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Use of LED light for Brussels sprouts postharvest conservation

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ABSTRACT

Yellowing is the most evident symptom of senescence in green vegetables during postharvest storage. As chlorophyll synthesis is promoted by blue light, illumination would be a clean alternative to maintain healthier vegetables for longer. In this paper was assessed the effect of white-blue light-emitting diodes (WB LED) on outer and inner leaves (OL, IL) of Brussels sprouts during 10 d storage at 22 °C. The treated sprouts showed lower respiration rate and remained greener with a better visual quality, with more than 10 times chlorophylls than controls in OL and 1.6 times in IL towards the end of the storage period. The OL had a higher content of antioxidants (DPPH• and $ABTS^{\bullet+}$ assays) than the IL, and the treatment increased the AOX only in the OL. Total flavonoids content was higher in OL than IL, and about 20% higher in treated samples at day 10 of storage. The storage of Brussels sprouts under continuous low intensity WB LED was effective in delaying the senescence. The effect of the treatment was visible not only in the leaves exposed to light (OL) but also in the IL. The WB LED lighting would be useful to maintain or improve the quality of Brussels sprouts for both storage and transport.

1. Introduction

One of the major postharvest problems of green vegetables is the yellowing of the tissues due to senescence, which leads to a loss of nutritional and commercial value. As other cruciferous, Brussels sprouts (*Brassica oleracea* var *gemmifera*) has been studied for its nutritional value (Viña et al., 2007a) and for the benefits against some diseases, included cancer (Fujioka et al., 2016; Liu et al., 2013). Yellowing of tissues is caused by chlorophyll degradation during tissue senescence. After the harvest, the outer leaves of Brussels sprouts suffer yellowing and dehydration, making the product to loose market value or diminish the yield, since the producers remove the outer yellowed leaves to continue selling the product. Some efforts have been made to delay postharvest yellowing in Brussels sprouts, such as heat treatment (Wang, 1998), edible coating (Viña et al., 2007b) or fluorescent lighting (Kasim and Kasim, 2007) with different results. Brussels sprouts are a group of imbricated leaves, and for its nature, it is possible to use low-disturbing methods with light to maintain the photosynthetic system active, extending the green color and enlarging the shelf life of sprouts. While conventional lighting could have an adverse effect by raising the temperature in the storage room, the use of narrowed light would avoid using wavelengths not useful for the photosynthesis as infrared band, wasting less energy when considering a postharvest continuous or photoperiodic exposure to light. It is known that the influence of blue light on the development of the photosynthetic apparatus as well as its relationship with chlorophyll biosynthesis (Fan et al., 2013; Terfa et al., 2013; Wang et al., 2015). Other studies have observed a delay in senescence by using blue LED light instead of white and red LED, due to an extension in the duration of active photosynthesis (Wang et al., 2015), and an increment of the photosynthetic capacity, increasing stomatal conductance and chlorophyll content (Hogewoning et al., 2010). Although the LED light has been used to a greater extent in vegetable seedlings (Li et al., 2010; Wu et al., 2007), studies in postharvest storage are also promissory (Hasperué et al., 2016; Jin et al., 2015).

Given the lack of background in the use of narrowed light sources in Brussels sprouts postharvest conservation, the aim of this work was to assess the effect of the continuous white and

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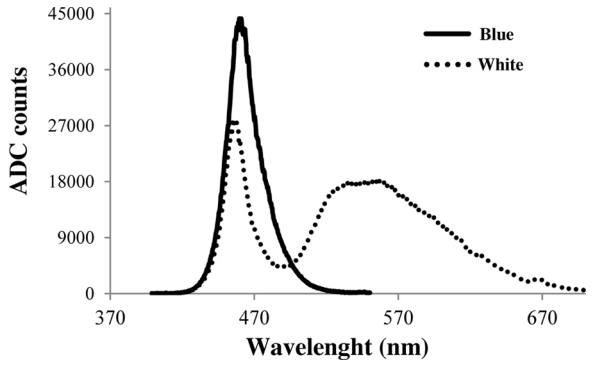

Fig 1. Spectral composition of blue and white LEDs used during the experiment.

blue LED exposition on quality of Brussels sprouts (inner and outer sprout leaves) stored at 22 °C.

