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Continuous white–blue LED light exposition delays postharvest senescence of broccoli

Joaquín H. Hasperué [a], Luciano Guardianelli [a], Luis M. Rodoni [a, b], Alicia R. Chaves [a], Gustavo A. Martínez [c, *]

[a] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA)-CONICET, calle 47 y 116, Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), 47 y 115, 1900, La Plata, Argentina
[b] Laboratorio de Investigación en Productos Agroindustriales (LIPA), Facultad de Ciencias Agrarias y Forestales-UNLP, calle 60 y 119, CP 1900, La Plata, Argentina
[c] Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús (IIB-INTECH), UNSAM-CONICET, Av. Intendente Marino Km 8,5, B7130IWA, Chascomús, Argentina

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ABSTRACT

In this work, the impact of low intensity (20 μmol m^{-2} s^{-1}) continue illumination with white and blue light-emitting diodes (WB LED) on the shelf life of broccoli heads stored at 5 °C or at 22 °C was assessed. At both temperatures, heads stored under WB LED showed the highest levels of chlorophylls, reflected in a higher Hue angle and lower L* values compared to controls stored in the darkness. Also, during storage at 22 °C the treated samples had a slower rate of sugar lost compared to dark stored controls. At 5 °C, glucose and fructose levels were maintained and sucrose was increased by the WB LED treatment. The dark stored controls showed the highest accumulation of antioxidant compounds, but there were no differences in ascorbic acid content except for the last storage day at 5 °C, being the WB LED samples which had a slightly higher level ($p < 0.05$). Finally, treated samples showed an increment in the total carotenoid content, mainly during storage at 22 °C. According to the results, WB LED treatment would be a feasible and low cost technology to enlarge the postharvest storage of whole broccoli heads.

1. Introduction

Broccoli is considered to be the vegetable with the highest nutritional value per edible unit weight. However, after harvest, broccoli heads trigger a quick senescence and cause the product deterioration, visible by the degreening of sepals. It is known that the cellular changes during senescence are induced by sugar starvation (Yu, 1999), and a common mechanism that regulates metabolic processes during sugar starvation and senescence has been explained by Dieuaide, Brouquisse, Pradet, & Raymond (1992) and Buchanan-Wollaston et al., (2005). Also, in many cases it was shown that postharvest storage in light has also been associated with an increase of sugars and the consequent delay of senescence (Noichinda, Bodhipadma, Mahamontri, Narongruk, & Ketsa, 2007; Toledo, Ueda, Imahori, & Ayaki, 2003; Zhan, Hu, Pang, Li, & Shao, 2014). Such effect was also described in broccoli, in which the storage under light during postharvest retards the chlorophyll degradation and extends the product shelf life (Büchert, Civello, & Martínez, 2011; Kasim & Kasim, 2007; Zhan, Hu, Li, & Pang, 2012, 2014).

The illumination with light emitting-diodes (LED) has lately become a more available technology and a more economic and energetically efficient way for light treatments on vegetables (Morrow, 2008). Light from LEDs provide a narrow output spectrum that allow determined wavelengths to match more specifically to plant photoreceptors (Lin et al., 2013; Massa, Kim, Wheeler, & Mitchell, 2008). For the photosynthesis, the absorption quantum yield curve only has 2 broad maxima, centered at 620 (red) and near 450 nm (blue), with a shoulder at 670 nm (McCree, 1972). The white phosphor types LEDs have peaks of emission from 450 to 500 nm and from 550 to 600 nm (Pimputkar, Speck, DenBaars, & Nakamura, 2009). In the case of postharvest treatments, the addition of green or red LED light has been reported to improve the quality of broccoli florets (Jin, Yao, Xu, Wang, & Zheng, 2015; Ma et al., 2014), maintaining higher quantity of chlorophylls. Blue light has a variety of important roles in plants, such as

* Corresponding author.
E-mail address: gmartinez@intech.gov.ar (G.A. Martínez).

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photomorphogenesis, stomatal control (Assmann, Simoncini, & Schroeder, 1985), phototropism, development of photosynthetic apparatus and chlorophyll biosynthesis (Senger, 1982), among others. Moreover, short-duration blue LED treatments before harvest significantly increased shoot tissue carotenoid and glucosinolates content in sprouting broccoli (Kopsell & Sams, 2013).

Even though there are consistent works on plantlets grown under LED lighting, up to the best of our knowledge the effect of narrowed wavelengths on postharvest quality of whole broccoli heads at different storage temperatures has not been studied. In this work we assessed the effect of combined white and blue LED light on postharvest visual and chemical quality parameters of broccoli stored at 5 °C or at 22 °C.

