Research Article

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Effect of visible light treatments on postharvest senescence of broccoli (*Brassica oleracea* L.)

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Abstract

BACKGROUND: Broccoli (*Brassica oleracea* L.) is a rapidly perishable vegetable crop. Several postharvest treatments have been applied in order to delay de-greening. Since light has been shown to have an effect on pigment accumulation during development and darkness is known to induce senescence, the effect of continuous and periodic exposure to low-intensity white light at 22 °C on postharvest senescence of broccoli heads was assayed.

RESULTS: Exposure to a constant dose of 12 μmol m^{-2} s^{-1} was selected as the most suitable treatment and was employed for subsequent experiments. During the course of the treatments, hue and *L values as well as chlorophyll content and visual observation of florets indicated an evident delay in yellowing in treated samples compared with controls. No statistically significant differences in total protein content were found, but soluble protein content was higher in treated samples. Total and reducing sugar as well as starch levels decreased during postharvest senescence, with lower values in control samples.**

CONCLUSION: The results of this study indicate that storage under continuous low-intensity light is an efficient and low-cost treatment that delays postharvest senescence while maintaining the quality of harvested broccoli florets.

Keywords: broccoli; postharvest; senescence; light; chlorophyll

INTRODUCTION

Fresh broccoli (*Brassica oleracea* L.) has become a popular vegetable in many parts of the world owing to its high nutritional value. It has low caloric and high dietary fibre content, important levels of ascorbic acid and a wide range of anticarcinogenic and antioxidant compounds.[1]

Broccoli has a short postharvest shelf life since it is harvested while its inflorescences are still immature, prior to sepal opening. Harvesting and handling cause severe stress conditions that result in water loss and variations in nutrient and hormone content, inducing early onset of senescence and accelerating this process significantly. As a consequence, a loss of the superficial green colour of the product is observed, which decreases the commercial approval of broccoli florets. Moreover, senescence accelerates loss of sugars and proteins and lipid peroxidation, leading to a loss of nutritional quality.[1,2] Senescence implies a programmed degradation of macromolecules that allows the plant to remobilise nutrients from dying to developing tissues. One of these catabolic processes is proteolysis, which is highly activated during senescence. The degradation of proteins occurs rapidly in postharvest broccoli, and the expression pattern of several protease genes has been shown to be enhanced in senescence.[2] Sugars have been implicated in regulating senescence, as they are needed to provide a carbon source to maintain high respiration rates in harvested immature tissues.[3] Some reports have shown a decrement in sugar levels during postharvest senescence of broccoli.[2,4]

Several different techniques have been applied in order to delay the emergence of postharvest senescence symptoms in broccoli, including refrigeration, modified atmospheres, thermal shock treatments and different types of packaging, among others.[1,2]

Light is one of the most crucial factors involved in plant growth and development, and its role in pigment accumulation has been documented.[5–7] Several studies have shown that darkness induces senescence,[8–10] and research on *Arabidopsis* has demonstrated up-regulation of the ethylene biosynthetic genes in darkness,[11] thus accelerating the process. Broccoli and many other horticultural products are harvested when they are still photosynthetically active. However, in many cases, light is not controlled during postharvest storage and products are commercially stored in darkness, which induces senescence and accelerates this process significantly. Although the senescence-inducing effect of postharvest light deprivation on many crops has been acknowledged and extensively described, there is little information regarding the effect of visible light exposure on fruits or horticultural products during postharvest storage. In tomato, for example, exposure to red light increases

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accumulation of lycopene,[12] whereas, in raspberries, storage under visible light causes higher levels of soluble solids and lower values of titratable acidity.[13] In green tissues such as Chinese kale[9] and spinach,[10] storage under light can delay chlorophyll degradation and improve ascorbic acid accumulation respectively.

Literature concerning the effects of visible light quality on postharvest senescence of green horticultural products is not abundant. In this study we analysed the effect of different exposures to low intensities of visible light on broccoli postharvest senescence and quality during storage at 22 °C.

