Spectral composition from led lighting during storage affects nutraceuticals and safety attributes of fresh-cut red chard (Beta vulgaris) and rocket (Diplotaxis tenuifolia) leaves

SPECTRAL COMPOSITION FROM LED LIGHTING DURING STORAGE AFFECTS NUTRACEUTICALS AND SAFETY ATTRIBUTES OF FRESH-CUT RED CHARD (*BETA VULGARIS*) AND ROCKET (*DIPLOTAXIS TENUIFOLIA*) LEAVES

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ABSTRACT

The main objective of this study was to evaluate the physiological and quality changes of fresh-cut red chard (*Beta vulgaris*) and rocket (*Diplotaxis tenuifolia*) leaves illuminated during storage with monochromatic light emitting diode (LED) lamps, featuring different spectral component (red, green, yellow, white, blue and far-red) and same light intensity (35 μmol m^{-2} s^{-1}). As control, storage in darkness was assayed. Biomass, colorimetric and microbiological changes were determined up to 10 d of storage at 5 ºC. In addition, total antioxidant activity and bioactive compounds changes along the shelf-life were also monitored. Microbial counts were reduced by yellow and blue light in red chard, and by yellow and green light in rocket. Green and white light enabled to preserve colorimetric indexes and chlorophylls content mostly in rocket and, eventually, increasing carotenoids in red chard. Total antioxidant capacity and total phenols content were stimulated in response to red or blue light application for both species. On the other hand, LED light supply increased weight losses during storage as compared to darkness, although more limitedly in response to yellow and far red light. The study provides solid ground for further exploration on how LED lighting treatment during storage of red chard and rocket may foster product qualitative properties, suggesting that different spectral wavebands may alternatively enhance antioxidant properties and reduce microbiological risks.

Keywords: *Beta vulgaris*; *Diplotaxis tenuifolia*; postharvest; bioactive compounds; antioxidant capacity; phenols.

HIGHLIGHTS

- LED lighting improved postharvest quality of leafy vegetables
- LED light increased weight loss in stored samples
- Yellow, blue and green light reduced microbiological load
- Red and blue light increased antioxidant compounds

47 # 1. INTRODUCTION

48 ## 1.1. Postharvest losses and horticultural food waste

49 Reducing food loss and waste is gathering increased consideration within the worldwide
50 effort against food insecurity and toward the implementation of sustainable systems
51 (Porat et al., 2018). While global estimates account food waste for about a third of the
52 total production (Okawa, 2015), such value raises to 50 % when only horticultural goods
53 are considered (Beausang et al., 2017). Moreover, while globally food waste occurs all
54 along the agricultural supply chain, in developed countries about 80 % of the losses are
55 experienced during the postharvest life of the products (Porat et al., 2018). A clear
56 indication on the effort required to reduce food losses has been reflected in the ambitious
57 'Development Goal' set by United Nations to reduce by 50 % food waste by 2030 (Grosso
58 and Falasconi, 2018), a decision that was also integrated by local governments, including
59 USA and the EU parliament, among others (Porat et al., 2018). Consequently, the
60 collaboration between research and logistics/technology suppliers has been advised
61 (Thyberg and Tonjes, 2016). Among horticultural goods, the so-called ready-to-eat sector
62 is raising relevant concern on the impact associated with food waste (Fadda et al., 2016),
63 due to further environmental and economic impact associated with processing and
64 packaging (Schott and Andersson, 2015). Accordingly, it was recently estimated that
65 waste prevention in the consumed ready-to-eat meals in Norway could reduce their
66 associated overall emissions of GHG by about 13 % and energy use by 16 % (Hanssen et
67 al., 2017), which could be extrapolated to other developed countries.

68

69 ## 1.2. Nutritional properties of ready-to-eat leafy vegetables during refrigerated
70 storage

71 Within fresh-cut produces, leafy vegetables including red chard (*Beta vulgaris*) and
72 rocket (*Diplotaxis tenuifolia*) have been targeted in a number of researches (Tomás-
73 Callejas et al., 2011; Mastrandrea et al., 2017). Red chard was recently described for its
74 antioxidant and anticancer activities (Zein et al., 2015) resulting from bioaccumulation of
75 phenolics and carotenoids, which also translated into elevate antioxidant activity.
76 Similarly, rocket features valuable sensory and nutritional characteristics (Pasini et al.,
77 2011), with elevate reported contents in functional metabolites, including carotenoids and
78 phenolic compounds (Bell and Wagstaff, 2014). During refrigerated storage of both

species, changes in physiological and visual quality have been earlier described (Tomás-Callejas et al., 2011; Spadafora et al., 2016). Furthermore, quality decay during shelf-life of fresh-cut vegetables is strongly related to potential microbiological outbreaks (Taban and Halkman, 2011; Söderqvist, 2017). Indeed, despite the described decay of nutritional properties over time (Hodges and Toivonen, 2018), to date, cold-chain preservation (Rediers et al., 2009) and the selection of the species-specific optimal temperature range for storage (Ferrante et al., 2004) have been widely described as key determinants of the safety and visual quality preservation of fresh cut produce.

1.3. Light as a strategy for quality preservation during storage

Current strategies for quality preservation of fresh-cut produce include washing and sanitizing agents (e.g., chlorine, ozone, hydrogen peroxide, chlorine dioxide and antioxidant solutions), as well as preservation techniques, like superatmospheric O_2, hot water treatments and exposure to UVB and UVC illumination (Artés-Hernández et al., 2017). Among other emerging strategies, the application of artificial light during storage is gaining relevance as a tool to preserve or even improve the nutritional properties of horticultural goods (Azuma et al., 2019; Xu et al., 2014). Previous applications of low intensity fluorescent lighting (ranging 6 to 16 μmol m^{-2} s^{-1}) during cold storage did not alter the antioxidant profile of *Brassica rapa* subsp. *sylvestris* (Barbieri et al., 2009) and fresh-cut Romaine lettuce (Martínez-Sánchez et al., 2011). As advances in light emitting diode (LED) technologies take place, their adoption in the horticultural sector gains relevance, thanks to their low heat dissipation, the limited energetic needs and the potentialities associated with fine tuning of both light intensity and spectral properties (Pennisi et al., 2019a). While LED application during storage of vegetables (e.g., broccoli, *Brassica oleracea* var. *Italica*, Ma et al., 2014, and tomato, *Solanum lycopersicum* Najera et al., 2018) is raising interest, their adoption on fresh-cut produces are actually mostly unexplored. It was shown that lamb's lettuce (*Lactuca sativa* L.) samples stored under a warm white LED lighting device supplying limited light intensity (1.4 μmol m^{-2} s^{-1}) preserved quality over time more efficiently than those stored in darkness (Braidot et al., 2014). A green LED light (12 μmol m^{-2} s^{-1}) during storage of broccoli florets was shown to preserve their visual quality and chlorophyll content, while increasing total phenols and glucosinolates as compared with storage in darkness (Jin et al., 2015). Although no

reference to blue light during storage of leafy vegetables were available at the time of this study, in strawberry stored at 5 °C, blue LED light (at 40 μmol m^{-2} s^{-1}), was shown to increase the content of both total anthocyanin and the associated enzymes (Xu et al., 2014). Furthermore, postharvest far red LED illumination of minimally processed broccoli sprouts was also recently shown to increase morphological development and the total antioxidant and scavenging activities while decreasing the microbial growth during 15 d at 5 ºC (Castillejo et al., 2021)

1.4. Aim of the research

Preliminary findings suggest that LED technology may provide a non-thermal efficient management tool to preserve or improve food health promoting properties during postharvest (Hasperué et al., 2016). It may also have complementary functions against bacterial or fungal outbreaks (Imada et al., 2014; Jin et al., 2015). However, despite the encouraging preliminary results on potential applicability of LED lighting during storage of fresh vegetables, knowledge still lacks on most efficient spectral regions. Therefore, the aim of the present research was to describe the effects of different monochromatic LED lights as postharvest treatments for fresh-cut red chard and rocket leaves. Content of main bioactive compounds, microbial counts and color changes were therefore periodically monitored in samples throughout 10 d at 5 °C under six different LED lighting treatments and a control in darkness.