2. Materials and methods

2.1. Plant material and light treatment

Broccoli (*Brassica oleracea* var. *Italica* cv. Legacy) were harvested in a farm in La Plata, Argentina (34° 59′ S and 58° 3′ W). The heads were harvested in the morning and transported within an hour to the laboratory. Heads were placed in plastic cups, wrapped with perforated PVC to avoid excessive dehydration and stored at 68 ± 1% humidity in chambers at 5 °C or at 22 °C under continuous white and blue LED light (WB LED). The disposition of the LED strips was pointed at the top side of broccoli heads, at the distance of 9 cm in order to achieve the desired dose of photons. The light intensity was selected according to previous researches (Büchert et al., 2011), maintaining a photosynthetic photon flux (PPF) level to 20 μmol m^{-2} s^{-1}, measured with a PAR meter (Radlogger RAD 1, Cavadevices, Argentina). Controls were stored in darkness at the same conditions of temperature and humidity.

The broccoli heads were stored at 5 °C for 0, 35 and 42 days or at 22 °C for 0, 2, 3 and 4 days. Samples were taken at the aforementioned times and immediately evaluated or processed in order to separate florets from stems. Florets were then frozen in liquid nitrogen and stored at −80 °C until analysis. The whole experiment was repeated twice.

2.2. Surface color and weight loss

Superficial color ($L^*a^*b^*$ system) was evaluated with a colorimeter (Minolta CR-400, Japan). Six measurements per head were done randomly at each storage time. The Hue angle (h°) was calculated as $h^\circ = \tan^{-1}\left(b/a\right)$ when a and $b > 0$ or $h^\circ = 180^o + \tan^{-1}\left(b/a\right)$ when $a < 0$ and $b > 0$.

For the weight loss determination, the broccoli heads were weighed during the mentioned sampling days and weight loss (WL) was calculated from initial (IW) and final weights (FW) as: WL (%) = (IW − FW/IW) × 100.

2.3. Chlorophyll and total carotenoids content

Frozen samples were processed in a mill and approximately 0.4 g of the obtained powder were added to 2.5 mL of acetone/water (80/20) and homogenized. The homogenate was vortexed for 1 min and centrifuged at 5500× *g* for 5 min. The supernatant was collected and the extraction procedure was repeated with the addition of 2.5 mL of acetone/water (80/20). Chlorophylls and total carotenoids content were determined with spectrophotometer (UV-Mini 1240 model, Shimadzu Corp., Japan) according to Lichtenthaler (1987) and expressed as mg kg^{-1} of chlorophyll in a fresh weight basis. All measurements were repeated twice.

2.4. Soluble sugars

Approximately 50 g of frozen broccoli samples were ground in a mill and 0.6 g of the obtained powder were homogenized with 5 mL of ethanol and vortexed for 1 min. The mixture was centrifuged at 5580× *g* for 10 min at 4 °C; the supernatant was recovered and filtered through 0.2 μm RC membrane (Cole-Parmer, USA). For sugar determination, a high-performance liquid chromatograph (HPLC, Waters 1525 Binary HPLC Pump) was used, equipped with a refractive index detector (Waters, IR 2414) and a Hypersil Gold Amino column (4.6 × 250 mm, 5 μm, Thermo Sci., USA). Samples were run with an isocratic flow rate of 1.0 mL min^{-1} of acetonitrile/water (70/30). Two extracts per sample and storage time were obtained and measurements were done in duplicate. Results were expressed as mg kg^{-1} of sugar in a fresh weight basis.

2.5. Ascorbic acid

Samples were frozen in liquid nitrogen, processed in a mill and approximately 1 g of the obtained powder was homogenized with 5 mL of 2.5% m/v metaphosphoric acid. The mixture was vortexed for 1 min and then centrifuged at 12,000× *g* for 10 min at 4 °C. The supernatant was recovered and filtered through 0.2 μm RC membrane and ascorbic acid (AsA) determination was done by using a high-performance liquid chromatograph (HPLC, Waters 1525 Binary HPLC Pump), fitted with a photo diode array detector and a C_{18} column (4.6 × 150 mm, 5 μm, Waters Corp., USA). The mobile phase was 0.5% m/v metaphosphoric acid/acetonitrile (93/7) at an isocratic flow rate of 1.0 mL min^{-1} and the wavelength for detection was 245 nm. For identification and quantitation a standard AsA solution was employed. Results were expressed as mass of AsA on a fresh weight basis, mg kg^{-1}. Two extracts per sample and storage time were obtained. All the measurements were done twice.

