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The Influence of Pre-Harvest LEDs on Phytochemical Constituents and Antioxidant Activity of Microgreens during Short-Term Storage

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Abstract: This study aims to evaluate the influence of the pre-harvest light-emitting diode (LED) spectrum on the metabolic indices in microgreens during post-harvest storage. Broccoli 'Micro Green' and kale 'Dwarf Blue Green' microgreens were cultivated in a growth chamber under the photosynthetic photon flux density (PPFD) of 200 µmol m^{-2} s^{-1} provided by violet (V, 405 nm), blue (B, 447 nm), green (G, 520 nm), and red (R_{638}, R_{665}, R—638 nm and 665 nm, or both, respectively) LEDs in combinations of BR_{638}, BR_{665}, BR, BRV, and BRG. We evaluated the total phenolic content (TPC), total protein (TP), chlorophyll (CHL), and carotenoid (CAR) contents, and the ferric-reducing antioxidant power (FRAP) and ABTS and DPPH free radical scavenging activities at harvest and during storage at 4 °C for five days in the dark. The results demonstrate that the influence of pre-harvest LEDs on the metabolic indices varied among microgreens species and decreased consistently throughout the post-harvest period. BRV treatment led to the highest TPC, CHL, and CAR in kale, and increased the DPPH radical scavenging activity in broccoli. The TP content was the highest in kale and broccoli under BR_{665} and BR lights, respectively. In addition, BR light had a similar impact on the antioxidant capacity at harvest day for both microgreens species. The TPC, CHL, and CAR contents were influenced by BR_{665} after one day from harvest.

Keywords: broccoli; grow light; kale; metabolites; pigments; shelf life

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1. Introduction

The WHO (World Health Organization) defines the increment of vegetables in daily diets as one of the primary tasks for a healthy society [1]. Green leafy vegetables are widely consumed worldwide due to high levels of health-promoting compounds such as fiber, vitamins, and mineral nutrients, which prevent chronic and non-infectious diseases [2]. However, fresh vegetables are highly perishable products, as plant tissue wounding due to detachment or cutting leads to changes in the physiological and biochemical processes and causes discoloration, increased respiration, loss of flavor and texture, loss of weight, and a decline in the levels of nutrition [3]. According to the Food and Agriculture Organization of the United Nations (FAO), about 33% of harvested vegetables are never consumed since they naturally have a short shelf life, leading to post-harvest loss and waste [4]. The wastage can be decreased by producing more high-quality vegetables with a longer shelf life, and both cultivation and post-harvest storage aspects are equally crucial for sustainable diets [5].

Microgreens are immature, fragile cotyledonary leafy greens that come in delicious flavors, various colors, and textures. In comparison to mature greens, microgreens typically

have higher quantities of phytonutrients. However, the shelf life of microgreens is only a few days. [6]. Recently, microgreen production has benefited from a variety of emerging cultivation systems in controlled environment agriculture (CEA), where artificial lighting is used. Greater attention has been paid to the influence of light-emitting diodes (LEDs) on fresh horticultural products. The higher intensity of lighting from blue (B), red (R), and far-red (FR) LEDs led to increased total phenolic contents (TPCs) in tatsoi and kohlrabi, red pak choi, and mustard microgreens [7]. A higher intensity of BRFR lighting led to higher total carotenoid (TC) contents in red pak choi, tatsoi, and mustard microgreens [8]. A supplemental green LED light increased the β-carotene content in mizuna [9], and increase the β-carotene and lutein contents of mustard and beet microgreens [10].

Although the lighting impact on CEA-cultivated microgreens was widely analyzed, the pre-harvest lighting effects on quality preservation after harvest remain unclear. This study aims to determine the effects of the pre-harvest light spectrum on the biochemical indices of microgreens and the impact on their shelf life. We postulated that crop production with added nutritional value and longer storability can be achieved by manipulating the pre-harvest lighting conditions.