EXPERIMENTAL

Plant material and light treatments

Broccoli (*B. oleracea* var. Iron) heads were obtained from a local producer in La Plata, Buenos Aires, Argentina and processed immediately. Heads were defoliated and placed in plastic cups containing a small amount of distilled water to prevent dehydration. To analyse the effects of visible light quality on postharvest senescence of broccoli, in a first set of experiments, detached heads were stored under different intensities of visible light. Assayed continuous light intensities were 12, 25 and 50 μmol m^{-2} s^{-1}, using 40 W white light fluorescent tubes, while experiments with discontinuous illumination were performed with a photoperiod of 16 h light/8 h dark using 25 μmol m^{-2} s^{-1} light. Superficial colour was measured daily in all broccoli heads. Samples were placed in a well-ventilated chamber isolated from external light sources at 22 °C. One half of the chamber with 35 broccoli heads was kept in complete darkness (<1 μmol m^{-2} s^{-1}) and the other half with the same number of samples was exposed to different continuous as well as periodic light intensities. The superficial colour of all heads was measured in order to select the best treatment. In a second set of experiments, 35 heads were either treated with the selected dose (12 μmol m^{-2} s^{-1}) or maintained in the dark. After reading superficial colour, seven heads from light or dark treatment were segmented and the inflorescences were frozen using liquid nitrogen and stored at −20 °C until use.

Superficial colour measurement

The superficial colour of samples was determined by measuring the parameters L^*, a^* and b^* using a chromameter (CR300, Minolta, Osaka, Japan). The hue angle 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 of each broccoli head were measured. Data of all heads receiving a given treatment were pooled and the means were calculated.

Chlorophyll content

Frozen broccoli florets were ground in liquid nitrogen and 0.5 g of the resulting powder was mixed with 5 mL of 800 mL L^{-1} acetone and centrifuged at 10 000 × *g* for 10 min at 4 °C. The chlorophyll content was measured in the supernatant according to Inskeep and Bloom[14] and results were expressed as g total chlorophyll kg^{-1} tissue fresh weight. All measurements were performed in triplicate.

Total and soluble protein content

For soluble protein content measurement, frozen broccoli florets were ground in liquid nitrogen and 0.5 g of the resulting powder was mixed with 5 mL of a buffer solution containing 50 mmol L^{-1} Tris-HCl, 0.4 mL L^{-1} β-mercaptoethanol and 2 mmol L^{-1} ethylenediaminetetraacetic acid (pH 7.5). The mixture was centrifuged at 10 000 × *g* for 10 min at 4 °C and the soluble protein content was determined in the supernatant according to Bradford[15] using bovine serum albumin (Sigma, St Louis, MO, USA) as standard. For total protein content measurement, 0.3 g of frozen broccoli powder was homogenised with 10 mL of 0.1 mol L^{-1} NaOH and 10 g L^{-1} sodium dodecyl sulfate (SDS) and heated at 100 °C for 10 min. Samples were centrifuged at 10 000 × *g* for 20 min at 4 °C. In order to precipitate proteins, 5 volumes of acetone was added to the supernatant, which was then incubated at −20 °C for 12 h and centrifuged at 13 000 × *g* for 10 min at 4 °C. The precipitate obtained was dissolved in 0.2 mL of 0.1 mol L^{-1} NaOH and 10 g L^{-1} SDS and the protein content was measured according to Lowry *et al.*[16] using bovine serum albumin (Sigma) as standard. All measurements were performed in triplicate and soluble as well as total protein content was expressed as g protein kg^{-1} tissue fresh weight.

Total and reducing sugar content

Frozen broccoli florets were ground in liquid nitrogen and 1 g of the resulting powder was suspended in 6 mL of ethanol. The mixture was centrifuged at 9 000 × *g* for 10 min at 4 °C and 1 mL of the supernatant was diluted to 5 mL using distilled water. The content of reducing sugars was determined spectrophotometrically at 540 nm using a modification of the Somogyi–Nelson method.[17] For total sugar determination, 0.1 mL of ethanol extract was mixed with 1 mL of 2 g L^{-1} anthrone in 706 g L^{-1} H_2SO_4. The mixture was incubated at 100 °C for 12 min, cooled in a water bath and the sugar content was measured spectrophotometrically at 625 nm. Measurements were performed in triplicate and results were expressed as g glucose kg^{-1} tissue fresh weight.