2. Materials and methods

2.1. Plant material and light treatment

Brussels sprouts (*Brassica oleracea* var. *gemmifera*) grown under conventional agricultural practices were harvested in a farm in La Plata, Argentina (34° 59′S and 58° 3′W) at 8:00 a.m. from plants of an age of approximately eight months. Harvested sprouts were transported within an hour to the laboratory. Sprouts were selected by their size and the first whorl of small leaves was discarded to uniform their quality. The sprouts were placed in plastics trays, wrapped with perforated PVC to avoid excessive dehydration and stored at 68% humidity at 22 °C under continuous white and blue LED light (WB LED) SMD 3020 model. Controls were stored in dark at the same humidity and temperature. 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 RAD1, Cavadevices, Argentina). The treatment was carried out by disposing white and blue LED strips at a distance of 9 cm from the sprouts, in order to achieve the desired dose of photons. The emission spectrum was measured with a spectrometer (AvaSpec-ULS3648-USB2-UA-25, Avantes) and the peak of blue and white LED was 458–467 nm and 450/525-558 respectively (Fig. 1).

Samples were taken after 0, 5, or 10 days and evaluated for color, respiration rate and weight loss or frozen in liquid nitrogen and stored at −80 °C for further analysis. Before freezing and to compare the effect of the treatment on the metabolism of different leaves of the sprout, the outer two whorls of leaves (OL), those exposed to daylight in the field, were separated from inner leaves (IL), the ones covered completely for the outer leaves. The whole experiment was repeated twice, in consecutive years.

2.2. Weight loss and respiration rate

For the weight loss determination, the entire sprouts were weighed during the sampling days mentioned above and weight loss (WL) was calculated from initial (IW) and final weights (FW) as:

$$WL\ (\%) = \frac{(IW - FW)}{IW} x\ 100$$

For respiration measurement, approximately 300 g of whole Brussels sprouts were placed inside 3 l flasks, closed hermetically and incubated for 10 min at 20 °C. The CO_2 concentration was determined using an infrared analyzer (CompuFlow Model 8650, Alnor, USA) and results were expressed as rate of CO_2 evolution in μg kg^{-1} s^{-1}. Four measurements were done for each treatment and storage day.

2.3. Surface color

The color evaluation was performed with a colorimeter (Minolta CR-400, Osaka, Japan) using the Hunter L*a*b* scale: L* varies from 0 (black) to 100 (white), a* positive values are red and negative values are green, and b* positive values are yellow and negative values, blue. The hue angle (h°) was calculated as $h^o = \tan^{-1}\left(b/a\right)$ when a and $b > 0$ or $h^o = 180^o + \tan^{-1}\left(b/a\right)$ when $a < 0$ and $b > 0$.

All measurements were made in the two outer leaves that wrap the sprout.

2.4. Chlorophylls and carotenoids

The OL and IL stored at −80 °C were ground separately with a grinder and the procedure was performed according to Hasperué et al. (2016). Chlorophylls and total carotenoids were determined with a spectrophotometer (U-1900, Hitachi Corp., Tokyo, Japan) according to Lichtenthaler (1987) and expressed as mg kg^{-1} in a fresh weight basis. All measurements were done by quadruplicate.

2.5. Total soluble sugars (TSS)

IL and OL were frozen with liquid nitrogen and ground in a grinder. Approximately 0.6 g of the obtained powder were homogenized in 5 ml ethanol, vortexed and centrifuged at 5500 × g for 8 min at 4 °C. Four extracts per sample and storage time were obtained. The supernatants were recovered and utilized to determine the content of TSS by the anthrone method. Briefly, one milliliter of 2 g l^{-1} anthrone prepared H_2SO_4 (98% w/w) was added to 10 μl ethanolic extract, mixed and held at 100 °C in a water bath for 10 min. The test tubes were cooled in water for 20 min and the absorbance at 620 nm was measured in a spectrophotometer. For quantification, a standard glucose solution was employed and results were expressed on a fresh weight basis as g kg^{-1}.

Table 1
Weight loss (WL%) and respiration rate (RR) values of treated and control samples during postharvest storage at 22 °C. Data are presented as mean (n = 4) ± standard error.

