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

1. INTRODUCTION

1.1. Postharvest losses and horticultural food waste

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

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

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

111 reference to blue light during storage of leafy vegetables were available at the time of this
112 study, in strawberry stored at 5 °C, blue LED light (at 40 µmol m^{-2} s^{-1}), was shown to
113 increase the content of both total anthocyanin and the associated enzymes (Xu et al.,
114 2014). Furthermore, postharvest far red LED illumination of minimally processed
115 broccoli sprouts was also recently shown to increase morphological development and the
116 total antioxidant and scavenging activities while decreasing the microbial growth during
117 15 d at 5 ºC (Castillejo et al., 2021)
118

119 **1.4. Aim of the research**

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

131

132 **2.- MATERIALS AND METHODS**

133 **2.1. Plant material**

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

upward) in order to guarantee uniform leaf illumination during storage. 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), which was manually perforated with a needle creating four holes (0.8 mm ϕ) to avoid an atmosphere modification and ensure air partial pressures.

2.2. Storage conditions and light treatments during shelf life

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

2.3. Weight loss

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

177

178 **2.4. Colour determinations**

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

188

189 **2.5. Microbial analyses**

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

202

203 **2.6. Extracts preparation**

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

210

211 **2.7. Total phenols**

212 Total phenols were determined as previously described by Singleton and Rossi (1965).
213 Briefly, 19 µL sample extract were placed on a flat-bottom PS 96-well plate (Greiner Bio-
214 One; Frickenhausen, Germany) and 29 µL of 1 mol L^{-1} Folin–Ciocalteu reagent were
215 added. The latter mixture was incubated for 3 min in darkness at room temperature. Then,
216 192 µL of 0.4 % Na_2CO_3 and 2 % NaOH were added. After 1 h incubation at room
217 temperature in darkness, the absorbance was measured at 750 nm using a microplate
218 reader (Tecan Infinite M200, Männedorf, Switzerland). Following the same methodology
219 applied for the samples, a calibration curve (R^2=0.996) was obtained by measuring the
220 absorbance of solutions of decreasing concentrations of chlorogenic acid, prepared by
221 diluting a concentrated solution (300 mg L^{-1}). Total phenols were expressed as mg
222 chlorogenic acid equivalents (CAE) kg^{-1} fresh weight (FW). Three replicates were
223 considered per lighting treatment in each experiment, sampling day and block.

224

225 **2.8. Total antioxidant capacity**

226 Total Antioxidant Capacity (TAC) was analysed by using three different methods: DPPH
227 (2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical method), FRAP (Ferric Reducing
228 Antioxidant Power), and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
229 radical scavenging assay) assays. DPPH assay was performed following the method
230 described by Castillejo et al. (2017). For that, 194 µL of DPPH (0.7 mM in methanol,
231 absorbance at 1.1±0.02) solution were added to 21 µL of leaf extract. The mixture was
232 incubated for 30 min at room temperature in darkness. The TAC by DPPH was measured
233 by changes in absorbance at 515 nm. The scavenging activity (%) was calculated using
234 the next formula: [(Abs DPPH − Abs Sample)/Abs DPPH] × 100. ABTS assay was

235 carried out following the method previously described by Rodríguez-Verástegui et al.
236 (2016). For that, 200 µL of the activated ABTS solution (32 µM) were added to 11 µL of
237 leaf extract in a 96-well plate and incubated for 30 min at room temperature in darkness.
238 The TAC by ABTS was measured by changes in absorbance at 414 nm. The scavenging
239 activity (%) was calculated using the next formula: [(Abs ABTS − Abs Sample)/Abs
240 ABTS] × 100. The FRAP method was also developed following the method described by
241 Castillejo et al. (2017). A daily reaction solution containing sodium acetate buffer (pH
242 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution (in 40 mM HCl) and 20 mM
243 $FeCl_3$ was prepared in a v/v/v proportion of 10:1:1 and incubated at 37 ºC for 2 h in
244 darkness. Then, 198 µL of FRAP solution were added to 6 µL of leaf extract and incubated
245 for 1 h at room temperature in darkness. The TAC by FRAP was measured by changes in
246 absorbance at 593 nm. Following the same methodology applied for the samples, three
247 calibration curves were calculated, one for DPPH (R^2=0.997), one for ABTS (R^2=0.969),
248 and one for FRAP (R^2=0.999) assays, by measuring the absorbance of solutions
249 containing decreased concentrations of Trolox, prepared by diluting a concentrated
250 solution (300 mg L^{-1}). Obtained data were expressed as mg of Trolox Equivalents
251 Antioxidant Capacity (TEAC) kg^{-1} FW. Total Antioxidant Capacity (TAC) index was
252 calculated using the next equation: (TAC_{DPPH} + TAC_{ABTS} + TAC_{FRAP})/3. Three replicates
253 were considered per lighting treatment in each experiment, sampling day and block.