2. Materials and Methods

2.1. Microgreen Cultivation

Experiments were performed in growth chamber with autonomously and independently controlled Phytotron Microclimate Control System with separate microcontrollers (AL-2-24MR-D, Mitsubishi Electric, Tokyo, Japan). The air temperature was measured with resistance temperature detectors (P-100; OMEGA Engineering Ltd., Norwalk, CT, USA). The relative humidity and CO_2 concentration were measured using capacitive sensors (CO2RT(-D); Regin, Sweden) and controlled using additional humidifiers.

Two grams of broccoli 'Micro Green' (*Brassica oleracea* var. *italica*) and kale 'Dwarf Blue Green' (*Brassica oleracea* var. *sabellica*) seeds were sown on the surface of a peat-based substrate (Terraerden, Rucava, Latvia) with NPK (100–160; 110–180; 120–200 mg L^{-1}) and with microelements Mn, Cu, Mo, B, Zn, and Fe (pH H_2O 5.5–6.5; electrical conductivity (EC) ms cm^{-1} < 1.10) in a 0.5 L (18 × 11 × 6 cm) plastic pot, which represented one replicate, and covered with a lightweight agro-textile fabric until the seeds started to germinate. Four pots were used under each lighting condition and were randomized daily. Seeds were germinated under a 16 h photoperiod with day/night temperatures (±SD) of 21/17 ± 2 °C and a relative air humidity of 60 ± 5%.

Broccoli microgreens were harvested after nine days, and kale microgreens were harvested after ten days of germination above the substrate surface.

2.2. Lighting Treatments

Microgreens were grown under five lighting treatments consisting of blue (B; peak = 447 nm), red (R; peak = 638 and 665 nm), green (G; peak = 520 nm), and violet (V, peak = 405 nm) (Philips Lumileds Co., San Jose, CA, USA) LEDs. All lighting treatments delivered the same photosynthetic photon flux density (PPFD, 400–700 nm) of 200 µmol m^{-2} s^{-1} (Table 1). The surface area under the LED fixture was approximately 0.23 m^2. The daily light integral (DLI) was 11.52 mol m^{-2} d^{-1}. The photon distributions of all lighting treatments were measured using a portable spectroradiometer (WaveGo, Wave Illumination, Oxford, UK).

2.3. Post-Harvest Storage Conditions

For the evaluation of changes in the phytochemical contents during the short-term post-harvest storage, fresh-cut microgreens from each lighting treatment were stored in clear 18 × 11 × 6 cm polyethene terephthalate (PET) containers in the dark at 4 °C for 1, 3, and 5 days.

Table 1. The lighting treatments used in the experiments.

	PPFD, μmol m^{-2} s^{-1}				
Treatment	**V**	**B**	**G**	**R_{638}**	**R_{665}**
BR_{638}		40		160	
BR_{665}		40			160
BR		40		80	80
BRV	20	20		80	80
BRG		20	20	80	80

PPFD—photosynthetic photon flux density; V—violet (peak = 405 nm); B—blue (peak = 447 nm); G—green (peak = 520 nm); R_{638}—red (peak = 638 nm); Red_{665}—red (peak = 665 nm); BR_{638}—blue and red (peak = 638 nm); BR_{665}—blue and red (peak = 665 nm); BR—blue and red (peaks = 638 and 665 nm); BRV—blue, red, and violet; BRG—blue, red, and green.

2.4. Water Content

Microgreens were cut at pot surface level, and the fresh weight (FW, g) and dry weight (DW, g) were measured using an analytical balance (AG245; Mettler Toledo, Columbus, OH, USA). Plants were dried in an oven (Venticell 222, MBT, BMT Medical Technology s.r.o., Brno—Zábrdovice, Czech Republic) at 70 °C for 48 h before DW measurements. The difference between the FW and DW in FW was used for recalculating the biochemical compound contents in the DW of microgreens.

