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POSTHARVEST LED LIGHTING: EFFECT OF RED, BLUE, AND FAR RED ON QUALITY OF MINIMALLY PROCESSED BROCCOLI SPROUTS

Running title: Role of postharvest Blue, Red and Far-Red LED lighting on broccoli sprouts

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POSTHARVEST LED LIGHTING: EFFECT OF RED, BLUE, AND FAR RED ON QUALITY OF MINIMALLY PROCESSED BROCCOLI SPROUTS

ABSTRACT

BACKGROUND: The main objective of this study was to evaluate physiological and quality changes of minimally processed broccoli sprouts illuminated during postharvest storage under Blue, Red, and Far-Red LED lighting as compared to Darkness or illumination with Fluorescent light, as control treatments. RESULTS: Morphological and microbiological changes were determined during 15 d at 5 °C. In addition, total antioxidant activity and bioactive compounds changes throughout the shelf-life were also monitored. Results showed that Far-Red LED lighting increased hypocotyl and sprout length, decreased the microbial growth and improved the total antioxidant and scavenging activities, compared to Darkness and Fluorescent lighting treatments. However, it did not stimulate the biosynthesis of phenolic acids. In contrast, Blue LED light reduced by 50 % the total antioxidant capacity of broccoli sprouts compared to Far-Red treatment, as well as its morphological development. In addition, total scavenging activity was increased under Far-Red LEDs regarding the remaining treatments in 12-10 % (Darkness and Fluorescence) and 33-31 % (Blue and Red LEDs). CONCLUSION: Our results suggest that minimally processed sprouts may benefit from LED lighting during shelf life in terms of quality although further experiments should be conducted to optimize a proper exposure cycle and intensity aimed to be used in the distribution chain. It also opens the way for further development towards the integration of this technology in the food distribution chain.

Keywords: *Brassica oleracea* var. *italica*; light emitting diode; fresh-cut; bioactive compounds; antioxidants; phenols.

1. INTRODUCTION

About a third of global food production is not consumed due to losses and waste representing 1,300 million tons, with about 40 % of that total losses being fruit and vegetables among other foods (1). Broccoli (*Brassica oleracea* var. *italica*), is a recognized nutritive food rich in natural antioxidants, including vitamins, minerals, glucosinolates, isothiocyanates, and phenolics compounds (2). Broccoli sprouts and microgreens are valued as nutraceutical and functional foods for their significant greater concentrations of bioactive compounds with high potential antioxidant, anti-inflammatory, and anticancer activities, as compared to the adult plant (3,4). These products are commonly distributed within the minimally processed and MAP (Modified Atmosphere Packaging), where on the other hand the associated environmental impact and economic costs are particularly relevant (5).

In recent years, the use of artificial light during storage was suggested as a way to preserve or even improve the nutritional properties of horticultural commodities (6,7). The technological advancements in the LED technology resulted in widespread application in horticultural production systems, due to its limited thermal dissipation, its low energy requirements and the possibility to finely customize the light intensity and spectral properties (8). For instance, during plant cultivation, modulating red and blue spectral components was recently shown to influence the antioxidant profile of basil (8), lettuce (9), rocket (10), dill and parsley (11). Moreover, quantity and quality of light were also shown to alter pathogenic plant interactions in strawberry, by acting both on plant tissues and pathogens (7). The use of LED light at certain wavelengths also reduces the incidence of damage by phytophagous insects both by inhibiting their morphological growth (12) and by improving the defence mechanisms in plant tissues (13). Accordingly, the use of lighting during storage has been suggested to inhibit the development of fungal infections, thus also resulting in a longer shelf life of the product and limiting its quality decline (14). For instance, the development of *Botrytis cinerea* was inhibited through the exposure to LED lighting treatments with a peak at 405 nm (15). The use of LED light (with peaks at 405 and 460 nm) was also proven effective as antibacterial, limiting the development of

species like *Vibrio parahaemolyticus* and *Staphylococcus aureus* (16). In brassica species, first applications of a low-intensity ($16 \mu\text{mol m}^{-2} \text{s}^{-1}$) white fluorescent light during cold storage was shown to contribute to the reduction of leaf nitrate contents (17), although without consequences on other nutritional parameters, like the leaf antioxidant profile. Similarly, no changes respect to control conditions in darkness were observed in the antioxidant profile of lettuce exposed to a low-intensity ($6 \mu\text{mol m}^{-2} \text{s}^{-1}$) white fluorescent light during storage (18). The potentials related to the use of lighting during storage have increased by recent innovations in LED technology, also in the face of the possibility of using spectral regions with a higher efficiency of conversion of electrical energy into light (19), the low thermal emissions (and therefore adaptability to cold cells), or the containment of the junction temperature of the diode (8).

These evidences suggest the considerable potential that these applications can represent, contributing both to the health safety of the products and to their shelf life and nutritional value. In particular, LED technology could provide a non-thermal control tool, able to improve the health promoting properties of some foods and complements, also for home use. It could also be seen as a valid complementary tool to the use of sanitizing treatments, while avoiding the emergence of bacterial resistance, as preliminarily observed in broccoli florets (20,21).

The use of light in postharvest has been explored very little due to the difficulties to choose the appropriate lighting (22). Conventionally, Darkness and Fluorescence lighting are used during the postharvest food chain steps, but to the best of our knowledge, the use of LED lighting in this process has not been deeply studied before. Due to the potential impact of Blue, Red, and Far-Red wavelengths on sprout development and secondary metabolite production, the objective of the present work was to study the effects in quality of several illumination treatments continuously applied in minimally processed broccoli sprouts during 15 d at 5 °C. For that purpose, morphological changes, microbial analyses and bioactive compounds were periodically monitored.

2.- MATERIALS AND METHODS

2.1. Plant material and sprouting conditions

Broccoli (*Brassica oleracea* var. *italica*) seeds were purchased in Intersemillas S.A. (Valencia, Spain) with an average seed count of 291 seeds g⁻¹. The sprouting process was performed according to Rodríguez-Hernández et al. (23) with slight modifications. Briefly, seeds were sanitized for 15 min in NaOCl (1 % v/v) solution and rinsed during 2 min with distilled water until neutral pH was reached. After soaking in water overnight at room temperature, broccoli seeds were transferred into sterilised Petri dishes (13.5 cm diameter) with moistened filter paper. Seeds were germinated in darkness at 20 °C in a plant growth chamber (Sanyo MLR-350 H, Japan). The sprouts were collected on the ninth day of germination, when reaching commercial length of 3.5-4 cm.

