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Effect of different light-emitting diode (LED) irradiation on the shelf life and phytonutrient content of broccoli (*Brassica oleracea* L. var. *italica*)

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ABSTRACT

Broccoli (*Brassica oleracea* L. var. *italica*) is largely cultivated in southern Italy. It is an important source of phytonutrients, which are partially lost during postharvest storage. The aim of this work was to evaluate the overall effect of five different low-intensity light-emitting diodes (LEDs) on the quality parameters of broccoli florets over 20 d of cold storage. The level of ascorbic acid, chlorophylls, carotenoids, phenolic compounds and soluble proteins, as well as colour analysis, were evaluated. Green LED increased the chlorophyll and ascorbic acid content; white, red and yellow LEDs had a positive effect on the redox status of broccoli. Globally, only green LED had a statistically significant positive effect when considering all analysed parameters and could be proposed to prolong the shelf life of broccoli during cold storage.

1. Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) is largely consumed all over the world and has a high economic importance. This vegetable belongs to the *Brassicaceae* family, and it is renowned for its mineral content and for containing a wide range of non-enzymatic bioactive compounds, including glucosinolates, carotenoids and total phenols, which have been correlated with the prevention of chronic diseases (Mahn & Reyes, 2012).

Broccoli storage and processing were reported to affect the shelf life, the overall appearance (OA) and the nutritional quality of broccoli (Serrano, Martinez-Romero, Guillen, Castillo, & Valero, 2006). In addition to altering the quality, changes in the content of the main bioactive compounds may impact the global health-promoting effect of this product (Mahn & Reyes, 2012).

For these reasons, current research is needed to develop post-harvest techniques, including controlled atmosphere (CA) packaging (Fernández-León, Fernández-León, Lozano, Ayuso, & González-Gómez, 2013) and 1-methylcyclopropene (1-MCP) treatments, that maintain the quality of broccoli heads (Ma et al., 2010). However, cost, efficacy and the actual practicability of these techniques have to be taken into account.

Light is one of the most important factors regulating cell function and metabolism in living organisms. In plants, light regulates a wide variety of pathways from germination to flowering and fruit development (Jiao, Lau, & Deng, 2007). The agri-food sector has been exploiting this resource (light potential) by developing farming structures and greenhouses equipped with light-emitting diodes (LEDs) to optimize product storage (D'Souza, Yuk, Khoo, & Zhou, 2015). Further, although the effect of light exposure on horticultural products' growth and development is largely known, little is known about the influence of light during postharvest storage. The use of red LED has been reported to improve the quality of broccoli florets (Ma et al., 2014), and white and blue LEDs showed the highest levels of chlorophylls (Hasperué, Guardianelli, Rodoni, Chaves, & Martínez, 2016).

In this work, the level of ascorbic acid, chlorophylls, carotenoids, phenolic compounds and soluble proteins, as well as colour analysis, were determined in broccoli before and after LED treatments. So far, only few LED treatments were evaluated simultaneously, and a global evaluation of the effect of LED treatment on several quality parameters lacks. The aim of the study is to evaluate the effect of specific LED exposure on the qualitative and biochemical parameters of broccoli during cold storage. With this regard, a robust global statistical analysis which evaluates all the analyzed positive and negative effects of each

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parameter was used in order to propose the best LED treatment to use in the postharvest storage of broccoli.

2. Materials and methods

2.1. Plant material and processing

Fresh, mature broccoli (*Brassica oleracea* L. var. *italica*) was produced in the Apulia region and harvested from a local commercial farm in Bari province, Italy. The broccoli was grown on loam soil under commercial growing conditions and harvested in June 2017 between 6:00 and 7:00 AM at 17 ± 2 °C and 65 ± 5% relative air humidity. Fresh broccoli heads, homogenous for type, weight and quality, were immediately placed in a domestic refrigerator (Panasonic mod. NR-BN34FW1) equipped with modular light systems (see Section 2.2) and stored under continuous light exposure. Experiments were performed at 4 ± 0.5 °C and 68 ± 2% relative air humidity, and the experiments extended from 0 to 20 d until overall appearance (OA) scores reached values = 1 (see Section 2.3). Controls were stored in darkness under the same temperature and humidity conditions. Since broccoli is not usually stored more than 12–15 d, samples were taken at the interval times of 5, 10, 15 and 20 d. For each treatment and sampling time, florets from three broccoli heads were removed from the stems and used for analytical determinations. Florets were finely grounded with a stainless-steel knife, frozen using liquid nitrogen and stored at −80 °C until analysis.

2.2. Light system

The modular lighting system was assembled on refrigerator shelves. Each modular lighting apparatus consists of three LINEAR light© modules (OSRAM GmbH, Germany) of 0.15 m each, equipped with 5 LEDs each and connected in parallel. The lighting modules emitted in the wavelength of blue (BL, 467 nm, 4.1 W/m, 40 lm/m, 9 lm/W), green (GL, 522 nm, 4.1 W/m, 120 lm/m, 29 lm/W), yellow (YL, 587 nm, 6.0 W/M, 75 lm/m, 12 lm/W), red (RL, 625 nm, 6.0 W/m, 80 lm/m, 14 lm/W) and white (WL, 3000 K colour temperature, 130 lm/m, 31 lm/W). The photosynthetic photon flux (PPF) level at the top of the broccoli surface was 21, 24, 27, 66 and 40 μmol $m^{-2} s^{-1}$ for BL, GL, YL, RL and WL, respectively, measured with a Delta OHM Photo-Radio meter (mod. HD2302.0, Pordenone, Italy).