254

255 **2.9. Chlorophylls and carotenoids**

256 Chlorophylls determination was performed following the methodology from Martínez
257 Hernández et al. (2011). Frozen samples (-80 °C) were grinded in darkness, and 0.5 g of
258 sample were dissolved in hexane and a dilution of methanol/acetone (1:2, v/v) in Falcon
259 tubes placed on an ice bed. The extracts were shacked at 200 × g for 4 h in darkness. The
260 extract was then added to 25 mL of NaCl 1 M and the mix was then shacked in a vortex
261 (Heidolph Reax Control, Kelheim, Germany). The supernatant was used to determine
262 absorbance at 662, 644 and 470 nm for chlorophylls and 470 nm for carotenoids, in a UV-
263 visible spectrophotometer (Hewlet Packard 8453, Columbia, MD, USA). Contents of
264 chlorophylls and carotenoids were calculated following the procedure from Wellburn
265 (1994), and expressed as mg kg^{-1} FW. Three replicates were considered per lighting
266 treatment in each experiment, sampling day and block.

267

268 **2.10. Statistical analyses**

269 The experiment used a randomized block design with two blocks, that were fully
270 randomized within the cold chamber when moving to the second experiment (**Fig. 1**). The
271 data presented are the mean ± standard deviation (SD) of at least 3 different replicates per
272 block in two independent experiments per each species. Weight loss was analyzed
273 through one-way ANOVA (considering significant differences at $P \leq 0.05$) by comparing
274 the weight loss in 7 trays per each treatment after 10 d of storage. For all qualitative
275 parameters, the effects of storing rocket and red chard leaves under different light
276 conditions were tested by performing t-tests to compare these parameters measured in
277 light conditions against their measures in products stored in darkness (considering
278 significant differences at $P \leq 0.05$). In detail, at each time point and for each light
279 treatment, the values were rescaled by subtracting the corresponding mean value at the
280 time zero from them. Given a light treatment and a variable, the rescaled values
281 corresponding to the different time points were compared by using paired t-tests against
282 those obtained in the dark control condition. Each variable was assigned a label of '+' or
283 '−' according to if it was desirable to increase or decrease its intensity, respectively. In
284 more detail, for x, the value of the variable in the 'dark' control condition, and y, the value
285 of the same variable in the 'light' condition, one-tail paired t-tests of the null hypothesis
286 against the alternative hypothesis was performed. The null hypothesis was that data of the
287 difference between x-y were a random sample from a normal distribution, a mean of 0
288 and an unknown variance; the alternative hypothesis was that the mean was smaller than
289 0 in the case of '+' label or greater than 0 in the case of '−' label (Loi et al., 2019). The
290 analysis was conducted using SPSS 15.0 (Statistical Package for the Social Science for
291 Windows, IBM, Armonk, New York, USA).

292

293 **3. RESULTS**

294 **3.1. Weight loss**

295 Samples of both red chard and rocket leaves stored in darkness lost about 4 % of their
296 weight along the 10 days of storage. A greater weight loss after 10 d at 5 ºC was associated
297 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

330 treated samples (**Fig. 4C**). Conversely, leaf chlorophylls resulted to be higher as
331 compared to darkness conditions in rocket stored under red (32.7 %), green (25.1 %),
332 yellow (21.1 %) and blue (17.9 %) light for 7 d or under yellow and white light (14.6 and
333 19.3 %, respectively) for 10 d (**Fig. 4D**).

334

335 **3.3. Microbiological load**

336 The microbial growth was affected by light treatments (**Fig. 5**). The initial counts of
337 enterobacteria were very low (1.58 and 1.65 log CFU g^{-1} in rocket and red chard,
338 respectively), indicating the effectiveness of disinfection by chlorine. Differences
339 between treatments were only evident after 10 d of storage in red chard, with a statistically
340 significant reduction in the load as compared to control samples stored in darkness (3.94
341 log CFU g^{-1}) in response to yellow (2.64 log CFU g^{-1}) and blue light (2.90 log CFU g^{-1})
342 (**Fig. 5A**). Enterobacteria load was reduced in rocket leaves after 7 d at 5 ºC under yellow
343 light (**Fig. 5B**), while after 10 d only green light enabled to reduce the enterobacteria load
344 (**Fig. 5B**), as compared to control samples. Conversely, an increase from dark stored
345 samples was evidenced in rocket when far red light was supplied (4.19 vs 5.08 log CFU
346 g^{-1}) (**Fig. 5B**). In red chard, the use of yellow and blue light resulted in a reduction of
347 psychrophilic bacteria count as compared to samples stored in darkness at 5 °C after 10
348 d, while no differences were evidenced after 7 d of storage (**Fig. 5C**). On the other hand,
349 a lower psychrophilic growth in rocket leaves was evidenced after 7 d at 5 ºC under yellow
350 light as compared to control samples stored in darkness (3.95 vs 4.81 log CFU g^{-1}) (**Fig.
351 5D**). Mould and yeasts counts were always low, as expected for leafy vegetables, and
352 increased in red chard after 7 d of storage under red light as compared to control samples
353 (3.08 vs 2.52 log CFU g^{-1}) , whereas they featured a decreased growth after 10 d in red,
354 yellow, white, and blue illuminated samples (**Fig. 5E**). Finally, mould and yeast counts
355 provided were not affected by the applied lighting regimes on rocket leaves (**Fig. 5F**).