2.- MATERIALS AND METHODS

2.1. Plant material

Commercial sealed bags (75 g each) of fresh-cut red chard (*Beta vulgaris* cv. Red Bull) and wild rocket (*Diplotaxis tenuifolia*) leaves were provided by a local company (Kernel Export S.L., Murcia, Spain) just after fresh-cut processing. Sanitation used by the company was a 2 min washing at 5 ºC with a solution containing 75 ppm NaClO at pH=6.5. Packages were immediately transported 20 km to the Universidad Politécnica de Cartagena under refrigerated conditions (5°C, 95% RH). Bags were opened under the hook and leaves selected for absence of diseases and visual or mechanical damages. Samples (7.0 ± 0.1 g) of leaves of both red chard and rocket were placed in polypropylene trays (173 x 120 x 38 mm; 750 mL), distributed over one only layer (adaxial surface

143 upward) in order to guarantee uniform leaf illumination during storage. Then, trays were
144 thermally sealed on the top with a bioriented polypropylene (BOPP) film of 40 µm
145 thickness (Plásticos del Segura S.L., Murcia, Spain), which was manually perforated with
146 a needle creating four holes (0.8 mm ϕ) to avoid an atmosphere modification and ensure
147 air partial pressures.

148

149 **2.2. Storage conditions and light treatments during shelf life**

150 Packaged samples were stored at 5 °C and 85 % RH in a cold room of 7 m^3, where 7
151 treatments were applied in individual metallic containers (0.3 x 0.6 x 0.6 m, W x H x D)
152 (**Fig. 1**). Lamps were installed on the top of the containers, which was the only opened
153 part allowing for air circulation. Six lighting treatments were applied, using LED lamps
154 featuring red (peak at 660 nm), green (peak at 517 nm), yellow (peak at 600 nm), white
155 (peak at 610 nm), blue (peak at 465 nm) or far red (peak at 730 nm) diodes (**Fig. 2**).
156 Furthermore, a control treatment in darkness was included within the same cold storage.
157 Trays were placed at 30 cm from the light source and were continuously (24 h d^{-1})
158 illuminated using a photosynthetic photon flux density (PPFD) of 35 ± 2.5 µmol m^{-2} s^{-1}.
159 PPFD was measured using a PAR Photon Flux Sensor model QSO (Apogee instruments,
160 Logan, UT, USA) connected with a ProCheck handheld reader, manufactured by
161 Decagon Devices Inc. (Pullman, WA, USA). The spectral characteristics were determined
162 using an illuminance spectrophotometer (CL-500A, Konica Minolta, Chiyoda, Tokyo,
163 Japan) as previously described in Castillejo et al. (2021). Each lighting treatment was
164 replicated in two individual metallic containers organized in two blocks, and each
165 container hosted 7 sample trays. Leaves were sampled (5-7 g per replicate) for quality
166 analyses on processing day (day 0), and after 7 and 10 d at 5 ºC, with three replicates per
167 lighting treatment in each experiment, sampling day and block. On such days, samples
168 were removed from the trays, weighed and immediately frozen in liquid nitrogen, freeze-
169 dried and stored at -80 ºC until further analysis, with exclusion of samples used for
170 microbiological analyses.

171

172 **2.3. Weight loss**

Weight loss was calculated along the experiment as the difference between the initial weight of the samples at the beginning of storage and their final weight at the end of the experiment (after 10 d of storage), on each individual tray. To normalize data, weight loss values were expressed as % of the initial value.

2.4. Colour determinations

Leaf colour was determined through identification of L*, a* and b* values with a colorimeter (Chroma Meter CR-400, Minolta, Tokyo, Japan). L* values represent the lightness value, a* values correspond to the green-red component (green for negative values and red for positive values), while b* values denote the blue-yellow component (blue for negative values and yellow for positive values) (Minolta, 1998). The hue angle (h°) was calculated as $h^0 = tan^{-1}\, b/a$ when a and b > 0, or $h^0 = 180 + tan^{-1}\, b/a$ when a < 0 and b > 0. The instrument collects average values from 3 measures performed on the same leaf. In each sampling time, ten leaves were measured per lighting treatment per block in each experiment.

2.5. Microbial analyses

Standard enumeration methods were used to determine mesophilic, psychrophilic, enterobacteria, yeasts, and moulds growth (Martínez-Hernández et al., 2013; Castillejo et al., 2017). One leaf per species and per sample was mixed with a peptonated saline solution in a Stomacher circulator (Seward, London, UK), during 60 s. For each microbial group, 10-fold dilution series were prepared in 9 mL sterile peptone saline solution. All used microbial media was obtained from Scharlau Chemie (Barcelona, Spain). The following media and incubation conditions were used: plate count modified agar for psychrophilic aerobic bacteria with incubations 5 ºC/7 d; Violet Red Bile Dextrose (VRBD) Agar for enterobacteria incubated at 37 °C/48 h; Rose Bengal (RB) Agar for moulds and yeasts incubated at 24 °C/7 d. All microbial counts were reported as log colony forming units per gram of product (log CFU g^{-1}). Three replicates were considered per lighting treatment in each experiment, sampling day and block.

2.6. Extracts preparation

Samples of 0.5 g of freeze-dried leaves were placed in tubes and 3 mL methanol were added. The extraction was carried out in an orbital shaker (Stuart, Stone, UK), where samples were strongly shaken for 1 h in darkness inside a polystyrene box with an ice bed. The extracts were centrifuged at 3220 × g for 15 min at 4 ºC. The supernatant was collected and kept at -80 ºC until analysis of total phenols, and Total Antioxidant Capacity (TAC).

2.7. Total phenols

Total phenols were determined as previously described by Singleton and Rossi (1965). 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 % Na_2CO_3 and 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). Following the same methodology applied for the samples, a calibration curve (R^2=0.996) was obtained by measuring the absorbance of solutions of decreasing concentrations of chlorogenic acid, prepared by diluting a concentrated solution (300 mg L^{-1}). Total phenols were expressed as mg chlorogenic acid equivalents (CAE) kg^{-1} fresh weight (FW). Three replicates were considered per lighting treatment in each experiment, sampling day and block.

2.8. Total antioxidant capacity

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. (2017). For that, 194 µL of DPPH (0.7 mM in methanol, absorbance at 1.1±0.02) solution were added to 21 µL of leaf 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: [(Abs DPPH − Abs Sample)/Abs DPPH] × 100. ABTS assay was

carried out following the method previously described by Rodríguez-Verástegui et al. (2016). For that, 200 µL of the activated ABTS solution (32 µM) were added to 11 µL of leaf extract in a 96-well plate and incubated for 30 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: [(Abs ABTS − Abs Sample)/Abs ABTS] × 100. The FRAP method was also developed following the method described by Castillejo et al. (2017). A daily reaction solution containing sodium acetate buffer (pH 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (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 ºC for 2 h in darkness. Then, 198 µL of FRAP solution were added to 6 µL of leaf extract and incubated for 1 h at room temperature in darkness. The TAC by FRAP was measured by changes in absorbance at 593 nm. Following the same methodology applied for the samples, three calibration curves were calculated, one for DPPH (R^2=0.997), one for ABTS (R^2=0.969), and one for FRAP (R^2=0.999) assays, by measuring the absorbance of solutions containing decreased concentrations of Trolox, prepared by diluting a concentrated solution (300 mg L^{-1}). Obtained data were expressed as mg of Trolox Equivalents Antioxidant Capacity (TEAC) kg^{-1} FW. Total Antioxidant Capacity (TAC) index was calculated using the next equation: $(TAC_{DPPH} + TAC_{ABTS} + TAC_{FRAP})/3$. Three replicates were considered per lighting treatment in each experiment, sampling day and block.

2.9. Chlorophylls and carotenoids

Chlorophylls determination was performed following the methodology from Martínez Hernández et al. (2011). Frozen samples (-80 °C) were grinded in darkness, and 0.5 g of sample were dissolved in hexane and a dilution of methanol/acetone (1:2, v/v) in Falcon tubes placed on an ice bed. The extracts were shacked at 200 × g for 4 h in darkness. The extract was then added to 25 mL of NaCl 1 M and the mix was then shacked in a vortex (Heidolph Reax Control, Kelheim, Germany). The supernatant was used to determine absorbance at 662, 644 and 470 nm for chlorophylls and 470 nm for carotenoids, in a UV-visible spectrophotometer (Hewlet Packard 8453, Columbia, MD, USA). Contents of chlorophylls and carotenoids were calculated following the procedure from Wellburn (1994), and expressed as mg kg^{-1} FW. Three replicates were considered per lighting treatment in each experiment, sampling day and block.

