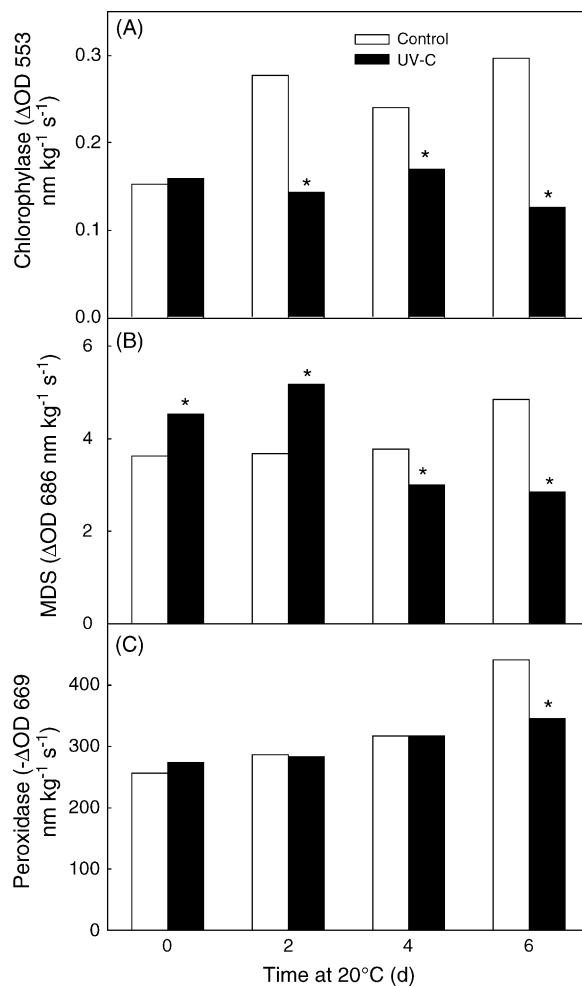


Fig. 2. Change of chlorophyll degrading enzymes activities in control and UV-C treated broccoli ( $10 \text{ kJ m}^{-2}$ ) during storage at  $20^\circ\text{C}$ . (A) chlorophyllase; (B) Mg-dechelatase and (C) peroxidase. The asterisk indicates that the value is significantly different from the corresponding control at  $P < 0.05$ .

### 3.2.2. Chlorophyll degrading enzymes

Chlorophyllase activity increased in control broccoli during storage at  $20^\circ\text{C}$ . Otherwise, the chlorophyllase activity of UV-C treated broccoli did not change during storage and remained below the enzyme activity of control broccoli throughout the storage period (Fig. 2A). Mg-dechelatase activity did not show any change until 4 d of storage at  $20^\circ\text{C}$  in control samples, but increased thereafter (Fig. 2B). UV-C treated florets showed an increase in MDS activity immediately after the treatment and after 2 d at  $20^\circ\text{C}$ . However, after that MDS activity dropped to lower levels than those found in control broccoli. Chlorophyll peroxidase activity increased in both control and UV-C treated broccoli heads along the storage period (Fig. 2C). No differences in activity were found either immediately after the treatment or after 4 d at  $20^\circ\text{C}$ . However after 6 d at  $20^\circ\text{C}$  treated broccoli showed lower peroxidase activity than control samples.

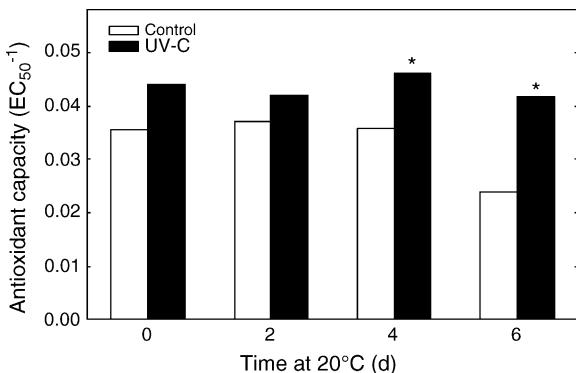


Fig. 3. Antioxidant capacity in control and UV-C treated broccoli ( $10\text{ kJ m}^{-2}$ ) during storage at  $20^\circ\text{C}$ . The asterisk indicates that the value is significantly different from the corresponding control at  $P < 0.05$ .

