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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

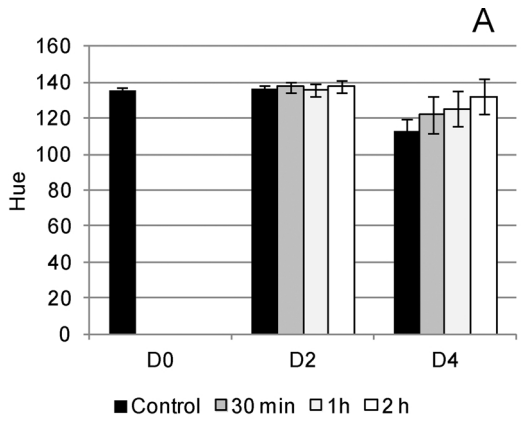
Insoluble and soluble reducing sugar content was determined using Somogy Nelson (Southgate, 1976; Hasperué et al., 2011). Approximately 20 g of frozen broccoli florets were crushed in a mill and samples of 0.15 g were homogenized twice with 1 mL of 96% ethanol (v/v). The extract was centrifuged at 9,000 × *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



B

	Control	30 min	1 h	2 h
Yellowing (Hue D0 – Hue D4)	21.5 a	13.3 b	9.5 b	2.5 c

Fig. 1. A) External color parameter (Hue) of broccoli heads after 0 (D0), 2 (D2) and 4 (D4) d of storage. Florets either had no light treatment (Control) or were treated with low intensity white light pulses (20–25 μmol m^{-2} s^{-1}) for 30 min, 1 h or 2 h every day and then stored in darkness at 20 °C. The results are expressed as the mean (n = 7) ± the standard deviation. Different letters indicate significant differences ($p < 0.05$) among treatments. B) Yellowing of broccoli calculated as the difference of Hue between D4 and D0 for each treatment. Different letters indicate significant differences ($p < 0.05$) among treatments.

ANOVA, and the means were compared with Tukey's Test at a significance level of 0.05.

3. Results

3.1. Effect of low intensity white light treatment on postharvest senescence of broccoli

To select the most suitable time of white light treatment we analyzed the effect of different time pulses of irradiation on postharvest senescence of broccoli. Broccoli has a very short shelf life at room temperature due to the yellowing of its florets. Initial H° values were approximately 140 and decreased in all florets during storage at 20 °C (Fig. 1A). After 4 d, H° decreased less in all light treatments than in control, indicating that light treatment delayed the broccoli yellowing (Fig. 1B, Supplementary Fig. 1). The lowest change in H° was found in heads exposed to 2 h of low intensity light every day.

Control florets had a weight loss around 6% after 4 d at 20 °C, while weight loss increased with duration of treatment with the pulse of low intensity white light (Fig. 2). The highest weight loss, around 11%, was found in 2 h treatment. Since there were considerable differences in weight loss among treatments, analytical determinations data were expressed based on dry mass.

A significant decrease of total chlorophyll was observed during postharvest storage of broccoli heads. Approximately 40% of the initial chlorophyll content was lost after 4 d in control florets (Fig. 3A). The effect of low intensity white light treatment on chlorophyll degradation depended on the pulse duration time. While the chlorophyll content was similar in florets treated 30 min and in controls, only 22% of chlorophyll was lost in florets irradiated 1 h after 4 d. Finally, florets treated for 2 h did not lose chlorophyll contents after 4 d. Although total chlorophyll degradation was lower in 1 h treatment, there were no differences in chlorophyll a (Chla) degradation among control, 30 min and 1 h treatments (Fig. 3B). Chlorophyll b (Chlb) degradation was higher in control and 30 min treatment while both 1 and 2 h treatments retained high percentage of Chlb and this effect affected the chlorophyll

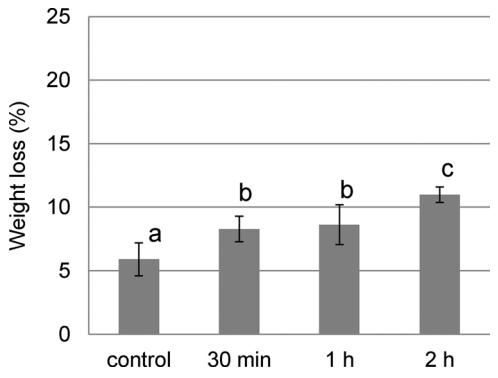


Fig. 2. Effects of treatments on weight loss of broccoli heads. Florets either had no light treatment (control) or were treated with low intensity white light pulses (20–25 μmol m^{-2} s^{-1}) for 30 min, 1 h or 2 h every day and then stored for 4 d in darkness at 20 °C. The results are expressed as the mean (n = 5) ± the standard deviation. Different letters indicate significant differences ($p < 0.05$) among treatments.

a/b ratio (Fig. 3C and D).

Total phenolics increased during storage at 20 °C (Fig. 4). After 4 d, total phenolics increased in both control and treated broccoli, but the increase was higher in the case of the 1 and 2 h treatments.

Another typical senescence symptom associated with disorganization of chloroplasts is protein degradation. After 4 d of storage at 20 °C, control and 30 min treated florets retained only 50% of initial proteins. However, florets treated with 1 h and 2 h retained 65 and 75% of proteins, respectively (Fig. 5), indicating that some light treatments can also delay protein degradation during postharvest senescence of broccoli.