356

357 **3.4. Antioxidant activity and total phenols content**

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

361 regimes as compared to control condition (**Fig. 6A**). In rocket, after 7 d of storage, ABTS
362 values resulted increased in red and blue treated samples (20.4 and 18.6 %, respectively)
363 as compared to control sample stored in darkness, whereas after 10 d, this increase in
364 ABTS resulted evident in green, yellow, white and blue lighting treatments (**Fig. 6B**).
365 Similarly, in both species, DPPH was increased under red and blue light already at 7 d
366 (**Fig. 6C** and **6D**) as compared to control sample stored in darkness, while at 10 d it was
367 increased by all lighting treatments in red chard (**Fig. 6C**), and under red, green, yellow
368 and blue light in rocket (**Fig. 6D**). The FRAP was higher at 7 d in leaves of red chard
369 stored under red, green, yellow, white and blue light (**Fig. 6E**), whereas in rocket the
370 increase was associated with red, green, yellow, and blue light (**Fig. 6F**). Moreover, after
371 10 d of storage, it was increased by all lighting treatments in red chard (**Fig. 6E**), and all
372 lighting treatments except yellow and far red in rocket (**Fig. 6F**). When TAC was
373 calculated, the trend observed in FRAP was also replicated, with increases at 7 d
374 associated with red, green, yellow, white, and blue light in red chard (**Fig. 6G**) and with
375 red, green, and blue light in rocket (**Fig. 6H**). Moreover, at 10 d at 5 ºC, all lighting
376 regimes increased TAC in both species as compared to control samples stored in dark
377 (**Fig. 6G** and **Fig. 6H**), with the exclusion of far red in rocket (**Fig. 6H**). When looking at
378 a single class of antioxidant components, total phenols were increased at 7 days under
379 red, yellow, white and blue light in both red chard (**Fig. 4E**) and rocket (**Fig. 4F**) (with
380 the exclusion of green light) as compared to darkness storage condition, whereas an
381 increase was observed in all lighting treatments after 10 days of storage in both species.

382

383 **4. DISCUSSION**

384 **4.1. Light during storage increases weight losses**

385 Senescence is a process that rapidly occurs in horticultural crops, beginning immediately
386 after harvesting and, besides compositional changes, weight loss is one of the most visible
387 symptoms (Loi et al., 2019). The elevate weight loss of leafy vegetables during storage is
388 generally associated with their low volume-to-surface ratio (Kasim and Kasim, 2012).
389 Observed weight losses during darkness storage were in the range of 4 % of the initial
390 biomass, in accordance with previous literature (Roura et al., 2000; Miceli and Miceli,
391 2014), resulting always greater in samples stored under light (**Table 1**). The decrease of
392 biomass in response to light during storage has been previously associated with the

393 preserved photosynthetic activity of plant tissues (Ferrante et al., 2003; Ogweno et al.,
394 2009), which may have resulted in greater water loss through leaf transpiration (Olarte et
395 al., 2009). Accordingly, weight losses up to 30 % of the initial weight were formerly
396 observed in lettuce leaves stored for 7 d while exposed to continuous white light
397 supplying 50 to 150 μmol m^{-2} s^{-1} (Charles et al., 2018). Similarly, Brussels sprout
398 (*Brassica oleracea* var. *gemmifera*) exposed to white and blue LED treatments featured
399 weight losses of around 15 % as compared to values of 6 % observed in control samples
400 stored in darkness (Hasperué et al., 2016). Dehydration was even higher when stored baby
401 mustard buds (*Brassica juncea* var. gemmifera) were exposed to continuous light for 6 d,
402 reaching values of 45 to 70 % of weight loss from the initial biomass (Sun et al., 2020).
403 Alternatively, when stomatal closure is induced by darkness, leaf transpiration is
404 minimized and therefore fresh weight is preserved in this condition (Roura et al., 2000).
405 However, transpiration rates are highly dependent on daily light integrals (Arve et al.,
406 2013), as previously observed when growing plants of lettuce and basil (*Ocimum*
407 *basilicum* L.) were exposed to variable light intensity (Pennisi et al., 2020a) or
408 photoperiod (Pennisi et al., 2020b). Accordingly, when light photoperiod during storage
409 is increased also weight loss increases are observed (Kasim and Kasim, 2012).
410 Interestingly, more evident weight losses were observed in those treatments (e.g., blue
411 and white) that included a blue fraction within their spectrum (**Table 1**). During crop
412 growth, the effect of blue light in fostering stomatal opening has been previously
413 evidenced e.g., in cucumber (Hogewoning et al., 2010), cherry tomato (XiaoYing et al.,
414 2011), basil (Pennisi et al., 2019b) and lettuce (Pennisi et al., 2019c). It may be therefore
415 advanced that also during storage, the blue spectral fraction allows for conserved stomatal
416 opening and results in increased transpiration fluxes which ultimately causes greater fresh
417 biomass losses. Albeit the observed weight losses were extremely high (and could result
418 in non-marketability of the samples), it should be acknowledged that the adopted
419 experimental conditions were functional to the research but different from standard
420 commercial management. For instance, opting for single leaf layers (to ensure uniformity
421 in illumination) or continuous lighting supply may have primarily resulted in the elevate
422 transpiration. Optimization of package size and headspace as well as photoperiod, light
423 spectral composition and light intensity should be achieved.