	WL (%)		RR (μg CO_2 kg^{-1} s^{-1})	
	C	WB LED	C	WB LED
0 d	0.00 ± 0.00	0.00 ± 0.00	31.29 ± 1.42 b	31.29 ± 1.42 b
5 d	3.19 ± 0.14 a	7.19 ± 0.17 b	35.69 ± 4.42 b	22.46 ± 1.46 a
10 d	6.45 ± 0.07 b	15.04 ± 0.82 c	32.60 ± 2.20 b	24.41 ± 2.72 a

Different letters indicate, in each parameter, differences at $P < 0.05$ among treatments and storage days (Duncanís test).

Table 2
Changes in Hue (°) and lightness (L*) values of treated and control samples during postharvest storage at 22 °C. Data are presented as mean (n = 35) ± standard error.

	Hue (°)		Lightness (L*)	
	C	WB LED	C	WB LED
0 d	119.4 ± 0.29 d	119.4 ± 0.29 d	58.5 ± 0.68 a	58.5 ± 0.68 a
5 d	104.4 ± 1.05 b	118.8 ± 0.3 d	76.74 ± 1.04 c	61.51 ± 0.71 b
10 d	96.2 ± 0.49 a	115.7 ± 0.91 c	77.64 ± 0.54 c	62.56 ± 1.04 b

Different letters indicate, in each parameter, differences at $P < 0.05$ among treatments and storage days (Duncanís test).

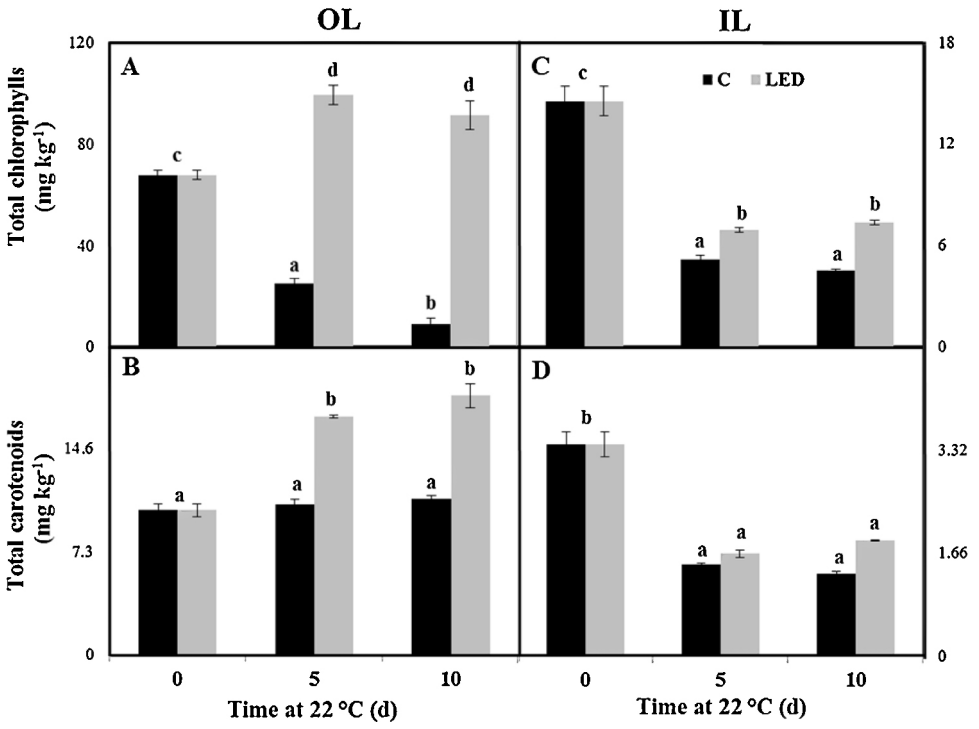

Fig. 2. Total chlorophylls and carotenoids in outer leaves (OL) (A, B) and in inner leaves (IL) (C, D) of treated (LED, grey columns) and not treated (C, black columns) Brussels sprouts stored for 0, 5 and 10 days at 22 °C. Vertical bars represent the standard errors of the means (n = 4). Within each graph, columns with different letters indicate differences at $P < 0.05$ based on Duncan's test.