### 3.2.3. Antioxidant capacity

Immediately after the treatment the treated broccoli had higher antioxidant capacity than control broccoli (Fig. 3). Antioxidant capacity maintained during storage until day 4 and decreased thereafter in control samples. No changes in  $\text{EC}_{50}^{-1}$  values were observed in treated samples through 6 d of storage.

### 3.2.4. Respiration rate

Immediately after treatment there were no differences in respiration rate between control and treated broccoli heads (Table 3). During storage at  $20^\circ\text{C}$   $\text{CO}_2$  production increased more markedly in control broccoli, and after 4 d of storage these samples respired at a greater rate than UV-C treated broccoli.

### 3.2.5. Total phenols and flavonoids

Total phenols increased after UV-C treatment (Table 3). During storage at  $20^\circ\text{C}$ , total phenols increased in both control and treated broccoli. However, the increment was higher in the case of untreated broccoli. After 4 and 6 d of storage at  $20^\circ\text{C}$  UV-C treated florets displayed lower levels of total phenols. Flavonoids also increased during storage in control and

UV-C treated broccoli as senescence took place, but lower levels were found in UV-C treated broccoli after 4 and 6 d at  $20^\circ\text{C}$  (Table 3).

## 4. Discussion

Fruits and vegetables are severely stressed after harvest due to a reduction in the sources of energy, nutrients, hormones and water and this leads to a rapid initiation of senescence (King and Morris, 1994). In the case of broccoli, one of the main symptoms of senescence is yellowing due to chlorophyll catabolism (Tian et al., 1994). Short UV-C treatments have been used mainly to reduce the incidence and severity of postharvest diseases (Stevens et al., 1996; Nigro et al., 1998, 2000), but beneficial physiological effects in vegetable tissues like retardation of some ripening-associated changes (Barka et al., 2000; Baka et al., 1999) and reduction of physiological disorders (González-Aguilar et al., 2004; Vicente et al., 2005) have also been reported. However, the effect of UV-C treatment on postharvest senescence has not been reported. Postharvest broccoli senescence is associated with yellowing, chlorophyll degradation, and pheophytin accumulation (Costa et al., 2005a). UV-C treatments ( $4\text{--}14\text{ kJ m}^{-2}$  doses) delay these processes. However, treatment with  $14\text{ kJ m}^{-2}$  reduced chlorophyll degradation but did not delay the increase of pheophytin content. This high level of pheophytins could be the reason that heads treated with  $14\text{ kJ m}^{-2}$  showed a superficial color similar to controls. It is possible that high doses of UV-C directly affect the process of chlorophyll molecules releasing  $\text{Mg}^{2+}$ . To analyze this possibility, we treated two chlorophyll solutions (in acetone and in water plus Triton X-100) with the same UV-C dose, but we did not find any pheophytin formation (data not shown). This indicates that there is not a direct effect of UV-C on chlorophyll molecules and that the accumulation of pheophytins in UV-C treated samples probably occurs through a biochemical mechanism that requires several components and possibly intact tissue. Previous studies have shown that excessive doses of UV light affect the chloroplast structure in pea leaves by producing membrane disorganization and progressive disruption of thylakoids (Kovács and Keresztes, 2002; He et al., 1994). This could liberate chlorophyll from the pigment–protein complexes, facilitating enzymatic and/or non-enzymatic formation of pheophytin.

Hue value in broccoli is higher when the content of chlorophyll increases and the amount of pheophytin decreases. Therefore, the influence of chlorophyll increase on hue can be counteracted by a simultaneous increase of pheophytins. The highest hue value was observed with a treatment of  $7\text{ kJ m}^{-2}$ , while the highest chlorophyll retention was found in the  $10$  and  $14\text{ kJ m}^{-2}$  treatments. However, the latter treatment provoked a higher accumulation of Pheo, which probably contributed to lowering the hue value.