Total phenolic compounds

Frozen broccoli florets were ground in liquid nitrogen and 1 g of the resulting powder was suspended in 6 mL of ethanol. The mixture was centrifuged at 9 000 × *g* for 10 min at 4 °C and the supernatant was used to determine total phenolic compounds according to Zieslin and Ben-Zaken.[18] A 100 μL aliquot of the extract was added to 1.11 mL of water and 200 μL of 0.5 mol L^{-1} Folin–Ciocalteu reagent. After 3 min of incubation at 25 °C, 1.5 mL of saturated Na_2CO_3 solution was added and the reaction mixture was incubated for 1 h at the same temperature. The absorbance was measured at 760 nm and the total phenolic content was calculated using phenol as standard. Measurements were performed in triplicate and results were expressed as g phenol kg^{-1} tissue fresh weight.

Starch content

Frozen broccoli florets were ground in liquid nitrogen and 0.5 g of the resulting powder was heated at 100 °C for 1 h. Starch was extracted, solubilised and hydrolysed according to Enzyme Method 2 of Rose *et al.*[19] The resulting glucose was quantified using an enzymatic glucose determination kit (Wiener Lab., Rosario, Argentina). Measurements were performed in triplicate and results were expressed as g glucose kg^{-1} tissue fresh weight.

Statistical analysis

Experiments were performed according to a factorial design, the factors being time (0, 2, 3, 4 and 5 days postharvest) and treatment

(light dose and darkness). Data were subjected to analysis of variance, and means of control (dark) and treated (light) samples for each storage day were compared by the Fisher least significant difference test at a significance level of 0.05.

RESULTS AND DISCUSSION

Selection of treatment

In order to follow the time course of postharvest senescence, the superficial colour of samples was determined. Initial hue values on the day of harvest ranged from 121 to 130° and decreased in all samples during storage, while initial L^* values were 34–40 and increased during storage (Table 1), indicating a loss of green colour and yellowing. At all intensities employed, the continuous light treatment delayed the loss of green colour in comparison with controls, which were kept in complete darkness. This delay was statistically significant starting on day 3 for the light dose of 50 μmol m^{-2} s^{-1} and on day 2 for all other light conditions assayed (data not shown). Statistically significant differences from day 0 in hue values caused by postharvest de-greening appeared 1 day later in treated samples than in controls, indicating that light treatment maintains the greenness of the tissue for an extra day during postharvest senescence (data not shown). Treated samples showed hue values approximately 9–13% higher than controls after 4 days. However, there were no differences in hue or L^* values after 4 days of storage among treatments with different intensities (Table 1) when compared with controls kept in darkness, a condition known to induce and accelerate postharvest senescence and the de-greening process. When samples were incubated in the presence of a 16 h light (25 μmol m^{-2} s^{-1})/8 h dark photoperiod, a delay in de-greening was also detected, but to a lesser extent than that detected under continuous light treatment. Since no

Table 1. Changes in hue and L^* of control (dark) and light-treated broccoli from day 0 to day 4 of storage at 22 °C

		Hue (°)		L^*	
Treatment	Sample	Day 0	Day 4	Day 0	Day 4
12 μmol m^{-2} s^{-1} continuous	Control	125.9 ± 1.8	110.5 ± 5.6	31.7 ± 2.3	46.9 ± 3.6
	Treated	126.6 ± 2.2	123.9 ± 2.8	33.7 ± 3.4	40.4 ± 2.3
25 μmol m^{-2} s^{-1} continuous	Control	129.9 ± 2.8	114.1 ± 4.3	39.8 ± 1.6	54.7 ± 3.1
	Treated	130.6 ± 2.9	125.1 ± 5.5	39.8 ± 2.4	48.0 ± 2.5
50 μmol m^{-2} s^{-1} continuous	Control	121.7 ± 7.5	104.4 ± 7.3	35.3 ± 2.5	49.7 ± 4.6
	Treated	124.6 ± 8.3	121.2 ± 9.5	35.5 ± 1.7	40.1 ± 2.3
25 μmol m^{-2} s^{-1} photoperiodic	Control	124.3 ± 4.5	102.0 ± 6.9	40.3 ± 2.9	56.4 ± 3.9
	Treated	123.7 ± 6.6	105.2 ± 7.5	42.0 ± 2.7	52.8 ± 3.6