2.6. Folin Ciocalteu (FC)-reacting substances

Ethanolic extracts were obtained and homogenized in 5 mL of ethanol as described above (2.4). Then, 50 μL of the supernatant was added to 950 μL of distilled water and 50 μL of 1:1 water diluted FC reagent. After 3 min, 100 μL of a solution containing 20% (m/v) Na_2CO_3 in 0.1 mol L^{-1} NaOH were added and the resulting solution was incubated at 25 °C for 90 min (Singleton, Orthofer, & Lamuela-Raventos, 1999). The absorbance was measured with spectrophotometer at 760 nm and the FC-reacting substances were calculated using gallic acid (GA) as standard. Results were expressed as mass of GA equivalents on a fresh weight basis, mg kg^{-1}. Four replicates were analyzed per storage time.

2.7. Antioxidant capacity against DPPH• and ABTS•+ radicals

To measure antioxidant capacity with DPPH• and ABTS•+ radicals, ethanolic extracts were obtained as described above (2.4). The DPPH• assay was done according to the method described by Brand-Williams, Cuvelier, and Berset (1995) with minor modifications. Test tubes containing 0, 25, 37.5, 50 and 75 μL of sample and ethanol to a final volume of 150 μL were prepared. After that, 1.0 mL of a 59.2 mg L^{-1} of the radical DPPH• dissolved in ethanol solution was added. Samples were vortexed and incubated in darkness at 20 °C for 90 min. The absorbance was measured at 515 nm and the equivalent mass of florets tissue required to consume 50% of the initial DPPH• was calculated (EC_{50}). The antioxidant capacity was expressed as EC_{50}^{-1} in g^{-1}. Four replicates were analyzed per storage

time.

The ABTS$^{\bullet+}$ assay was performed as described by Arnao, Cano, and Acosta (2001). The ABTS$^{\bullet+}$ stock solution was prepared by weighing 7 mmol of ABTS ammonium salt and 2.45 mmol of $K_2S_2O_8$, which were added to water to make 1 L and allowed to react overnight at 20 °C in darkness. ABTS$^{\bullet+}$ working solutions were prepared by diluting the stock solution to an absorbance of 0.700 ± 0.03 at 734 nm. Then, 20 μL of ethanolic extract were added to 1 mL of ABTS$^{\bullet+}$ working solution, vortexed, and incubated for 6 min and the absorbance at 734 nm was measured. Corresponding blanks without extract were used to determine the stability of the ABTS$^{\bullet+}$. Samples were measured in triplicate. Trolox® was used as antioxidant standard and results were expressed as Trolox equivalents antioxidant capacity (TEAC) on a fresh weight basis (mmol kg^{-1}).

2.8. Experimental design and statistical analysis

The experiments, at 5 or 22 °C, were designed according to a factorial design, being the factors the treatment and the storage time. Data were subjected to analysis of variance (ANOVA), and means were compared by a Fisher test by using the software InfoStat (Di Rienzo et al., 2012) at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Weight loss

One of the factors that determine the postharvest quality of vegetables is the tissue turgidity, which is mainly determined by the degree of dehydration during storage (Serrano, Martinez-Romero, Guillen, Castillo, & Valero, 2006). Weight loss (WL) of broccoli heads increased during storage at 5 °C and 22 °C (Table 1). At 22 °C the WL was higher in samples stored under WB LED compared to controls, but in both groups it was less than 10% from the day 0 (Table 1). In the case of the samples stored at 5 °C, although there was a little tendency to a greater WL in the light-stored samples, there were no significant differences until day 42 between treated and control samples, being the WL at the end of storage about 20% from the beginning of storage. The greater tendency to lose weight in light-exposed samples could be due to a possible higher stomata opening caused by blue light exposition (Kinoshita et al., 2001; Noichinda, Bodhipadma, Mahamontri, Narongruk, & Ketsa, 2007).

3.2. Color, chlorophylls and carotenoids

The main visible symptom of broccoli deterioration after the harvest is the floret yellowing, process that is visible since chlorophyll content starts to decrease (Costa, Civello, Chaves, & Martínez, 2005; Hasperué, Gómez-Lobato, Chaves, & Martínez, 2013). The progress of degreening is commonly followed by the measure of Hue angle and L* parameters (Costa, Vicente, Civello, Chaves, & Martínez, 2006; Hasperué, Chaves, & Martínez, 2011).

Exposing broccoli heads to continuous WB LED at both 5 °C and 22 °C helped to maintain the heads greener, as it can be observed by the values of L* and Hue parameters during the whole storage period (Table 1). In the case of the L*, illuminated samples showed lower L* values both at 5 and 22 °C in all sampling days, while the Hue angle was 26 and 12% higher in treated samples respectively. Concomitantly with color evolution, the content of chlorophyll *a* and *b* was higher in samples exposed to continuous WB LED (Fig. 1). The treated samples maintained the highest levels of chlorophyll towards the end of storage, with values 38% and 53% higher than controls in the experiments at 5 °C and at 22 °C respectively ($p < 0.05$).