Finally, taking into account that light treatments could maintain active photosynthesis, we also analyzed sugar content in broccoli samples. During storage at 20 °C the level of total sugars decreased around 50% in all florets except in 2 h treatment which caused retention of about 80% of total sugar (Table 1). However, differences among treatments in the content of total sugars are explained by differences in reducing sugars rather than in insoluble ones (Table 1).

Taken together, these results indicate that 2 h pulses of low intensity white light applied daily were effective to delay postharvest senescence of broccoli.

3.2. Effect of low intensity red and far red light treatment on postharvest senescence of broccoli

To analyze the mechanism of action of low intensity light treatment, in a second experiment we investigated if pulses of red light have the same effect as white light on postharvest senescence of broccoli and if pulses of far red light could revert this effect. Broccoli florets were irradiated each day with 2 h of low intensity red light using a LEE red filter positioned between the light source and broccoli trays.

Red light had similar effect on yellowing and total chlorophyll retention to white light treatment, while far red light was similar to that of control florets(Fig. 6A and B; Supplemental Fig. 2). The effect of red light treatment on Chla and Chlb degradation was different (Fig. 7). In the florets stored in darkness, Chla decreased and was significantly lower than florets exposed to white or red light. After 3 d, florets treated with white or red light retained 16% more Chla compared with that of florets in darkness or treated with far red light (Fig. 7A). Regarding Chlb, while white treatment caused retention of 75% in relation to initial levels, florets treated with red and far red light showed the same decrement of Chlb as controls (Fig. 7B). Therefore, the chlorophyll a/b ratio of heads treated with white and red light was different (Fig. 7C).

Protein degradation during postharvest senescence was delayed by red light treatment similar to that caused by white light treatment, and this effect was reversed with far red light treatment (Fig. 8). White light treatment resulted in higher total phenolic concentrations after 3d,

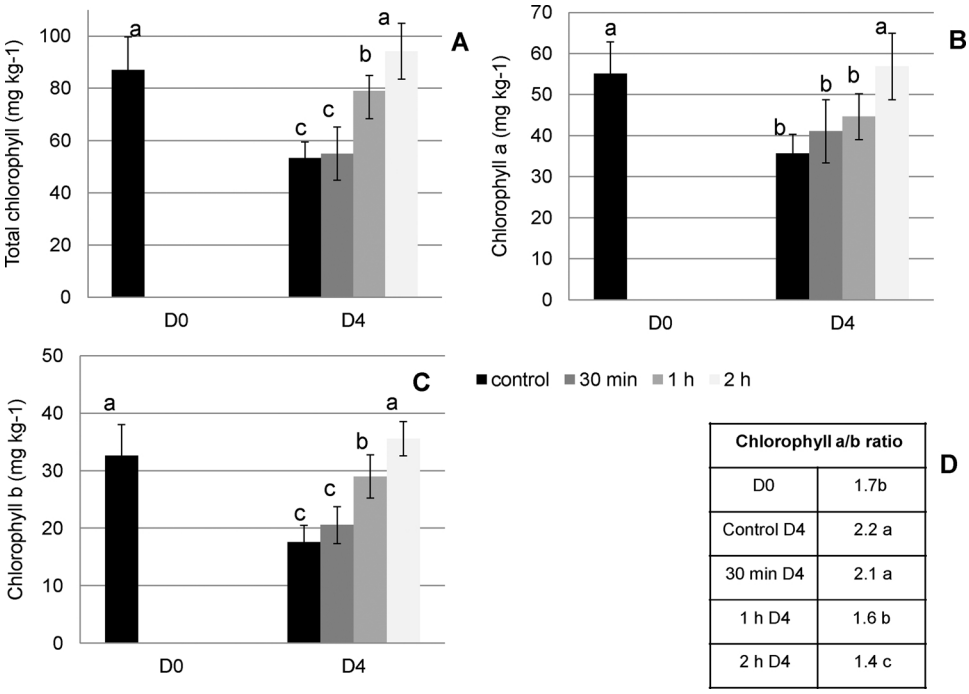


Fig. 3. Content of total chlorophylls (A); chlorophyll a (B) and chlorophyll *b* (C) at initials (D0) and after 4 d (D4) in broccoli florets without light treatment (control) or treated with low intensity light pulses (20–25 μmol m^{-2} s^{-1}) for 30 min, 1 h or 2 h every day and stored at 20 °C in darkness. Chorophyll a/b was calculated for each sample (D). The results are expressed as the mean (n = 5) ± the standard deviation (A-C). Different letters indicate significant differences ($p < 0.05$) among treatments.