2.3. Overall appearance

In order to measure the effect of light treatments on OA, samples were monitored during the storage period and individually scored by a group of 10 trained people (5 females and 5 males between the ages of 25 and 55 years old) on a 5 to 1 scale, defined as 5 = excellent, no defects; 4 = very good; 3 = fair, moderate defects; 2 = poor, major defects; and 1 = inedible. A score of 3 was defined as the limit of marketability, while a score of 2 was defined as the limit of edibility (Amodio, Cabezas-Serrano, Rinaldi & Colelli, 2007).

2.4. Determination of dry matter content

Dry matter content (DM) of florets was measured at time zero and at each time point for each light treatment. For the analysis, 5 g of fresh matter of each sample was dried at 60 °C in an oven until a constant weight was obtained. Dry matter content was calculated as a percentage of constant weight related to fresh matter.

2.5. Soluble protein determination

The material was homogenized according to Paciolla, D'Emerico, Tommasi and Scrugli (2010) with slight modifications. One gram of frozen broccoli florets was homogenized in 4 mL of solution containing 0.05 M Tris-HCl (pH 8.0), 0.001 M EDTA, 0.01 M $MgCl_2$ and 0.05% cysteine. The homogenate was filtered through four layers of cheesecloth and centrifuged at 20,000 × *g* for 20 min at 4 °C. The resulting supernatant was used for the determination of soluble protein content according to Bradford (1976) with serum albumin as a standard.

2.6. Chlorophyll and carotenoid analysis

Five grams of frozen florets was mixed with 20 mL of methanol and incubated overnight in darkness at room temperature (20 °C). Samples were centrifuged at 20,200 × *g* for 15 min. Absorbance was measured in the supernatant by a spectrophotometric assay (Ultraspec 3100pro, Amersham Pharmacia Biotech Italia – Cologno Monzese, Italy) at 653 and 666 for chlorophyll *a* and *b*, respectively, and at 470 nm for carotenoids. Chlorophyll *a* and *b*, total chlorophyll and carotenoid contents were calculated according to Wellburn (1994).

2.7. Colour analysis

The colour of the florets was non-destructively measured with a colorimeter (CR-400, Konica Minolta, Osaka, Japan) equipped with a D65 illuminant (6504 K) in the reflectance mode and on the CIE L^*, a^*, b^* colour scale. The colorimeter was calibrated with a standard reference having values of L^*, a^* and b^* corresponding to 97.5, 0.03 and 1.85, respectively. For each sample, the colour of the whole head was assessed at nine random points on the upper surface. Every colour measure was the result of the mean of three different replicates. The results were reported as lightness (L^*), hue angle (hab, Eq. (1)), which is related to the red/blueness (green/yellowness), and chroma (C^*, Eq. (2)), which is related to the quantitative qualities of colour.

$$h_{ab} = tan^{-1} \frac{b^*}{a^*} \quad (1)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

2.8. Total phenolic compounds

Frozen florets (0.6 g) were homogenized with 5 mL of ethanol and vortexed for 1 min. The mixture was centrifuged at 6000 × *g* for 10 min at 4 °C; 50 μL of the supernatant was added to 950 μL of distilled water and 50 μL of a 1:1 water diluted Folin-Ciocalteu reagent (Sigma Aldrich, Milan, Italy). After 3 min, 100 μL of a 0.1 M NaOH solution containing 20% (W/V) Na_2CO_3 was added, and the resulting solution was incubated at 25 °C for 90 min (Hasperué et al., 2016). The absorbance was measured with a spectrophotometer (Ultraspec 3100pro, Amersham Pharmacia Biotech Italia – Cologno Monzese, Italy) at 760 nm. The FC-reacting substances were calculated using gallic acid (GA) as the standard. The results were expressed as the mass of GA equivalents on a fresh matter basis (g kg^{-1}). Three replicates were analysed per storage time and light condition.

2.9. H_2O_2 content and lipid peroxidation analysis

One gram of frozen broccoli florets was homogenized in 4 mL of a 0.1 M sodium phosphate buffer, pH 6.8. The homogenate was filtered through four layers of cheesecloth to remove cellular debris and then centrifuged at 18,000 × *g* for 20 min at 4 °C. The H_2O_2 content was measured as reported by Lanubile et al. (2015). A supernatant aliquot of the reaction mixture was read at 436 nm, and its absorbance was compared to the extinction coefficient of an H_2O_2 standard.

For lipid peroxidation, 1 g of frozen broccoli florets was ground with 4 mL of 0.1% TCA. The homogenate was centrifuged at 10,000 × *g* for 10 min according to Veronico, Paciolla, Sasanelli, De Leonardis, and Melillo (2017). One mL of the supernatant was diluted with 1 mL of 20% TCA containing 0.5% thiobarbituric acid. The level of lipid

peroxidation was measured in terms of malondialdehyde (MDA) content determined by the thiobarbituric acid reaction as described by Zhang and Kirkham (1996).

2.10. Ascorbic acid measurement

One gram of frozen broccoli florets was homogenized in 4 mL of cold 5% metaphosphoric acid in a porcelain mortar with quartz sand. The homogenate was centrifuged at 20,000 × *g* for 15 min, and the supernatant was used to determine ascorbic acid (AsA) content as reported by Cozzi et al. (2013).