Carotenoids perform a variety of critical functions in light harvesting, and it was demonstrated that the major stimulus for carotenoid synthesis in greening leaf tissue is light (Oelmüller & Mohr‘ 1985). In addition to contributing to the functions mentioned above and from the point of view of human consumption, carotenoids are considered a good source of fat-soluble antioxidants (Edge, McGarvey, & Truscott, 1997; Palozza & Krinsky, 1992). In this work, it was detected the highest carotenoid content in samples stored under WB LED (Fig. 1). At 5 °C, the content remained almost constant, but at the end of storage a slight increment in treated samples was detected. On the other hand, at 22 °C the treated samples showed an important accumulation of carotenoids, resulting in amounts about 25% higher than controls. Lefsrud, Kopsell, Kopsell, and Curran-Celentano (2005) observed a linear increase of carotenoid concentration with increasing air temperatures. In our experiment, carotenoids showed an increase during storage in samples kept at 22 °C, while in those kept at 5 °C maintained the levels of the initial day.

3.3. Sugars

Plants use sugars as the main source of energy to perform numerous chemical reactions necessary to the maintenance of the tissue integrity and the synthesis of new compounds (De Vries, 1975). Broccoli inflorescences are immature organs with a high respiration rate and a high requirement of sugars (King & Morris, 1994). In addition, several works have associated the rate of broccoli senescence with the sugar level (Hasperué et al., 2011; Nishikawa et al., 2005; van Doorn, 2004). In this work, the experiment conducted at 22 °C showed a decrease in the total sugar content during the storage in all samples (Fig. 2). However, the broccoli heads stored under WB LED had a slower tendency in the

Table 1
Changes in weight loss (%), Hue (°), and lightness (L*) of florets stored in darkness and under WB LED during postharvest storage at 5 °C and 22 °C.

	Weight loss (%)		Hue (°)		Lightness (L*)	
	Control	WB LED	Control	WB LED	Control	WB LED
Storage at 5 °C						
0 d	0.0 ± 0,0	0.0 ± 0.0	123.3 ± 10.7	123.3 ± 10.7	37.9 ± 2.3	37.9 ± 2.3
35 d	6.2 ± 2.3	7.5 ± 2.2	108.2 ± 8.1	123.2 ± 3.1*	42.3 ± 2.5	37.5 ± 2.1*
42 d	7.7 ± 1.3	8.2 ± 2.5	91.6 ± 8.8	122.4 ± 2.9*	47.4 ± 4.1	39.7 ± 3.3*
Storage at 22 °C						
0 d	0.0 ± 0.0	0.0 ± 0.0	132.1 ± 3.0	132.1 ± 3.0	39.0 ± 2.8	39.0 ± 2.8
2 d	1.2 ± 0.1	2.4 ± 0.8	126.1 ± 2.4	131.1 ± 1.2*	43.8 ± 1.9	40.7 ± 1.8*
3 d	2.4 ± 0.2	4.5 ± 1.1*	112.4 ± 1.2	119.3 ± 1.7*	51.3 ± 2.3	47.0 ± 2.0*
4 d	3.8 ± 0.2	6.0 ± 1.0*	101.6 ± 3.2	115.9 ± 4.8*	56.4 ± 2.7	47.4 ± 3.0*

Asterisks show significant statistical differences between Control and WB LED samples at the same storage time ($P < 0.05$).