D

Chlorophyll a/b ratio	
D0	1.7b
Control D4	2.2 a
30 min D4	2.1 a
1 h D4	1.6 b
2 h D4	1.4 c

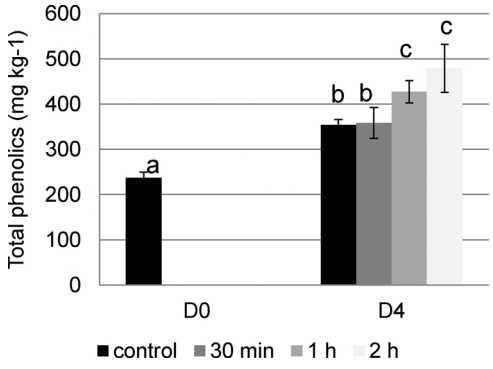


Fig. 4. Content of total phenolics at initials (D0) and after 4 d (D4) in broccoli florets either had no light treatment (Control) or were treated with low intensity white light pulses (20–25 μmol m^{-2} s^{-1}) for 30 min, 1 h or 2 h every day and then stored in darkness at 20 °C. The results are expressed as the mean (n = 5) ± the standard deviation. Different letters indicate significant differences ($p < 0.05$) between treatments.

while red light and far red light treatments were similar to those of untreated florets (Fig. 9). Total sugar concentrations decreased approximately 60% in florets without treatment after 3d of storage while but by only 30% in all light treated florets (Table 2).

Table 1
Reducing soluble, insoluble and total sugar concentrations of broccoli florets at the start of the experiment (D0) and after 4 d in darkness (D4). Florets either had no light treatment (Control) or were treated with low intensity white light pulses (20–25 μmol m^{-2} s^{-1}) for 30 min, 1 h or 2 h every day and then stored in darkness at 20 °C. The results are expressed as the mean (n = 5) ± the standard deviation. Different letters indicate significant differences ($p < 0.05$) between treatments.

	Reducing sugars (g Kg^{-1})	Insoluble sugars (g Kg^{-1})	Total sugars (g Kg^{-1})
D0	30.30 ± 2.0 a	3.70 ± 0.2 a	34.00 ± 2.2 a
D4 Control	10.20 ± 5.4 c	2.79 ± 0.7 b	12.99 ± 6.1 c
D4 30 min	8.55 ± 2.5 c	3.00 ± 0.2 b	11.55 ± 2.7 c
D4 1h	12.15 ± 5.5 c	2.96 ± 0.5 b	15.11 ± 6.0 c
D4 2h	20.45 ± 2.3 b	2.83 ± 0.2 b	23.28 ± 2.5 b

4. Discussion

The main goal of broccoli postharvest technology is to extend its shelf life and improve its visual and nutritional qualities. To achieve,-this, it is necessary delay senescence development of florets. In the last years, light treatments were successfully utilized as methodologies to delay postharvest senescence of green vegetables (Zhan et al., 2012; Costa et al., 2013b; Gergoff Grozeff et al., 2013; Ma et al., 2014; Jin et al., 2015). In the case of broccoli, particularly, continuous irradiation

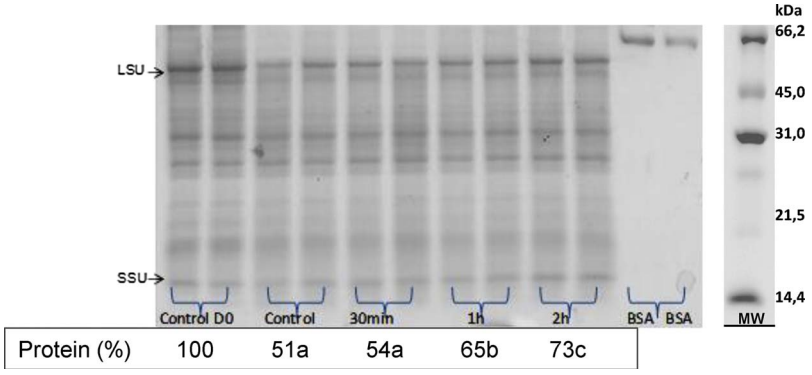


Fig. 5. Coomassie blue-stained gel of soluble proteins from broccoli florets. A quantitation of proteins in each gel line was done using known quantities of BSA as reference.% Protein was calculated for each gel line. Five data for each treatment was measurement. Different letters indicates significant differences ($p < 0.05$) among treatments. Additionally, molecular weight markers (MW) are showed and large subunit of RUBISCO (LSU) and small subunit of RUBISCO (SSU) were estimated on molecular weight bases: 56 and 15–16 kDa respectively.