The heads treated with  $10\text{ kJ m}^{-2}$  maintained higher chlorophyll levels than controls by delaying both chlorophyll

Table 3  
Respiration rate, total phenols and flavonoids in control and UV-C ( $10\text{ kJ m}^{-2}$ ) treated broccoli during storage at  $20^\circ\text{C}$

|  | Days at $20^\circ\text{C}$ |       |         |       |
|--|----------------------------|-------|---------|-------|
|  | 0                          | 2     | 4       | 6     |
| $\text{CO}_2$ production ( $\text{mg kg}^{-1} \text{s}^{-1}$ ) |                            |       |         |       |
| Control  | 90.42                      | 98.96 | 182.52  | ND    |
| UV-C   | 85.03                      | 91.23 | 147.52* | ND    |
| Total phenols ( $\text{g kg}^{-1}$ )                           |                            |       |         |       |
| Control  | 0.68                       | 0.93  | 1.18    | 1.26  |
| UV-C   | 0.81*                      | 0.89  | 1.05*   | 1.05* |
| Flavonoids ( $\text{g kg}^{-1}$ )                              |                            |       |         |       |
| Control  | 0.46                       | 0.62  | 0.92    | 1.04  |
| UV-C   | 0.50                       | 0.67  | 0.76*   | 0.67* |

The asterisk indicates that the value is significantly different from the corresponding control at  $P < 0.05$ .

*a* and *b* degradation. Chlorophyll degradation can be slowed in broccoli by other physical treatments like heat treatments (Funamoto et al., 2002). In our case, the higher chlorophyll content detected in UV-C treated samples correlated with the lower chlorophyllase and MDS activity. In the case of chlorophyll-peroxidase activity, the effect of UV-C treatment was evident only at the end of storage. This indicates that the delay in chlorophyll degradation could be due to the effect of the treatment on chlorophyllase and MDS activity rather than on peroxidase activity. Many reports have shown that UV-C light could affect the activity of enzymes involved in postharvest metabolism like polygalacturonase, cellulase and proteases (Barka et al., 2000), and our results show that this is also true in the case of the enzymes involved in chlorophyll catabolism (MDS, chlorophyllase and chlorophyll-peroxidase). Previous studies showed that all three enzymes are stimulated by ethylene (Maeda et al., 1998; Jacob-Wilk et al., 1999). This hormone is not necessary for senescence but accelerates the process (Buchanan-Wollaston et al., 2003). UV-C treatments reduce ethylene production in horticultural products (Stevens et al., 1996; Maharaj et al., 1999) and could slow the increase of ethylene responsive enzymes. However, other important aspects of senescence, like ethylene sensitivity and/or changes in protein synthesis and degradation, could be modified by the UV-C treatments and further research is needed to address this point.

In the case of MDS, higher activity was found in UV-C treated broccoli immediately after the treatment, indicating a short time effect of UV-C on the enzyme. It has been reported that MDS activity increases in the presence of  $H_2O_2$  and that the enzyme is inhibited by reduced glutathione and  $HgCl_2$ , suggesting that the SH groups are necessary for enzymatic activity and that the redox state of these groups is critical for the catalytic activity (Vicentini et al., 1995; Costa et al., 2002a). As UV light stimulates the production of reactive oxygen species (ROS) (A-H-Mackerness et al., 1999), it is possible that an increase of  $H_2O_2$  could modify the redox state of MDS and increase the enzyme activity in UV-C treated broccoli relatively rapidly.

UV-C treatment also increased the antioxidant capacity of the florets and the UV-C treated broccoli maintained higher antioxidant levels than control broccoli during storage. Many reports have described an increase in antioxidant levels in response to UV exposure (Douillet-Breuil et al., 1999; Adrian et al., 2000). Thus, the application of UV-C to delaying broccoli senescence could be beneficial from a nutritional perspective. According to respiration rate data, the UV-C treatments also delayed tissue damage. The results found in this work are in accordance with Vicente et al. (2005) who found lower levels of respiration, phenols and electrolyte leakage in UV-C treated peppers during storage. In the case of phenols, higher amounts were found immediately after the UV-C treatment. It is well known that phenylpropanoid compounds are synthesized in response to UV-C treatments and it is believed that this could be a protective mechanism of plant tissues against excessive radiation (Bieza and Lois,