2.5. Total Phenolic Content

For the determination of total phenolic content (TPC), 500 mg of plant material was frozen in liquid nitrogen and homogenized with 5 mL of 80% ice-cold methanol (Riedel-de Haën, Honeywell International, Inc., Charlotte, NC, USA) in a 15 mL polypropylene conical centrifuge tube (Thermo Fisher Scientific Inc., Waltham, MA, USA). The extracts were incubated at 4 °C for 24 h. After the incubation, the samples were centrifuged for 15 min at 3000 rpm and filtered through Whatman Grade 1 qualitative filter paper. The TPC of samples was determined using the spectrophotometric method [11]. An amount of 100 μL of the extract was diluted with 200 μL of 10% (*v*/*v*) Folin and Ciocalteu's phenol reagent (Sigma-Aldrich Chemie GmbH, St. Louis, MO, USA) and vortexed thoroughly. Then, 280 μL of 700 mM sodium carbonate (Sigma-Aldrich Chemie GmbH, St. Louis, MO, USA) was added. After 20 min, the absorbance of the samples was measured using a SPECTROstar Nano Microplate Reader from BMG LABTECH (Ortenberg, Germany) at 765 nm.

2.6. Antioxidant Capacity

The 80% methanol extracts were used to evaluate non-enzymatic antioxidant activity.

The DPPH (2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich Chemie GmbH, St. Louis, MO, USA) free radical scavenging activity was evaluated according to the spectrophotometric method with modifications [12,13]. An amount of 20 μL of extract was diluted with 280 μL of 60 μM DPPH solution. Absorbance at 515 nm was measured after 16 min (SPECTROstar Nano Microplate Reader from BMG LABTECH, Ortenberg, Germany). The ability of plant extracts to scavenge DPPH free radicals was calculated using the DPPH solution as a blank. Data are presented as the mean of three analytical samples to scavenge DPPH free radicals in μmol g^{-1} DW.

The ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) free radical scavenging activity was evaluated using the spectrophotometric method according to Re et al. [14]. The ABTS (Thermo Fisher Scientific Inc., Branchburg, NJ, USA) radical cation (ABTS+) was generated by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (K2S2O8; ≥99% purity; Carl Roth GmbH + Co. KG, Karlsruhe, Germany). An amount of 50 μL of the extract was mixed with 2 mL of ABTS solution (ABTS stock solution was diluted 1:10), and the absorbance was measured after 20 min at 734 nm (UV-1280, Shimadzu, Kyoto, Japan). The ABTS scavenging activity in samples was calculated as the difference between the initial

absorbance and after reacting for 20 min. Samples were measured in triplicate. A calibration curve was determined using Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; 97% purity; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) as an external standard. It was expressed as mM of Trolox equivalent antioxidant capacity (TEAC) per 1 g of dry weight (mM TEAC g^{-1} DW).

The ferric-reducing antioxidant power (FRAP) was determined using the method described by Benzie and Strain [15]. To prepare FRAP reagent, the 300 mM acetate buffer at pH 3.6 (≥99.0% purity; Sigma-Aldrich Chemie GmbH, St. Louis, MO, USA), 10 mM TPTZ (2,4,6-Tris(2-pyridil)-s-triazine) (≥98% purity; Sigma-Aldrich Chemie GmbH, St. Louis, MO, USA) solution in 40 mM hydrochloric acid, and 20 mM iron (III) chloride (97% purity; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) were mixed in a 10:1:1 ratio. An amount of 2 mL of FRAP reagent was mixed with 50 µL of the sample, and the absorbance was measured at 593 nm using a spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan). Samples were measured in triplicate. Standard curve of Trolox (97% purity; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was prepared using the similar procedure from which the regression formula was derived. Results are expressed as mM of Trolox equivalent antioxidant capacity (TEAC) per 1 g of dry weight (mM TEAC g^{-1} DW).

2.7. Total Protein Content

Total protein content (TP) was determined using the spectrophotometric method [16]. Plant material was ground with liquid nitrogen and extracted with 50 mM phosphate buffer containing 1 mM EDTA and 1 mM dithiothreitol. The extract was centrifuged for 10 min at 4000 rpm- and then 200 µL of supernatant was mixed with 1 mL distilled water and 1.2 mL Bradford reagent. The protein calibration curve used bovine albumin as a standard. Absorbance was read at 595 nm (Analytik Jena SPECORD® 210 PLUS, Jena, Germany). Data are presented as the mean of three analytical samples in mg g^{-1} DW.