2.6. Water soluble antioxidants

Ethanolic extracts for water soluble antioxidants determination were obtained and homogenized as described in 2.5. For Folin-Ciocalteu (FC) reacting substances, antioxidant capacity (AOX) against DPPH• and $ABTS^{\bullet+}$ radicals determination were followed the procedures described by Hasperué et al. (2016). Four replicates were analyzed per storage time. Results were expressed as g gallic acid kg^{-1}, $1/EC_{50}$ and g trolox kg^{-1} (Trolox Equivalent Antioxidant Capacity, TEAC) for phenols, DPPH• and $ABTS^{\bullet+}$ respectively.

2.7. Flavonoids

Total flavonoid content was determined as Shin et al., 2007 with slight modifications. A volume of 30 μl 5% $NaNO_2$ were added to 150 μl of ethanolic extract and 550 μl of distilled water to bring volume. The mixture was allowed to stand for 5 min at room temperature and 30 μl 10% $AlCl_3$ were added. After 6 min were mixed with 80 μl NaOH 1 M and the absorbance of the solution versus a blank at 510 nm was measured immediately. Since the quercetin content is relatively high in many cruciferous, it was used as standard for the calibration curve. Results were expressed as g quercetin kg^{-1}.

2.8. Experimental design and statistical analysis

The experiment was designed according to a factorial design, being the factors the treatment, the storage time and the leaf position in the sprouts (OL or IL). Data were analyzed with either a two-way or three-way ANOVA using the software InfoStat (Di Rienzo et al., 2012) and means were compared by the Duncanís multiple range test at a significance level of $P < 0.05$.

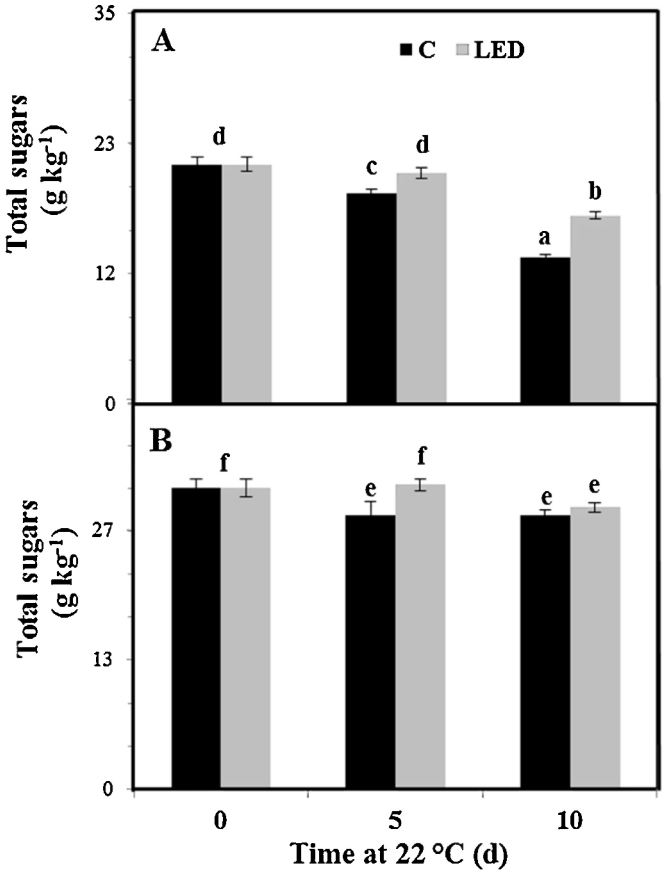

Fig. 3. Total sugars in outer (A) and inner (B) leaves of treated (LED, grey columns) and not treated (C, black columns) Brussels sprouts stored for 0, 5 and 10 days at 22 °C. Vertical bars represent the standard errors of the means (n = 5). In both graphics, columns with different letters indicate differences at $P < 0.05$ based on Duncanís test.