2001). Previous studies have found that UV-C treatments induce PAL, which is a key regulatory enzyme of phenylpropanoid metabolism (Stevens et al., 1990; Chalutz et al., 1992). Total phenols increased in both control and UV-C treated broccoli during storage, with the increase being higher in untreated broccoli. An increase in total phenols has been reported during broccoli development (Vallejo et al., 2003), and the lower level found in the case of UV-C treated broccoli in this work could be due to a less advanced developmental stage.

## 5. Conclusions

Short UV-C treatments (4, 7, 10 and 14  $\text{kJ m}^{-2}$ ) delayed chlorophyll degradation in broccoli, with 10 and 14  $\text{kJ m}^{-2}$  dose cases showing the greatest delay. However, only 4, 7 and 10  $\text{kJ m}^{-2}$  doses reduced Pheo accumulation. The UV-C treatment with a dose of 10  $\text{kJ m}^{-2}$  delayed not only chlorophyll *a* and *b* degradation but also the increase of chlorophyllase and chlorophyll-peroxidase activity. In the case of MDS, higher activity was found immediately after the treatments, but after 4 and 6 d at 20 °C UV-C treated broccoli maintained lower MDS level than controls. The UV-C treatments also reduced tissue damage and disruption according to data obtained from respiration rate and phenolic compound content. The antioxidant capacity was increased by UV-C treatments and this could be useful from the nutritional point of view. Results suggest that short UV-C treatments could be a useful non-chemical method to delay senescence in broccoli.

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## References

- Able, A.J., Wong, L.S., Prasad, A., O'Hare, T.J., 2002. 1-MCP is more effective on a floral brassica (*Brassica oleracea* var. *italica* L.) than a leafy brassica (*Brassica rapa* var. *chinensis*). *Postharvest Biol. Technol.* 26, 147–155.
- Adrian, M., Jeandet, P., Douillet Breuil, A.C., Tesson, L., Bessis, R., 2000. Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. *J. Agric. Food Chem.* 48, 6103–6105.
- A-H-Mackerness, S., Surplus, S.L., Blake, P., John, C.F., Buchanan-Wollaston, V., Jordan, B.R., Thomas, B., 1999. Ultraviolet-B induced stress and changes in gene expression in *Arabidopsis thaliana*: role of signaling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. *Plant Cell Environ.* 22, 1413–1423.
- Baka, M., Mercier, J., Corcuff, F., Castaigne, F., Arul, J., 1999. Photochemical treatment to improve storability of fresh strawberries. *J. Food Sci.* 64, 1068–1072.
- Barka, E.A., Kalantari, S., Makhoul, J., Arul, J., 2000. Impact of UV-C irradiation on the cell wall-degrading enzymes during ripening of tomato (*Lycopersicon esculentum* L.) fruit. *J. Agric. Food Chem.* 48, 667–671.