Sample number was 35 broccoli heads for each treatment and day. Values are average of five measurements on each of 35 heads ± standard deviation.

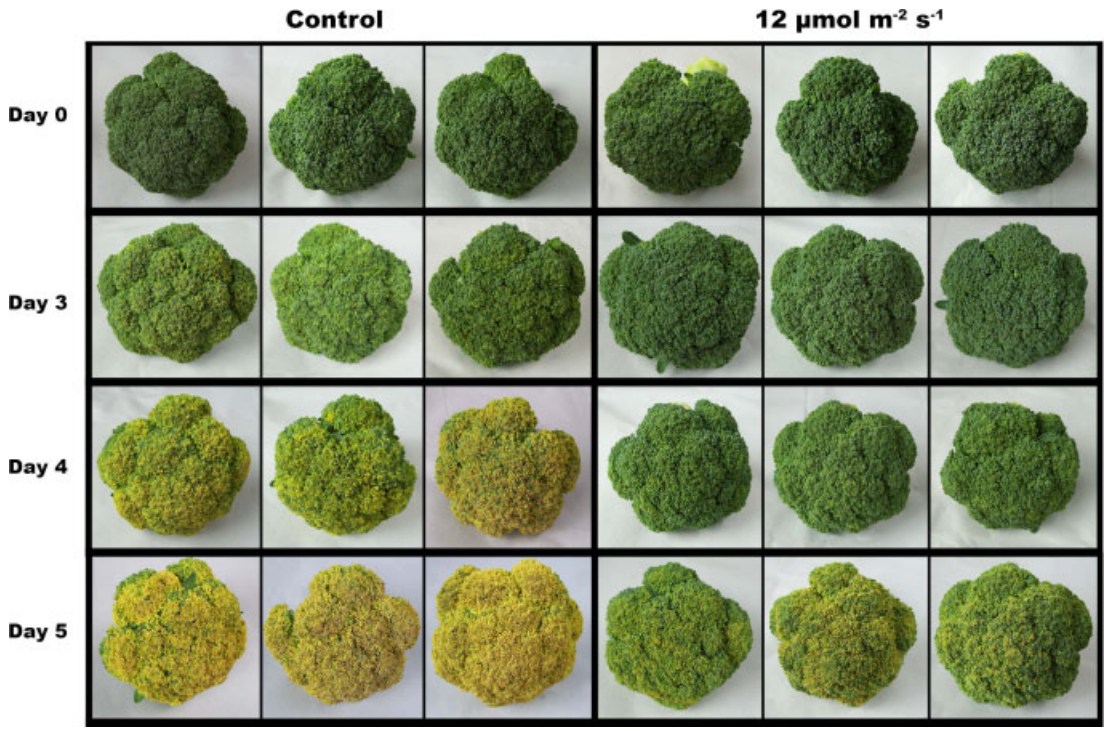

Figure 1. Visual external aspect of broccoli heads stored at 22 °C in darkness (control) or under continuous visible light (12 μmol m^{-2} s^{-1}).

major differences were found between the three continuous light doses applied, the dose of 12 µmol m^{-2} s^{-1} was selected as the best, considering that from a technological point of view it would require a lower dose, thus reducing costs. Therefore this treatment was employed for a second set of experiments, performed only on these samples, in order to assay different postharvest quality parameters.