2.11. Statistical analysis

The data presented are the mean ± standard deviation of at least 3 different replicates of four independent experiments. The effects of storing broccoli under different visible light conditions on the qualitative parameters were tested by performing t-tests to compare these parameters measured in light conditions against their measures in broccoli stored in darkness. In detail, at each time point and for each light treatment, the measures of each variable were averaged across the different replicates. The mean values were rescaled by subtracting the corresponding mean value at the time zero from them. Given a light treatment and a variable, we compared the rescaled mean values corresponding to the different time points by using paired t-tests to compare these values with those obtained in the dark control condition. Each variable was assigned a label of "+" or "−" according to if it was desirable to increase or decrease its intensity, respectively. In more detail, for x, the value of the variable in the 'dark' control condition, and y, the value of the same variable in the 'light' condition, we performed one-tail paired *t*-tests of the null hypothesis against the alternative hypothesis. The null hypothesis was that data of the difference between x-y are a random sample from a normal distribution, a mean of 0 and an unknown variance; the alternative hypothesis was that the mean is smaller than 0 in the case of '+' label or greater than 0 in the case of '−' label. We applied the Fisher's method to combine the *P*-values obtained from the different variables for a given light treatment. This analysis, integrating the effects of the measured variables, allowed for us to test whether there is a global effect of the light treatment.

3. Results

3.1. Changes in overall appearance

The evolution of OA in samples stored at 4 °C under the respective light conditions is reported in Table 1. The samples stored under dark and BL reached the threshold of marketability after 10 d, while samples stored under GL, RL, WL and YL reached the OA score of 3 after 15 d. The limit of edibility was reached at 15 d for samples stored under dark and BL, while the limit of edibility was reached at 20 d for samples stored under GL, RL, WL and YL.

Table 1
Overall appearance scores of samples stored in the dark (control) or under blue (BL), green (GL), red (RL), yellow (YL) or white (WL) LED lights. The limit of marketability (3) is indicated in bold.

Days	Control	BL	GL	RL	YL	WL
0	5	5	5	5	5	5
5	4	4	4	4	4	4
10	**3**	**3**	4	4	4	4
15	2	2	**3**	**3**	**3**	**3**
20	2	2	2	2	2	2

3.2. Effect of light wavelengths on dry matter content

The initial dry matter (DM) content of the samples was of 14% (Fig. 1, Panel A). Under the control condition, the increase in DM was constant, reaching a maximum value of 19% after 20 d of storage. Under all the light wavelengths tested, the DM content at 20 d was significantly higher compared to the control condition, indicating a water loss induced by the light treatment, with YL and RL having the highest water loss (Fig. 1, Panel A).

3.3. Influence of light on soluble protein content

After 5 d, a significant increase ($P < 0.05$) in the protein content was observed under GL, RL, YL, and WL treatments with respect to the dark control and compared to time 0, whereas it decreased under BL exposure. No significant change occurred at 10 d. Thereafter, RL caused a significant decrease at all time points, while BL, YL and WL significantly decreased the protein content only at 20 d. Finally, GL induced a significant increase in the protein content at 15 d (Fig. 1, Panel B).

3.4. Colour analysis

Broccoli is characterized by a dark green pigmentation. During postharvest, a loss of the superficial green colour of the product is observed, which decreases the commercial acceptance of the broccoli florets. Colour measurements were taken after 0, 5, 10, 15 and 20 d in exposed and control samples. The degree of colour saturation (C^*) increased during storage under all the lightning conditions; however, it was significantly lower after 10 d of exposure under RL and WL ($P < 0.05$) with respect to the dark control (Fig. 2) and not significantly different from the initial value (time 0).

No significant differences were registered for L^* values in all LED light treatments (data not shown). Regarding tonality values, after 5 d they decreased in the control as well as in all exposed samples, which is in agreement with the visible yellowing of the florets. However, no significant difference was observed for any of the LED light treatments during the whole storage period (data not shown).

3.5. Changes in chlorophyll and carotenoid content during storage under light exposure

Initial total chlorophyll content in samples was 1.19 g kg^{-1} of fresh matter (Fig. 3, Panel A). A general increase in both chlorophyll *a* (Fig. 3, Panel B) and *b* (Fig. 3, Panel C) was measured during the whole storage period for the dark control and all exposed samples. A statistically significant chlorophyll *a* and *b* accumulation at 10, 15 and 20 d was induced by the exposure to the GL. After 10 d, total chlorophyll content reached the highest value (3.60 g kg^{-1}) and the highest increase ($P < 0.05$). WL was only able to increase chlorophyll *b* content at 5 d. The other LED treatments were either ineffective or detrimental in increasing chlorophyll *a* or *b* content (Fig. 3, Panel B and C). Initial carotenoids content (time zero) was 0.63 g kg^{-1} and gradually increased to 0.8 g kg^{-1} in control sample stored in dark after 20 d of cold storage in control as well as in LED treated. No difference in the carotenoid content emerged among all LED lights and the control (data not shown).