424

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

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

509

510 **4.4. Red and Blue light more effectively increase antioxidant properties during**
511 **storage**

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

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

532

533 **5. CONCLUSIONS**

534 Our results confirmed that light during storage of red chard and rocket leaves may foster
535 beneficial effects in terms of epiphytic microflora growth and total antioxidant activity
536 up to 10 d at 5 ºC. Reduced microbial counts were mainly observed in samples stored
537 under yellow and blue lights, while green and white lights enabled to preserve visual
538 quality by reducing chlorophyll degradation in rocket leaves and by enhancing
539 carotenoids concentration in red chard, as compared to control samples stored in darkness.
540 Although a general increase of total antioxidant activity was observed among all light
541 treatments, the most consistent increases were those associated with red and blue light.
542 Accordingly, future research should target an exploration on how to finely tune
543 combinations between light spectral composition (e.g., on yellow, green or red light) and
544 light integrals in order to target specific improvements of quality and storability of
545 different ready-to-eat vegetable species. This would allow to confirm the potential of LED
546 supply during storage for improving quality of baby leaves, by specifically targeting the
547 reduction of the hereby observed dehydration, which was higher under the white or blue
548 treatments. The observed physiological adaptations (e.g., weight loss due to dehydration
549 and water loss) to light during storage may also affect leaf gas concentration and
550 equilibrium between O_2 and CO_2 in the headspace of the tray, which may overall alter
551 sample storability. Accordingly, further research should specifically target the application
552 of shorter lighting periods, pulsed lighting technology or the combination of light with
553 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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884 **TABLES**

885 **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
886 (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

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

888

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 $P \leq 0.05$ 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 $P \leq 0.05$ 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 $P \leq 0.05$ 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).

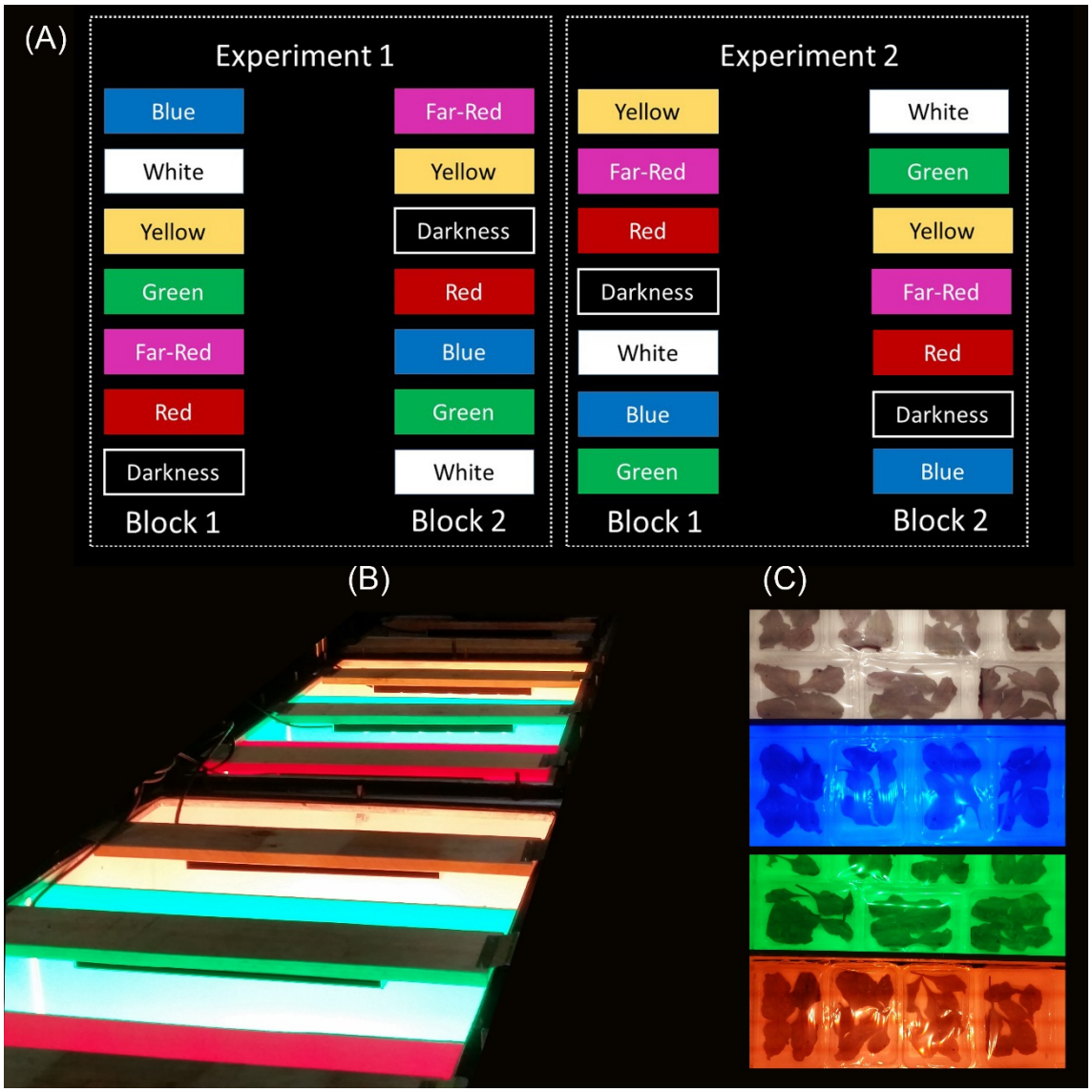
922

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)