External aspect, superficial colour and pigment content

Horticultural crops are severely stressed after harvest owing to a reduction in sources of energy, nutrients, hormones and water, which leads to the rapid initiation of senescence.[1] In the case of broccoli, one of the main symptoms of senescence is yellowing due to chlorophyll catabolism.[20,21] Broccoli heads were harvested completely green and turned yellow as senescence progressed during storage. However, this change was much less evident in those samples stored under continuous light (Fig. 1). After 3 days, samples stored in darkness showed symptoms of yellowing, but those under light were completely green. After 5 days, controls became totally yellow, while light-treated samples remained partially green. Superficial colour parameters and pigment content were also evaluated throughout the senescence period. Hue values decreased in control and treated samples during storage at 22 °C, but smaller changes were observed in samples maintained under continuous light, in agreement with the visible yellowing delay (Fig. 2A). In contrast, L^* values increased during storage, but treated samples presented a lower increase than controls (Fig. 2B).

Total chlorophyll content was also determined in broccoli heads as a senescence parameter. A constant decrease was found in treated as well as in control samples, indicating postharvest senescence. In the case of heads exposed to continuous light, a delay in chlorophyll degradation was found, with values approximately 40% higher compared with controls at the end of the experiment (Fig. 2C).

Initiation of senescence is highly dependent on light supply, among other things. It was largely demonstrated that detached leaves develop senescence and suffer chlorophyll degradation when they are stored in darkness.[22–24] Light can delay chlorophyll degradation during postharvest, as shown in kale[9] and in leaves of *Zantedeschia* and *Hosta*, plants of commercial importance utilised by florists in cut flower arrangements.[25] It has been found that postharvest storage in light can induce fresh weight loss and reduce leaf nitrate content in *Brassica rapa* as well as reduce leaf yellowing in Brussels sprouts (*B. oleracea* var. *gemmifera*).[26–28]

Soluble and total protein content

The total protein content of non-treated control broccoli florets decreased over the course of postharvest senescence, showing values at the end of the experiment corresponding to approximately 50% of the initial values for controls as well as for treated tissues (Fig. 3A). No statistically significant differences in total protein content were found until day 5 for treated *versus* control tissues. In the case of soluble protein content the value obtained on day 4 of the experiment was 59% of that on day 0 for controls and 83% for light-treated samples (Fig. 3B). It is worth mentioning that after 4 days of storage the treated florets were still acceptable for consumption (see Fig. 1). These results indicate that exposure of broccoli florets to constant light reduces the degradation of soluble proteins, but this effect is not reflected in total protein content, suggesting a possible selective down-regulation of some metabolic pathways related to protein degradation during postharvest senescence.