3.6. Changes in total phenolic compounds during storage under light exposure

The initial total phenolic content of broccoli florets was 0.99 g kg^{-1} of GA equivalents and significantly increased during the whole storage time under the RL and YL ($P < 0.05$) LED light exposure. Both RL and YL LED lights increased the phenolic content up to 2.5 times after five days of storage and maintained a significantly higher (nearly doubled) phenolic content until the end of the storage time with respect to the

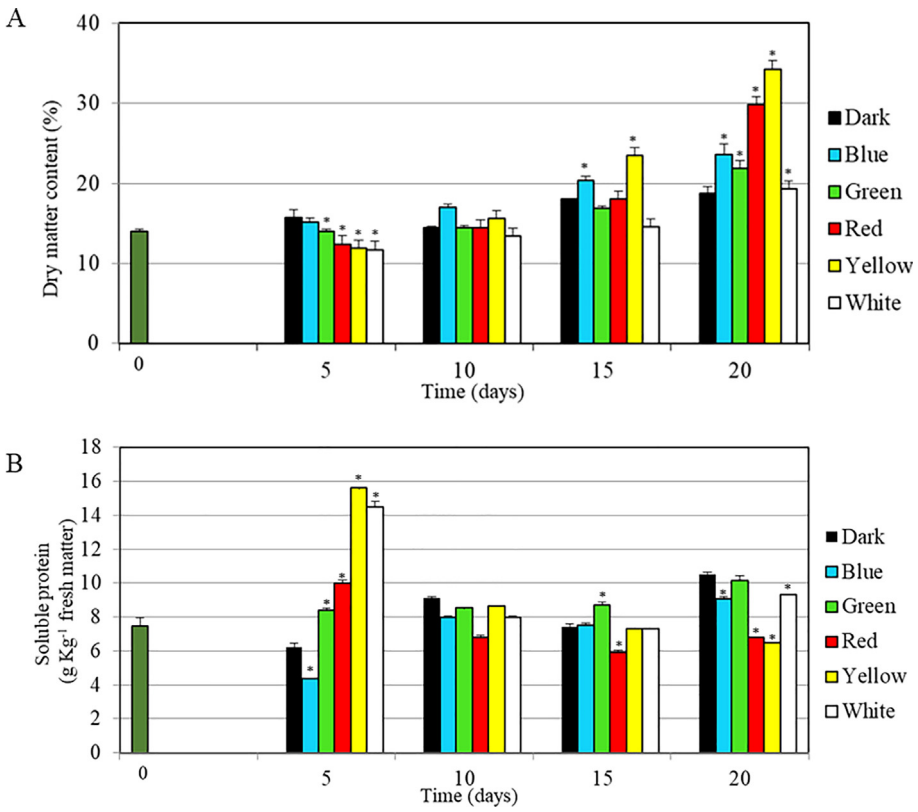

Fig. 1. Dry matter (Panel A) and soluble protein (Panel B) contents of samples stored in the dark (control) or under blue, green, red, yellow or white LED lights at time zero and after 5, 10, 15 and 20 d. Error bars represent the standard error measured for three independent replicates of four independent experiments. Asterisk (*) indicates a significant difference between the dark (control) and each LED treatment with a P-value < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

control (Fig. 3, Panel D). After 15 d, both RL and YL induced the highest phenol accumulation, which accounted for 3.04 and 3.17 g kg^{-1} of GA equivalents, respectively. Additionally, a significant increase was also observed for BL and GL at 5 and 20 d and for WL at 10 and 20 d.

3.7. Effects of light on ascorbic acid

When irradiated with GL, a significant ($P < 0.05$) increase in AsA content at each time point was observed with the respect to the dark control (Fig. 4, Panel A). There was also an increase in AsA content until 15 d for both RL and YL, whereas no differences were observed during the remaining period of analysis and in the presence of WL. Interestingly, a significant ($P < 0.05$) decrease in AsA content occurred under BL after 10 d and until the end of the storage compared to the dark control.

3.8. Influence of light on H_2O_2 and lipid peroxidation

Compared to the dark, a significant decrease in H_2O_2 under RL, YL and WL occurred at 5 and 10 d. Conversely, a significant increase at 10 and 15 d was observed for BL and GL. At 20 d, a significant ($P < 0.05$) increase was also recorded for all LED treatments (Fig. 4, Panel B).

The level of lipid peroxidation was evaluated as an indicator of the integrity of the biological membrane. Under RL, YL and WL, the levels of lipid peroxidation significantly decreased until 10 d but then increased at 20 d of storage ($P < 0.05$). An increase in lipid peroxidation was also observed at 10 d under BL and GL exposure (Fig. 4, Panel C).

3.9. Statistical analysis

Paired t-tests were performed to evaluate the effects on the different qualitative parameters of each light treatment compared with the control condition (broccoli stored in darkness) (Table 2). BL resulted in having a significant positive influence on the phenolic compound content ($P < 0.05$). A stronger influence on the same parameter was found in samples exposed to RL and YL, increasing phenolic compound content at all the time points ($P < 0.01$). GL had a significant positive influence on chlorophyll *a* ($P < 0.05$), chlorophyll *b* ($P < 0.01$), AsA

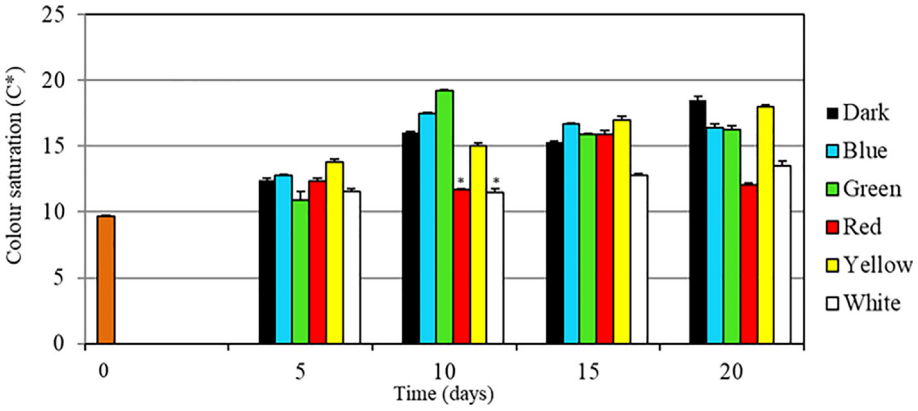

Fig. 2. Colour saturation of samples stored in the dark (control) or under blue, green, red, yellow or white LED lights at time zero and after 5, 10, 15 and 20 d. Error bars represent the standard error measured for three independent replicates of four independent experiments. Asterisk (*) indicates a significant difference between the dark (control) and each LED treatment with a P-value < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

