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Pulses of low intensity light as promising technology to delay postharvest senescence of broccoli

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ABSTRACT

Visible light irradiance may be a useful technology to delay postharvest senescence of green vegetables. In this work, we studied the effects of low-intensity white, red and far red light pulses on postharvest senescence of broccoli stored in the dark at 20 °C. Daily exposure for 2 h to 20–25 μmol m^{-2} s^{-1} of white light delayed yellowing and retained chloroplast components (chlorophyll and soluble proteins). The utilized light intensity was insufficient to re-initiated photosynthesis since total sugar content was lower than initials in irradiated florets. Light treatment resulted in a slower loss of sugars in comparison with the untreated samples, but was not affected by light quality. The effects of red light treatment on chlorophyll a and soluble protein degradation were similar to white light, and opposite to far red light. However, these treatments did not delay chlorophyll *b* degradation, suggesting that phytochromes could be involved in molecular mechanism of chlorophyll a and soluble protein degradation, but not of chlorophyll *b*.

1. Introduction

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) consumption has increased markedly in the last few decades, in part due to its high concentrations of vitamins, antioxidants and anticarcinogenic compounds as glucosinolates (Yuan et al., 2010). For commercial purpose, broccoli inflorescences are harvested when they are still immature, and are highly perishable products with a high senescence rate. The main symptoms of plant tissue senescence are photosynthetic apparatus dismantling which leads to massive chlorophyll and protein degradation and the loss of chloroplast functioning (Buchanan-Wollaston et al., 2003; Page et al., 2001; Costa et al., 2013a). The typical visual change detected during postharvest senescence of broccoli is yellowing, but it is accompanied by other changes in several metabolic pathways that also affect its organoleptic and nutritional qualities (Page et al., 2001; Nishikawa et al., 2005; Costa et al., 2006).

The rate of postharvest senescence of broccoli heads can be modulated by storage conditions. Refrigeration at 0 °C with 98 to 100% relative humidity is recommended conditions for broccoli storage, under which its shelf life can reach 20 d (Toivonen and Forney, 2016). However, cooling and refrigeration facilities are not easily available in many countries, and frequently postharvest storage, handling, transportation and spending phases take place at ambient temperature (Jones et al., 2006; Yuan et al., 2010). At 20–25 °C, the shelf life of broccoli decreases to 3 d, and it is necessary to develop strategies to delay senescence at these high temperatures. Different treatments have been widely investigated as technologies to delay broccoli senescence, including heat treatments, UV-C radiation, controlled atmosphere and 1- MCP (Costa et al., 2005, 2006; Jones et al., 2006; Jia et al., 2009; Yuan et al., 2010; Perini et al., 2017). None of these technologies are still being applied in the productive sector of Argentina.

More recently, visible light irradiance, which is an environmental friendly treatment, has been investigated as a means of delaying postharvest senescence of green vegetables. Darkness induces senescence in detached leaves of green vegetables, and therefore, light exposure during storage could delay senescence development. The effectiveness of light treatment on postharvest quality of vegetables depends of light intensity and photoperiod used. Lester et al. (2010) showed that treatment with low intensity light preserves nutritional qualities of spinach. Moreover, when leaves of spinach were treated with light pulses and were transferred to a chamber at 4 °C under continuous dark, senescence was delayed and ascorbic acid and glutathione contents were kept higher (Gergoff Grozeff et al., 2013). Combinations of continuous low intensity light exposure with refrigeration during storage

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preserved nutritional quality and prolonged shelf-life of fresh-cut broccoli (Zhan et al., 2012). Light quality can also influence the senescence process; Ma et al. (2014) found that continuous irradiation with 50 μmol m^{-2} s^{-1} from red LED light was effective in delaying senescence, but in contrast, a similar blue LED light treatment had little effect. Most studies about postharvest light treatment have been focused on senescence symptoms of vegetables, but less is known about the physiological mechanism mediated by light. Postharvest senescence of fresh basil was delayed by low light pulses (Costa et al., 2013b). The intensity used as postharvest treatment during storage of basil was lower than the photosynthesis light compensation point of basil leaves and the same effect was observed with white or red light. From these results, it seems that the light effect on delaying senescence would be mediated by phytochromes (photoreceptors sensitive to red light) signal rather than by photosynthesis.

The aim of this work was to found a suitable low intensity white light pulses treatment to delay broccoli postharvest senescence at room temperature. The possible role of photosynthesis or phytochromes in the control of this process is also discussed.