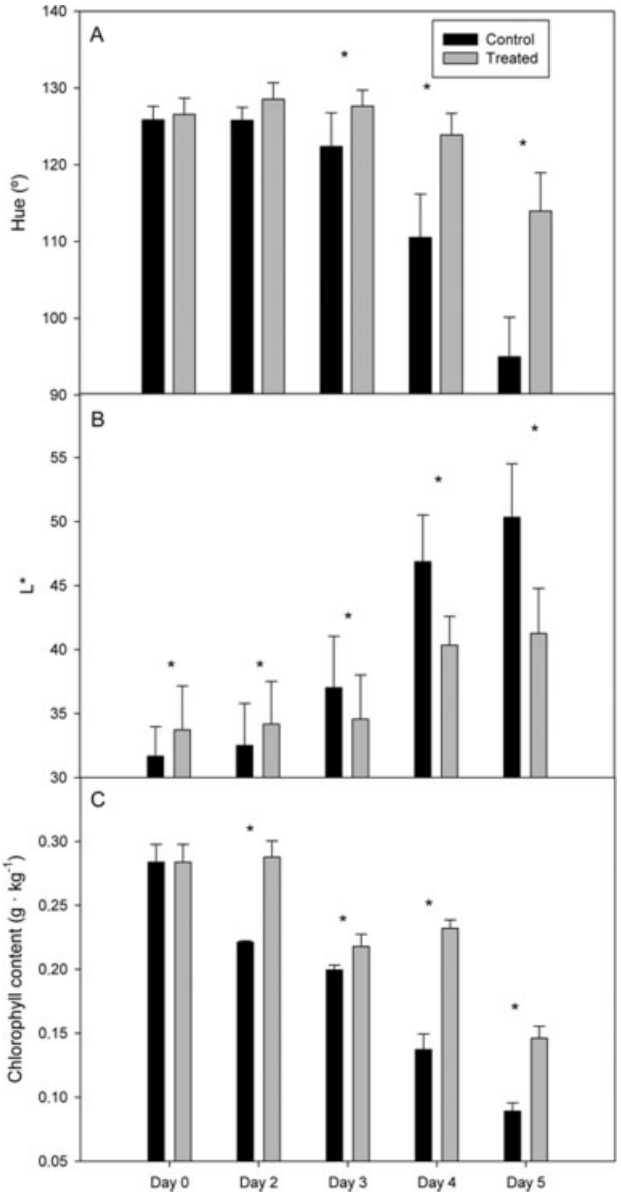

Figure 2. Postharvest senescence parameters measured in light-treated broccoli samples (12 µmol m^{-2} s^{-1}) and dark-stored controls (<1 µmol m^{-2} s^{-1}). (A) Superficial colour (hue, $n = 35$). (B) Superficial lightness (L^*, $n = 35$). (C) Total chlorophyll content expressed as g total Chl kg^{-1} tissue fresh weight. Values represent the average of three measurements, which were obtained from a pool of seven broccoli heads. Asterisks indicate statistically significant differences between control (dark) and treated (light) tissues for each storage day ($P < 0.05$).

Reducing and total sugar content

In our experiments, control and light-treated broccoli heads showed a decrease in the content of total and reducing sugars during storage (Figs 4A and 4B respectively). The sugar content in control heads was always lower than that in light-treated heads, particularly on days 3 and 4, when treated broccoli florets were still acceptable for consumption, while controls showed considerable de-greening and yellowing, as seen in Fig. 1. By the final time point analysed, the sugar content in treated samples was 15% higher than in controls for reducing sugars and 13% for total soluble sugars. It has been shown that treatments with simple sugars can delay postharvest senescence of broccoli,[29] probably

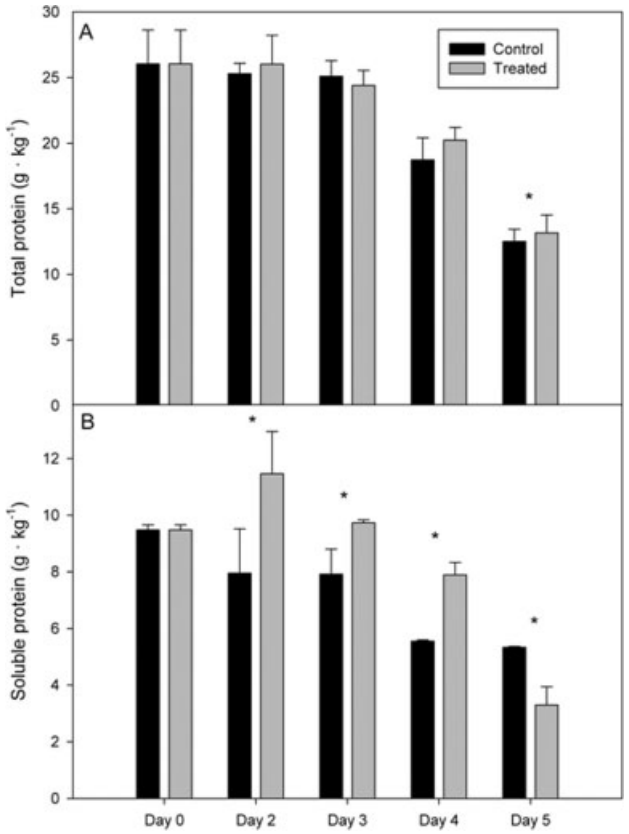

Figure 3. (A) Total and (B) soluble protein contents analysed in extracts of broccoli treated by light exposure (12 µmol m^{-2} s^{-1}) and dark-stored controls (<1 µmol m^{-2} s^{-1}). Data are expressed as g protein kg^{-1} tissue fresh weight. Values represent the average of three measurements, which were obtained from a pool of seven broccoli heads. Asterisks indicate statistically significant differences between control (dark) and treated (light) tissues for each storage day ($P < 0.05$).