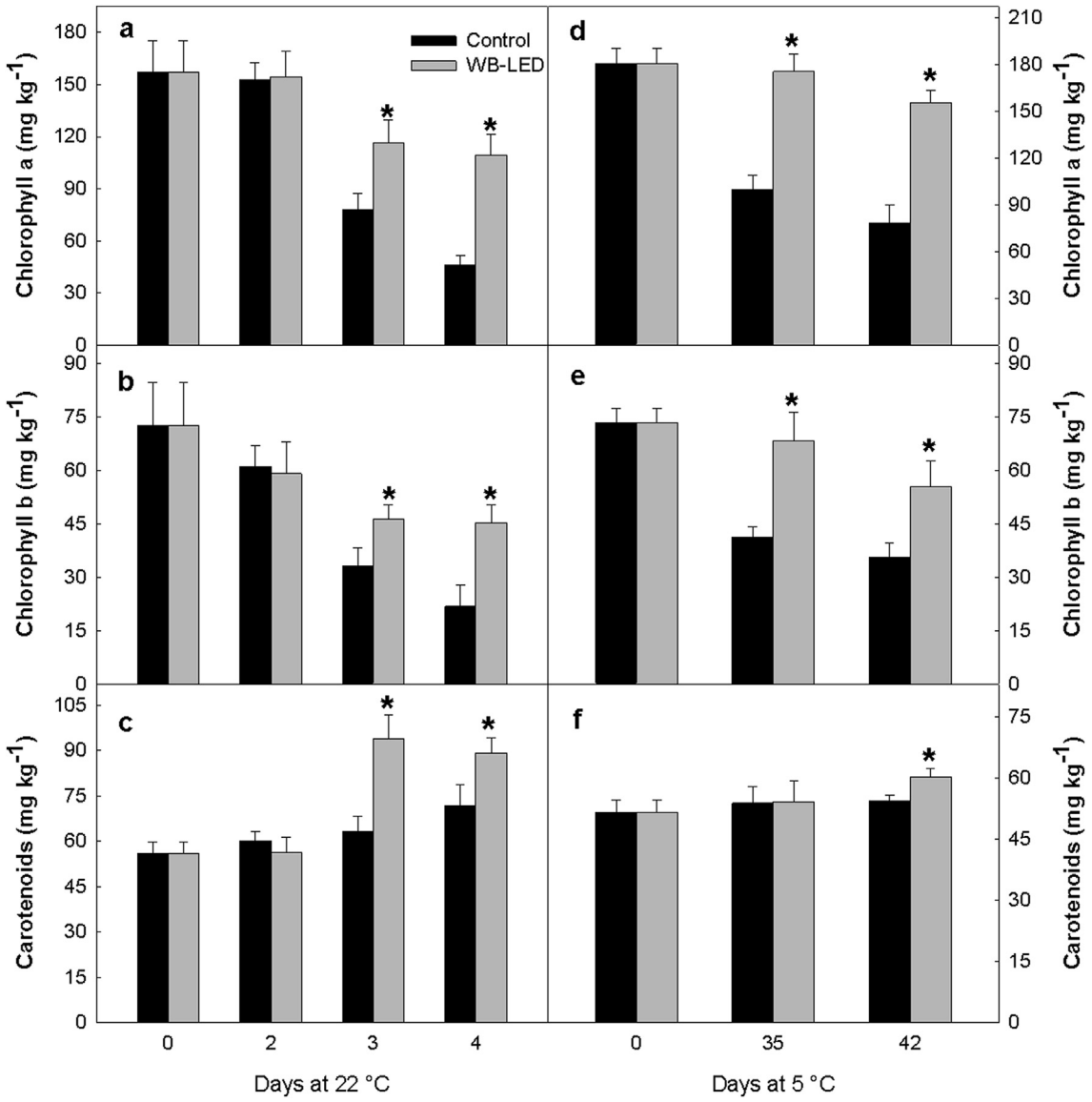

Fig. 1. Changes in the content of Chlorophyll *a* (plots a and d), Chlorophyll *b* (plots b and e) and Carotenoids (plots c and f) during storage of broccoli heads maintained at 22 °C (plots a, b and c) and at 5 °C (plots d, e and f). Columns with asterisks indicate significant statistical differences between Control and WB LED samples at the same storage time ($P < 0.05$).

decrease and showed higher amounts of glucose and fructose after 2 and 3 days respectively, and higher amounts of sucrose after 4 days of storage (Fig. 2).

In the experiment at 5 °C, in the controls stored in darkness the level of fructose and glucose decreased, while sucrose increased slightly after day 42 (Fig. 2). On the contrary, samples stored under WB LED maintained the fructose and glucose levels and increased significantly the sucrose level ($p < 0.05$). Treated samples contained higher levels of fructose after 35 and 42 days of storage and higher levels of glucose after 42 days compared to controls. Sucrose accumulated more than 3-fold in relation to their initial levels by 35 days in samples exposed to WB LED, being at the end of storage two times higher than those of controls. Previous works showed that the starch and sugars generated by photosynthesis are starved faster at dark and high temperature conditions (Lu, Gehan, & Sharkey, 2005; Pilkington et al., 2015). The high sugar accumulation in samples stored under WB LED at 5 °C could be due to the lower energy needed for the maintenance of metabolism under refrigerated conditions in comparison to that at 22 °C. The increase in sucrose level during refrigerated storage even in the control samples might be due to an increase in the activity of enzymes related to sucrose synthesis like sucrose phosphate synthase, in order to increment the tolerance to freezing stress by adjusting the metabolism to the low nonfreezing temperature (Guy, Huber, & Huber, 1992).