2.2. Minimal processing, storage conditions, and light treatments during shelf life

Freshly harvested sprouts were sanitized (NaOCl, 0.1 g L⁻¹; 5 °C; pH = 6.5; 2 min), rinsed (tap water; 5 °C; 1 min), and dewatered with absorbent paper. Samples (5.0 ± 0.1 g) of sanitized sprouts were placed in polypropylene trays (120 x 78 x 30 mm; 250 mL). Then, trays were thermally sealed on the top with a bioriented polypropylene (BOPP) film of 40 µm thickness (Plásticos del Segura S.L., Murcia, Spain). The permeability at 23 °C and 0 % relative humidity (RH) according to DIN 53380 was 800 cm³ m⁻² d⁻¹ atm⁻¹ for both O₂ and CO₂ gases (data provided by the supplier) with a coefficient of permeability equal to 1. Packaged broccoli sprouts were stored at 5 °C and 85 % RH in a cold room of 5 m³, where 5 lighting treatments were applied in individual metallic containers (0.3 × 0.6 × 0.6 m; W × H × D) provided with lamps on the top, which was the only opened part of the containers. Trays were placed at 30 cm from the light source and were continuously illuminated using a photosynthetic photon flux density (PPFD) of 35 ± 2.5 µmol m⁻² s⁻¹. PPFD (µmol m⁻² s⁻¹) was measured using a PAR Photon Flux Sensor model QSO (Apogee instruments, Logan, UT, USA) connected with a ProCheck handheld reader, manufactured by Decagon Devices Inc. (Pullman, WA, USA) and spectral characteristics were determined using an illuminance spectrophotometer (CL-500A, Konica Minolta, Chiyoda, Tokyo, Japan).

Three LED lighting treatments were applied, using LED lamps featuring Blue diodes (peak at 465 nm), Red diodes (peak at 660 nm) or Far-Red diodes (peak at 730 nm), developed by Flytech s.r.l. (Belluno, Italy). Furthermore, a lighting treatment using Fluorescent lamps with white spectrum (0.31 W m^{-2} ; Philips 36W/54-765) and a control treatment in Darkness (resembling standard postharvest storage procedure used by the industry), were included within the same cold storage room in similar containers. Figure 1 shows the spectral composition of these LED treatments and the fluorescent light. Sprouts were sampled for quality analyses on processing day (0 d of storage) and after 4, 8, and 15 d at 5°C , with three replicates per lighting treatment and sampling day. On such days, samples were removed from the trays and immediately frozen in liquid nitrogen, freeze-dried and stored at -80°C until further analysis.

2.3. Gas changes within packages

O_2 and CO_2 partial pressures within packages were periodically monitored during storage at 5°C . Samples of 1 mL were withdrawn from the headspace gas of the packages and analysed in a gas chromatograph (GC; PerkinElmer Precisely Clarus500, Massachusetts, USA). The GC conditions for O_2 and CO_2 determinations were those previously described by Tomás-Callejas et al. (24).

2.4. Characterization of broccoli sprouts

Morphological measurements were carried out using ImageJ, Version 1.52v for Windows. Images of broccoli sprouts from each treatment were used to measure with a ruler (cm) hypocotyl (H), root (R), and total sprout lengths (H + R). H/R ratio was also calculated. Three repetitions of 15 sprouts per each treatment were measured on each sampling day.

2.5. Microbial analyses

Standard enumeration methods were used to determine mesophilic, psychrophilic, enterobacteria, yeasts, and moulds growth (25, 26). All used microbial media was obtained from Scharlau Chemie (Barcelona, Spain). The following media and incubation

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conditions were used: Plate Count Modified Agar for mesophilic and psychrophilic aerobic bacteria with incubations at 30 °C for 48 h and 5 °C for 7 d, respectively; Violet Red Bile Dextrose Agar for enterobacteria incubated at 37 °C for 48 h; Rose Bengal Agar for moulds and yeasts incubated at 22 °C for 7 d. All microbial counts were reported as log CFU g⁻¹. Three repetitions per treatment were analysed on each sampling day.

2.6. Extracts preparation

Samples of 25 mg of freeze-dried sprouts were placed in tubes and 3 mL methanol were added. The extraction was carried out in an orbital shaker (Stuart, Stone, UK) where the samples were vigorously shaken for 1 h in darkness inside a polystyrene (PS) box with an ice bed. The extracts were centrifuged at 3220 g for 10 min at 5 °C. The supernatant was collected and kept at -80 °C until analysis of Total Phenolic Content (TPC), Total Antioxidant Capacity (TAC), and Individual Phenolic Content.

2.7. Total Phenolic Content

TPC was determined as previously described by Singleton and Rossi (26). Briefly, 19 µL sample extract were placed on a flat-bottom PS 96-well plate (Greiner Bio-One; Frickenhausen, Germany) and 29 µL of 1 mol L⁻¹ Folin-Ciocalteu reagent were added. The latter mixture was incubated for 3 min in darkness at room temperature. Then, 192 µL of 0.4 % Na₂CO₃ 2 % NaOH were added. After 1 h incubation at room temperature in darkness, the absorbance was measured at 750 nm using a microplate reader (Tecan Infinite M200, Männedorf, Switzerland). The TPC was expressed as g chlorogenic acid equivalents (CAE) kg⁻¹ dry weight (DW). Each sample was analysed in triplicate.

2.7. Total antioxidant capacity

TAC was analysed by using three different methods: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical method), FRAP (Ferric Reducing Antioxidant Power), and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay) assays. DPPH assay was performed following the method described by Castillejo et al. (25). For that, 194 µL of DPPH (0.7 mM) solution were added to 21 µL of sprout extract.

The mixture was incubated for 30 min at room temperature in darkness. The TAC by DPPH was measured by changes in absorbance at 515 nm. The scavenging activity (%) was calculated using the next formula: $[(\text{Abs DPPH} - \text{Abs Sample})/\text{Abs DPPH}] \times 100$. ABTS assay was carried out following the method previously described by Llorach et al. (27). For that, 200 μL of the activated ABTS solution (32 μM) were added to 11 μL of sprout extract in a 96-well plate and incubated for 20 min at room temperature in darkness. The TAC by ABTS was measured by changes in absorbance at 414 nm. The scavenging activity (%) was calculated using the next formula: $[(\text{Abs ABTS} - \text{Abs Sample})/\text{Abs ABTS}] \times 100$. FRAP method was also developed following the method described by Castillejo et al. (25). A daily reaction solution containing sodium acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl) and 20 mM FeCl_3 was prepared in a v/v/v proportion of 10:1:1 and incubated at 37 °C for 2 h in darkness. Then, 198 μL of FRAP solution were added to 6 μL of sprout extract and incubated for 14 min at room temperature in darkness. The TAC by FRAP was measured by changes in absorbance at 593 nm. Obtained data were expressed as g of Trolox Equivalents (TE) kg^{-1} dry weight (DW). Each sample was analysed in triplicate.

Total Antioxidant Capacity Index was calculated using the next equation: $(\text{TAC}_{\text{DPPH}} + \text{TAC}_{\text{ABTS}})/2$. Total Scavenging Activity (%) was calculated using next equation: $(\% \text{ Scavenging}_{\text{DPPH}} + \% \text{ Scavenging}_{\text{ABTS}})/2$.