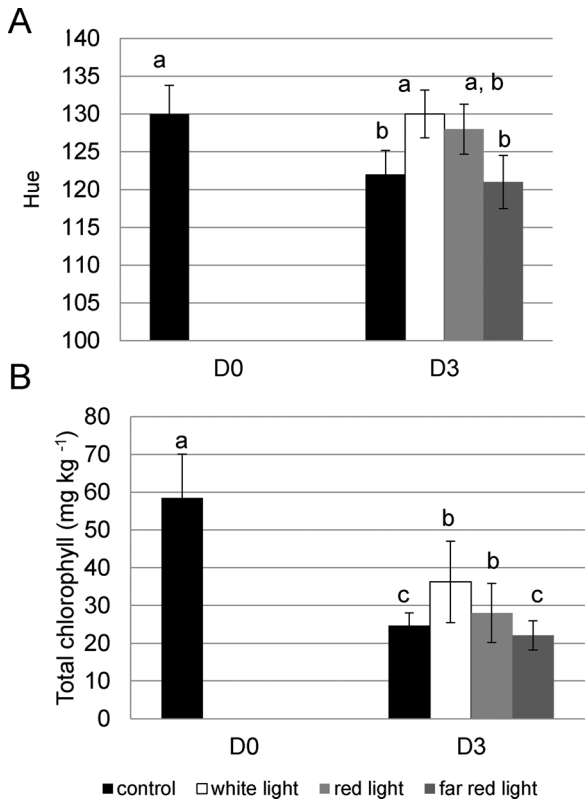
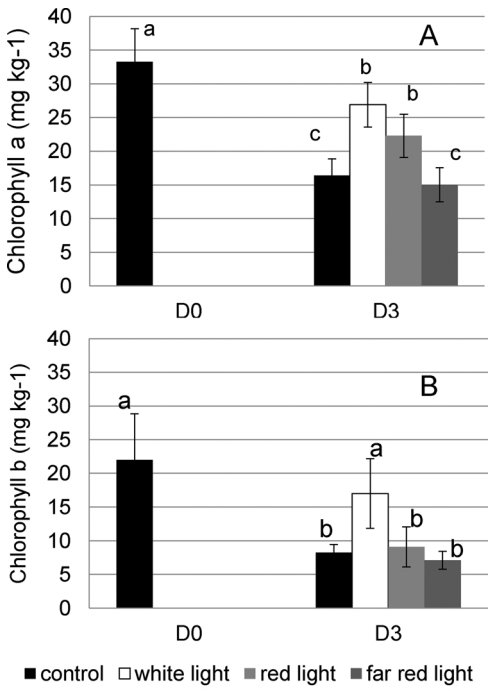


Fig. 6. External color (A) and content of total chlorophyll (B) in broccoli florets at initials (D0) and after 3 d (D3) without light treatment (control) or treated with low intensity white, red or far red light pulses (20–25 μmol m^{-2} s^{-1}) for 2 h every day and stored in darkness at 20 °C. For superficial color, each data represents the mean of 7 measurements. For chlorophyll determination, five independent extracts were made for each sampling date and treatment. Bars indicate the standard deviation and different letters indicate significant differences ($p < 0.05$) among treatments.



Chlorophyll a/b ratio	
D0	1.9a
Control D3	2.0a
White light D3	1.6b
Red light D3	2.5c
Far red light D3	2.1c

C

Fig. 7. Content of chlorophyll a (A) and b (B) in broccoli florets at initials (D0) and after 3 days (D3) without light treatment (control) or treated with low intensity (20–25 μmol m^{-2} s^{-1}) white, red or far red light for 2 h every day and stored in darkness at 20 °C. Chorophyll a/b was calculated for each sample (C). The results are expressed as the mean ($n = 5$) ± the standard deviation. Different letters indicate significant differences ($p < 0.05$) among treatments.

during storage can delay postharvest yellowing (Büchert et al., 2011; Zhan et al., 2012). In the present study we analyzed the possibility of using pulses of low intensity white light during storage of broccoli to delay postharvest senescence. Yellowing of green tissues, a consequence of chlorophyll degradation is the most evident symptom of senescence. We applied pulses of white light with different duration and found that the pulse duration was inversely proportional to the extent of yellowing of broccoli stored at 20 °C (Fig. 1B), and this was also consistent with chlorophyll degradation results (Fig. 3). Treatment with pulse of 30 min every day resulted insufficient to delay broccoli senescence since contents of chlorophylls, proteins, phenolics and sugars were similar to the control after 4 d of storage (Figs. 3–5; Table 1). On the other hand, treatments with pulses of 1 and 2 h daily were efficient to delay senescence (Supplemental Fig. 1 and Figs. 1–5 and Table 1); but it is noteworthy that 2 h treatment resulted the most suitable time to retain chlorophylls, proteins and sugars (Figs. 3 and 5; Table 1). Similar results were described by Costa et al. (2013b) in basil leaves stored in darkness and subjected to light pulses. Although light treatments were efficient to delay senescence, irradiation causes a slightly higher weight loss in treated broccoli and this fact must be taken into account. In plants, transpiration occurs through the stomata pores of leaves. Stomata opening are modulated by light, and it is well known that white and blue light are the main signals that affect the guard cells, which in turn are the responsible of regulation of stomata aperture (Eckert and Kaldenhoff, 2000). The percentage of weight loss resulted directly proportional to the duration of the pulse. Thus, it is possible to consider that stomata maintained opened during light treatment, so that the longer the treatment, the greater the weight loss (Fig. 2).