3.4. Antioxidants

Several works have shown that, after harvest, the antioxidant (AOX) capacity of broccoli is maintained or slightly increased during storage (Toivonen & Sweeney, 1998; Leja, Mareczek, Starzyńska, & Rozek, 2001; Hasperué, Lemoine, Vicente, Chaves, & Martínez, 2015). In the present work, the AOX capacity, measured with ABTS and DPPH assays increased during storage at 22 °C in all samples but mainly in the controls, which showed at the end of the storage values from 11 to 17% higher for DPPH• and $ABTS^{\bullet+}$

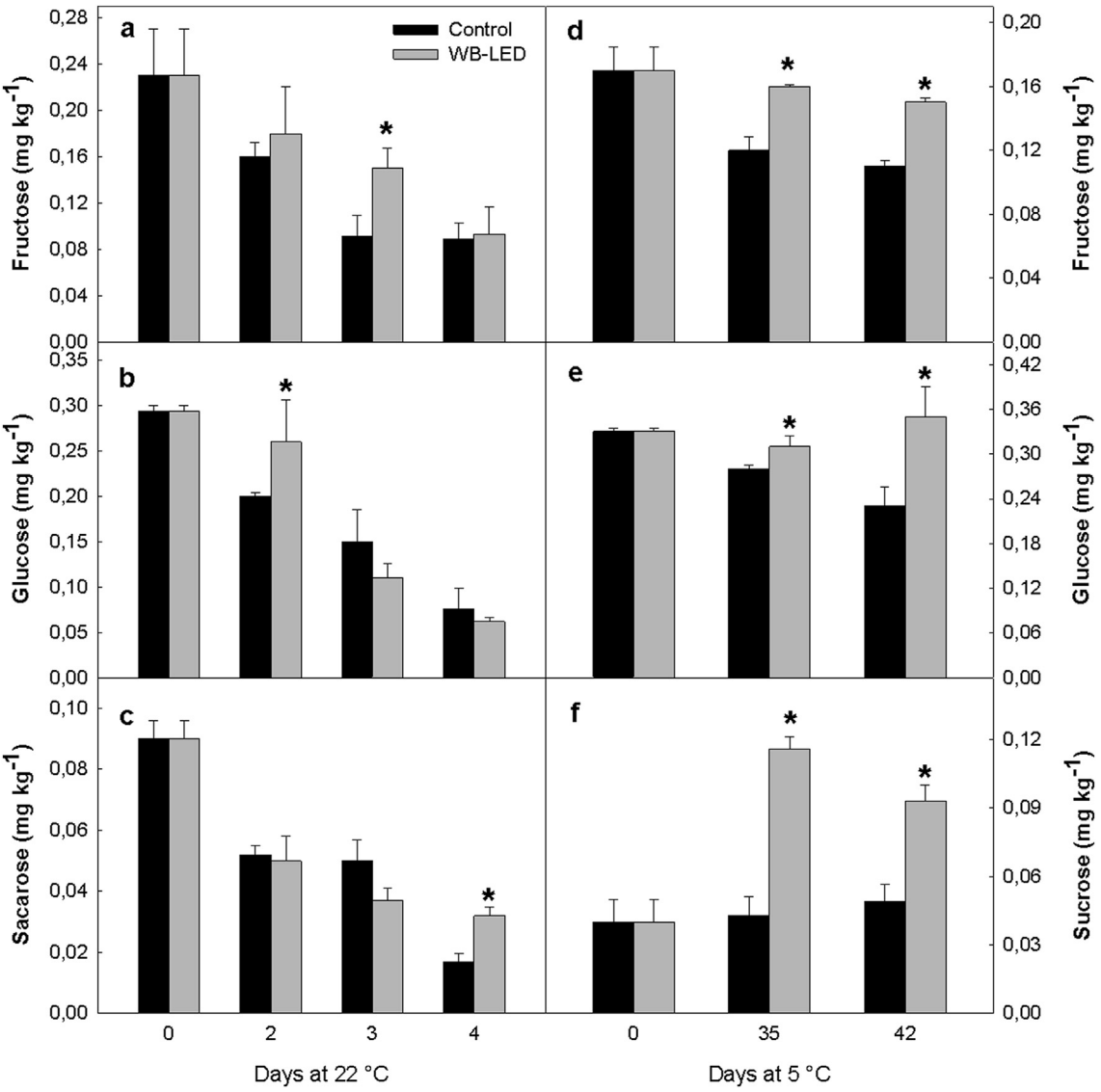

Fig. 2. Changes in the content of Fructose (plots a and d), Glucose (plots b and e) and Sacarose (plots c and f) during storage of broccoli heads maintained at 22 °C (plots a, b and c) and at 5 °C (plots d, e and f). Columns with asterisks indicate significant statistical differences between Control and WB LED samples at the same storage time ($P < 0.05$).

respectively (Fig. 3). At 5 °C the increase in the AOX capacity during the storage was lower and could only be observed in the controls, which showed towards the end of storage values from 20 to 12% higher for DPPH• and $ABTS^{\bullet+}$ respectively compared to samples treated with WB LED.