2.8. Individual phenolic content analyses

A sample of 1 mL of the extracted solution was collected and filtered using 0.2 μm PTFE membrane filters. Analysis and identification of individual phenolic compounds were conducted according to Moreira-Rodríguez et al. (4). An Ultra High-Performance Liquid Chromatography instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater, and SPDM-20A photodiode array detector was used. Chromatographic analyses were carried out into a Gemini C18 column (250 mm \times 4.6 mm, 5 μm particle size; Phenomenex, Torrance CA, USA). Phenolic acids were quantified as equivalents of

chlorogenic acid (5-caffeoylquinic acid; Sigma, St Louis, MO, USA) and sinapic acid (Sigma, St Louis, MO, USA). The results were expressed as mg kg⁻¹ dry weight (DW). Each sample was analysed in triplicate.

2.9. Statistical analyses

The experiment was a two-factor (light treatment × storage time) design subjected to analysis of variance (ANOVA) using SPSS 15.0 (Statistical Package for the Social Science for Windows (IBM, Armonk, New York, USA). Statistical significance was assessed at $p < 0.05$, and Tukey's multiple range test was used to separate means.

3. RESULTS

3.1. Headspace gas composition changes within packages

As it happens in other plant tissues stored, a decrease in the headspace O₂ partial pressure, along with an increase in the headspace CO₂ partial pressure, was observed throughout storage. After 4 d at 5 °C, the partial pressure ranged from 11 to 13 kPa and 8 to 10 kPa for O₂ and CO₂ respectively for all treatments. The steady state was reached after 12 d with gas partial pressures of 11 kPa CO₂ and 10 kPa O₂ without remarkable differences among lighting treatments (data not shown). This fact can be justified by the low storage temperature (5 °C) typically used in the fresh-cut market, which decreases the respiration rate (28), even under lighting conditions, and, therefore, gas partial pressures did not depict significant differences at the steady state.

3.2. Broccoli sprouts characterization

Table 1 shows the results of broccoli sprouts characterization obtained during 15 d at 5 °C under different postharvest light treatments. As expected, broccoli sprouts stored under Far-Red LEDs exhibited an increase in hypocotyl growth and full sprout (hypocotyl + root) throughout the shelf life ($p < 0.05$) regarding Fluorescence treatment (Table 1). In contrast, Blue LED lighting presented the lowest sprout and hypocotyl lengths, even lower than in Darkness and Fluorescent treatments (Figure 2). In this way, broccoli sprouts illuminated with Red and Far-Red LEDs reported 35-37 % and 44-46 % higher

full sprout length compared to Darkness and Fluorescent treatments, respectively, throughout the storage period. Moreover, a higher growth increase was reported by Red and Far-Red regarding Blue treatment with 44 % and 53 %, respectively. Hence, the illumination with Red and Far-Red LEDs showed a positive physiological effect, which can be explained due to the fact that LEDs wavelength influences plant photoreceptors, which are related to changes in plant morphology and metabolism (31). Otherwise, there were no significant differences between samples under different lighting treatments with regards to H/R Ratio. Weight losses monitored were very low (0.0010 g), without significant ($p < 0.05$) differences among treatments, due to the high RH within packages (data not shown).

3.3. Microbial analyses

The microbiological growth was directly affected by light treatments (Table 2). After 4 d, Far-Red treated sprouts reported the lowest mesophilic bacteria growth ($p < 0.05$) as compared to the other light regimes, with comparable values detected under darkness conditions. On 8 and 15 d, less marked differences between the treatments were found. Furthermore, broccoli sprouts illuminated with Far-Red LEDs reported the lowest counts of psychrophilic at 4 d, with comparable values observed in red and fluorescence treated samples, and at 15 d, with similar values as compared to sprouts stored under Red light. After 15 d of storage, Enterobacteria growth was reduced in lighted stored samples as compared to darkness conditions without differences among the different lighting regimes. After 4 d of storage, Darkness, Fluorescence and Red LEDs promoted moulds and yeasts growth, which after 15 d of storage resulted reduced under Far-Red light condition, even if with significant differences only if compared with Red treatment.

3.4. Total phenolic content and antioxidant capacity

Table 3 shows the obtained results of total phenolic content and antioxidant capacity measured by DPPH• free radical scavenging method and ABTS•+ radical cation assay. Although FRAP method was performed (data not shown; $R = 0.229$, $p > 0.05$), a much higher correlation was appreciated between reported results by DPPH and ABTS assays

and total phenolic content measured by Folin-Ciocalteu method ($R = 0.820$, $p < 0.01$; $R = -0.720$, $p < 0.01$, respectively).

Although the illumination with Red and Far-Red positively stimulates the growth of broccoli sprouts, these treatments did not produce an increase in the biosynthesis of phenolic compounds, which presented similar (4 d) or even lower (15 d) values as compared to Darkness conditions. Furthermore, TPC was the lowest in Blue treated samples (at 4, 8, 15 d) and Red treated samples (at 8 and 15 d). While TAC measured by DPPH did not present statistically significant differences among lighting regimes at 4 d, it resulted the lowest in sprouts stored under Blue and Red LEDs at 8 and 15 d. At 4 d, TAC (measured by ABTS) resulted the highest in samples stored under Far-Red LEDs, with a 37.6 % increase as compared to Darkness conditions. Besides, during the storage (at 8 and 15 d), TAC (measured by ABTS) of Far-Red treated samples resulted comparable to that found in samples under Darkness condition, but always higher as compared to values obtained in Fluorescent, Blue and Red LEDs treated samples.

Results on TPC and TAC can also be related with TAC Index and Total Scavenging Activity (%). In fact, TAC Index was increased by application of Far-Red LEDs in 10.1 %, 12.4 %, 49.5 %, and 50.3 % with regard to the remaining treatments: Darkness, Fluorescence, Blue, and Red LEDs, respectively. Consequently, Total Scavenging Activity measured by DPPH and ABTS methods was also significantly increased ($p < 0.05$) after application of Far-Red LEDs in relation to the rest of light treatments in 12.1 %, 9.9 %, 33.3 %, and 31.1 %, respectively.

Also, an increasing trend was observed for individual phenols (Table 4). Chlorogenic acid was the major compound found in broccoli seeds, while phenols derived from synapic acid experienced an increase from 2 to 9 times as compared to the initial content of the seeds. Broccoli sprouts treated with Blue LEDs presented the lowest amount of all phenolic compounds ($p < 0.05$), while sprouts in Darkness, Fluorescence, and Far-Red LED showed the highest values without significant differences between them. From the other side, broccoli sprouts preserved under Blue LEDs presented a decrease in the

concentration of all phenolic compounds identified, especially in phenolics derived from the sinapic acid: 1-sinapoyl-2-feruloylgentiobiose, 1,2,2-trisinapoylgentiobiose, and 1,2-disinapoyl-1'-feruloylgentiobiose.