2.10. Statistical analyses

The experiment used a randomized block design with two blocks, that were fully randomized within the cold chamber when moving to the second experiment (**Fig. 1**). The data presented are the mean ± standard deviation (SD) of at least 3 different replicates per block in two independent experiments per each species. Weight loss was analyzed through one-way ANOVA (considering significant differences at $P \leq 0.05$) by comparing the weight loss in 7 trays per each treatment after 10 d of storage. For all qualitative parameters, the effects of storing rocket and red chard leaves under different light conditions were tested by performing t-tests to compare these parameters measured in light conditions against their measures in products stored in darkness (considering significant differences at $P \leq 0.05$). In detail, at each time point and for each light treatment, the values were rescaled by subtracting the corresponding mean value at the time zero from them. Given a light treatment and a variable, the rescaled values corresponding to the different time points were compared by using paired t-tests against 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, one-tail paired t-tests of the null hypothesis against the alternative hypothesis was performed. The null hypothesis was that data of the difference between x-y were a random sample from a normal distribution, a mean of 0 and an unknown variance; the alternative hypothesis was that the mean was smaller than 0 in the case of '+' label or greater than 0 in the case of '−' label (Loi et al., 2019). The analysis was conducted using SPSS 15.0 (Statistical Package for the Social Science for Windows, IBM, Armonk, New York, USA).

3. RESULTS

3.1. Weight loss

Samples of both red chard and rocket leaves stored in darkness lost about 4 % of their weight along the 10 days of storage. A greater weight loss after 10 d at 5 ºC was associated with the presence of light (**Table 1**). Statistically significant differences were observed

among lighting treatments, with the lowest reductions occurred in yellow and far red light treated samples (13 and 11 % respectively in red chard, and 11 and 10 % respectively in rocket), and the highest biomass decreases observed under blue and white light (38 and 31 % respectively in red chard, and 31 and 27 % respectively in rocket). Also, under green and red light relevant reductions of biomass were observed (24 and 27 % respectively in red chard, 20 and 22 % respectively in rocket), which were not statistically different from those observed under white light (**Table 1**).

3.2. Colour determinations, carotenoids and total chlorophyll

Modifications in both lightness (L*) and hue angle (h°) in response to different spectral components were observed (**Fig. 3**). L* index of all illuminated red chard samples did not show statistically significant differences as compared to control samples stored in darkness for both sampling time (**Fig. 3A**). However, in rocket L* index was increased after 7 d under red and blue light as compared to control condition (7.9 and 6.4 % respectively), and after 10 d under red, yellow, white, blue and far red light (**Fig. 3B**). With reference to h° index, a statistically significant decrease was found after 7 d in red chard leaves from control samples stored in darkness when green and white lights were used (17.9 and 19.9 %, respectively) (**Fig. 3C**). After 10 d, the decrease was confirmed for samples stored under green light, but also observed in those stored under red, yellow, blue, and far red light (**Fig. 3C**). In rocket, only green and white light preserved h° value at the same level of control samples stored in darkness in both sampling dates, while the adoption of other lighting treatments resulted in a reduction (**Fig. 3D**). Carotenoids content was initially increased in red chard after 7 d in all lighting treatments, except far red, but only remained higher at 10 d in samples stored under green and blue light (20.7 and 18.2% as compared to control samples stored in darkness, respectively) (**Fig. 4A**). Concurrently, a decrease (19.4 %) in rocket leaves was associated with blue light only after 10 d at 5 ºC (**Fig. 4B**). With reference to leaf chlorophylls content, initial values were higher in red chard than in rocket. However, while for red chard leaves it markedly decreased during storage, values for rocket leaves changed slightly. No effects of lighting regimes were evidenced in red chard leaves after 7 d at 5 ºC (**Fig. 4C**), but total chlorophyll concentration resulted lowered in all treatments as compared to control samples stored in darkness at the end of storage, with the only exclusion of white light

treated samples **(Fig. 4C**). Conversely, leaf chlorophylls resulted to be higher as compared to darkness conditions in rocket stored under red (32.7 %), green (25.1 %), yellow (21.1 %) and blue (17.9 %) light for 7 d or under yellow and white light (14.6 and 19.3 %, respectively) for 10 d (**Fig. 4D**).

3.3. Microbiological load

The microbial growth was affected by light treatments (**Fig. 5**). The initial counts of enterobacteria were very low (1.58 and 1.65 log CFU g^{-1} in rocket and red chard, respectively), indicating the effectiveness of disinfection by chlorine. Differences between treatments were only evident after 10 d of storage in red chard, with a statistically significant reduction in the load as compared to control samples stored in darkness (3.94 log CFU g^{-1}) in response to yellow (2.64 log CFU g^{-1}) and blue light (2.90 log CFU g^{-1}) (**Fig. 5A**). Enterobacteria load was reduced in rocket leaves after 7 d at 5 ºC under yellow light (**Fig. 5B**), while after 10 d only green light enabled to reduce the enterobacteria load (**Fig. 5B**), as compared to control samples. Conversely, an increase from dark stored samples was evidenced in rocket when far red light was supplied (4.19 vs 5.08 log CFU g^{-1}) (**Fig. 5B**). In red chard, the use of yellow and blue light resulted in a reduction of psychrophilic bacteria count as compared to samples stored in darkness at 5 °C after 10 d, while no differences were evidenced after 7 d of storage (**Fig. 5C**). On the other hand, a lower psychrophilic growth in rocket leaves was evidenced after 7 d at 5 ºC under yellow light as compared to control samples stored in darkness (3.95 vs 4.81 log CFU g^{-1}) (**Fig. 5D**). Mould and yeasts counts were always low, as expected for leafy vegetables, and increased in red chard after 7 d of storage under red light as compared to control samples (3.08 vs 2.52 log CFU g^{-1}) , whereas they featured a decreased growth after 10 d in red, yellow, white, and blue illuminated samples (**Fig. 5E**). Finally, mould and yeast counts provided were not affected by the applied lighting regimes on rocket leaves (**Fig. 5F**).

3.4. Antioxidant activity and total phenols content

Light during storage resulted in statistically significant increases of antioxidant compounds in both red chard and rocket leaves (**Fig. 6**). Specifically, in red chard increases were evident after both 7 and 10 d for ABTS under all the considered lighting

regimes as compared to control condition (**Fig. 6A**). In rocket, after 7 d of storage, ABTS values resulted increased in red and blue treated samples (20.4 and 18.6 %, respectively) as compared to control sample stored in darkness, whereas after 10 d, this increase in ABTS resulted evident in green, yellow, white and blue lighting treatments (**Fig. 6B**). Similarly, in both species, DPPH was increased under red and blue light already at 7 d (**Fig. 6C** and **6D**) as compared to control sample stored in darkness, while at 10 d it was increased by all lighting treatments in red chard (**Fig. 6C**), and under red, green, yellow and blue light in rocket (**Fig. 6D**). The FRAP was higher at 7 d in leaves of red chard stored under red, green, yellow, white and blue light (**Fig. 6E**), whereas in rocket the increase was associated with red, green, yellow, and blue light (**Fig. 6F**). Moreover, after 10 d of storage, it was increased by all lighting treatments in red chard (**Fig. 6E**), and all lighting treatments except yellow and far red in rocket (**Fig. 6F**). When TAC was calculated, the trend observed in FRAP was also replicated, with increases at 7 d associated with red, green, yellow, white, and blue light in red chard (**Fig. 6G**) and with red, green, and blue light in rocket (**Fig. 6H**). Moreover, at 10 d at 5 ºC, all lighting regimes increased TAC in both species as compared to control samples stored in dark (**Fig. 6G** and **Fig. 6H**), with the exclusion of far red in rocket (**Fig. 6H**). When looking at a single class of antioxidant components, total phenols were increased at 7 days under red, yellow, white and blue light in both red chard (**Fig. 4E**) and rocket (**Fig. 4F**) (with the exclusion of green light) as compared to darkness storage condition, whereas an increase was observed in all lighting treatments after 10 days of storage in both species.