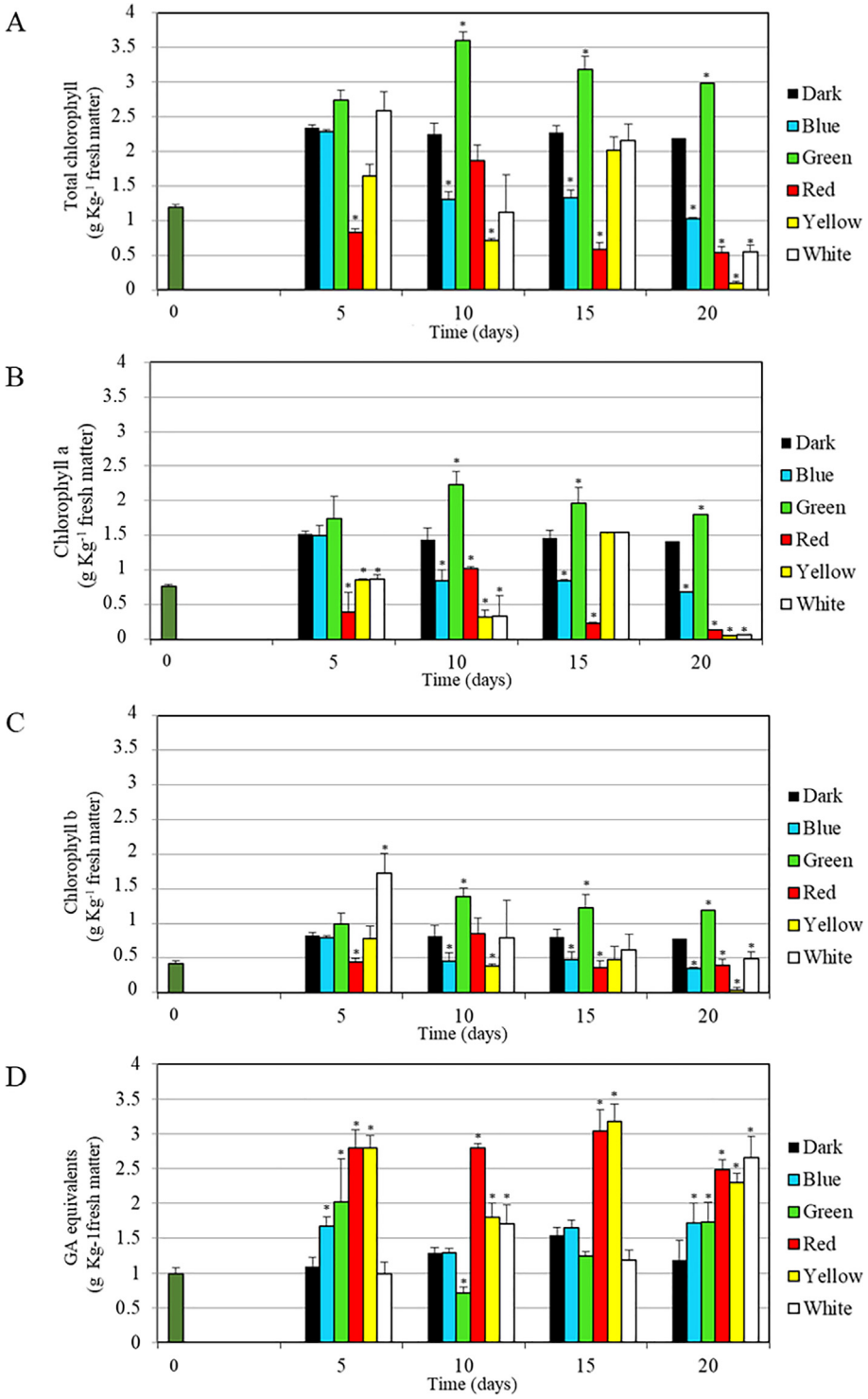

Fig. 3. Total chlorophyll (Panel A), chlorophyll *a* (Panel B), chlorophyll *b* (Panel C) and total phenol (Panel D) contents of samples stored in the dark (control) or under blue, green, red, yellow or white LED lights at time zero and after 5, 10, 15 and 20 d. Error bars represent the standard error measured for three independent replicates of four independent experiments. Asterisk (*) indicates a significant difference between the dark (control) and each LED treatment with a P-value < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

content ($P < 0.05$) and soluble proteins ($P < 0.05$). Finally, the WL treatment significantly increased the water content during the whole time course ($P < 0.05$).

P-values obtained from all the statistical analyses were combined in order to evaluate the global effect of each light treatment on all the parameters. Although RL and YL had a very strong effect on phenolic compound content, only GL resulted in having a significant positive global effect considering all analysed parameters with a Fisher's combined *P*-value of 0.015.