In line with these findings, throughout the storage at 22 °C the FC-reacting substances increased only in controls. At 5 °C, although there was an increase in FC-reacting substances in all samples ($p < 0.05$), there were no differences between controls and treated with WB LED (Fig. 4).

Under high-light conditions plants suffer an increase of reactive oxygen species production and the efficiency of photosynthesis is reduced by a photoinhibition process (Barber & Andersson, 1992). This is followed by an increase of enzymatic and non-enzymatic antioxidant system to neutralize the potential harmful effect on tissues (Montillet et al., 2005; Agati, Azzarello, Pollastri, & Tattini, 2012; Suzuki, Koussevitzky, Mittler, & Miller, 2012). In the current experiment, the illumination during storage was of low intensity; therefore, it should not be considered a stressful condition. In addition, reactive oxygen species also increase with the senescence (Zimmermann & Zentgraf, 2005), and this increase should also be compensated with higher levels of antioxidant compounds. Therefore, samples subjected to continuous light could be considered with a less advanced senescence, a lower oxidative stress and consequently, less accumulation of antioxidants.

A significant decrease in AsA content was found during the storage at both temperatures (5 °C and 22 °C) with or without WB LED ($p < 0.05$) (Fig. 4). There were no differences in AsA content between WB LED and control samples stored at 22 °C, but, in the experiment conducted at 5 °C WB LED treated samples showed a slightly higher level of AsA at the end of the storage period ($p < 0.05$) (Fig. 4). It is known from literature that AsA content diminishes during the storage in harvested broccoli (Nishikawa et al., 2003; Mori, Terai, Yamauchi, & Suzuki, 2009; Raseetha, Leong, Burritt, & Oey, 2013) and that its level could be increased by light (Zhan et al., 2012). However, in the present work, in most of the cases, no significant differences were detected between controls and treated samples. This result seems to suggest that the light intensity used in the conditions of the assay was not high enough to enhance AsA accumulation.

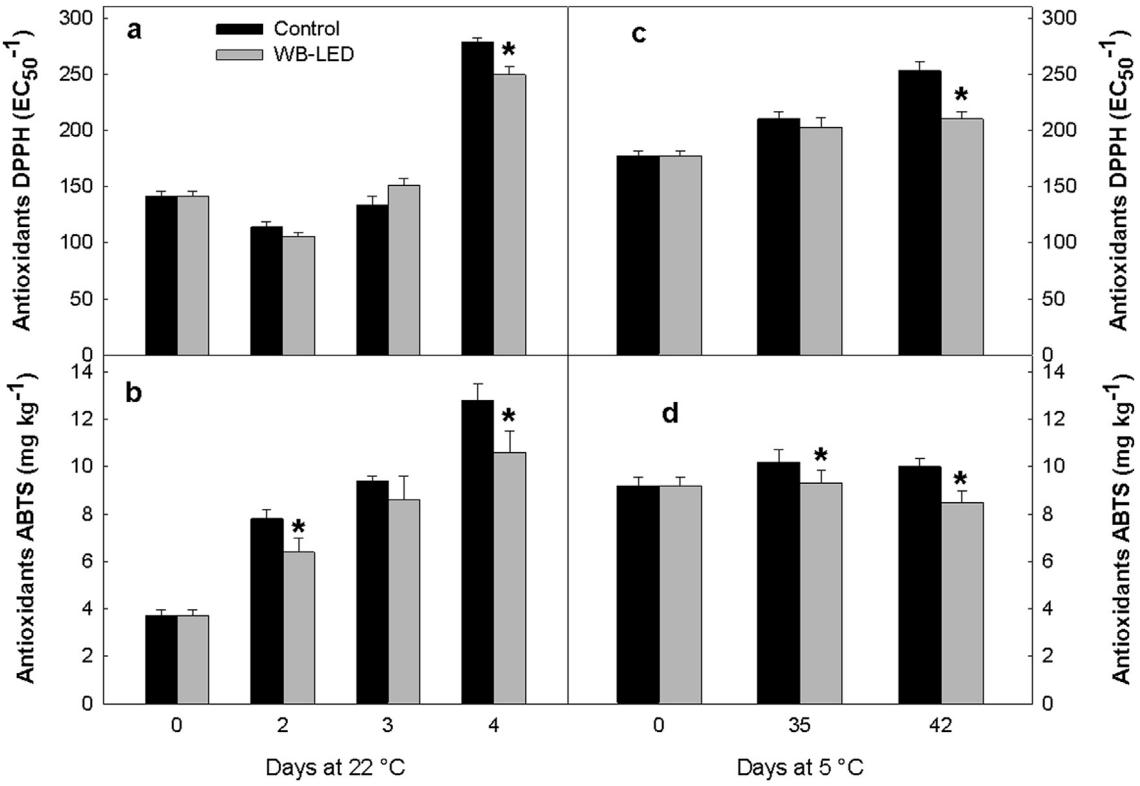

Fig. 3. Changes in the content of antioxidants measured with DPPH (plots a and c) or with ABTS (plots b and d) during storage of broccoli heads maintained at 22 °C (plots a and b) and at 5 °C (plots c and d). Columns with asterisks indicate significant statistical differences between Control and WB LED samples at the same storage time ($P < 0.05$).