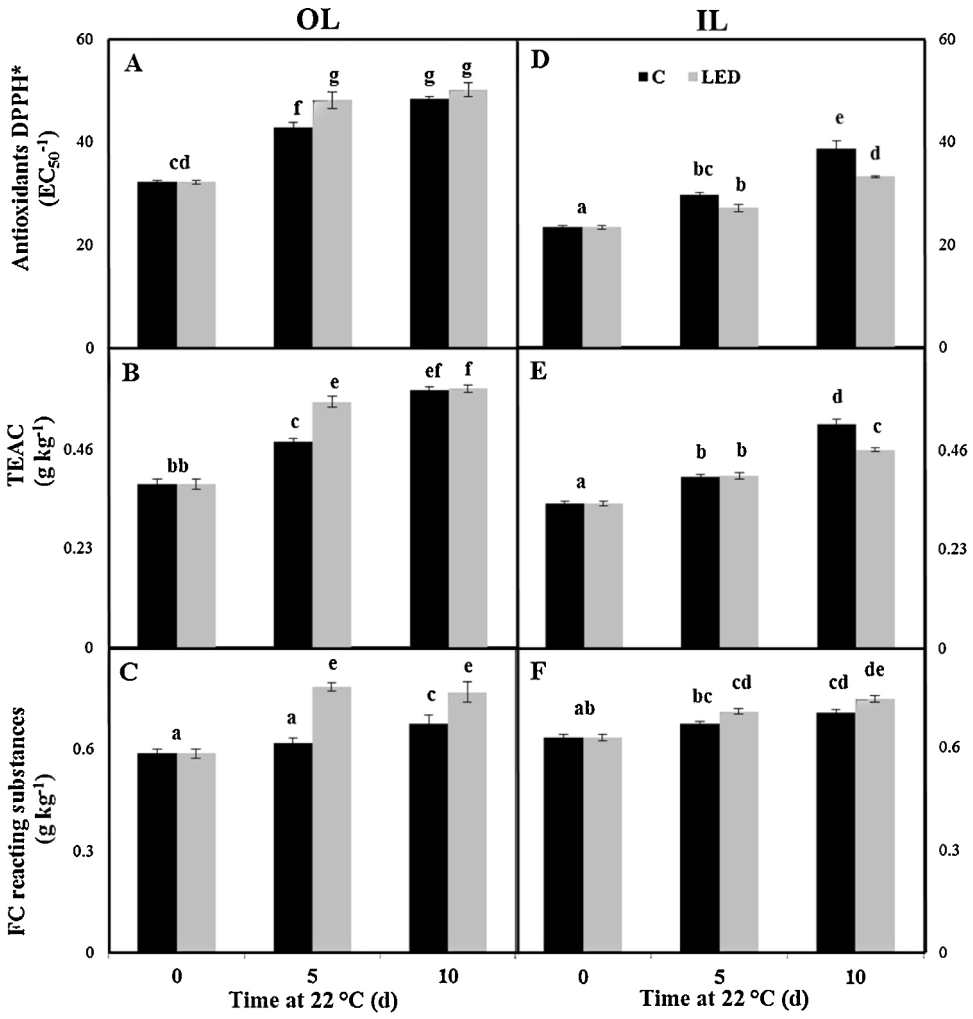

Fig. 4. DPPH• scavenging capacity, TEAC (Trolox Equivalents Antioxidant Capacity), FC-reacting substances in outer leaves (OL) (A, B, C) and in inner leaves (IL) (D, E, F) of treated (LED, grey columns) and not treated (C, black columns) Brussels sprouts stored for 0, 5 and 10 days at 22 °C. Vertical bars represent the standard errors of the means (n = 3). Within each assay, columns with different letters indicate differences at $P < 0.05$ based on Duncanís test.

3. Results and discussion

3.1. Weight loss and respiration rate

During the storage at 22 °C, samples exposed to continuous WB LED showed higher WL compared to controls stored in dark until both 5 and 10 d storage, with values 4% and 8% higher respectively (Table 1).

It is known that the blue light is involved in the stomata opening (Kinoshita et al., 2001; Noichinda et al., 2007), therefore the continuous exposition to WB LED light would increase the WL by increasing transpiration rate. Regarding the respiration rate (RR), the controls maintained the initial levels and had higher RR at 5 and 10 d after harvest, while in the treated samples it was observed a decrease from the day 0 to 5 and maintained lower rates until 10 d (Table 1). Since there is an inverse relationship between respiration and the postharvest life of fresh vegetables (Kader and Saltveit, 2002), the higher respiration measured in the controls could be related to a more accelerated metabolism and thus a premature yellowing.