4. Discussion

In this study, various LED lights (BL, GL, RL, YL and WL) as well as dark conditions were applied during the cold storage of broccoli to

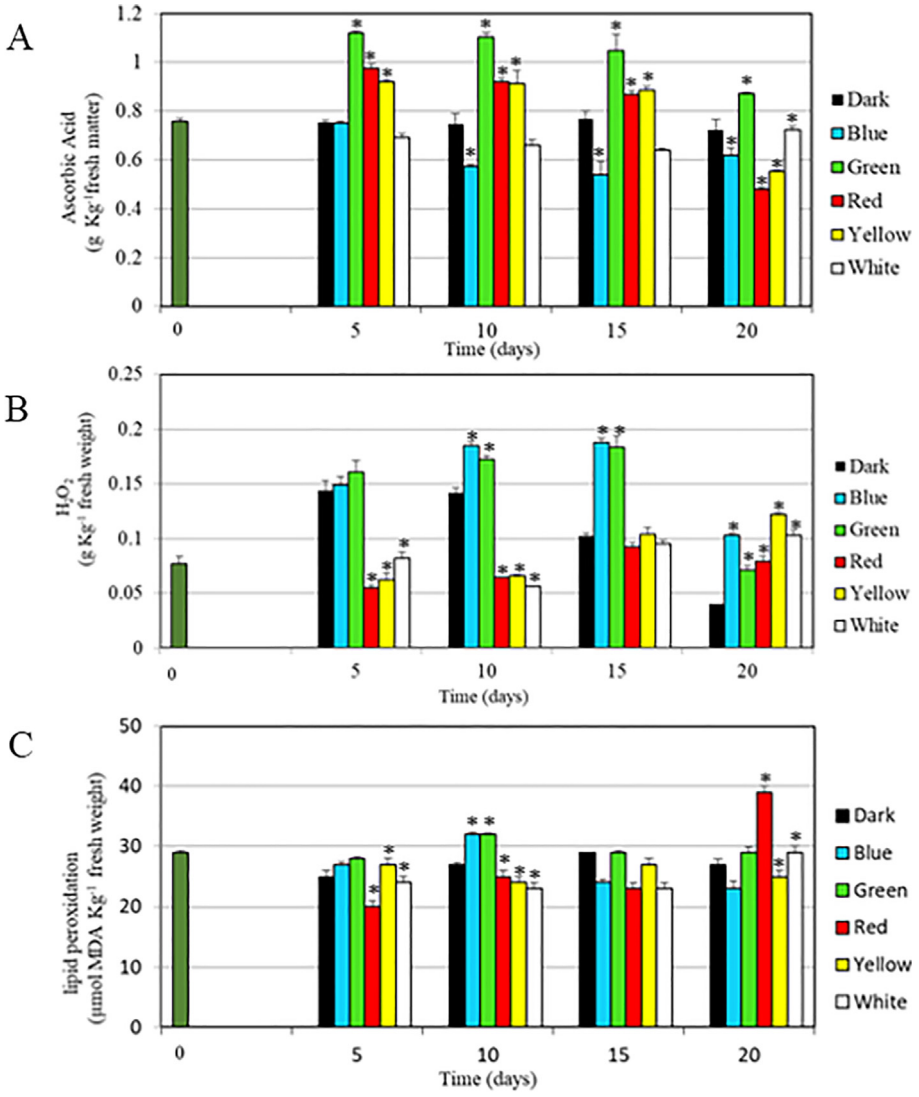

Fig. 4. Ascorbic acid (Panel A), hydrogen peroxide contents (Panel B) and lipid peroxidation level (Panel C) content of samples stored in the dark (control) or under blue, green, red, yellow or white LED lights at time zero and after 5, 10, 15 and 20 d. Error bars represent the standard error measured for three independent replicates of four independent experiments. Asterisk (*) indicates a significant difference between the dark (control) and each LED treatment with a P-value < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
P-values of paired *T*-test and Fisher's combined P-value of samples stored in the dark (control) or under blue (BL), green (GL), red (RL), yellow (YL) or white (WL) LED lights. *P*-values < 0.05 are indicated in bold.

	BL	GL	RL	YL	WL
Water content	0,916	0,502	0,735	0,851	**0,035**
Lightness (L^*)	0,956	0,934	0,826	0,996	0,931
Colour saturation (C^*)	0,200	0,402	0,889	0,393	0,987
Tonality (h_{ab})	0,831	0,788	0,765	0,776	0,789
Chlorophyll *a*	0,991	**0,013**	0,996	0,978	0,814
Chlorophyll *b*	0,992	**0,006**	0,973	0,856	0,671
Carotenoids	0,951	0,988	0,997	0,993	0,991
Total chlorophyll	0,992	**0,009**	0,992	0,953	0,758
Total phenols	**0,025**	0,451	**< 0,001**	**0,006**	0,106
Soluble proteins	0,976	**0,049**	0,783	0,359	0,263
H_2O_2	0,956	0,961	0,171	0,410	0,351
Malondialdehyde (MDA)	0,619	0,973	0,516	0,718	0,127
Ascorbic acid	0,982	**0,044**	0,581	0,392	0,888
Fisher's combined P-value	0,988	**0,015**	0,433	0,803	0,645

evaluate their effects on the biochemical and physiological parameters occurring in broccoli during postharvest storage. Different light intensities were used in this work. However, we did not find a direct and unambiguous correlation between light intensity and each specific parameter, meaning that light overall quality (intensity and wavelength) has a specific impact on quality parameter and that it has to be considered as a whole (Jin, Yao, Xu, Wang, & Zheng, 2015).