4. DISCUSSION

Regarding headspace gas composition, a previous research using broccoli sprouts packaged and stored by using films of polyester-polypropylene with different thickness showed an increase of CO₂ and a decrease in O₂ partial pressures during the 16 d of cold storage period (29). However, their results suggested that a modified atmosphere packaging of 18 kPa O₂ + 0.36 kPa CO₂ was the best option to avoid anaerobic metabolism. Our results indicate that a greater atmosphere modification, with lower O₂ and higher CO₂ partial pressures, may be more appropriate to decrease the aerobic respiration ratio and release other physiological and biochemical degradative reactions. That atmosphere is markedly above the anaerobic compensation point, without causing any physiological disorder on the sprouts. Gil and Garrido (22) showed that in green and red baby leaves stored for 9 d at 7 °C under a PPFD of 20 μmol m⁻² s⁻¹ (although no further description of the light source used nor the spectral composition adopted were provided), CO₂ production was compensated by photosynthetic activity under light even at low temperature, thus leaving almost unaltered the atmospheric composition during the storage period. Similarly, Hasperué et al. (30) reported a decrease of respiration rate from 0 d to 5 d of storage and maintained lower rates until 10 d at 22 °C in Brussels sprouts exposed to continuous white and blue LED.

In Figure 2, the hypocotyl growth of broccoli sprouts under the different LED treatments at 4 d during refrigerated storage is shown. The response of broccoli sprouts under Far and Far-Red treatments may be due to their light spectral (Figure 1). In fact, light spectral quality directly influences morphological and physiological changes in plants, increasing photosynthesis, controlling flowering time, or modulating plant growth (32). Plants photomorphogenetic responses to changes in light intensity, quality, direction, and time of exposure are mediated by photoreceptors, like phytochromes (phys), which are

responsible for absorbing Red (600-700 nm) and Far-Red (700-750 nm) regions of the spectrum (33). Plants are able to control several processes of the plant physiology, such as induction of germination, seedling de-etiolation, flowering time, leaf development, root elongation, and tolerance to biotic and abiotic stressors (31).

Comparing with both Red and Far-Red light treatments, the lower growth of Blue light exposed sprouts can be explained by the fact that Blue light (390-500 nm) is mediated by the cryptochromes (crys), phototropins (phot), and members of the Zeidler family (ztl, fkl1, and lkp2). In this case, phototropins are involved in the inhibition of hypocotyl elongation and regulation of plant growth, as well as the directional light orientation (34).

Similarly to our results, Park and Runkle (35) have recently reported the influence of Far-Red radiation to promote growth of seedlings by increasing plant expansion. In their study, Far-Red supplementation during preharvest decreased phytochrome photoequilibrium and increased yield photon flux. Therefore, Far-Red radiation may foster plant elongation and affect physiological mechanisms in plants. The same authors have also shown that Blue radiation attenuates the effects of Red and Far-Red radiation on extension growth, but it did not influence subsequent flowering (36). Thus, the postharvest application of Blue illumination reduces plant size even more than Darkness and Fluorescence treatments, while Far-Red wavelengths can regulate phytochrome-mediated morphological and developmental plant responses.

Microbial results (Table 2) can be justified because Far-Red light is involved in the photosensory system of the plant (31,33) and it acts as protector of a wide range of cellular organelles and cell functions, including cell membrane, cytoplasmic enzymes, and nucleic acids.

In a previous research, Baenas et al. (37) obtained slightly higher results than ours regarding the microbiological growth of mesophilic (10.1 ± 0.2 and 10.04 ± 0.18 log CFU g⁻¹) and psychrophilic (9.58 ± 0.01 and 10.19 ± 0.17 log CFU g⁻¹) bacteria, enterobacteria (9.15 ± 0.62 and 9.60 ± 0.53 log CFU g⁻¹), moulds, and yeasts (8.47 ± 0.56 and 8.59 ± 0.01 log CFU g⁻¹) in broccoli sprouts preserved at 5 and 10 °C, respectively, for 14 d.

These results resemble those found in the present study, because we reaffirm the idea of developing safe broccoli sprouts with constant microbiological values, suitable for human consumption after a rather long storage period of 14 d.

Our results can be also supported by those previously obtained by Mihaly Cozmuta et al. (38), which demonstrated the significant reduction of moulds and yeasts in tomatoes stored under Far-Red lighting ($25 \mu\text{mol m}^{-2} \text{s}^{-1}$) regarding Darkness treatment. In fact, these results can be also justified by the relation between Far-Red light and the synthesis of bioactive compounds with potential antimicrobial capacity. Therefore, these results suggest the impact of Far-Red light on the modulation of growth and replication of microorganisms which should be further elucidated in futures studies.

In contrast, other authors have suggested the possibility of the inhibitory effect of Blue LED on microorganism growth. For instance, Maclean et al. (39) and Wang et al. (40) have reviewed the last scientific evidences of the inactivation of a wide range of microbial species after application of Blue LED lighting (405 nm). This effectiveness can be related to our results at 4 d (Table 2). However, in the present study, these values are not consistent enough to appreciate the antimicrobial effect of Blue light on broccoli sprouts.

Regarding phenolic content and antioxidant capacity determined, our results differ from those obtained by Kopsell et al. (3), who concluded that stimulation of primary and secondary metabolic pathways associated with phenolic compounds (as nutritional benefits) were increased after application of Blue LEDs in broccoli microgreens growing sprouts. However, such difference could be due to the intensity and the duration of the lighting treatment. Accordingly, in our study Blue LEDs light was applied continuously at $33.31 \mu\text{moles m}^{-2} \text{s}^{-1}$ during 15 d at 5°C , while Kopsell et al. (3) used a combination of 12 % Blue and 88 % Red light at $350 \pm 10 \mu\text{mol m}^{-2}\text{s}^{-1}$ (10 times higher than our study) for 24 h, on the fifth day after sowing the seeds. After that, broccoli plantlets were again radiated by 12 % Blue and 88 % Red LEDs at $350 \pm 10 \mu\text{mol m}^{-2}\text{s}^{-1}$ or Blue LED at $41 \pm 2 \mu\text{mol m}^{-2}\text{s}^{-1}$ treatment for 5 d before harvest (starting on the thirteenth day after sowing). As well as Kwack et al. (41), who also showed the increase of phenolic content

after treatment with Blue LEDs at different intensities (0, 12.5, 25, 50, and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

The phenolic compounds content is an important quality index of sprouts and microgreens, and the accumulation of phenolic phytochemicals can be stimulated by preservation under different LEDs. This effect of the light quality on the synthesis of phenolics in the present study contrasts with that obtained by Lobiuc et al. (42), who showed an increase of phenolic compounds under Blue treatment and a decrease under Red LEDs in different coloured basil sprouts and microgreens. Thus, the green cultivars were the most stimulated by higher proportion of Red light, while the red cultivars reacted the most by higher ratios of Blue light. Pennisi et al. (9) showed as a combination of lights RB_{0.5} (30 % Red, 58 % Blue) decreased the antioxidant activity and the total bioactive compounds (phenolics and chlorophyll), while a higher application of Red light, RB₃ (>70 % Red, 23 % Blue), showed a promising increase of flavonoids and total phenolic content. This behaviour can explain the results obtained in the present study since broccoli sprouts are mostly green and phytochemicals were stimulated by Far-Red LEDs, but not under Blue light treatments. Furthermore, the synthesis of phenolics has demonstrated to be influenced by light, specially phenylalanine ammonia lyase (PAL) and other key enzymes related to the production and accumulation of phenols (43,44), which can justify the direct relation between LED radiation and phenolic content. Hence, Blue and Red LED treatments could have a significant potential of improving growth of different cultivars and microgreens, while increasing also the contents of phenolic compounds as valuable phytochemicals; however, it depends on the pigmentation of the cultivar/species.