2.8. Total Chlorophyll and Carotenoid Content

The total chlorophyll (CHL) and carotenoid (CAR) contents were determined using the spectrophotometric method according to Wellburn [17]. Pigments were extracted from microgreen tissue with acetone (SIGMA-ALDRICH Chemie GmbH). Light absorption was measured at 470, 645, and 662 nm (Shimadzu UV-1280, Kyoto, Japan). Data are presented as the mean of three analytical samples in mg g^{-1} DW.

2.9. Statistical Analysis

Microsoft Excel 2016 and Addinsoft XLSTAT 2019.1 XLSTAT statistical and data analysis solution (Long Island, New York, NY, USA) were used to conduct the statistical analysis. For multiple comparisons, one-way analysis of variance (ANOVA) was used followed by Tukey's honestly significant difference test ($p < 0.05$).

3. Results

On harvest day (HD), the highest total phenolic content (TPC) was measured in kale microgreens grown under the treatment of blue, red, and violet light (BRV) (Figure 1). The kale microgreens under the BRV treatment had from 22% to 42% higher TPC compared to the other lighting treatments. The lowest TPC was determined in the kale microgreens grown under the blue, red, and green light (BRG) treatment. Compared to BRV, the kale microgreens under BRG had a 42% lower TPC on HD. On the contrary, the highest TPC in broccoli microgreens on HD was under the BRG or BR_{665} treatment. Compared to the other treatments, the plants grown under both treatments had from 7% to 10% higher TPC. Although the highest TPC in kale was under the BRV, it decreased the most during the post-harvest storage. The results show that the TPC decreased by 52% on average during the post-harvest storage period from one to three and five days after the harvest (1D-, 3D-, and 5D-PH, respectively) compared to HD. The decrement in the TPC was observed in kale

grown under the other lighting treatments at 3D-PH and did not significantly differ from the result at 5D-PH.

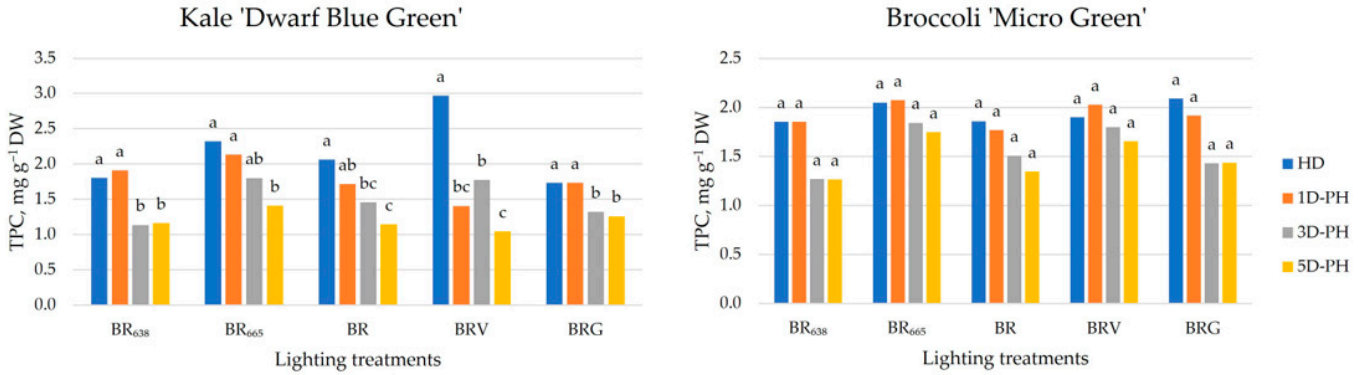

Figure 1. The total phenolic content (TPC) in kale and broccoli microgreens under different lighting treatments on harvest day and during post-harvest storage. BR_{638}—blue (B, peak = 447 nm) and red (R_{638}, peak = 638 nm); BR_{665}—blue and red (R_{665}, peak = 665 nm); BR—blue and red (R, peaks = 638 and 665 nm); BRV—blue, red, and violet (V, peak = 405 nm); BRG—blue, red, and green (G, peak = 520 nm); HD—harvest day; 1D-PH—one day after the harvest; 3D-PH—three days after the harvest; 5D-PH—five days after the harvest; DW—dry weight. Means with different letters significantly differ from the control (HD) at $p < 0.05$.