2. Materials and methods

2.1. Plant material and experimental design

Broccoli heads (*Brassica oleracea* L. var. *italica* Plenck "Legacy") were harvested early in the morning from local producer Los Hornos, La Plata, Argentina, (34°54′45.69″S, 57°55′50.39″O) and immediately transported to the laboratory. Heads were placed in PVC trays with perforated cover to decrease water loss (one head per tray). In the first experiment, different times of light treatment were used to select the most appropriate duration to delay senescence. Four treatments were performed with seven trays for each and other seven trays were used as initial samples. Treatments consisted of control (without light treatment), 30 min, 1 h or 2 h of irradiation at 20–25 μmol m^{-2} s^{-1} (different times of white light provided by fluorescent lamps) for each day of storage. In a second experiment, the effect of different light qualities was analyzed. Again, four treatments were performed; control (without light treatment), white light, red light and far red light. To irradiate broccoli heads with red and far red light, the respective LEE filters were placed between lamps and florets so that irradiance reached 20–25 μmol m^{-2} s^{-1} as described in Costa et al. (2013b). The irradiance was measured with a photosynthetically active radiation quantum sensor (RADIAPAR, Cavadevices, Argentina) and the spectral qualities were analyzed with a spectrometer (USB650, Red Tide, Ocean Optics, USA). After treatment all trays were stored at 20 °C in darkness. Each broccoli head was weighed every day and the percentage of weight loss was determined. Florets were taken at the beginning of the experiment and after 3 d or 4 d of storage (D0, D3 and D4 in figures and tables). Florets of five heads per treatment were frozen at −80 °C and stored at −20 °C until analysis. To measure dry weight, some florets (2 or 3) of five heads per each treatment were dried at 60 °C. Each experiment was repeated two times, and the same trend was found.

2.2. Color measurement

External color was determined by measuring L^*, a^*, and b^* with a chromameter (Minolta CR300, Osaka, Japan). The hue angle (H°) 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 $b > 0$. Five positions on each of 5 heads were measured for each treatment and storage time.

2.3. Chlorophyll content

Pigment content was determined spectrophotometrically according to Lichtenthaler (1987). Approximately 20 g of frozen broccoli florets were crushed in a mill and samples of 0.250 g were homogenized twice with 1.5 mL of 80% acetone (v/v), and then centrifuged at 6,000 × *g* for 10 min at 4 °C. The supernatant was used to determine the absorbance at 663.2 and 646.8 nm for chlorophylls and 470 nm for total carotenoid content. Total chlorophyll and chlorophyll a and b contents are expressed as mg of pigment per kg on a dry weight basis. Five replicates per treatment were analyzed.

2.4. Total phenolics

Total phenolic concentrations were determined spectrophotometrically according to Costa et al. (2006) with slight modifications. Approximately 20 g of frozen broccoli florets were crushed in a mill and samples of 0.50 g were homogenized twice with 1.5 mL of 96% ethanol (v/v). The mixture was centrifuged at 9000 × *g* for 10 min at 4 °C. The extracts were used to determine total phenolics; 150 μL of extract was added to 500 μL water and 200 μL of Folin–Ciocalteau reagent. After 3 min at 25 °C, 500 μL of saturated solution of Na_2CO_3 was added, and the reaction mixture was incubated for 30 min at 25 °C. The absorbance was measured at 760 nm and total phenolics were calculated by using phenol as standard. Results were expressed as mg of phenol per kg on a dry weight basis. Five replicates per treatment were analyzed.

2.5. Sugar content

Insoluble and soluble reducing sugar content was determined using Somogy Nelson (Southgate, 1976; Hasperué et al., 2011). Approximately 20 g of frozen broccoli florets were crushed in a mill and samples of 0.15 g were homogenized twice with 1 mL of 96% ethanol (v/v). The extract was centrifuged at 9,000 × *g* for 5 min at 4 °C. The supernatant was used for analysis of soluble reducing sugars. The pellet obtained after centrifugation was hydrolyzed with 1.5 mL of 1.1% HCl at 100 °C during 30 min. After cooling the suspension obtained was centrifuged at 9000 × *g* for 5 min at 4 °C and the supernatant was used to analyze insoluble sugars. After Somogy Nelson reaction the absorbance was measured at 520 nm. Glucose was used as standard, and total sugar content was calculated by adding up the soluble sugar and ethanol-insoluble (starch) fractions. Results were expressed as g of sugars per kg on a dry weight basis. Five replicates per treatment were performed.

2.6. Soluble protein content

Approximately 20 g of frozen broccoli florets were crushed in a mill and samples of 0.50 g were homogenized with 1.5 mL of buffer (50 mM Tris hydroxy-methylaminomethane–HCl, pH 7, with 1 mM EDTA and 1 mM PMSF) and centrifuged at 10,000 × *g* for 10 min at 4 °C. For SDS-PAGE analysis, one volume of the supernatant from protein extraction was mixed with one volume of 2× solubilization buffer (125 mM Tris pH 6.8; 4% w/v SDS; 10% v/v glycerol; 10% v/v β-mercaptoethanol), boiled for 5 min and separated in 1.5 mm thick, 12% acrylamide concentration minigels as in Laemmli (1970). Proteins were visualized by staining with Coomassie Brilliant BlueR-250. Gels were photographed with a digital camera, and the protein content was calculated by using the SIGMA gel analysis software. Different concentrations of bovine serum albumin (BSA) or molecular weight (Sigma) were included in each gel to serve as standard. Results were expressed as percentage of initial level of proteins on a dry weight basis. Five replicates per treatment were analyzed. Molecular markers (BIORAD, low range) were used as standard weight and large subunit of RUBISCO (LSU) and small subunit of RUBISCO (SSU) were estimated on molecular weight bases: 56 and 15 kDa respectively (Parry et al., 1987).

2.7. Statistical analysis

Each experiment was repeated two times. Data were analyzed by