- Bieza, K., Lois, R., 2001. An *Arabidopsis* mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. *Plant Physiol.* 126, 1105–1115.
- Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* 28, 25–30.
- Brosché, M., Fant, C., Bergkvist, S.W., Strid, H., Svensk, A., Olsson, O., Strid, A., 1999. Molecular markers for UV-B stress in plants: alteration of the expression of four classes of genes in *Pisum sativum* and the formation of high molecular mass RNA adducts. *Biochim. Biophys. Acta* 1447, 185–198.
- Buchanan-Wollaston, V., Earl, S., Harrison, E., Mathas, E., Navabpour, S., Page, T., Pink, D., 2003. The molecular analysis of leaf senescence—a genomics approach. *Plant Biotechnol. J.* 1, 3–22.
- Chalutz, E., Droby, S., Wisniewski, M.E., 1992. UV-induced resistance to postharvest diseases of citrus fruits. *J. Phytochem. Photobiol.* 15, 367–374.
- Clarke, S.F., Jameson, P.E., Downs, C.G., 1994. The influence of 6-benzylaminopurine on post-harvest senescence of floral tissues of broccoli (*Brassica oleracea* var Italica). *Plant Growth Regul.* 14, 21–27.
- Costa, M.L., Civello, P.M., Chaves, A.R., Martínez, G.A., 2002a. Characterization of Mg-dechelatase activity obtained from *Fragaria* × *ananas* fruit. *Plant Physiol. Biochem.* 40, 111–118.
- Costa, H., Gallego, S.M., Tomaro, M.L., 2002b. Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons. *Plant Sci.* 162, 939–945.
- Costa, M.L., Civello, P.M., Chaves, A.R., Martínez, G.M., 2005a. Effect of ethephon and 6-benzylaminopurine on chlorophyll degrading enzymes and a peroxidase-linked chlorophyll bleaching during postharvest senescence of broccoli (*Brassica oleracea* L.) at 20 °C. *Postharvest Biol. Technol.* 35, 191–199.
- Costa, M.L., Civello, P.M., Chaves, A.R., Martínez, G.A., 2005b. Effect of hot air treatments on senescence and quality parameters of harvested broccoli (*Brassica oleracea* L. var *italica*) heads. *J. Sci. Food Agric.* 85, 1154–1160.
- Danon, A., Gallois, P., 1998. UV-C radiation induces apoptotic-like changes in *Arabidopsis thaliana*. *FEBS Lett.* 437, 131–136.
- D'hallewin, G., Schirra, M., Pala, M., Ben-Yehoshua, S., 2000. Ultraviolet-C irradiation at 0.5 kJ m<sup>-2</sup> reduces decay without causing damage or affecting postharvest quality of star ruby grapefruit (*C. paradisi* Macf.). *J. Agric. Food Chem.* 48, 4571–4575.
- Douillet-Breuil, A.C., Jeandet, P., Adrian, M., Bessis, R., 1999. Changes in phytoalexin content of various *Vitis* spp. in response to ultraviolet-C elicitation. *J. Agric. Food Chem.* 47, 4456–4461.
- Funamoto, Y., Yamauchi, N., Shigenaga, T., Shigyo, M., 2002. Effects of heat treatment on chlorophyll degrading enzymes in stored broccoli (*Brassica oleracea* L.). *Postharvest Biol. Technol.* 24, 163–170.
- Gonzalez-Aguilar, G., Wang, C.Y., Buta, G.J., 2004. UV-C irradiation reduces breakdown and chilling injury of peaches during cold storage. *J. Sci. Food Agric.* 84, 415–422.
- He, J., Hung, L.K., Whitecross, M.L., 1994. Chloroplast ultrastructure changes in *Pisum sativum* associated with supplementary ultraviolet (UV-B) radiation. *Plant Cell Environ.* 17, 771–775.
- Hörtensteiner, S., 1999. Chlorophyll breakdown in higher plants and algae. *Cell. Mol. Life Sci.* 56, 330–347.
- Jacobsson, A., Nielsen, T.M., Sjoholm, I., Wendin, K., 2004. Influence of packaging material and storage condition on the sensory quality of broccoli. *Food Qual. Pref.* 15, 301–310.
- Jacob-Wilk, D., Holland, D., Goldschmidt, E.E., Riov, J., Eyal, Y., 1999. Chlorophyll breakdown by chlorophyllase: isolation and functional expression of the *Chlase1* gene from ethylene-treated *Citrus* fruit and its regulation during development. *Plant J.* 20, 653–661.
- King, G.A., Morris, S.C., 1994. Early compositional changes during postharvest senescence of broccoli. *J. Am. Soc. Hort. Sci.* 119, 1000–1005.