A possible biological mechanism associated to the delay of senescence due to light pulses could be related to the role of light in photosynthesis. As a consequence of re-initiated photosynthesis sugars concentrations increase; therefore greater photoassimilate availability could delay senescence. The effect of sugars on delaying senescence of broccoli has been previously shown (Hasperué et al., 2011). In our case, utilized irradiations (20–25 μmol m^{-2} s^{-1}) are probably below compensation point of most of vegetables, and therefore would not have induced photosynthesis (Costa et al., 2013b). Moreover, total sugars decreased after 4 d of storage in florets of all light treatments (Table 1), which confirmed the idea that the intensity light was insufficient to maintain photosynthetic activity. We analyzed only reduced sugar (and not total sugars) in the soluble fraction since hexoses represent the higher percentage of soluble sugars in broccoli (Rosa et al., 2001) and sucrose content declines by 50% during the first hour of harvest in broccoli (Downs et al., 1997; King and Morris, 1994). After 4 d of storage, the light treatments only had effect on soluble sugar, but there were no differences among treatments on insoluble sugar level, which include starch and cell walls components (Table 1). Starch degradation is rapid at the beginning of senescence because this process provides sugar for the high respiration activity of florets during senescence (King and Morris, 1994). It is probable that the decrease observed on insoluble sugars after 4 d could be due mainly to starch degradation, regardless of treatment. Higher level of reducing sugar on 2 h light treatment may be due to a lower respiration activity during storage. In

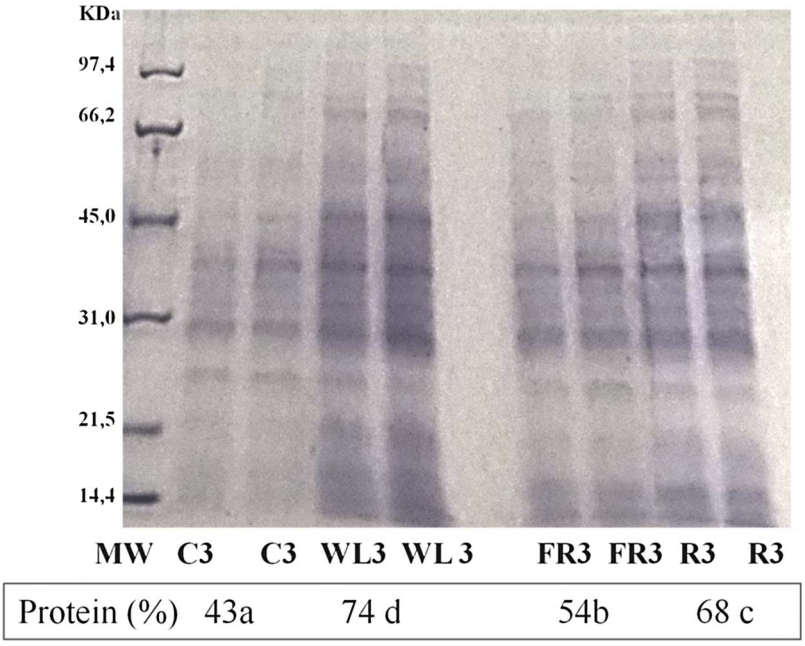


Fig. 8. Coomassie blue-stained gel of soluble protein from broccoli florets after 3 d of storage. A quantitation of proteins in each gel line was done using known quantities of molecular weight (MW).% Proteins was calculated for each gel line. C3, WL3, FR3 and R3 mean control, white light, red light and far red light treatments. Five data for each treatment was measurement. Different letters indicates significant differences ($p < 0.05$) among treatments.

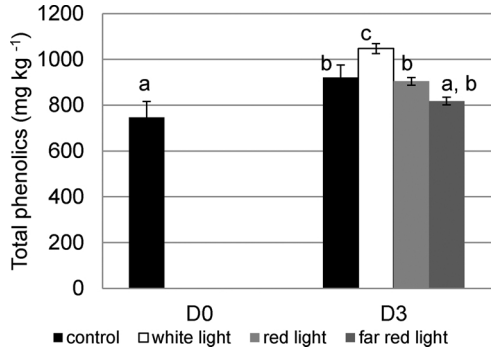


Fig. 9. Content of total phenolics at initials (D0) and after 3 d in broccoli florets (D3) without light treatment (control) or treated with low intensity white, red or far red light pulses (20–25 μmol m^{-2} s^{-1}) for 2 h every day and stored in darkness at 20 °C. The results are expressed as the mean (n = 5) ± the standard deviation. Different letters indicate significant differences ($p < 0.05$) among treatments.

Table 2
Reducing soluble, insoluble and total sugar concentrations of broccoli florets at the start of the experiment (D0) and after 3 d in darkness (D3). Florets either had no light treatment (Control) or were treated with low intensity White light or Red light or Far red light (FRL) pulses (20–25 μmol m^{-2} s^{-1}) for 2 h every day and then stored in darkness at 20 °C. The results are expressed as the mean (n = 5) ± the standard deviation. Different letters indicate significant differences ($p < 0.05$) between treatments.

	Reducing sugars (g Kg^{-1})	Insoluble sugars (g Kg^{-1})	Total sugars (g Kg^{-1})
D0	16.50 ± 1.7 a	4.37 ± 1.3 a	20.87 ± 3.0 a
D3 Control	6.00 ± 1.4 c	1.90 ± 0.5 b	7.90 ± 6.1 c
D3 White light	13.61 ± 0.9 b	2.40 ± 0.7 b	16.01 ± 1.6 b
D3 Red light	12.70 ± 1.3 b	1.70 ± 0.4 b,c	14.40 ± 1.7 b
D3 Far red light	12.90 ± 2.0 b	1.48 ± 0.2 c	16.38 ± 2.2 b

this sense, the high sugar levels may represent one of the signals of delayed postharvest senescence, (Hasperué et al., 2011). The effect of soluble sugar levels on leaf senescence is complex; at the beginning of *Arabidopsis thaliana* senescence, endogenous sugar concentrations increase and can change the expression of some senescence associated genes, which could delay symptoms of later senescence (Gibson 2005; Wingler and Roitsch, 2008). Light treated florets had higher sugar level than not irradiated heads after 4 d storage (Table 1) and this higher hexose availability could help to delay senescence. Our results agree with Hasperué et al. (2011) and Irving and Joyce (1995) who demonstrated that hexoses delayed senescence of broccoli.