4. DISCUSSION

4.1. Light during storage increases weight losses

Senescence is a process that rapidly occurs in horticultural crops, beginning immediately after harvesting and, besides compositional changes, weight loss is one of the most visible symptoms (Loi et al., 2019). The elevate weight loss of leafy vegetables during storage is generally associated with their low volume-to-surface ratio (Kasim and Kasim, 2012). Observed weight losses during darkness storage were in the range of 4 % of the initial biomass, in accordance with previous literature (Roura et al., 2000; Miceli and Miceli, 2014), resulting always greater in samples stored under light (**Table 1**). The decrease of biomass in response to light during storage has been previously associated with the

preserved photosynthetic activity of plant tissues (Ferrante et al., 2003; Ogweno et al., 2009), which may have resulted in greater water loss through leaf transpiration (Olarte et al., 2009). Accordingly, weight losses up to 30 % of the initial weight were formerly observed in lettuce leaves stored for 7 d while exposed to continuous white light supplying 50 to 150 µmol m^{-2} s^{-1} (Charles et al., 2018). Similarly, Brussels sprout (*Brassica oleracea* var. *gemmifera*) exposed to white and blue LED treatments featured weight losses of around 15 % as compared to values of 6 % observed in control samples stored in darkness (Hasperué et al., 2016). Dehydration was even higher when stored baby mustard buds (*Brassica juncea* var. gemmifera) were exposed to continuous light for 6 d, reaching values of 45 to 70 % of weight loss from the initial biomass (Sun et al., 2020). Alternatively, when stomatal closure is induced by darkness, leaf transpiration is minimized and therefore fresh weight is preserved in this condition (Roura et al., 2000). However, transpiration rates are highly dependent on daily light integrals (Arve et al., 2013), as previously observed when growing plants of lettuce and basil (*Ocimum basilicum* L.) were exposed to variable light intensity (Pennisi et al., 2020a) or photoperiod (Pennisi et al., 2020b). Accordingly, when light photoperiod during storage is increased also weight loss increases are observed (Kasim and Kasim, 2012). Interestingly, more evident weight losses were observed in those treatments (e.g., blue and white) that included a blue fraction within their spectrum (**Table 1**). During crop growth, the effect of blue light in fostering stomatal opening has been previously evidenced e.g., in cucumber (Hogewoning et al., 2010), cherry tomato (XiaoYing et al., 2011), basil (Pennisi et al., 2019b) and lettuce (Pennisi et al., 2019c). It may be therefore advanced that also during storage, the blue spectral fraction allows for conserved stomatal opening and results in increased transpiration fluxes which ultimately causes greater fresh biomass losses. Albeit the observed weight losses were extremely high (and could result in non-marketability of the samples), it should be acknowledged that the adopted experimental conditions were functional to the research but different from standard commercial management. For instance, opting for single leaf layers (to ensure uniformity in illumination) or continuous lighting supply may have primarily resulted in the elevate transpiration. Optimization of package size and headspace as well as photoperiod, light spectral composition and light intensity should be achieved.

425 **4.2. Green and white light help preserving color properties and pigment content**
426 **during storage**

427 The economic value of horticultural commodities is negatively affected by the presence
428 of visible symptoms of senescence, given that consumer preference mainly builds on the
429 visual appearance (Kader, 2001). In previous research, white cool fluorescent light during
430 storage was shown to alter colorimetric properties of rocket (*Eruca sativa* Mill.), Swiss
431 chard (*Beta vulgaris* L.) and chicory (*Chicorium intybus*) as compared with control
432 samples stored in darkness, due to increased chlorophyll degradation in illuminated
433 samples, concurrent with changes in other pigments (e.g., carotenoids) (Ferrante et al.,
434 2004). In green leafy species, a loss of visual appearance due to senescence was
435 previously associated with an increase in lightness index and a decrease in hue angle,
436 which results in leaf yellowing (Conversa et al., 2014). In the current experiment, green
437 light was the lighting regime which did not affect L* index and, also together with white
438 light, h° of rocket in both sampling dates (**Fig. 3B, 3D**). Senescence in harvested green
439 organs generally involves the massive degradation of chlorophylls and chloroplast
440 proteins (Bárcena et al., 2020). Besides, light is considered one of the most important
441 exogenous factors which can regulate senescence progress, due to the fact that harvested
442 leaves maintain their capacity to respond to light stimuli activating biological responses
443 mediated by photoreceptors, and these responses depend on light quality and intensity
444 (Noodén and Schneider, 2004). Indeed, photo-oxidative damage may occur when elevate
445 light intensity results in chlorophyll breakdown (Muñoz and Munné-Bosch, 2018). In our
446 study, total chlorophylls content of rocket leaves was not altered or even increased upon
447 lighting (**Fig. 4D**). It emerges that appropriate light management may enable to finely
448 tune the balance between functional metabolism (e.g., preservation of chlorophylls and
449 carotenoids) and leaf senescence (e.g., modifications of visual appearance). Alternatively,
450 when referring to red leafy species as red chard, a reduction in hue angle values stands
451 for color change from green to red, altogether with unaltered lightness, is considered as a
452 positive attribute, and is also commonly related to an increase in specific secondary
453 metabolites (e.g., anthocyanins and carotenoids) (Conesa et al., 2015). Red chard leaves
454 lightness (L*) was not modified during both sampling dates by lighting regimes (**Fig.**
455 **3A**), and h° values was reduced by green and white light after 7 d of storage, and by red,
456 green, yellow, blue and far red light after 10 d (**Fig. 3C**). This variation in h° values can
457 be associated with the increase in carotenoids experienced under all light regimes (with

458 the exclusion of far red light) after 7 d of storage, and under green and blue light after 10
459 d (**Fig. 4A)**. The quite similar effects emerging from the application of white and green
460 lights can be attributed to the fact that white light used in the current experiments
461 contained 40 % of green light (500-600 nm). The differential effect of light may suggest
462 that senescence and colorimetric changes result from more complex causes than only
463 transpiration fluxes and biomass losses, and may find explanations in the biochemical and
464 microbiological changes that take place in response to light (Noichinda et al., 2007), as
465 further addressed in the following sections.

466

467 **4.3. Light during storage reduces microbiological load in red chard and rocket**
468 **leaves**

469 In the current research, specific spectral regions were found to limit microbiological
470 growth during storage, contrasting with previous evidences (e.g., on broccoli and
471 cauliflower, *Brassica rapa* var *botrytis*), where no changes in the microbial counts were
472 found comparing a control in darkness and the application of white cool fluorescent
473 lighting during storage (Olarte et al., 2009). The most consistent trend resulted in the
474 inhibition of epiphytic microflora growth associated with yellow and blue light (**Fig.5**).
475 This antimicrobial effect of LED light, also known as photodynamic inactivation, is due
476 to the activity of endogenous photosensitizers that absorb visible light wavelengths for its
477 activation to form reactive oxygen species, whose action results in biomolecules
478 oxidation and cells lysis (Purushothaman and Mol., 2021). This is the case of porphyrins
479 with blue light, whose antibacterial effect is well known in literature (Lipovsky et al.,
480 2010; Maclean et al., 2014; Wang et al., 2017). However, some results where blue light
481 did not affect microbiological load already exist (Castillejo et al., 2021). As compared to
482 blue light, yellow light has been less studied, and while previous evidences have already
483 suggested the potential of yellow light in inhibiting fungal growth (Velmurugan et al.,
484 2010), no former studies have, to our knowledge, targeted its effect on bacterial growth.
485 In the current research, also the adoption of red light resulted in a decreasing effect against
486 mould and yeast growth in red chard (at 10 d) (**Fig. 5E**). Similar results are available in
487 literature, with counts of mould and yeast previously shown to decrease in response to
488 red light during storage in tomato (Fuller et al., 2013) and broccoli sprouts (Castillejo et
489 al., 2021). In addition, bacterial growth was successfully reduced by application of red