5. CONCLUSIONS

The present study reported positive results regarding the exposure to LED lights during postharvest of broccoli sprouts. Particularly, Far-Red LEDs increased hypocotyl and sprout length in contrast with Fluorescence and Darkness treatments, as it is conventionally done during the postharvest food chain steps. In addition, Far-Red LEDs decreased the microbial growth of psychrophilic, enterobacteria, moulds and yeasts

regarding Darkness and Fluorescence treatments, presenting a better quality after 15 d at 5 °C. Despite these promising results, Far-Red LEDs did not stimulate the synthesis of phenolic acids. Nevertheless, the total antioxidant and scavenging activities were improved in relation to the remaining light treatments, which included Darkness and Fluorescence and Blue and Red LED lighting. In conclusion, postharvest Blue LEDs application during postharvest did not report any relevant improvement in morphological, microbial, and bioactive compounds under the assayed conditions. However, the application of postharvest Far-Red LED illumination in minimally processed broccoli sprouts during refrigerated storage seems to be an interesting tool for further investigation to elucidate if other horticultural commodities would benefit in terms of quality from this lighting treatment. It also would pave the way for further development towards the integration of the LED technology in the food distribution chain.

DECLARATIONS

Author contributions statement

Noelia Castillejo: performed the experiments; analyzed and interpreted the data; wrote the paper. Lorena Martínez-Zamora: performed the experiments; analyzed and interpreted the data; wrote the paper. Perla A. Gómez: analyzed and interpreted the data; reviewed the paper. Giuseppina Pennisi: contributed materials; wrote and reviewed the paper. Francesco Orsini: contributed materials; interpreted the data; reviewed the paper. Andrea Crepaldi: contributed in R&D of led materials. Juan A. Fernández: interpreted the data; reviewed the paper. Francisco Artés-Hernández: responsible of the financed project and the paper; conceived and designed the experiment; interpreted the data; contributed reagents, materials, analysis tools and/or data; analyzed and interpreted the data; wrote and reviewed the paper.

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Competing interest statement

The authors declare no conflicts of interest.

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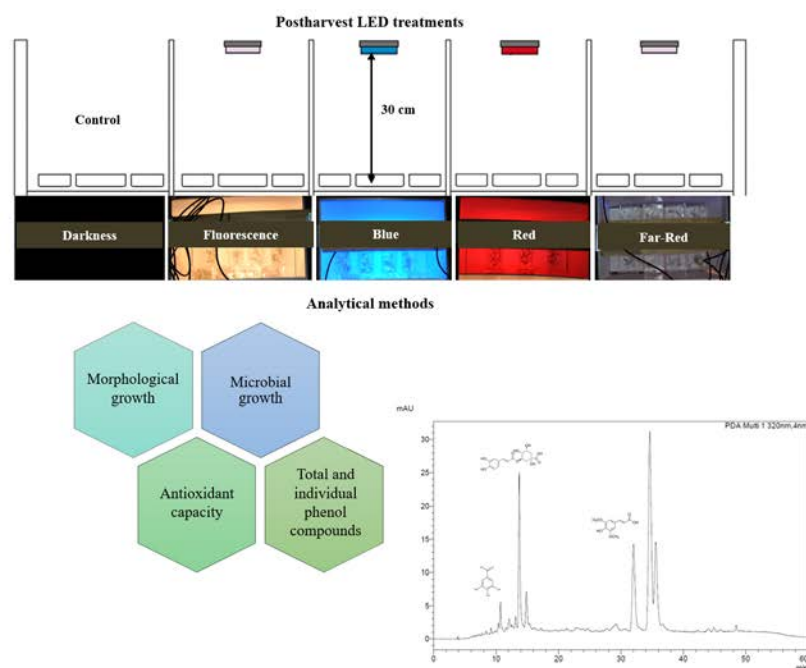
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Graphical abstract

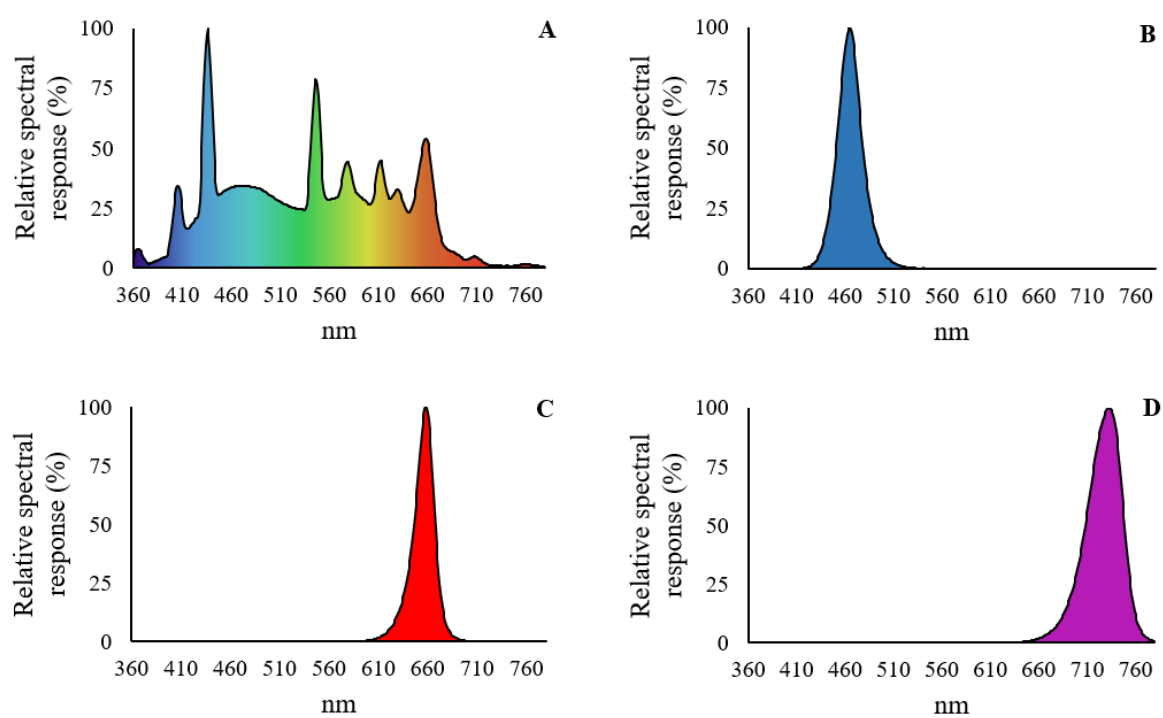


Figure 1. Spectral composition of Fluorescent lighting (A), Blue (B), Red (C), and Far Red (D) LEDs used during refrigerated storage.