On HD, the higher (an average of about 10%) TPC was determined in the broccoli grown under the BR_{665} and BRG treatments compared to the BR_{638}, BR, and BRV lighting treatments. Similar to kale, the TPC decreased with the increasing post-harvest storage time. The lowest TPC was determined in the broccoli grown under the BR_{638} treatment five days after the harvest (5D-PH). However, the TPC did not change significantly over the post-harvest storage period (Figure 1).

Very strong and strong positive correlations (from $r = 0.699$ to $r = 0.990$) between the ABTS free radical scavenging activity, the ferric-reducing antioxidant power (FRAP), and the DPPH free radical scavenging activity in the kale and broccoli microgreens were observed (Tables S1 and S2). However, there were moderate positive correlations (from $r = 0.430$ to $r = 0.586$) between the ABTS and DPPH free radical scavenging activities in kale under the BRV lighting treatment and in broccoli under the BR_{665} and BR lighting treatments. In addition, a moderate correlation ($r = 0.602$) between the FRAP and DPPH free radical scavenging activity in broccoli under the BR_{665} lighting treatment was observed.

The highest DPPH free radical scavenging activity was seen in the kale under blue and red light (BR) and in the broccoli under the BRV light treatment (Figure 2). However, the decrement in DPPH started to be significantly lower on 1D-PH. Compared to HD, it decreased by 55% in the kale under BR and by 18% in the broccoli under BRV on 1D-PH. However, at the end of the post-harvest storage (5D-PH), significantly lower DPPH radical scavenging activity was seen in the kale and broccoli microgreens grown under blue and red 638 nm light (BR_{638}). Compared to HD, the antiradical activity decreased by 66% on average in both microgreens species. There were no significant changes in the DPPH radical scavenging activity in the kale microgreens under blue and red 665 nm light (BR_{665}) from the 1D-PH to 5D-PH period compared to the HD.

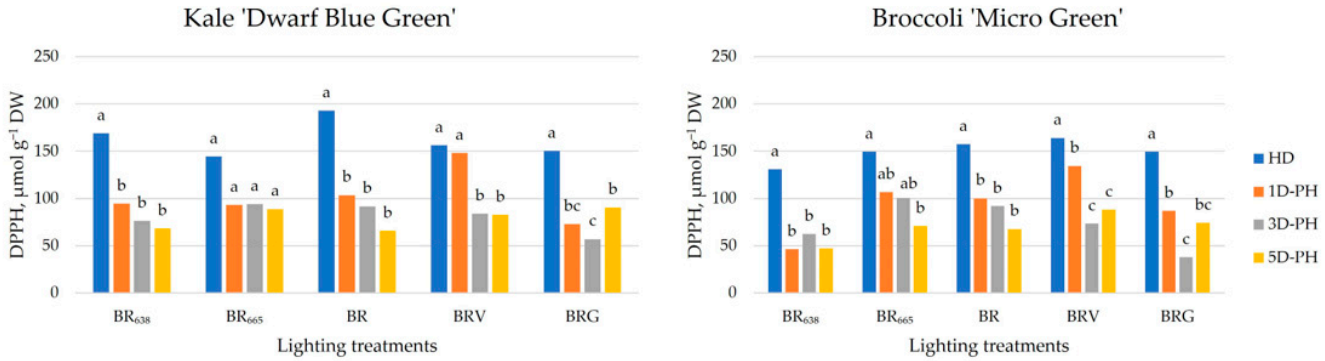

Figure 2. The DPPH free radical scavenging activity in microgreens under different lighting treatments on harvest day and during post-harvest storage. BR_{638}—blue (B, peak = 447 nm) and red (R_{638}, peak = 638 nm); BR_{665}—blue and red (R_{665}, peak = 665 nm); BR—blue and red (R, peaks = 638 and 665 nm); BRV—blue, red, and violet (V, peak = 405 nm); BRG—blue, red, and green (G, peak = 520 nm); HD—harvest day; 1D-PH—one day after the harvest; 3D-PH—three days after the harvest; 5D-PH—five days after the harvest; DW—dry weight. Means with different letters significantly differ from the average at $p < 0.05$.