Senescence is a rapid process occurring in horticultural crops after harvest. During senescence, water loss and compositional changes occur. However, we have to take into consideration that the laboratory conditions represent ideal storage conditions, and they are quite far from what happens during the shelf life of a commercialized broccoli sample, which is not consistently stored at 4 °C or in complete darkness. In addition, before being commercialized, broccoli can be pre-stored for some period (for cleaning, packaging and analysis) in local markets under non-ideal temperature and light conditions. In broccoli, water is the primary component of fresh matter. We found a general loss of water throughout the whole storage period and especially at 20 d under all the tested lights, probably due to an increase in stomatal opening during postharvest. Greater transpiration under light was reported to occur in leaves of Chinese kale (Noichinda, Bodhipadma, Mahamontri,

Narongruk, & Ketsa, 2007), Romaine lettuce (Martínez-Sánchez, Tudela, Luna, Allende, & Gil, 2011), green Asparagus (Mastropasqua, Borraccino, Bianco, & Paciolla, 2012) and in broccoli (Hasperué et al., 2016). In addition, during postharvest storage, plants might still produce components (such as proteins, starch, cell wall components, lipids and mineral salts) as a consequence of their persisting metabolism and physiological activity. Indeed, an increase in protein content, at least in the first five days, seems to contribute to the increase in dry matter for GL, RL, YL and WL.

Broccoli heads were harvested when they were completely green. The main visible evidence of broccoli deterioration after the harvest is the florets yellowing. The progress of degreening is commonly monitored by the decrease in tonality (h_{ab} value). However, in our experimental conditions, no evident yellowing was detected in all samples as also reported by Gao et al. (2018) and no statistically significant differences were recorded for these parameters in any of the samples. In contrast, RL and WL had positive effects on the degree of colour saturation (C^*), which resulted in a degree of colour saturation comparable to the initial value (time 0) after 10 d of exposure to those lights as reported by Volden et al. (2009) and Büchert et al. (2011) for the WL treatment. In regard to OA, floret overmaturation was less evident in samples stored under YL, RL and WL, in accordance with the limit of marketability and edibility reached later for those treatments than for the other samples.

High chlorophyll is a characteristic required by consumers for a quality parameter. Florets visibly yellow when chlorophyll content starts to decrease (Hasperué, Gomez-Lobato, Chaves, & Martínez, 2013). The initial chlorophyll increase (from 0 to 5 d) can be explained as a result of the presence of metabolic activity of the broccoli heads and development of the immature floral buds (Carr & Irish, 1997). Noichinda et al. (2007) found that light exposure resulted in higher chlorophyll content. Braidot et al. (2014) demonstrated that short-period white-light application at 6 °C allowed for the maintenance of an appreciable amount of chlorophyll *a* and *b* in lamb's lettuce after 6-day storage. Under our conditions, a constant increase in both chlorophylls *a* and *b* and of total chlorophyll content was found in treated as well as in control broccoli heads, but it resulted in a much higher and statistically significant difference in samples exposed to GL at almost all time points. However, since water is loss during the storage, a concentration process cannot be excluded. This data is also in accordance with Jin et al. (2015), who found that GL was able to increase chlorophyll content after 2 days of storage at 25 °C. In contrast, Hasperué et al. (2016) reported that chlorophyll increased under WL treatment; in our case WL did not have a positive effect on chlorophyll, with respect to the control and GL sample. The higher chlorophyll content increases the photosynthetic process, resulting in an increased production of photosynthates, which are known to be generally beneficial for the human diet.

In addition, carotenoid content was evaluated throughout the storage period. Carotenoids are considered a good source of fat-soluble antioxidants (Hasperué et al., 2013). Their content is influenced by light (Noichinda et al., 2007). In tomatoes, red light exposure increases lycopene accumulation (Liu, Zabaras, Bennett, Aguas, & Woonton, 2009). However, under our experimental conditions, carotenoid content was not influenced by light exposure. Most likely, the low irradiance used in our experiment was ineffective in increasing them. In fact, a high irradiance is required to increase the carotenoid content in Chinese kale (Noichinda et al., 2007). On the other hand, BL of 20 $\mu mol\, m^{-2}\, s^{-1}$ of PPF at 22 °C after 3 d increased the carotenoid content in broccoli (Hasperué et al., 2016). Despite the fact that we did not record a statistically significant increase in carotenoid content, the level of carotenoids we found appears to be suitable to preserve the photooxidation of chlorophyll molecules (Goodwin, 1980; Simkin, Zhu, Kuntz, & Sandmann, 2003).

Phenolic compounds are considered important components since they contribute to the maintenance of the antioxidant cell status. Light has an inductive effect on phenolic production in plants. We found a correlation between increased phenolic compounds and type and light intensity. Compared to the control sample, a constant and significant increase in phenolic compounds content was observed in YL and RL at all the detection points. The higher increase in RL with the respect to YL was probably due to a higher light intensity utilized for RL (66 *vs* 27 $\mu mol\, m^{-2}\, s^{-1}$); this confirms the positive inductive effect of light on phenolic metabolism. During senescence, pigments and various compounds that originate from phenylpropanoid metabolism that produces phenols, tannins, lignins and flavonoids accumulate in tissues. The general increase in phenols with all light treatments at higher time points (20 d) might indicate that senescence is starting in the broccoli tissues.