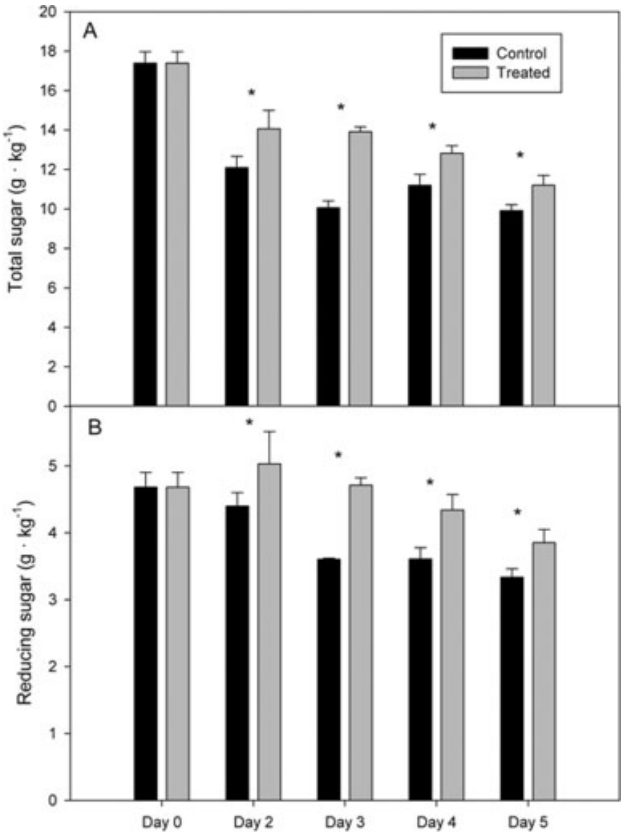

Figure 4. (A) Total and (B) reducing sugar contents in broccoli samples stored under continuous light (treated) and in darkness (control). Data are expressed as g glucose kg^{-1} tissue fresh weight. Values represent the average of three measurements, which were obtained from a pool of seven broccoli heads. Asterisks indicate statistically significant differences between control (dark) and treated (light) tissues for each storage day ($P < 0.05$).

by retaining high levels of these compounds necessary to maintain the energy supply. Other postharvest treatments that can delay senescence in broccoli, such as heat treatment or UV-C,[20,30] also reduce consumption of sugars. In the present work, samples stored under light presented a lower rate of senescence and probably maintained photosynthetic activity for a longer time, which in turn caused higher levels of sugars. Moreover, in spinach leaves it was suggested that continuous illumination by white light supports the photosynthetic capacity during postharvest, thereby increasing the availability of soluble carbohydrates and thus enabling them to contribute to the control of the ascorbic acid pool.[10] During senescence of broccoli, antioxidant power decreases,[20] accelerating deterioration of tissue. Delayed senescence in light-treated broccoli could be caused not only by the higher sugar level but also by a possibly enhanced content of antioxidants such as ascorbic acid.

Starch content

Owing to the decrease in sugar levels during postharvest senescence of broccoli, an important process to consider is the mobilisation of soluble sugars from other sources, which could help to overcome this decrease. Since a decline in sugar content was observed in our experiments and *Brassica* vegetables show relatively high levels of starch, we hypothesised that starch metabolism might provide soluble sugars. Thus starch levels in harvested broccoli florets were measured during storage. Control florets exhibited a minor decrease in starch content, which remained almost constant from the beginning of the experiment up to day 4, followed by a fourfold drop on day 5 (Fig. 5). In samples stored under continuous light, starch content remained constant throughout the experiment, showing levels always slightly higher than those in control florets until day 4 and noticeably higher on day 5. In these samples the lack of a significant decrease in sugar levels through senescence might be related to the high starch content found. Although starch degradation is probably the main source of soluble sugars, gluconeogenesis from lipids and cellulose breakdown from the cell wall should not be discarded as other sources of soluble sugar renovation during senescence of broccoli.[31,32]