light during in-vitro experiments (Yu and Lee, 2013). Such results confirm the previously observed antibacterial (Ghate et al., 2013) and antifungal (Alferez et al., 2012) potentialities of red light, formerly associated with increased phospholipase D and octanal biosynthesis within the plant tissues, both involved in the resistance to fungal infection and growth (Alferez et al., 2012; Yamaga et al., 2015). As formerly reported, white light had little effect on microbiological load (Olarte et al., 2009), with significant decreases observed in mould and yeast counts on 10 d stored red chard (**Fig. 5E**). Finally, an increased load of enterobacteria after 10 d of rocket storage was found in response to far red light (**Fig. 5B**). It is commonly acknowledged that far red light – involved in plant photomorphogenesis and acting as protector of cellular components and functionalities (Mihaly Cozmuta et al., 2016; Bantis et al., 2018) – may counteract bacterial growth during storage, as previously found in tomatoes supplied with 25 µmol m^{-2} s^{-1} of far red lighting. Indeed, far red growth inhibition of both bacteria (Gan and Bryant, 2015) and fungi (Mooney and Yager, 1990) was also shown to depend on their capability to acclimate and restore functionalities. On the other hand, far led light during cultivation was also shown to reduce plant capacity to deal with microbiological infections (Cerrudo et al., 2012; Courbier et al., 2020), therefore suggesting that amount of far red light supplied should be carefully quantified in order to avoid undesired effects on crop health status (Demotes-Mainard et al., 2016).

4.4. Red and Blue light more effectively increase antioxidant properties during storage

Major effects of postharvest light treatments on total phenols concentration in both red chard and rocket leaves were associated with either red or blue light (**Fig. 4E, F**). Consistently, red and blue applications were found to significantly increase TAC values by all methods (ABTS, DPPH, and FRAP) in both studied species throughout storage (**Fig. 6**), as compared with samples stored in darkness. The efficacy of red light towards promoting antioxidant properties of plant products has been previously observed in Chinese kale (*Brassica oleracea* var. *alboglabra* Bailey) sprouts (Deng et al., 2016). Further evidences on pea (*Pisum sativum* L.) sprouts (Liu et al., 2016) exposed to darkness or to a light intensity of 30 µmol m^{-2} s^{-1} of either white, red or blue light suggested that blue light more largely contributed to increased antioxidant properties.

Conversely, red light during postharvest was found to more largely contribute than blue light in promoting antioxidant properties (expressed as FRAP) of blueberry (*Vaccinium corymbosum* L.) leaves (Routray et al., 2018). However, the existence of a genotypic variability in the antioxidant response to either red, blue or white light was confirmed in different typologies of fresh-cut pepper (*Capsicum annuum*) (Maroga et al., 2019), overall constraining the possibility to drive univocal recommendations on the light spectrum to be used. Similarly, it appears difficult from the hereby presented experiments to discriminate whether red or blue light is more efficient in preserving antioxidant properties of the studied leafy vegetables, also in comparison with other adopted lighting treatments.

5. CONCLUSIONS

Our results confirmed that light during storage of red chard and rocket leaves may foster beneficial effects in terms of epiphytic microflora growth and total antioxidant activity up to 10 d at 5 ºC. Reduced microbial counts were mainly observed in samples stored under yellow and blue lights, while green and white lights enabled to preserve visual quality by reducing chlorophyll degradation in rocket leaves and by enhancing carotenoids concentration in red chard, as compared to control samples stored in darkness. Although a general increase of total antioxidant activity was observed among all light treatments, the most consistent increases were those associated with red and blue light. Accordingly, future research should target an exploration on how to finely tune combinations between light spectral composition (e.g., on yellow, green or red light) and light integrals in order to target specific improvements of quality and storability of different ready-to-eat vegetable species. This would allow to confirm the potential of LED supply during storage for improving quality of baby leaves, by specifically targeting the reduction of the hereby observed dehydration, which was higher under the white or blue treatments. The observed physiological adaptations (e.g., weight loss due to dehydration and water loss) to light during storage may also affect leaf gas concentration and equilibrium between O_2 and CO_2 in the headspace of the tray, which may overall alter sample storability. Accordingly, further research should specifically target the application of shorter lighting periods, pulsed lighting technology or the combination of light with optimized package design and the use of modified atmosphere.

DECLARATIONS

Author contributions statement

Giuseppina Pennisi: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; wrote the paper.

Noelia Castillejo: contributed to lab analyses; reviewed the paper.

Perla A. Gómez: contributed to technical design of the experiments; interpreted the data; reviewed the paper.

Andrea Crepaldi: contributed in R&D of LED materials.

Juan A. Fernández: reviewed the paper.

Catalina Egea-Gilabert: reviewed the paper.

Giorgio Gianquinto: Interpreted the data; reviewed the paper.

Francisco Artés–Hernández: interpreted the data; contributed reagents, materials, analysis tools and/or data; reviewed the paper.

Francesco Orsini: contributed materials; analyzed and interpreted the data; wrote the paper.

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Competing interest statement

Andrea Crepaldi was employed by company Flytech s.r.l. The remaining authors declare no competing interests.

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TABLES

Table 1. Relative weight loss (% from initial weight) of fresh-cut red chard and rocket leaves after 10 d at 5 °C in response to monochromatic light (35 μmol m^{-2} s^{-1}) in different spectral regions (red, green, yellow, white, blue and far red) or a control in darkness.

	Dark		Red		Green		Yellow		White		Blue		Far Red	
Red Chard	4.3 ±1.2	d	26.8 ±2.0	b	24.1 ±5.0	b	13.2 ±2.3	c	31.5 ±4.9	ab	38.2 ±5.6	a	11.2 ±2.1	c
Rocket	3.9 ±0.9	d	22.0 ±2.0	b	19.8 ±4.2	b	11.4 ±1.9	c	27.1 ±4.0	ab	31.0 ±4.5	a	10.0 ±1.7	c

Mean values ± SD from 7 independent trays per lighting treatment per each experiment (n=14). Different letters indicate significant differences between treatments at P≤0.05.

889

890 **FIGURE CAPTIONS**

891 **Fig. 1.** (A) Randomized block experimental design used for the experiments and images
892 taken in the cold room of the containers (B) and from above the lamp, showing the layout
893 of trays within each container (C). The same experimental design was adopted in each of
894 the two crops under assessment.

895 **Fig. 2.** Spectral features of the LED lamps used in the experiments. Relative spectral
896 composition in red (A), green (B), yellow (C), white (D), blue (E) and far red (F)
897 treatments. All lamps continuously supplied a photosynthetic photon flux density (PPFD)
898 of 35 μmol m^{-2} s^{-1}.

899 **Fig. 3.** Main colorimetric indexes expressed as L* (A, B) and h° (C, D) in red chard (A,
900 C) and rocket leaves (B, D) in response to LED lighting (35 μmol m^{-2} s^{-1}) during 7 and
901 10 days at 5 °C storage. Per each light treatment, * stands for significant differences at
902 P≤0.05 calculated through t-test by comparing values under light conditions against
903 samples in darkness, based on the difference from the measured value at the beginning of
904 the experiment. Mean values ±SD from 10 replicates in 2 blocks in 2 independent
905 experiments (n=40).

906

907 **Fig. 4.** Carotenoids (A, B), total chlorophylls (C, D) and total phenols (E, F) in red chard
908 (A, C, E) and rocket (B, D, F) leaves in response to LED lighting (35 μmol m^{-2} s^{-1}) during
909 7 and 10 days at 5 °C storage. Per each light treatment, * stands for significant differences
910 at P≤0.05 calculated through t-test by comparing values under light conditions against
911 samples in darkness, based on the difference from the measured value at the beginning of
912 the experiment. Mean values ±SD from 3 replicates in 2 blocks in 2 independent
913 experiments (n=12).

914

915 **Fig. 5.** Enterobacteria (A, B), psychrophilic microbial loads (C, D), and moulds and yeasts
916 (E, F), respectively in red chard (A, C, E) and rocket leaves (B, D, F) in response to LED
917 lighting (35 μmol m^{-2} s^{-1}) during 7 and 10 days at 5 °C storage. Per each light treatment,
918 * stands for significant differences at P≤0.05 calculated through t-test by comparing
919 values under light conditions against samples in darkness, based on the difference from

920 the measured value at the beginning of the experiment. Mean values ±SD from 3
921 replicates in 2 blocks in 2 independent experiments (n=12).