935 **Figure 2.**

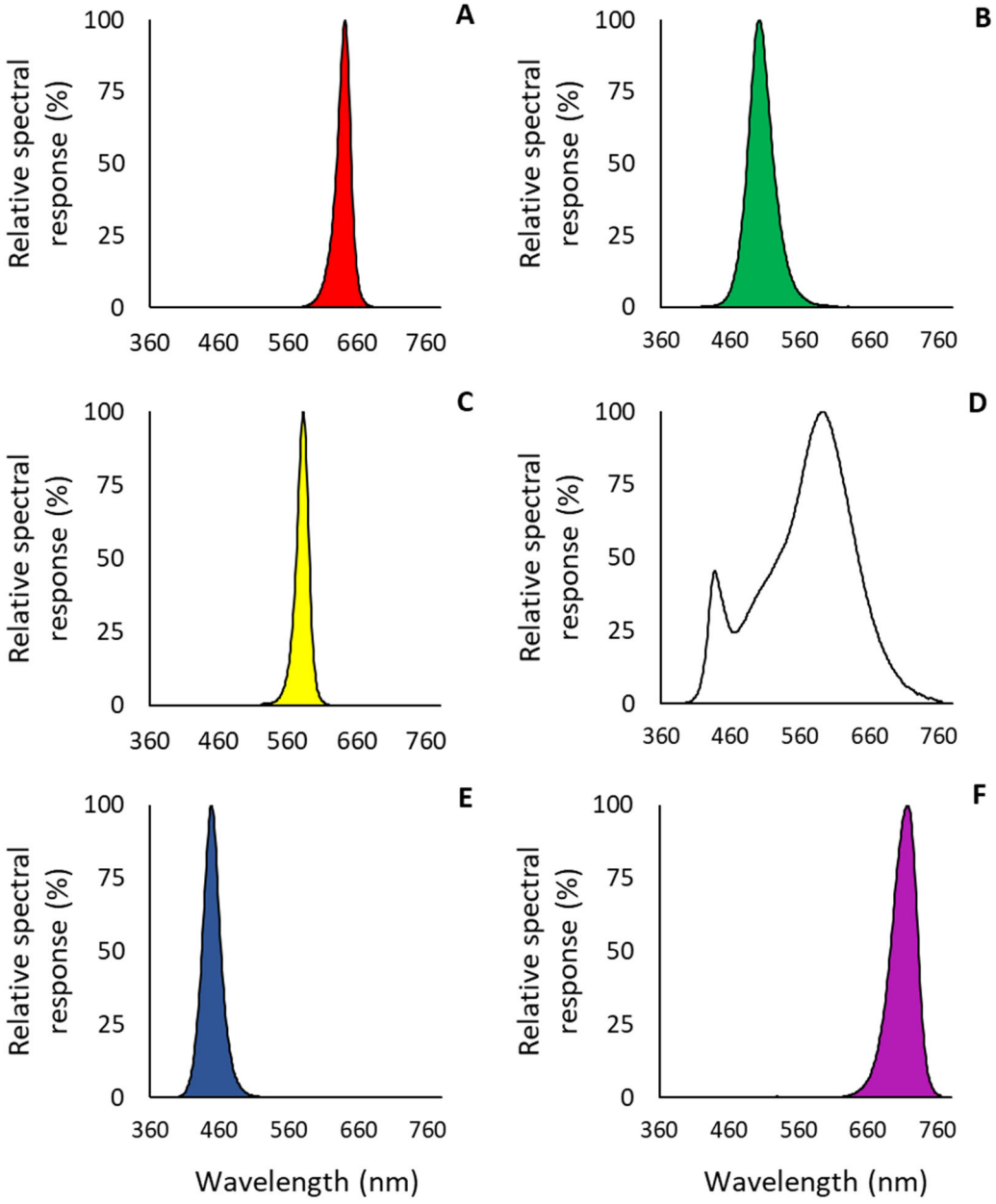


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