- Kovács, E., Keresztes, A., 2002. Effect of δ and UV-B/C radiation on plant cell. *Micron* 33, 199–210.
- Ku, V.V.V., Wills, R.B.H., 1999. Effect of 1-methylcyclopropene on the storage life of broccoli. *Postharvest Biol. Technol.* 17, 127–132.
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Method Enzymol.* 148, 350–382.
- Luckey, T.D., 1980. Hormesis with Ionizing Radiation. CRC Press, Boca Raton.
- Maeda, Y., Kurata, H., Adachi, M., Shimokawa, K., 1998. Chlorophyll catabolism in ethylene-treated *Citrus unshiu* fruits. *J. Jpn. Soc. Hortic. Sci.* 67, 497–502.
- Maharaj, R., Arul, J., Nadeau, P., 1999. Effect of photochemical treatment in the preservation of fresh tomato (*Lycopersicon esculentum* cv Capello) by delaying senescence. *Postharvest Biol. Technol.* 15, 13–23.
- Matile, P., 1980. Catabolism of chlorophyll. Involvement of peroxidase. *Z. Pflanzenphysiol.* 99, 475–478.
- Matile, P., Hörtensteiner, S., Thomas, H., 1999. Chlorophyll degradation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 67–95.
- Nigro, F., Ippolito, A., Lima, G., 1998. Use of UV-C light to reduce *Botrytis* rot of table grapes. *Postharvest Biol. Technol.* 13, 171–181.
- Nigro, F., Ippolito, A., Lattanzio, V., Venere, D.D., Salerno, M., 2000. Effect of ultraviolet-C light on postharvest decay of strawberry. *J. Plant Pathol.* 82, 29–37.
- Page, T., Griffiths, G., Buchanan-Wollaston, V., 2001. Molecular and biochemical characterization of postharvest senescence in broccoli. *Plant Physiol.* 125, 718–727.
- Pan, J., Vicente, A., Martínez, G., Chaves, A., Civello, M., 2004. Combined use of UV-C irradiation and heat treatment to improve postharvest life of strawberry fruit. *J. Sci. Food Agric.* 84, 1831–1838.
- Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol.* 299, 152–153.
- Stevens, C., Khan, V.A., Tang, A.Y., Lu, J.Y., 1990. The effect of ultraviolet irradiation on mold rots and nutrients of stored sweet potatoes. *J. Food Protect.* 53, 223–226.
- Stevens, C., Wilson, C.L., Lu, J.Y., Khan, V.A., Chalutz, E., Droby, S., Kabue, M.K., Haung, Z., Adeyeye, O., Pusey, L.P., Wisniewski, M.E., West, M., 1996. Plant hormesis induced by ultraviolet light-C for controlling postharvest diseases of tree fruits. *Crop Protect.* 15, 129–134.
- Suzuki, Y., Uji, T., Terai, H., 2004. Inhibition of senescence in broccoli florets with ethanol vapor from alcohol powder. *Postharvest Biol. Technol.* 31, 177–182.
- Tian, M.S., Downs, C.G., Lill, R.E., King, G.A., 1994. A role for ethylene in the yellowing of broccoli. *J. Am. Soc. Hortic. Sci.* 119, 276–281.
- Toivonen, P.M.A., 1997. The effects of storage temperature, storage duration, hydro-cooling, and micro-perforated wrap on shelf life of broccoli (*Brassica oleracea* L., Italica Group). *Postharvest Biol. Technol.* 10, 59–65.
- Vallejo, F., García-Viguera, C., Tomás-Barberá, N., 2003. Changes in broccoli (*Brassica oleracea* L. Var. *italica*) health-promoting compounds with inflorescence development. *J. Agric. Food Chem.* 51, 3776–3782.
- Vicente, A., Repice, B., Martínez, G., Chaves, A., Civello, M., Sozzi, G., 2004. Maintenance of fresh boysenberry fruit quality with UV-C light and heat treatments combined with low storage temperature. *J. Hortic. Sci. Biotech.* 79, 246–251.
- Vicente, A.R., Pineda, C., Lemoine, L., Civello, P.M., Martínez, G.A., Chaves, A.R., 2005. UV-C treatments reduce decay, keep quality and alleviate chilling injury in pepper. *Postharvest Biol. Technol.* 35, 69–78.
- Vicentini, F., Iten, F., Matile, P., 1995. Development of an assay for Mg-dechelatase of oilseed rape cotyledons, using chlorophyllin as the substrate. *Physiol. Plant.* 94, 57–63.
- Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64, 555–559.