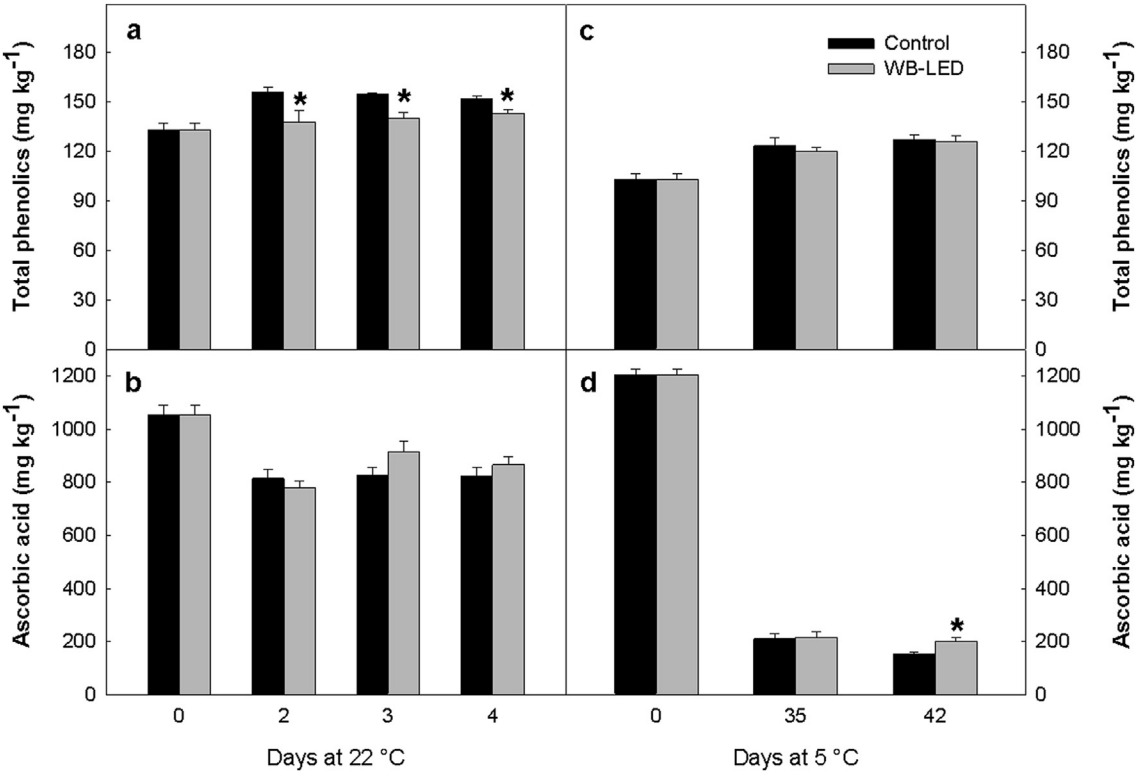

Fig. 4. Changes in the content of Ascorbic acid (plots a and c) and Total phenolics (plots b and d) during storage of broccoli heads maintained at 22 °C (plots a and b) and at 5 °C (plots c and d). Columns with asterisks indicate significant statistical differences between Control and WB LED samples at the same storage time ($P < 0.05$).

4. Conclusions

The white–blue LED treatment on entire broccoli heads during all the storage at 5 or at 22 °C was effective in delaying the senescence and extending the product shelf life. At the end of storage two times more chlorophyll was observed in treated samples than in the dark stored controls, which were visibly more yellow. Considering the further advance of the senescence in the controls, at the end of storage time, the AOX content in the controls was higher than treated samples. Similar results were found in the phenols content but only at 22 °C. At 22 °C temperature, the WB LED treatment increased the glucose, fructose and sucrose content at days 2, 3 and 4 respectively. On the other hand, at 5 °C the light treatment promoted the accumulation of all sugars during all storage. At both temperatures, it was observed also higher levels of carotenoids in the treated samples. Even though it is believed that the light exposition increases the AsA level in plants, in these experiments were found only slightly higher AsA levels in treated samples at the end of storage at 5 °C.

The combination of W–B LED light sources used in this work would be a clean and cheap technology to enlarge the postharvest shelf life of entire broccoli heads.

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