Phenolic compounds

Phenolics are considered components of relevance from the nutraceutical point of view, since they contribute to maintenance of the antioxidant status. During broccoli development an increase in total phenolic content has been reported.[33] In our case a slight but statistically significant increment in the content of these compounds was detected in light-treated samples compared with controls after 2 days of storage. After 4 and 5 days an increment of approximately 0.5 g phenolic compounds kg^{-1} tissue was detected in both control and treated heads, but no statistically

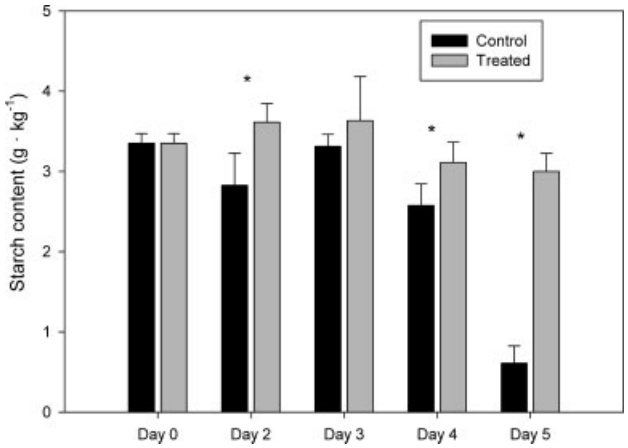

Figure 5. Starch content in treated and control broccoli florets. Data are expressed as g glucose (resulting form *in vitro* starch hydrolysis) kg^{-1} tissue fresh weight. Values represent the average of three measurements, which were obtained from a pool of seven broccoli heads. Asterisks indicate statistically significant differences between control (dark) and treated (light) tissues for each storage day ($P < 0.05$).

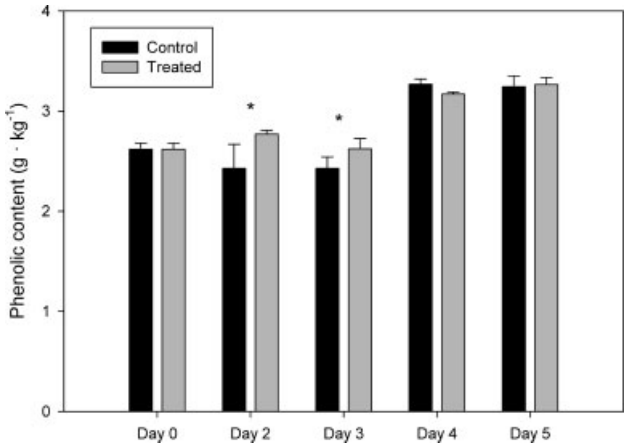

Figure 6. Phenolic content in treated and control broccoli florets. Data are expressed as g phenol kg^{-1} tissue fresh weight. Values represent the average of three measurements, which were obtained from a pool of seven broccoli heads. Asterisks indicate statistically significant differences between control (dark) and treated (light) tissues for each storage day ($P < 0.05$).

significant differences were detected among treatments in the final days of the experiment (Fig. 6).

CONCLUSIONS

Broccoli heads stored under continuous low intensities of white light showed an important delay in their senescence at 22 °C. The effect of white light treatments at intensities ranging from 12 to 50 μmol m^{-2} s^{-1} seems to be comparable, though the effect of light intensities outside this range remains to be evaluated. Treated broccoli had a higher retention of green colour and chlorophyll. The levels of total proteins were not affected by light, but light treatment allowed higher levels of total and reducing sugars as well as starch to be maintained, which in turn probably also contributed to the delay in tissue deterioration. These results indicate that a simple and economic postharvest treatment can delay senescence and contribute to maintaining the quality of broccoli heads.

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