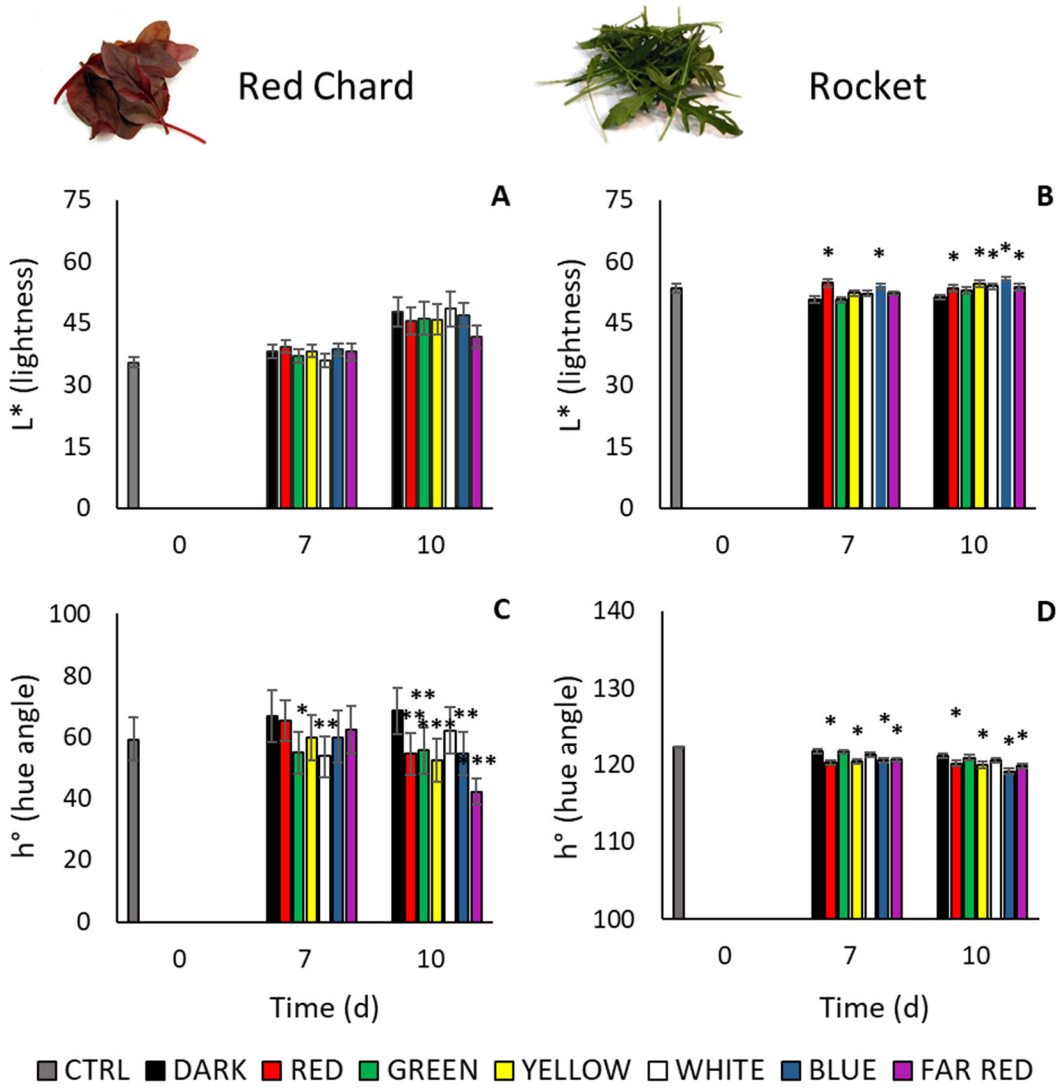
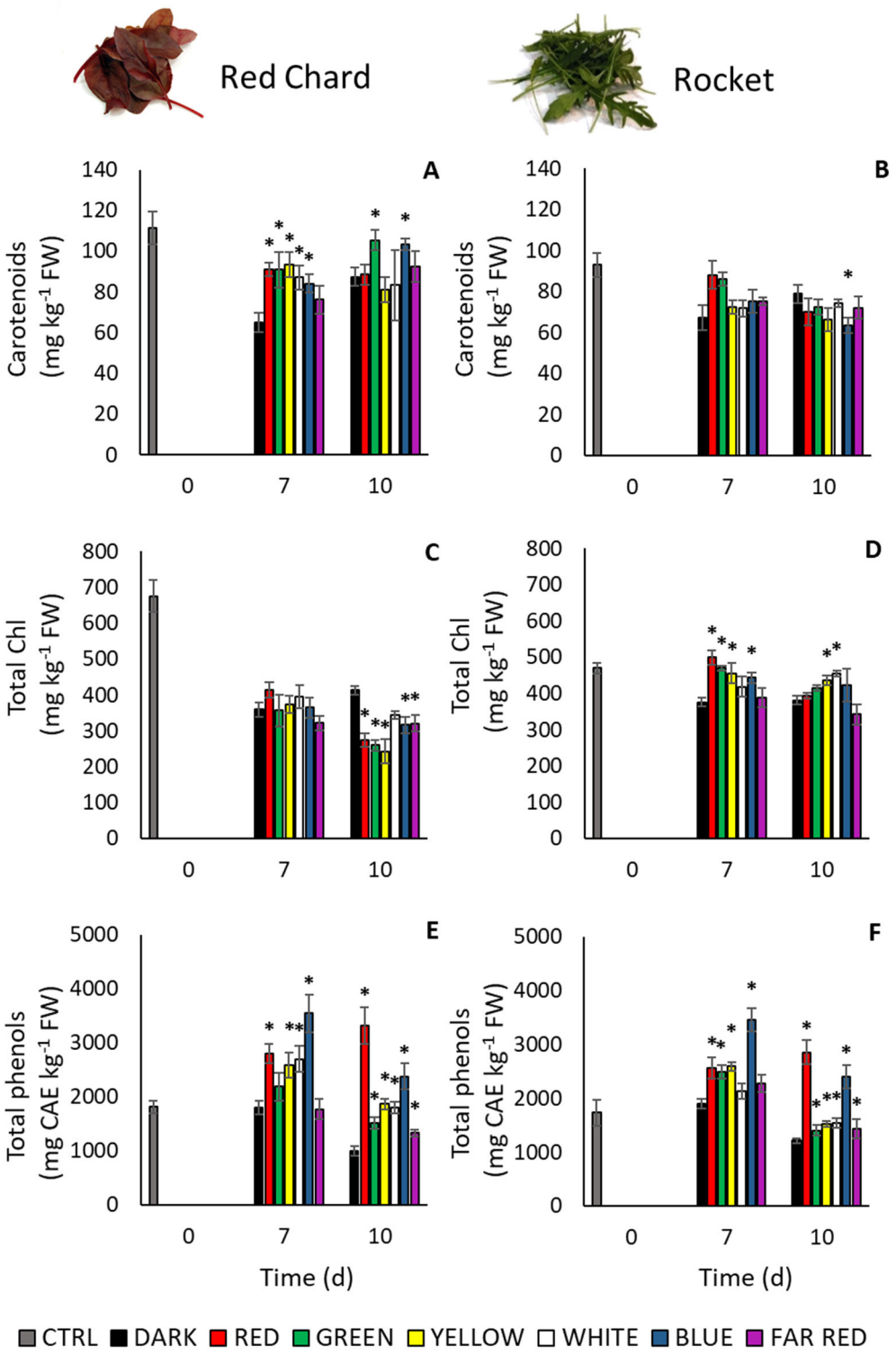
Figure 3.