Higher plants contain two types of chlorophyll, Chla and Chlb, associated with proteins as chlorophyll-protein complexes localized in thylakoid membranes and involved in light harvesting. Chlorophyll degradation was delayed by light treatments and this effect depended on the pulse time (Fig. 3). In addition, Chlb declined more than Chla in darkness control samples, which agrees with the fact that the first step of Chlb degradation is conversion to chlorophyll a (Hörtensteiner, 2006). Low intensity white light treatment delayed degradation of both types of chlorophyll but the effect was markedly higher on Chlb level and therefore the treatments also modified chlorophyll a/b ratio in relation to controls (Figs. 3C and D). The lower degradation of chlorophyll *b* in the treated florets could indicate an effect of light on the expression of coding genes for chlorophyll *b* reductase. Our results are similar to those shown by Zhan et al. (2012) for chlorophyll a and b degradation in fresh-cut broccoli.

Chlorophyll degradation was accompanied by soluble protein degradation in broccoli florets stored 4 d at room temperature (Fig. 5). Light treatment of 1 and 2 h had a positive effect on delay of protein degradation since these treatments retained 65 and 73% of initial level respectively; while darkness florets retained only approximately 50%. The most abundant soluble proteins of green tissues is Rubisco; a stromal chloroplast protein that is a key enzyme of photosynthetic carbon assimilation (Lodish et al., 2000). During senescence stromal chloroplast proteins are degraded early leading to the decline of photosynthetic capacity (Costa et al., 2013a). Despite its physiological importance, the mechanism of Rubisco degradation is not completely elucidated (Carrión et al., 2013) but senescence associated proteases are involved (Hörtensteiner and Feller, 2002). Moreover, it is highly probable that light could be a regulating factor of many senescence associated genes (SAGs) (Liebsch and Keech, 2016). We found that low intensity light treatment delayed Rubisco degradation. However, although Rubisco is necessary to maintain photosynthetic activity, the integrity of photosynthetic apparatus involves the presence of other components. Based on our results of decrease in the content of sugars in all treated florets (Table 1), it is unlikely that photosynthesis remains active even with high levels of Rubisco.

Our results suggest that the light intensity used in the light pulse treatments was not sufficient for net synthesis of sugars in florets; therefore the light retarding effect on senescence might occur via activation of phytochromes. Similar results were described previously for basil leaves (Costa et al., 2013b). Phytochromes exist in two different conformations, the inactive Pr one localized in the cytosol and the active Pf form able to reach to nucleus. Pr can absorb red light and become Pf, which, in turn, can absorb far red light and convert to Pr again. Thus, phytochrome responses are classically defined by their red/far red reversibility (Quail, 2002). Interaction phytochromes-light can act as a signal to activate transcription factors which modulate SAGs (senescence associated genes) expression (Piao et al., 2015); many of which are involved in chloroplast degradation (Page et al., 2001). Indeed, the last reports about leaf senescence showed that phytochromes and phytochromes interacting factors proteins (PIF) are involved in regulation of: genes involved in chloroplast maintenance and chlorophyll catabolism, genes involved in ethylene biosynthesis and ethylene and ABA signaling (Piao et al., 2015; Liebsch and Keech, 2016).

We analyzed whether or not the effects of light were mediated by phytochromes by investigating the red/far-red responses of broccoli florets during senescence. Responses obtained were different for each parameter measured. Apparently the effect of light treatment involves different mechanisms and only some of them were mediated by phytochromes. Red and far red light have the opposite effect on chlorophyll and protein degradation, which suggest that phytochromes are implicated in the degradation of these components (Figs. 6–8). This result was similar to that found in Costa et al. (2013b) in basil leaves. However, it must be noted that Chla and Chlb degradation had different patterns (Fig. 7). Chlorophyll a had the typical behavior of phytochrome mediated response; the level after 3 d was similar to white light treatment and the chlorophyll a retention was reverted by far red light (Fig. 7). The opposite happened with chlorophyll *b* degradation; there were no retention in red treatment and the effect was similar in red and far red light treatments. These results suggest that if there is some kind of regulation of chlorophyll *b* reductase mediated by light, as outlined above, it is not mediated by phytochromes.

Treatments performed with red light, far red light and darkness (controls) affected phenolic concentrations similarly, while they were higher in florets treated with white light. These results suggest that phytochromes are not involved in regulation of phenolics, and there are other signals induced by light treatments involved in increased phenolic concentrations.