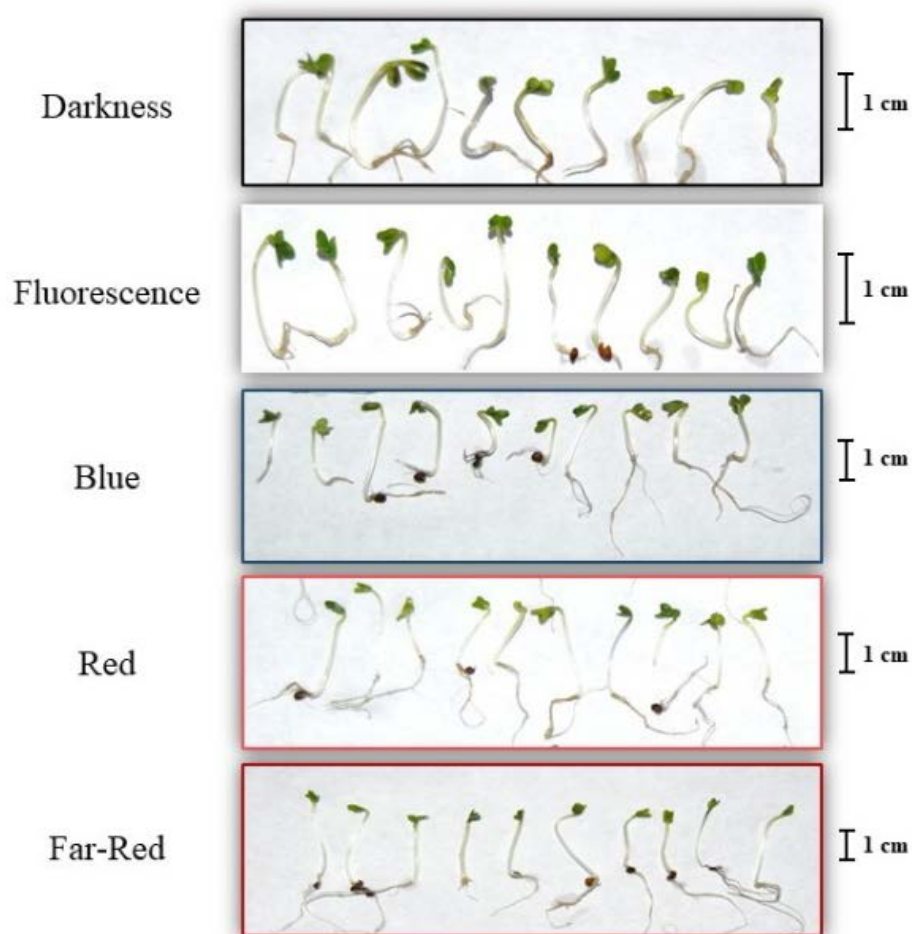


Figure 2. Broccoli sprouts illuminated under several treatments at 4 d at 5 °C (n=10).

Table 1. Characterization of broccoli sprouts illuminated under several treatments during 15 d at 5 °C (mean \pm sd) (n=45).

<i>Treatments</i>	<i>Days at 5 °C</i>			
	<i>0</i>	<i>4</i>	<i>8</i>	<i>15</i>
Hypocotyl lenght (cm)				
Darkness	2.01 \pm 0.17	1.60 \pm 0.15 ^B	1.85 \pm 0.10	2.17 \pm 0.09 ^{AB}
Fluorescence		1.68 \pm 0.12 ^B	1.73 \pm 0.15	1.77 \pm 0.08 ^B
Blue		1.65 \pm 0.10 ^B	1.77 \pm 0.14	2.13 \pm 0.08 ^{AB}
Red		1.98 \pm 0.21 ^{AB}	2.48 \pm 0.18	2.01 \pm 0.14 ^{AB}
Far-Red	ab	2.08 \pm 0.13 ^{A ab}	2.51 \pm 0.16 ^{ab}	2.87 \pm 0.18 ^{A a}
H/R Ratio[†]				
Darkness	1.37 \pm 0.10	0.91 \pm 0.04	1.45 \pm 0.14	1.24 \pm 0.12
Fluorescence	a	0.82 \pm 0.02 ^{bc}	0.68 \pm 0.04 ^c	1.29 \pm 0.14 ^{ab}
Blue		1.21 \pm 0.09	1.27 \pm 0.03	0.85 \pm 0.06
Red	a	0.89 \pm 0.02 ^b	1.18 \pm 0.01 ^{ab}	1.20 \pm 0.15 ^{ab}
Far-Red	a	1.00 \pm 0.04 ^{bc}	0.71 \pm 0.01 ^c	1.15 \pm 0.13 ^{ab}
Sprout length H+R (cm)				
Darkness	3.64 \pm 0.26	4.05 \pm 0.30 ^{AB a}	2.72 \pm 0.25 ^{C b}	4.04 \pm 0.10 ^{AB a}
Fluorescence		3.82 \pm 0.13 ^{AB}	3.68 \pm 0.34 ^B	3.19 \pm 0.31 ^B
Blue		3.26 \pm 0.30 ^B	3.20 \pm 0.31 ^{BC}	3.76 \pm 0.10 ^{AB}
Red		5.33 \pm 0.40 ^A	4.67 \pm 0.19 ^A	4.71 \pm 0.07 ^{AB}
Far-Red	b	5.25 \pm 0.29 ^{A a}	5.06 \pm 0.42 ^{A ab}	5.33 \pm 0.28 ^{A a}

[†]Hypocotyl/Radical Ratio.

Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

Table 2. Microbiological growth (log CFU g⁻¹) of minimally processed broccoli sprouts illuminated under several treatments during 15 d at 5 °C (mean ± sd) (n=3).