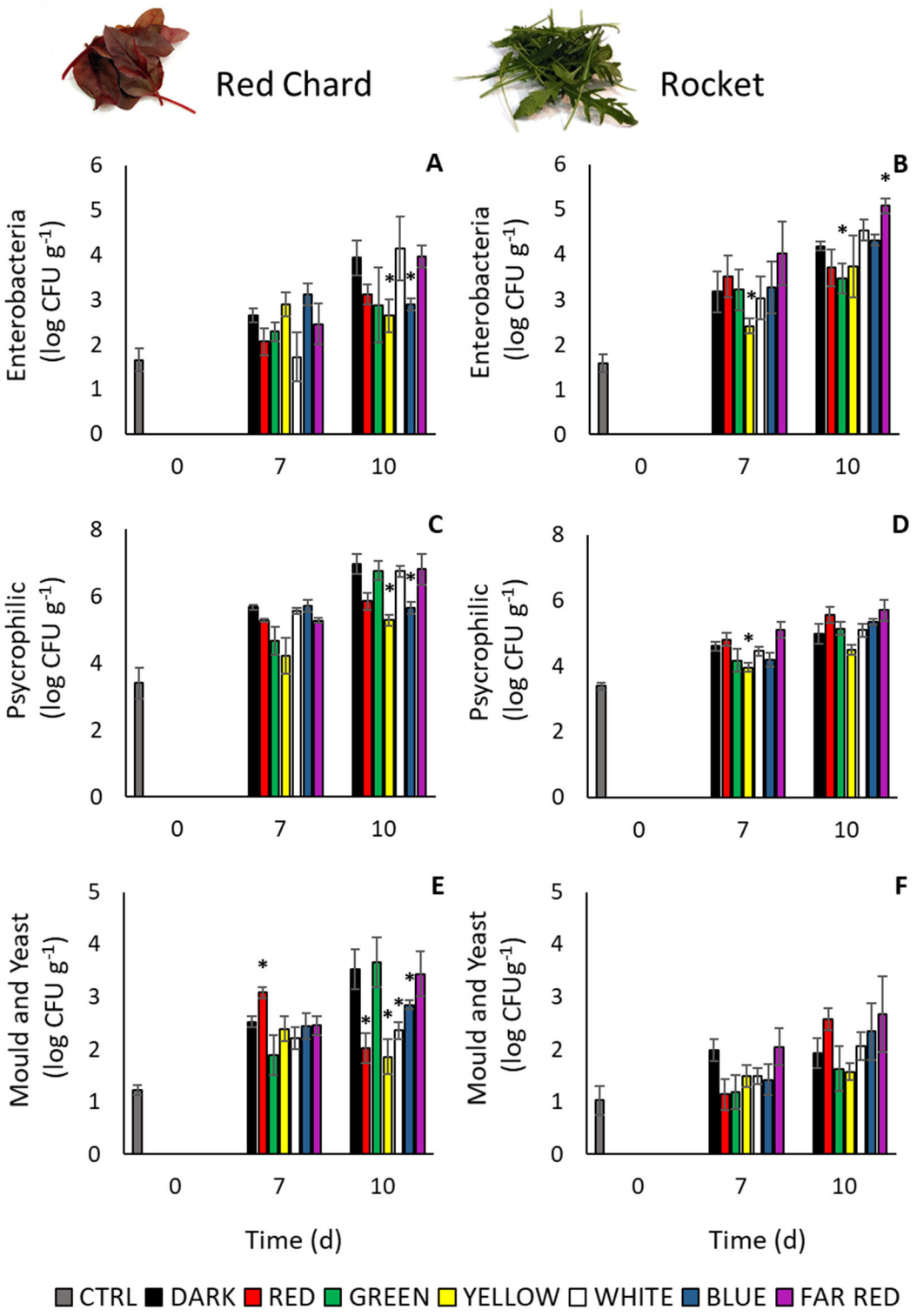

Red Chard
Rocket
A
B
C
D
L* (lightness)
h° (hue angle)
Time (d)
CTRL
DARK
RED
GREEN
YELLOW
WHITE
BLUE
FAR RED

940 **Figure 4.**



941