The differences between white and red light treatments suggest that there are other photoreceptors involved. Cryptochromes are blue light receptors that mediate diverse light-induced responses in plants, such as flowering induction and stomata opening (Li and Yang, 2007). Recently, Hasperué et al. (2016) used the combination of low intensity continuous illumination with white and blue light-emitting diodes (LEDs) during increased the storage life of broccoli heads stored at 5 °C or at 22 °C.

Finally, sugars were retained and sugar level was similar in all light treatment after 3 d respect to the control (Table 2). Therefore the retention of sugars was independent of quality of light used in light treatment. In *Arabidopsis thaliana*, it has been shown that later senescence associated genes as SAG12 are repressed by high level of sugars (Wingler et al., 2006). Possibly, the effect of light treatment is related with delay sugar consumption by a lower respiration rate during storage. Phytochromes are involved in regulation of genes of ethylene biosynthesis and signaling which could impact on delaying respiration. Anyway, more investigations are needed to understand the mechanism of low intensity light treatment on postharvest senescence.

5. Conclusion

Daily irradiation with pulses of 2 h of white light of low intensity (20–25 μmol m^{-2} s^{-1}) could be a promising technology to delay postharvest senescence of broccoli stored at room temperature. All florets showed reduced total sugars, but concentrations were higher in treated than in untreated florets, independent of the light quality utilized. Treatment with white and red light had similar effect on chlorophyll a and proteins degradation during postharvest senescence of broccoli but red light was insufficient to retained chlorophyll *b* and phenolics compared with white light treatment. The light intensity used was insufficient to maintain photosynthesis and the effects detected were at least partly mediated by phytochromes, although there are some effects that could depend of other light signals.

Author contribution statement

Favre, Bárcena and Vera Bahima did all experiments for this paper; Martínez and Costa designed experiments and wrote this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.postharvbio.2017.11.006.

References

Büchert, A.M., Gómez Lobato, M.E., Villarreal, N.M., Civello, P.M., Martínez, G.A., 2011. Effect of visible light treatments on postharvest senescence of broccoli (*Brassica oleracea* L.). J. Sci. Food Agric. 91, 355–361.

Buchanan-Wollaston, V.E.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.

Carrión, C.A., Costa, M.L., Martínez, D.E., Mohr, C., Humbeck, K., Guiamet, J.J., 2013. In vivo inhibition of cysteine proteases provides evidence for the involvement of senescence-associated vacuoles in chloroplast protein degradation during dark-induced senescence of tobacco leaves. J. Exp. Bot. 64, 4967–4980.

Costa, M.L., Civello, P.M., Chaves, A.R., Martínez, G.A., 2005. 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.

Costa, M.L., Vicente, A.R., Civello, P.M., Chaves, A.R., Martínez, G.A., 2006. UV-C treatment delays postharvest senescence in broccoli florets. Postharvest Biol. Technol. 39, 204–210.

Costa, M.L., Martínez, D.E., Gomez, F.M., Carrión, C., Guiamet, J.J., 2013a. Chloroplast protein degradation: involvement of senescence-associated vacuoles. In: Biswal, B., Krupinska, K., Biswal, U.C. (Eds.), Advances in Photosynthesis and Respiration Series. Springer Science + Business Media, Dordrecht, The Netherlands, pp. 417–433.

Costa, L., Montano, Y., Carrión, C., Rolny, N., Guiamet, J.J., 2013b. Application of low intensity light pulses to delay postharvest senescence of *Ocimum basilicum* leaves. Postharvest Biol. Technol. 86, 181–191.

Downs, C.G., Somerfield, S.D., Davey, M.C., 1997. Cytokinin treatment delays senescence but not sucrose loss in harvested broccoli. Postharvest Biol. Technol. 11, 93–100.

Eckert, M., Kaldenhoff, R., 2000. Light – induced stomatal movement of selected *Arabidopsis thaliana* mutants. J. Exp. Bot. 51 (349), 1435–1442.

Gergoff Grozeff, G., Chaves, A., Bartoli, C., 2013. Low irradiance pulses improve postharvest quality of spinach leaves (*Spinacia oleraceae* L. cv Bison). Postharvest Biol. Technol. 77, 35–42.

Gibson, S., 2005. Control of plant development and gene expression by sugar signaling. Curr. Opin. Plant Biol. 8, 93–102.

Hörtensteiner, S., Feller, U., 2002. Nitrogen metabolism and remobilization during senescence. J. Exp. Bot. 53, 927–937.

Hörtensteiner, S., 2006. Chlorophyll degradation during senescence. Annu. Rev. Plant Biol. 57, 55–77.

Hasperué, J.H., Chaves, A.R., Martínez, G.A., 2011. End of day harvest delays postharvest senescence of broccoli florets. Postharvest Biol. Technol. 59, 64–70.

Hasperué, J.H., Guardianelli, L., Rodoni, L.M., Chaves, A.R., Martínez, G.A., 2016. Continuous white-blue LED light exposition delays postharvest senescence of broccoli. LWT – Food Sci. Technol. 65, 495–502.

Irving, D.D., Joyce, D.C., 1995. Sucrose supply can increase longevity of broccoli (*Brassica oleracea*) branchlets kept at 22 °C. Plant Growth Regul. 17, 251–256.