Days at 5 °C					
Treatments	0		4	8	15
Mesophilic					
Darkness	6.5 ± 0.1	b	8.0 ± 0.1 ^{BC a}	7.6 ± 0.4 ^{AB a}	8.0 ± 0.1 ^{AB a}
Fluorescence		c	8.4 ± 0.3 ^{AB a}	7.2 ± 0.3 ^{B b}	7.4 ± 0.2 ^{B b}
Blue		b	8.9 ± 0.3 ^{A a}	8.2 ± 0.7 ^{AB a}	8.2 ± 0.3 ^{A a}
Red		c	8.8 ± 0.4 ^{A a}	8.5 ± 0.3 ^{A a}	7.5 ± 0.3 ^{B b}
Far-Red		c	7.4 ± 0.1 ^{C b}	8.2 ± 0.1 ^{AB a}	8.0 ± 0.2 ^{AB a}
Psychrophilic					
Darkness	6.1 ± 0.1	c	8.8 ± 0.1 ^{AB ab}	8.7 ± 0.2 ^b	9.2 ± 0.3 ^{A a}
Fluorescence		b	8.7 ± 0.2 ^{ABC a}	8.6 ± 0.3 ^a	8.8 ± 0.1 ^{AB a}
Blue		b	9.2 ± 0.2 ^{A a}	8.8 ± 0.2 ^a	8.9 ± 0.0 ^{AB a}
Red		b	8.2 ± 0.2 ^{C a}	8.5 ± 0.1 ^a	8.4 ± 0.3 ^{BC a}
Far-Red		b	8.4 ± 0.3 ^{C a}	8.6 ± 0.2 ^a	8.1 ± 0.2 ^{C a}
Enterobacteria					
Darkness	3.3 ± 0.5	c	4.6 ± 0.5 ^{AB a}	4.7 ± 0.6 ^{AB a}	7.5 ± 0.0 ^{A a}
Fluorescence		c	5.3 ± 0.4 ^{A a}	4.3 ± 0.5 ^{B b}	5.3 ± 0.3 ^{B a}
Blue		c	4.5 ± 0.5 ^{AB b}	4.1 ± 0.2 ^{B b}	6.0 ± 0.4 ^{B a}
Red		c	4.7 ± 0.7 ^{AB b}	4.9 ± 0.4 ^{AB b}	6.1 ± 0.3 ^{B a}
Far-Red		b	3.7 ± 0.6 ^{B b}	5.9 ± 0.5 ^{A a}	5.8 ± 0.1 ^{B a}
Moulds and Yeasts					
Darkness	4.8 ± 0.7	b	7.1 ± 0.2 ^{A a}	6.5 ± 0.9 ^a	6.3 ± 0.1 ^{AB a}
Fluorescence		b	7.1 ± 0.3 ^{A a}	6.8 ± 0.7 ^a	6.1 ± 0.9 ^{AB a}
Blue			5.3 ± 0.5 ^B	5.5 ± 0.5	5.9 ± 0.2 ^{AB}
Red		b	6.5 ± 0.5 ^{AB a}	6.9 ± 0.5 ^a	6.9 ± 0.4 ^{A a}
Far-Red			5.5 ± 0.7 ^B	6.4 ± 0.6	5.3 ± 0.2 ^B

Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

Table 3. Total phenolic content and antioxidant activity changes of minimally processed broccoli sprouts illuminated under several treatments during 15 d at 5 °C (mean \pm sd) (n=3).

Treatments	Days at 5 °C			
	0	4	8	15
TPC[†] (g chlorogenic acid kg⁻¹ DW)				
Darkness	17.9 \pm 1.2	b 35.7 \pm 1.9 ^{AB a}	35.2 \pm 1.9 ^{A a}	38.2 \pm 2.1 ^{A a}
Fluorescence		c 37.1 \pm 1.3 ^{A a}	30.2 \pm 2.1 ^{B b}	35.3 \pm 1.0 ^{AB a}
Blue		ab 20.0 \pm 0.4 ^{C a}	17.5 \pm 1.6 ^{D ab}	16.1 \pm 1.4 ^{D c}
Red		b 28.9 \pm 2.5 ^{B a}	25.5 \pm 1.5 ^{C a}	26.5 \pm 1.1 ^{C a}
Far-Red		b 33.7 \pm 5.4 ^{AB a}	32.9 \pm 0.5 ^{AB a}	33.7 \pm 2.0 ^{B a}
TAC[‡] \rightarrow DPPH (g TE[§] kg⁻¹ DW[¶])				
Darkness	7.5 \pm 0.5	b 8.1 \pm 0.7 ^{ab}	9.2 \pm 0.7 ^{A a}	7.0 \pm 0.3 ^{A b}
Fluorescence		b 9.6 \pm 0.6 ^a	8.7 \pm 0.5 ^{AB ab}	7.4 \pm 0.7 ^{A b}
Blue		ab 7.8 \pm 0.6 ^a	7.4 \pm 0.5 ^{BC ab}	4.0 \pm 0.3 ^{B b}
Red		b 9.2 \pm 0.6 ^a	6.5 \pm 0.2 ^{C b}	3.7 \pm 0.4 ^{B c}
Far-Red		b 9.3 \pm 0.7 ^a	8.2 \pm 0.6 ^{AB b}	8.1 \pm 0.3 ^{A b}
TAC \rightarrow ABTS (g TE kg⁻¹ DW)				
Darkness	19.9 \pm 1.0	a 13.2 \pm 0.1 ^{B c}	13.3 \pm 1.4 ^{AB c}	15.9 \pm 0.3 ^{A b}
Fluorescence		a 13.1 \pm 0.6 ^{B b}	13.0 \pm 1.2 ^{BC b}	13.2 \pm 1.1 ^{B b}
Blue		a 9.5 \pm 0.5 ^{D ab}	11.1 \pm 0.7 ^{BC b}	9.1 \pm 0.3 ^{C c}
Red		a 10.5 \pm 0.4 ^{C b}	10.5 \pm 0.5 ^{C b}	8.2 \pm 0.8 ^{C c}
Far-Red		a 18.2 \pm 1.4 ^A	15.7 \pm 0.8 ^A	13.8 \pm 0.9 ^A
Total Antioxidant Capacity Index (g TE kg⁻¹ DW)				
Darkness	13.7 \pm 0.5	a 10.4 \pm 0.6 ^{BC b}	11.2 \pm 0.8 ^{A b}	11.5 \pm 0.1 ^{A b}
Fluorescence		a 11.3 \pm 0.0 ^{B b}	10.9 \pm 0.9 ^{AB b}	10.3 \pm 0.9 ^{A b}
Blue		a 8.7 \pm 0.4 ^{D b}	9.3 \pm 0.4 ^{BC b}	6.5 \pm 0.3 ^{B c}
Red		a 9.9 \pm 0.4 ^{CD b}	8.5 \pm 0.3 ^{C c}	6.0 \pm 0.5 ^{B d}
Far-Red		a 13.6 \pm 0.6 ^{A a}	12.0 \pm 0.6 ^{A b}	11.0 \pm 0.6 ^{A b}
Total Scavenging Activity (%)				
Darkness	37.8 \pm 0.9	a 33.9 \pm 1.0 ^{B bc}	36.7 \pm 2.1 ^{A ab}	33.2 \pm 0.4 ^{B c}
Fluorescence		34.2 \pm 2.9 ^B	36.5 \pm 2.1 ^A	35.2 \pm 1.8 ^B
Blue		a 28.4 \pm 0.9 ^{C c}	32.2 \pm 0.8 ^{B b}	26.8 \pm 0.8 ^{C c}
Red		a 31.7 \pm 0.4 ^{BC b}	31.4 \pm 1.0 ^{B b}	25.6 \pm 1.0 ^{C c}
Far-Red		ab 39.8 \pm 1.0 ^{A a}	39.6 \pm 0.9 ^{A ab}	37.0 \pm 1.4 ^{A b}

[†]Total Phenolic Content; [‡]Total Antioxidant Capacity. [§]Trolox Equivalents. [¶]Dry Weight.