3.2. Surface color, chlorophylls and carotenoids

Surface color is one of the most visual quality attributes, and the luminosity (L*) and hue angle are related to the amount of chlorophylls in the tissue. Significant differences were found in color parameters during the storage between WB LED treated samples and controls (Table 2).

The yellowing was delayed in treated samples, according to the lower L* values observed. The hue angle, which indicates the greenness of the tissue, remained almost unchanged in treated samples until 10 d and decreased faster in controls. There was a correlation between the color parameters and chlorophyll content. Under exposition to WB LED, chlorophyll and carotenoids contents in OL were 5 and 3 times higher respectively than in IL (Fig. 2).

Exposition to a lower light intensity compared to the preharvest field conditions, can promote chlorophyll synthesis (Danesi et al., 2004). In the OL, controls showed a drastic decrease in chlorophyll content until 10 d storage, but in WB LED samples increased about 35% after 10 d storage, containing at the same day 10 times more chlorophylls than controls. Since carotenoids perform a variety of functions as accessory pigments for light harvesting, treated samples also showed higher amount of total carotenoids than controls, but only in OL. Controls had a steady level until 10 d but treated samples reached values 40% higher to the same storage time.

Despite the IL were not exposed directly to the LED light because they were covered by the OL, in the IL of illuminated sprouts it was observed a higher chlorophyll content compared to controls through all the storage time. A decrease was observed in the chloro-

phyll content in all samples, but the treated ones maintained higher levels at 5 and 10 d. The IL had higher carotenoids content after harvest respect to OL, but concerning to the treatment, there were no significant differences between treated and control samples ($P > 0.05$).

Carotenoids are permanently synthesized under light, but photo-oxidation apparently destroys large amounts of them (Simkin et al., 2003; Tracewell et al., 2001). It is for this reason that the carotenoid levels in illuminated leaves reach a certain level, which is lower when the light level increases. Since in this experiment it was used a low light intensity, the higher pigment content after 10 d of WB LED light exposition would be also explained for the positive effect of the blue light on the carotenoid and chlorophyll synthesis (Giliberto et al., 2005; Lintig et al., 1997; Poudel et al., 2008).

3.3. Total soluble sugars (TSS)

Sugars are the main source of energy and their starvation may result in senescence of tissues (Buchanan-Wollaston et al., 2005; Fujiki et al., 2001; Yu, 1999). Higher level of sugars in plant tissues also was associated with a delay in senescence in broccoli (Büchert et al., 2011; Hasperué et al., 2011; Nishikawa et al., 2005). TSS decreased from the day of harvest to the end of storage period in all samples, mostly in OL, which had a lower amount respect to IL (Fig. 3).

In the OL, the treated samples showed higher TSS both at 5 and 10 d storage ($P < 0.05$). In the IL treated samples maintained higher TSS than controls until 5 d but not at 10 d ($P > 0.05$). Even though the light intensity used in this experiment is near the light compensation point of the photosynthetic tissues, we guess that the lower RR and a photosynthetic activity during the WB light exposition could have improved the carbohydrate levels. Comparing OL to IL, similar results were obtained in other works on different lettuce varieties (Baslam et al., 2013; Poulsen et al., 1995), increasing TSS from OL to IL.

3.4. Antioxidants

As in other studies about postharvest storage (Hasperué et al., 2015, 2016; Leja et al., 2001; Toivonen and Sweeney, 1998), it was observed an increase of the AOX capacity during the storage of Brussels sprouts at 22 °C in both OL and IL (Fig. 4).

From the day of harvest until 10 d storage, the total AOX capacity was higher in OL than IL but not for the FC-reacting substances. The effect of the treatment was observed in both OL and IL. In the OL, FC-reacting substances were higher in treated samples at 5 and 10 d ($P < 0.05$), and the AOX capacity measured with DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays also was higher in the treated samples but only after 5 d ($P < 0.05$). In the IL, there were no differences in AOX capacity until 10 d, being the controls which had higher DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging capacity. After illuminating fresh-cut celery with a fluorescent source, Zhan et al. (2013) found an increase in the AOX capacity and total phenolics, and this increase was supposed to be due the higher activity of the phenylalanine ammonia lyase enzyme in treated samples. Similar results were obtained by Büchert et al. (2011) and Jin et al. (2015), with an increase of AOX and FC-reacting substances after illuminating broccoli heads with the same fluorescent source or with green LED. The present results of AOX capacity and FC-reacting substances in OL of Brussels sprouts were consistent with those previous studies using fluorescent light but not with our previous work using WB LED in broccoli florets (Hasperué et al., 2016).