Jia, C.G., Xu, C.J., Wei, J., Yuan, J., Yuan, G.F., Wang, B.L., 2009. Effect of modified atmosphere packaging on visual quality and glucosinolates of broccoli florets. Food Chem. 114 (1), 28–37.

Jin, P., Yao, D., Xu, F., Wang, H., Zheng, Y., 2015. Effect of light on quality and bioactive compounds in postharvest broccoli florets. Food Chem. 172, 705–709.

Jones, R.B., Faragher, J.D., Winkler, S., 2006. A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. Postharvest Biol. Technol. 41, 1–8.

King, G.A., Morris, S.C., 1994. Early compositional changes during postharvest senescence of broccoli. J. Amer. Soc. Hort. Sci. 119 (5), 1000–1005.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277, 680–685.

Lester, G.E., Makus, D.J., Hodges, D.M., 2010. Relationship between fresh-packaged spinach leaves exposed to continuous light or dark and bioactive contents: effects of cultivar, leaf size, and storage duration. J. Agric. Food Chem. 58, 2980–2987.

Li, Q.-H., Yang, Q., 2007. Cryptochrome signaling in plants. Photochem. Photobiol. 83, 94–101.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382.

Liebsch, D., Keech, O., 2016. Dark-induced leaf senescence: new insights into a complex light-dependent regulatory pathway. New Phytol. 212, 563–570.

Lodish, H., Berk, A., Zipursky, S.L., et al., 2000. Molecular Cell Biology, 4th ed. W.H. Freeman, New York Section 16.5, CO_2 Metabolism during Photosynthesis. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21472/.

Ma, G., Zhang, L., Setiawan, C.K., Yamawaki, K., Asai, T., Nishikawa, F., Maezawa, S., Sato, H., Kanemitsu, N., Kato, M., 2014. Effect of red and blue LED light irradiation on ascorbate content and expression of genes related to ascorbate metabolism in postharvest broccoli. Postharvest Biol. Technol. 94, 97–103.

Nishikawa, F., Kato, M., Hyodo, H., Ikoma, Y., Sugiera, M., Yano, M., 2005. Effect of sucrose on ascorbate level and expression of genes involved in the ascorbate biosynthesis and recycling pathway in harvested broccoli florets. J. Exp. Bot. 56, 65–72.

Page, T., Griffiths, G., Buchanan-Wollaston, V., 2001. Molecular and biochemical characterization of postharvest senescence in broccoli. Plant Physiol. 125, 718–727.

Parry, M.A.J., Schmidt, C.N.G., Cornelius, M.J., Millard, B.N., Burton, S., Gutteridge, S., Dyer, T.A., Keys, A.J., 1987. Variations and properties of ribulose-1, 5-bisphosphate carboxylase from various species related to differences in amino acid sequences. J. Exp. Bot. 38, 1260–1271.

Perini, M., Sin, I., Reyes Jara, A., Gomez Lobato, M.E., Civello, P.M., Martínez, G.A., 2017. Hot water treatments performed in the base of the broccoli stem reduce postharvest senescence of broccoli (*Brassica oleracea* L. var *italic*) heads stored at 20 °C. LWT–Food Sci. Technol. 77, 314–322.

Piao, W., Kim, E., Han, S., Sakuraba, Y., Paek, N., 2015. Rice phytochrome B (OsPhyB) negatively regulates dark- and starvation-Induced leaf senescence. Plants 4, 644–663.

Quail, P.H., 2002. Photosensory perception and signalling in plant cells: new paradigms? Curr. Opin. Cell Biol. 14, 180–188.

Rosa, E., David, M., Gomes, M., 2001. Glucose, fructose and sucrose content in broccoli, white cabbage and Portuguese cabbage grown in early and late seasons. J. Sci. Food. Agric. 81, 1145–1149.

Southgate, D.A.T., 1976. Determination of Food Carbohydrates. Appl. Science Publ. Ltd., London, pp. 128–129.

Toivonen, P.M., Forney, Ch., 2016. Broccoli in the commercial storage of fruits, vegetables, and florist and nursery stocks. In: Gross, Kenneth C., Wang, Chien Yi, Saltveit, Mikal (Eds.), Agriculture Handbook Number 66. Agricultural Research Service. Revised February 2016. https://www.ars.usda.gov/ARSUserFiles/oc/np/CommercialStorage/CommercialStorage.pdf. (Accessed 31 October 2017).

Wingler, A., Roitsch, T., 2008. Metabolic regulation of leaf senescence: interactions of sugar signalling with biotic and abiotic stress responses. Plant Biol. 10 (1), 50–62.

Wingler, A., Purdy, S., MacLean, J.A., Pourtau, N., 2006. The role of sugars in integrating environmental signals during the regulation of leaf senescence. J. Exp. Bot. 57, 391–399.

Yuan, G., Sun, B., Yuan, J., Wang, Q., 2010. Effect of 1-methylcyclopropene on shelf life, visual quality antioxidant enzymes and health-promoting compound in broccoli florets. Food Chem. 118, 774–781.

Zhan, L.J., Hu, J.Q., Li, Y., Pang, L.G., 2012. Combination of light exposure and low temperature in preserving quality and extending shelf-life of fresh-cut broccoli (*Brassica oleracea* L.). Postharvest Biol. Technol. 72, 76–81.