Different capital letters denote significant differences (p < 0.05) among different treatments for the same sampling time. Different lowercase letters denote significant differences (p < 0.05) among different sampling times for the same treatment.

Table 4. Influence of different illumination treatments on phenolic compounds (mg sinapic acid kg⁻¹ DW[†] or mg chlorogenic acid kg⁻¹ DW) of minimally processed broccoli sprouts during 15 d at 5 °C (mean ± sd) (n=3).

<i>Treatments</i>	<i>Day of analysis</i>	<i>Sinapic Acid</i>	<i>1-sinapoyl-2-feruloylgentiobiose</i>	<i>1,2,2-trisinapoylgentiobiose</i>	<i>1,2-disinapoyl-1'-feruloylgentiobiose</i>	<i>Gallic Acid</i>	<i>Chlorogenic Acid</i>	<i>Neochlorogenic Acid</i>
	Seed	1.46 ± 0.05	4.88 ± 0.36	2.03 ± 0.15	1.13 ± 0.05	0.21 ± 0.01	82.44 ± 1.31 ***	1.72 ± 0.08
	At harvest	1.71 ± 0.06 ^a	8.00 ± 0.57 * ^c	19.54 ± 0.87 ** ^c	9.74 ± 0.47 *** ^d	0.22 ± 0.00 ^c	15.16 ± 0.71 ^b	1.71 ± 0.10 ^d
Darkness	4	0.44 ± 0.02 ^{A b}	13.12 ± 0.19 ^{B b}	35.14 ± 1.16 ^{B b}	19.74 ± 1.02 ^{B a}	0.29 ± 0.01 ^{AB b}	17.62 ± 1.74 ^{A a}	4.33 ± 0.14 ^{A a}
	8	0.44 ± 0.03 ^{B b}	12.36 ± 1.02 ^{C b}	36.07 ± 0.49 ^{B b}	20.45 ± 0.20 ^{B a}	0.32 ± 0.02 ^{A b}	10.61 ± 0.12 ^{AB b}	1.84 ± 0.03 ^{ABC c}
	15	0.31 ± 0.01 ^{D b}	15.01 ± 0.78 ^{A a}	40.72 ± 1.98 ^{A a}	20.82 ± 1.23 ^{A a}	0.36 ± 0.00 ^{A a}	8.97 ± 0.90 ^{BC b}	2.35 ± 0.05 ^{A b}
Fluorescence	4	0.46 ± 0.03 ^{A b}	17.00 ± 0.44 ^{A a}	44.54 ± 1.43 ^{A a}	24.13 ± 0.08 ^{A b}	0.27 ± 0.02 ^{AB b}	15.45 ± 1.02 ^{B a}	2.59 ± 0.26 ^{BC a}
	8	0.41 ± 0.04 ^{B b}	16.60 ± 0.25 ^{A a}	42.74 ± 0.60 ^{A a}	26.22 ± 0.62 ^{A a}	0.33 ± 0.02 ^{A a}	11.47 ± 1.09 ^{AB b}	1.99 ± 0.16 ^{AB b}
	15	0.48 ± 0.00 ^{C b}	15.75 ± 0.83 ^{A a}	37.41 ± 1.37 ^{AB b}	20.63 ± 0.89 ^{B c}	0.36 ± 0.00 ^{A a}	7.31 ± 0.34 ^{C c}	2.06 ± 0.20 ^{B b}
Blue	4	0.31 ± 0.01 ^{B c}	12.63 ± 0.09 ^{B a}	29.29 ± 0.07 ^{C a}	15.16 ± 0.94 ^{C a}	0.23 ± 0.02 ^{B a}	9.13 ± 0.24 ^{D a}	2.13 ± 0.04 ^{C a}
	8	0.90 ± 0.02 ^{A b}	12.87 ± 0.84 ^{C a}	27.25 ± 0.77 ^{C a}	13.15 ± 0.98 ^{D b}	0.26 ± 0.01 ^{B a}	10.75 ± 1.05 ^{AB b}	1.74 ± 0.10 ^{BC b}
	15	0.64 ± 0.01 ^{B b}	12.46 ± 1.09 ^{BC a}	26.87 ± 2.48 ^{CD a}	10.58 ± 0.21 ^{C c}	0.18 ± 0.02 ^{C b}	9.12 ± 0.29 ^{BC b}	1.61 ± 0.06 ^{C b}
Red	4	0.31 ± 0.00 ^{B c}	11.86 ± 0.27 ^{C b}	29.57 ± 1.93 ^{C ab}	14.48 ± 0.63 ^{C a}	0.31 ± 0.03 ^{A a}	13.66 ± 0.00 ^{C a}	2.34 ± 0.29 ^{BC a}
	8	0.41 ± 0.03 ^{B c}	13.12 ± 0.72 ^{BC a}	33.63 ± 1.96 ^{B a}	13.10 ± 0.41 ^{D a}	0.28 ± 0.03 ^{AB ab}	12.13 ± 0.88 ^{A ab}	1.69 ± 0.02 ^{C b}
	15	0.72 ± 0.03 ^{A b}	10.94 ± 0.25 ^{C b}	26.05 ± 1.43 ^{D b}	6.81 ± 0.66 ^{D b}	0.27 ± 0.01 ^{B ab}	10.30 ± 0.64 ^{B b}	1.50 ± 0.04 ^{C ab}
Far-Red	4	0.28 ± 0.00 ^{B b}	9.74 ± 0.24 ^{D c}	27.24 ± 2.08 ^{C a}	10.93 ± 0.86 ^{D b}	0.31 ± 0.04 ^{AB a}	12.86 ± 1.79 ^C	2.79 ± 0.18 ^{B a}

8	0.39 ± 0.03 ^{B b}	15.03 ± 0.48 ^{AB a}	30.18 ± 1.72 ^{C a}	17.74 ± 1.01 ^{C c}	0.28 ± 0.02 ^{AB ab}	9.67 ± 0.41 ^B	2.09 ± 0.09 ^{A b}
15	0.14 ± 0.01 ^{E b}	12.92 ± 0.25 ^{B b}	32.29 ± 3.12 ^{BC a}	16.51 ± 0.26 ^{B c}	0.33 ± 0.03 ^{A a}	12.71 ± 1.13 ^A	1.99 ± 0.00 ^{B b}

^tDry Weight. Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment. *, **, ***: significant differences ($p < 0.05$, $p < 0.01$; $p < 0.001$) among broccoli sprouts (day 0) and seeds.