922

923 **Fig. 6.** Total antioxidant capacity by ABTS (A, B), DPPH (C, D), FRAP (E, F) and TAC
924 (G, H) methods, respectively in red chard (A, C, E, G) and rocket leaves (B, D, F, H) in
925 response to LED lighting (35 μmol m^{-2} s^{-1}) during 7 and 10 days at 5 °C storage. Per each
926 light treatment, * stands for significant differences at $P \leq 0.05$ calculated through t-test by
927 comparing values under light conditions against samples in darkness, based on the
928 difference from the measured value at the beginning of the experiment. Mean values ±SD
929 from 3 replicates in 2 blocks in 2 independent experiments (n=12).

930

931

Figure 1.

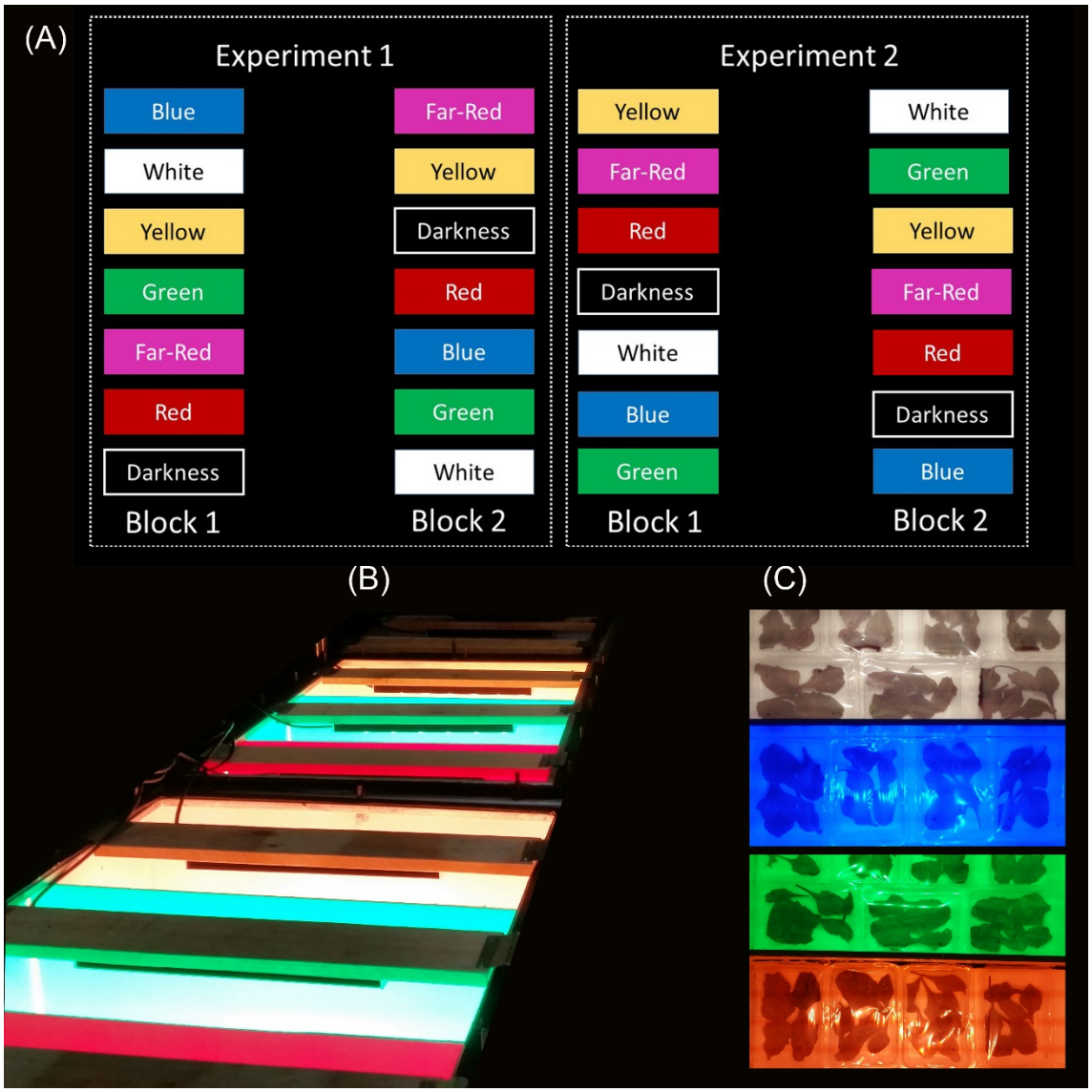
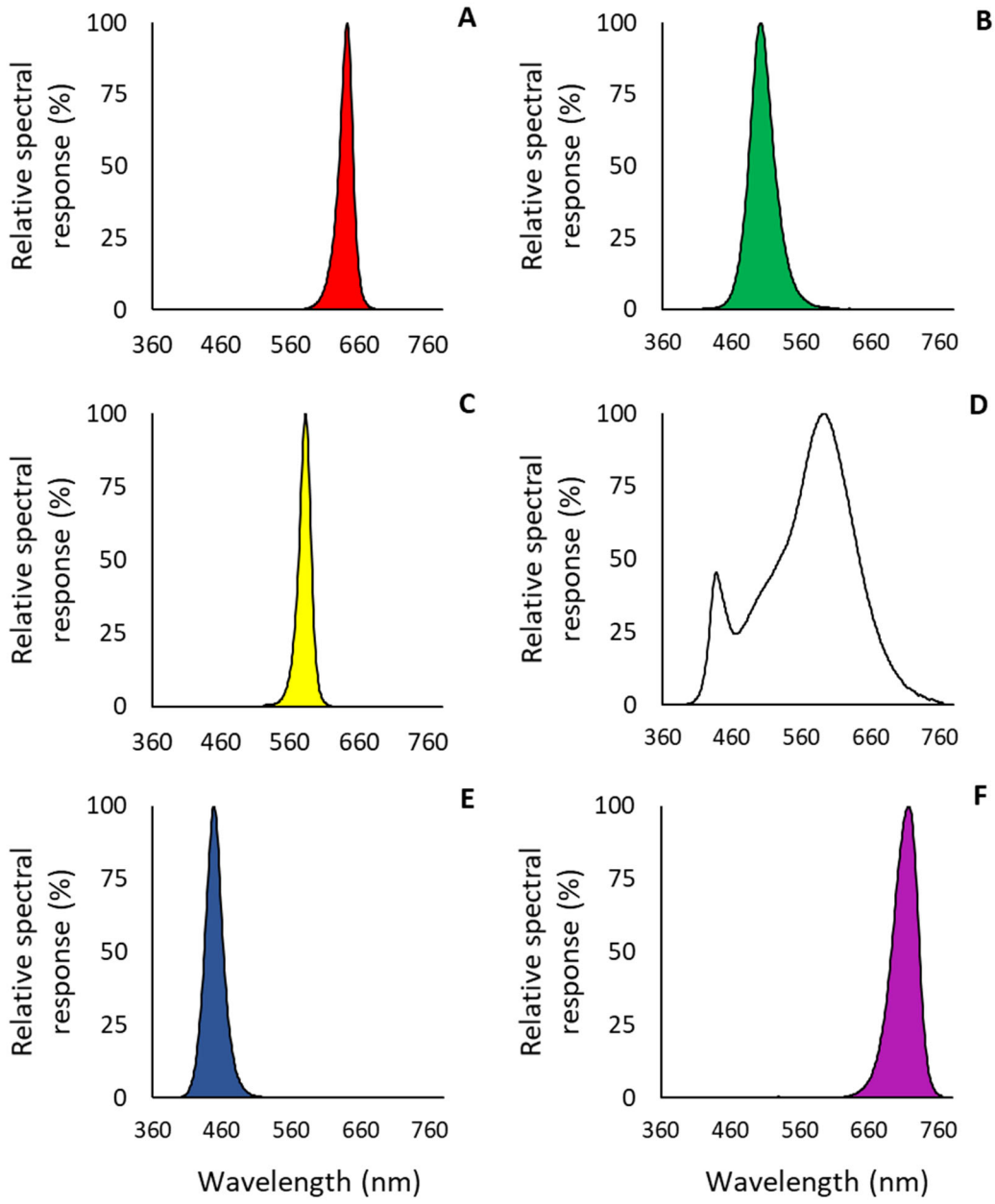
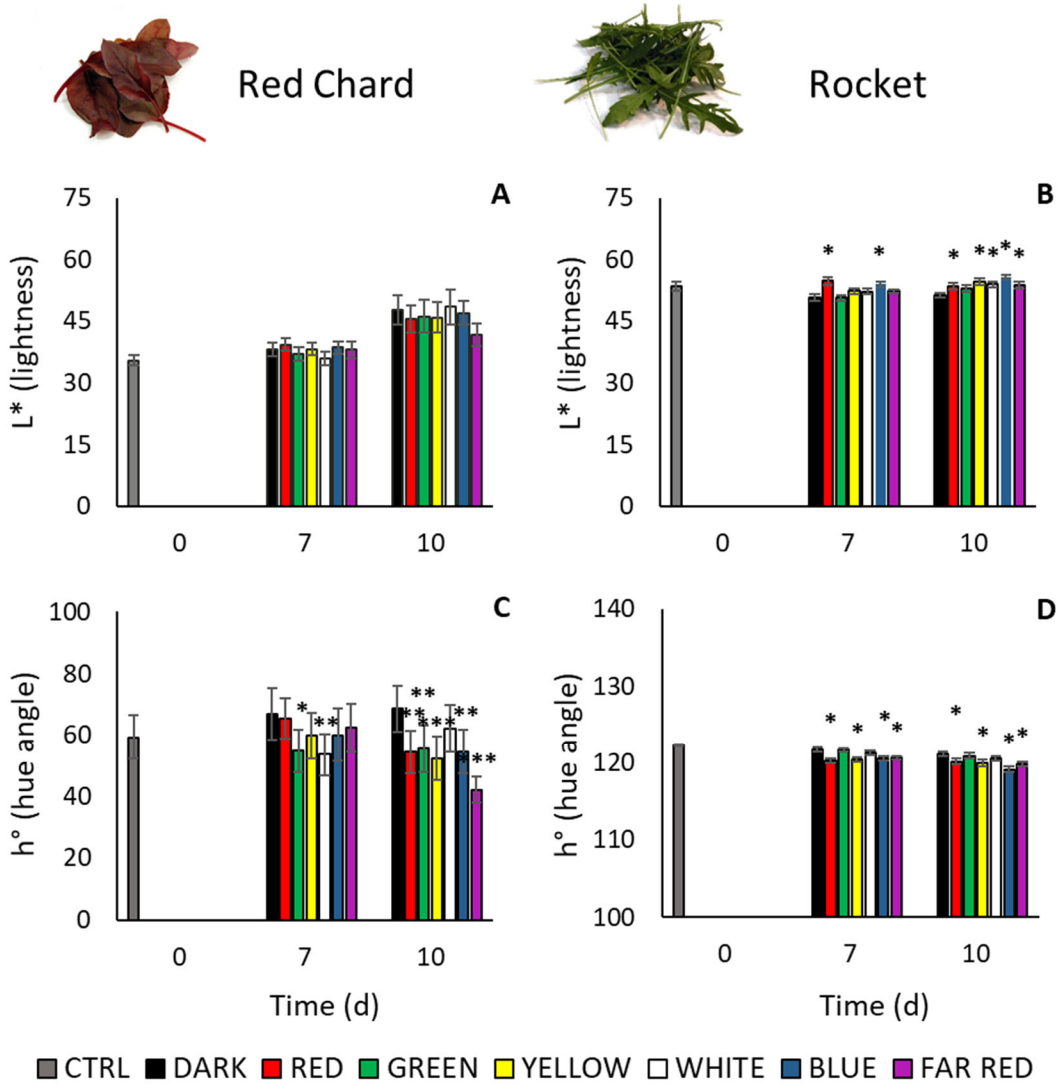