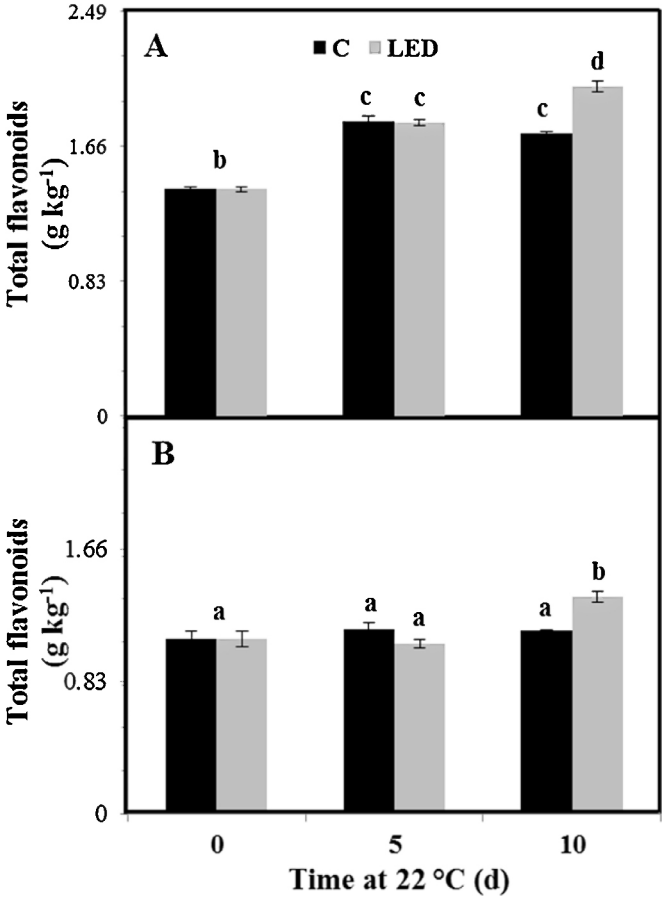

Fig. 5. Total flavonoids in outer (A) and inner (B) leaves of treated (LED, grey columns) and not treated (C, black columns) Brussels sprouts stored for 0, 5 and 10 days at 22 °C. Vertical bars represent the standard errors of the means (n = 3). Columns with different letters indicate differences at $P < 0.05$ based on Duncanís test.

3.5. Flavonoids

As reported in lettuce (Crozier et al., 1997; Hohl et al., 2001), total flavonoids content in Brussels sprouts was higher in OL than IL (Fig. 5).

In OL, flavonoids increased during storage in all samples, but after 10 d were the exposed to WB light which had the highest contents. In the case of the IL, the flavonoids did not increase in controls, but treated samples had higher contents after 10 d of storage. Changes in reactive oxygen species (ROS) activate the biosynthesis of flavonoids, which would scavenge singlet oxygen and stabilize the chloroplasts membranes (Agati et al., 2012). Flavonoid synthesis has been reported to be increased by exposure to light (Gliszczyńska-Świgło et al., 2007; Hohl et al., 2001) and mainly in the blue spectrum (Taulavuori et al., 2016). The higher flavonoids content in the treated samples may be a result of the light quality used, since the WB LED source was especially enriched in the blue spectrum.

4. Conclusions

The senescence and yellowing of Brussels sprouts was effectively delayed by the continuous exposition to low intensity WB LED. The WB LED treatment helped to maintain the quality and the green color in the tissues exposed to light (OL), and the effect of the treatment was visible also in the IL. The WB LED lighting would be useful to maintain or improve the quality of Brussels sprouts for

both storage and long distance transport, even with a potential use in combination with refrigeration.

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