942 **Figure 5.**



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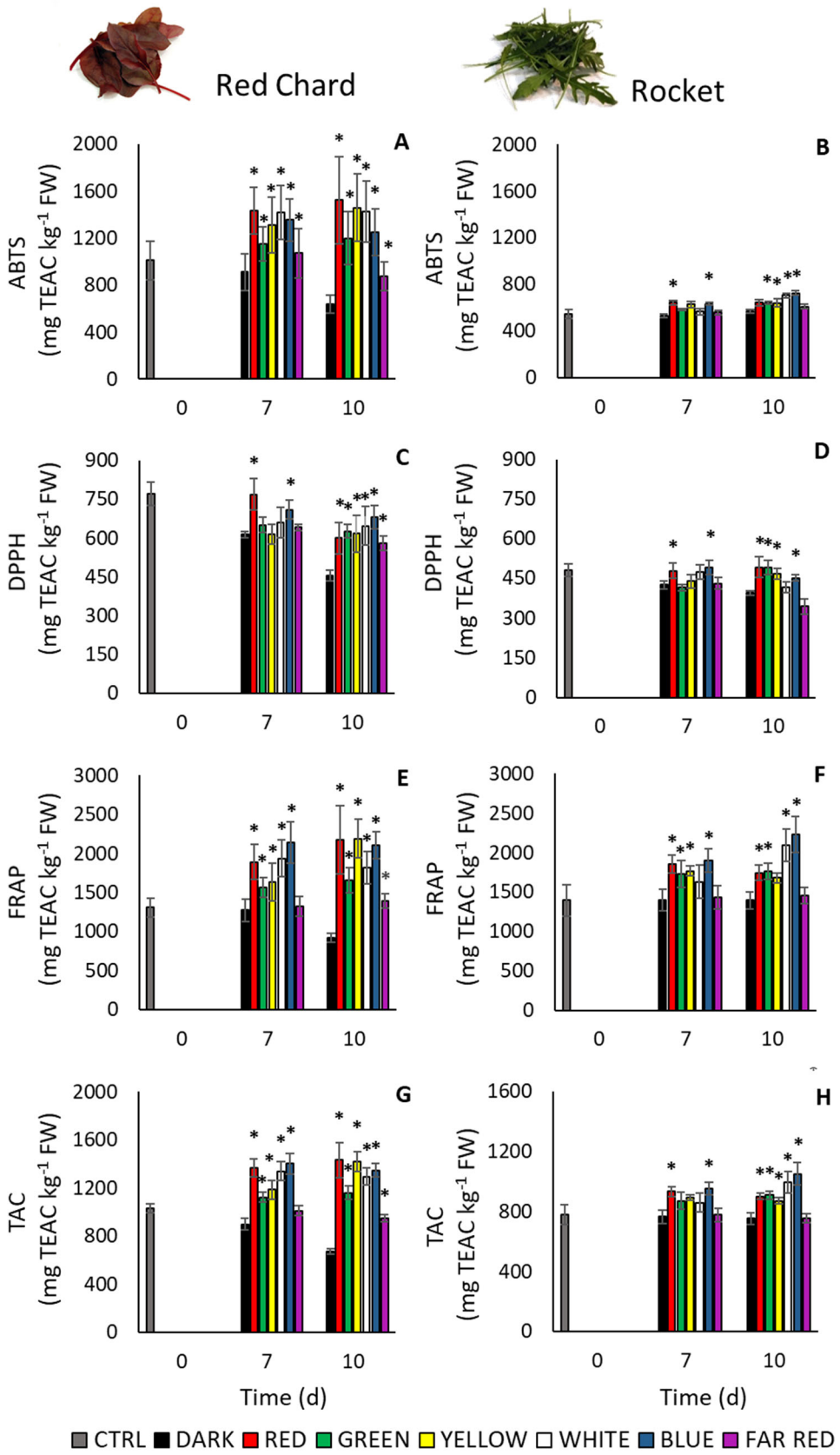
945 **Figure 6.**



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