(A)
Experiment 1
Blue
White
Yellow
Green
Far-Red
Red
Darkness
Block 1
Far-Red
Yellow
Darkness
Red
Blue
Green
White
Block 2
Experiment 2
Yellow
Far-Red
Red
Darkness
White
Blue
Green
Block 1
White
Green
Yellow
Far-Red
Red
Darkness
Blue
Block 2
(B)
(C)

Figure 2.


A
B
C
D
E
F
Relative spectral response (%)
100
75
50
25
0
360
460
560
660
760
Wavelength (nm)

937 **Figure 3.**



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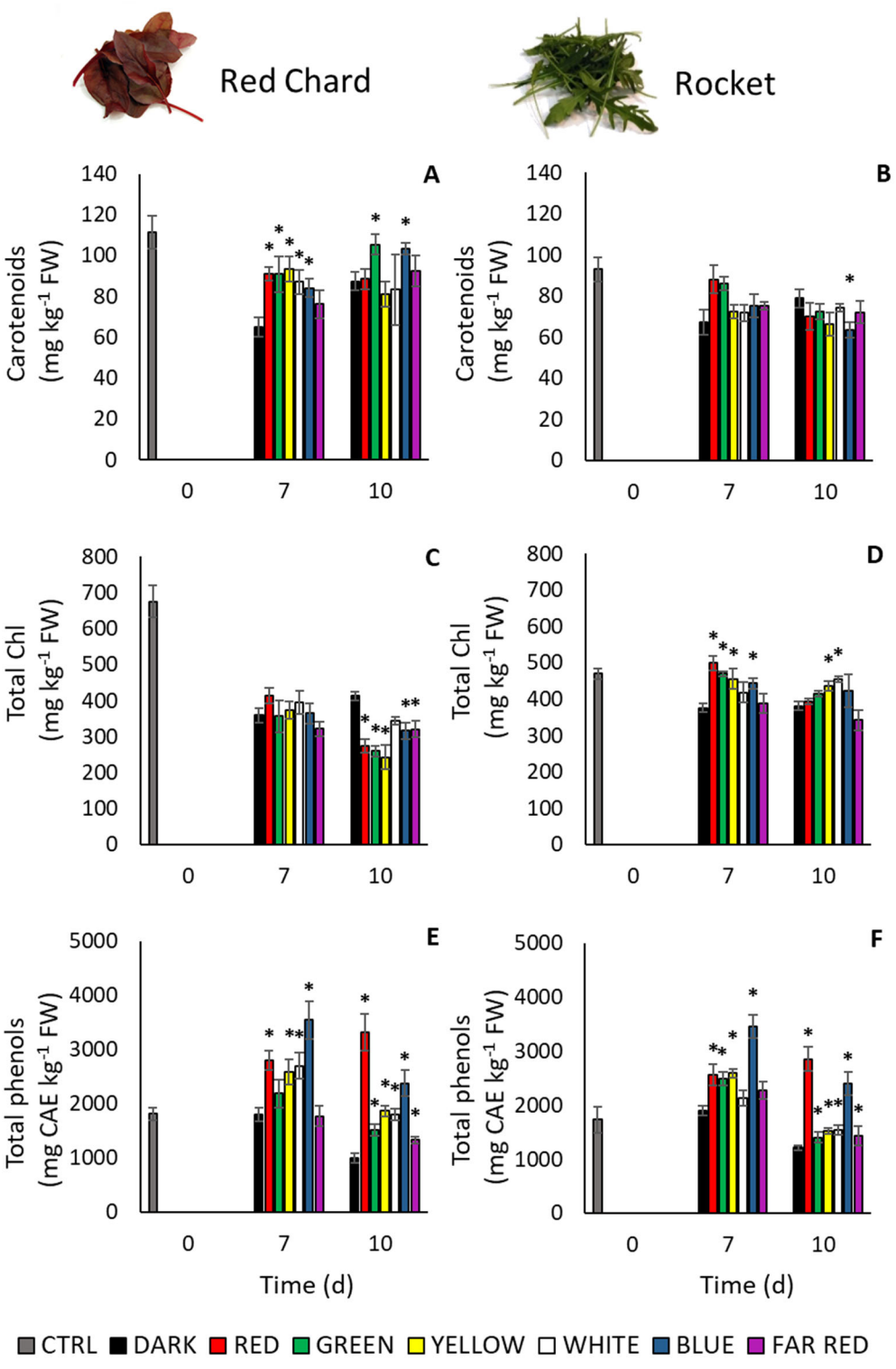
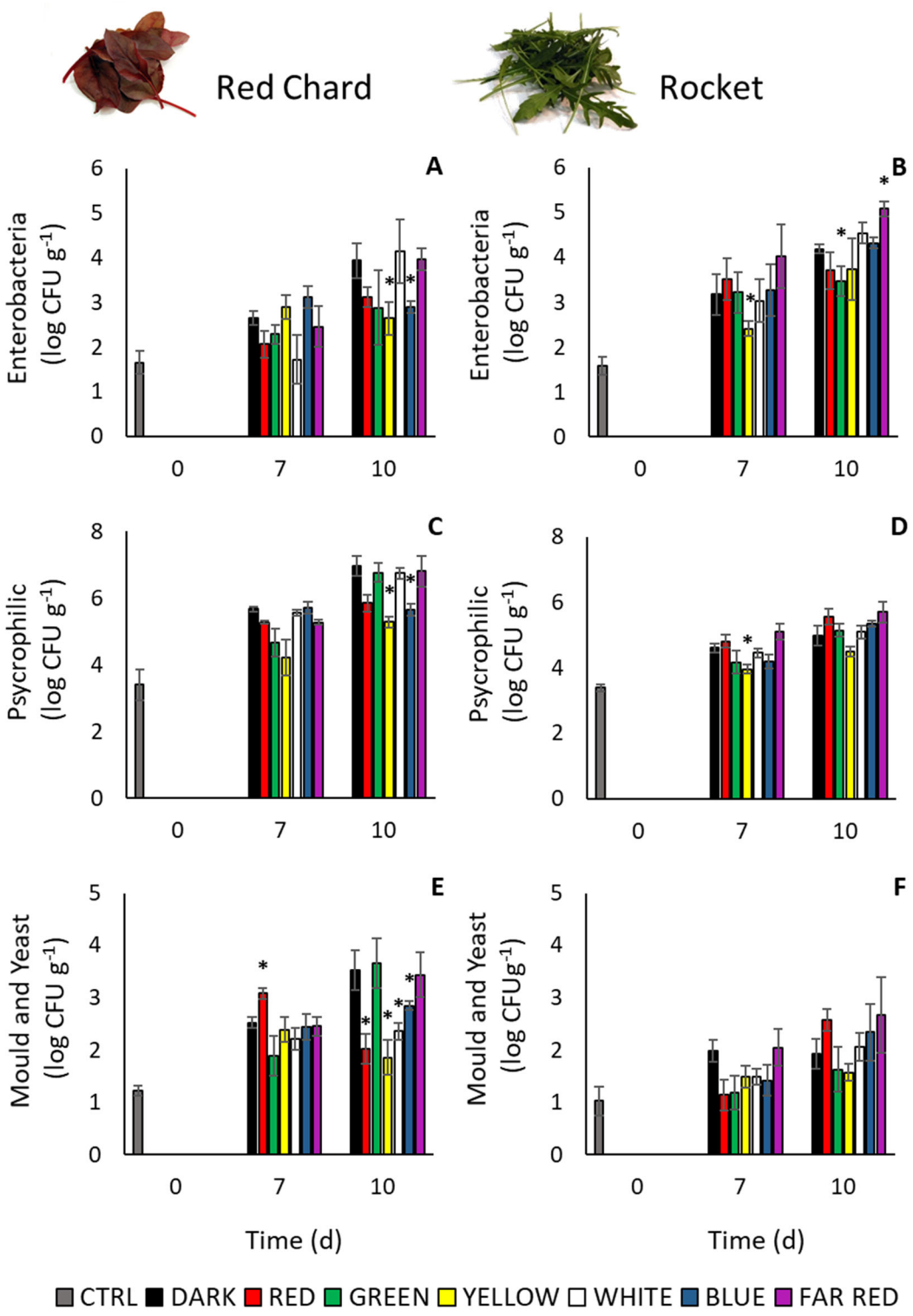
939

940 **Figure 4.**



941

942 **Figure 5.**



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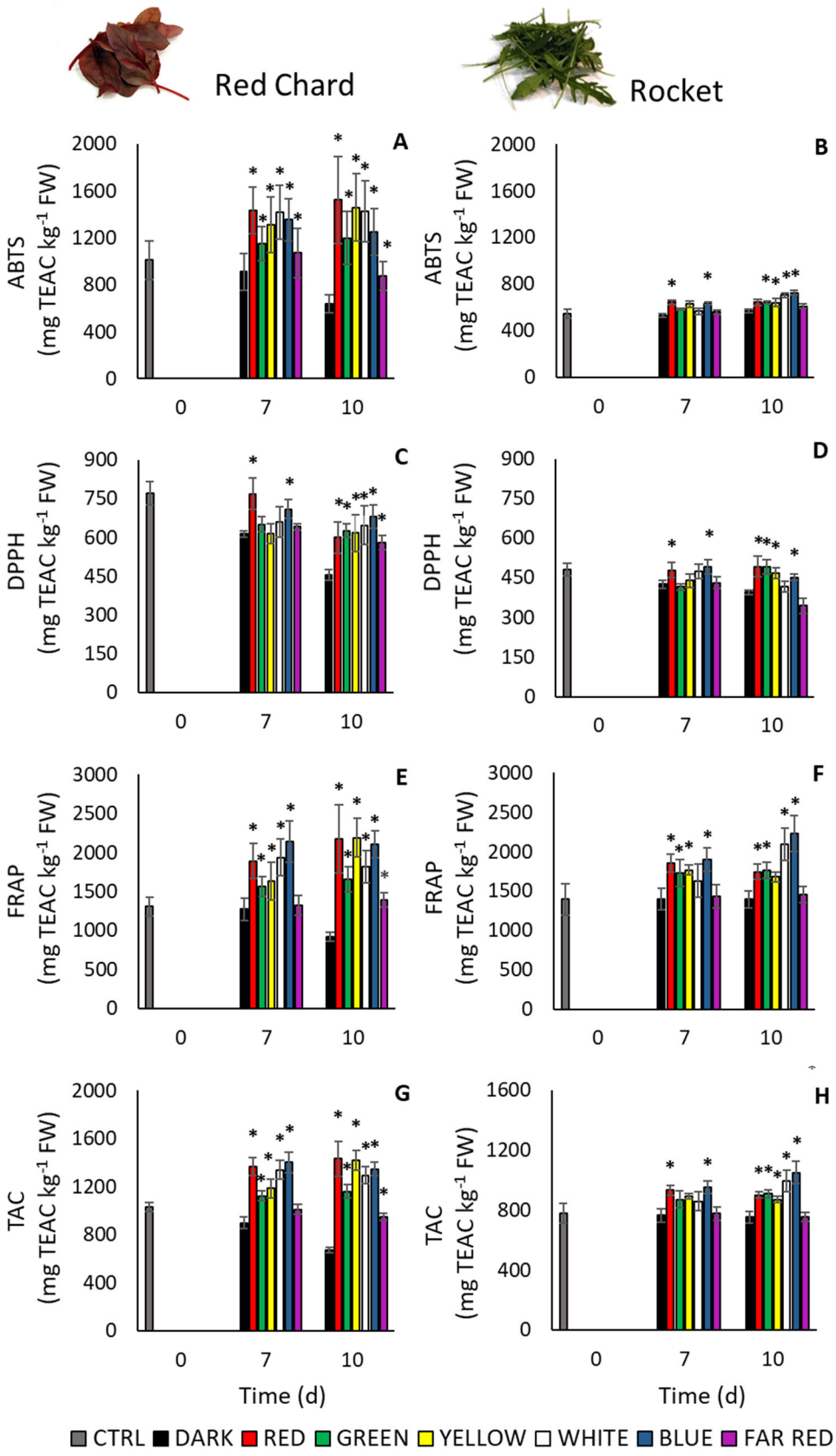
944

945 **Figure 6.**



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