Fresh broccoli is rich in ascorbic acid, which is one of the most important nutritional quality factors in many horticultural crops and has many biological uses in the human body (Lee & Kader, 2000). The synthesis of ascorbate depends on the quantity and spectral properties of light. Low irradiance caused an increase in ascorbic acid in oat leaves (Mastropasqua et al., 2012). However, during postharvest storage of broccoli, ascorbate was reported to decrease (Raseetha, Leong, Burritt, & Oey, 2013) and then to increase after light (Zhan, Hu, Li, & Pang, 2012) and RL exposure (Ma et al., 2014). It is known that in photosynthetically active plant tissues, such as in harvested broccoli, an active metabolism is present (Heyes, Burton, & de Vré, 1998; Mastropasqua et al., 2012). Therefore, the increase in AsA observed under GL, RL and YL could be due to the persisting metabolic and physiological activity during postharvest and *de novo* synthesis. Indeed, it is reported that light plays probable role in controlling the ascorbate pool in the terminal step of ascorbate synthesis (Toledo, Ueda, Imahori, & Ayaki, 2003).

With regards to BL, the decrease in AsA level observed throughout the time period in our experimental conditions (20 $\mu mol\, m^{-2}\, s^{-1}$ and 5 °C) confirms what Hasperué et al. (2016) have reported. Compared to controls stored in darkness, BL did not affect AsA content in broccoli heads stored at 5 °C except for an increase recorded after a longer storage time (45 d) (Hasperué et al., 2016). On the other hand, when broccoli was irradiated with BL with a higher intensity (50 $\mu mol\, m^{-2}\, s^{-1}$), the ascorbate content was similar to that in the control (Ma et al., 2014). Our data seem to confirm that BL has no positive effects on AsA levels during postharvest storage of broccoli. Likely, AsA levels are maintained at a constant level as a consequence of decreased biosynthesis or/and increased consumption.

AsA is involved in plant development, growth and in the defence mechanisms as a scavenger of reactive oxygen species (ROS), such as H_2O_2, O_2^- and OH^-, during biotic and abiotic stress (Davey et al., 2000; Foyer Lelandais, Galap & Kunert, 1991). The regulation of the senescence process in fruit and vegetables is based on changes in the synthesis and removal ratio of the ROS by antioxidant systems. Therefore, to counteract the toxic effect of the ROS, an increase in chemical and enzymatic antioxidant systems occurs. The observed increase in AsA under GL, RL and YL could be useful to control the hydrogen peroxide and lipid peroxidation levels that are considered indexes of oxidative stress. Indeed, these indexes increased at the longer storage times when a simultaneous strong decrease in AsA occurred. Compared to the dark control, RL, YL and WL reduced lipid peroxidation and hydrogen peroxide until 10 d of storage, delaying the cell oxidative processes that typically occurs quickly at ambient temperature in postharvest broccoli. In contrast, GL could prevent oxidative stress at higher time points.

The decrease in H_2O_2 levels with RL, YL and WL indicates that those light intensities preserve broccoli from oxidation reactions. On the other hand, the H_2O_2 increase in BL and GL cannot be considered toxic for the plant cells as only a slight increase in the malondialdehyde content was registered at 10 d. Indeed, H_2O_2 is also considered to be a signal molecule that regulates the activity of antioxidant systems (Antunes & Brito, 2017).

Since LED lights have different effects on the quality parameters of broccoli, a data integration approach was implemented in order to evaluate whether a global positive or negative effect was produced on broccoli florets by each light tested. Starting with a single parameter, a comprehensive evaluation could be performed in order to identify GL as the type of light that could be the most useful in maximizing the shelf life of broccoli florets during cold storage. Indeed, GL is involved in the photosynthetic process through pigment photoreceptor proteins in phytochromes and cryptochromes, stimulating plant growth, photosynthetic capacity and phytochemical production (Zhong, Qi, & Wen, 2015). In addition, green light can penetrate the plant canopy more effectively than red or blue light and it was found to promote growth in red-leaf lettuce (Kim, Goins, Wheeler, & Sager, 2004). Absorption of green light is used to stimulate photosynthesis deep within the leaf and canopy profile, contributes to carbon gain and likely to crop yield (Hayley, McAusland, & Murchie, 2017).

5. Conclusions

During postharvest, physiological and biochemical changes occur in broccoli. It is well known that light influences plant growth and physiology. However, high-light conditions induce a strong increase in ROS production in plants. Therefore, in this study, we used low LED light intensities in order not to cause any abiotic stress. The results from this study highlight the fact that LED light exposure could be a useful, non-chemical method to reduce biochemical alterations of phytonutrients in broccoli post-harvest. After dark or LED exposure, the analysed parameters were modified in different ways. Compared to darkness, GL increased the chlorophyll content; RL and YL increased the phenolic compound content and the OA of broccoli florets; GL, RL and YL caused an increase in the ascorbic acid content, and all lights increased the soluble protein content (at 5 d). We confirmed that low-intensity LED lights (20 $\mu mol\ m^{-2}\ s^{-1}$) are sufficient to preserve broccoli from postharvest senescence. A comprehensive, global data integration showed that GL was the best treatment to improve the overall shelf life and quality of broccoli. The effectiveness of GL might be mediated by the action of green light photoreceptors in a cryptochrome-independent or dependent manner. H_2O_2 increase to non-toxic concentration might act as signal molecule in this molecular mechanism(s) and therefore to be involved in the production of beneficial antioxidant compounds.

Declaration of interests

None.

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