The highest ABTS free radical activity was determined in the kale and broccoli microgreens under the BRV treatment on HD (Figure 3). However, it did not significantly differ from the antiradical activity after one day of post-harvest storage (1D-PH). In addition, no significant differences in the ABTS free radical scavenging activity on HD and 1D-PH in broccoli under all lighting treatments or in kale under the BR_{638} and BRG lighting treatments were determined. A significant decrease (about 32% and 37%) in the ABTS free radical scavenging activity was observed in the kale at 1D-PH under the BR_{665} and BR treatments, respectively. The BR_{638}, BRV, and BRG lighting treatments led to a decrease in the ABTS free radical activity starting at 3D-PH, and a lower antiradical activity by 45%, 41%, and 47%, respectively, at 5D-PH. No significant changes in the ABTS free radical scavenging activity were observed in the broccoli under the BR_{638}, BR_{665}, and BR lighting treatments on HD and during post-harvest storage. As in the kale, the BRG led to significantly lower ABTS free radical scavenging activity in the broccoli at 3D-PH and 5D-PH (42% and 51%, respectively).

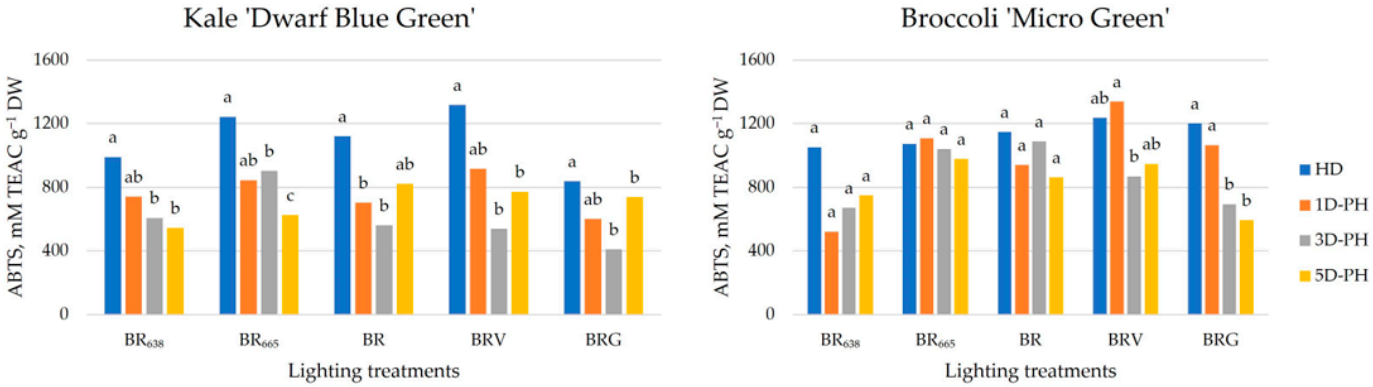

Figure 3. The ABTS free radical scavenging activity in microgreens under different lighting treatments on harvest day and during post-harvest storage. BR638—blue (B, peak = 447 nm) and red (R638, peak = 638 nm); BR665—blue and red (R665, peak = 665 nm); BR—blue and red (R, peaks = 638 and 665 nm); BRV—blue, red, and violet (V, peak = 405 nm); BRG—blue, red, and green (G, peak = 520 nm); HD—harvest day; 1D-PH—one day after the harvest; 3D-PH—three days after the harvest; 5D-PH—five days after the harvest; DW—dry weight. Means with different letters significantly differ from the average at $p < 0.05$.

On HD, the highest ferric-reducing antioxidant power (FRAP) was determined in the kale microgreens under the BR_{665} lighting treatment (Figure 4). However, it decreased by an average of 49% from 1D-PH to 5D-PH. A similar trend in the FRAP decrement in kale under other lighting treatments was observed, except for BRV, when the FRAP at 1D-PH did not significantly differ compared to HD. As in kale, the highest FRAP was measured in the broccoli under the BR_{665} treatment, and no significant changes were observed during post-harvest storage. Also, the same trend was observed in the broccoli under the BR and BRV lighting treatments. The BRG treatment led to a significant decrease in the FRAP at 3D-PH and 5D-PH (by 39% and 60%, respectively).

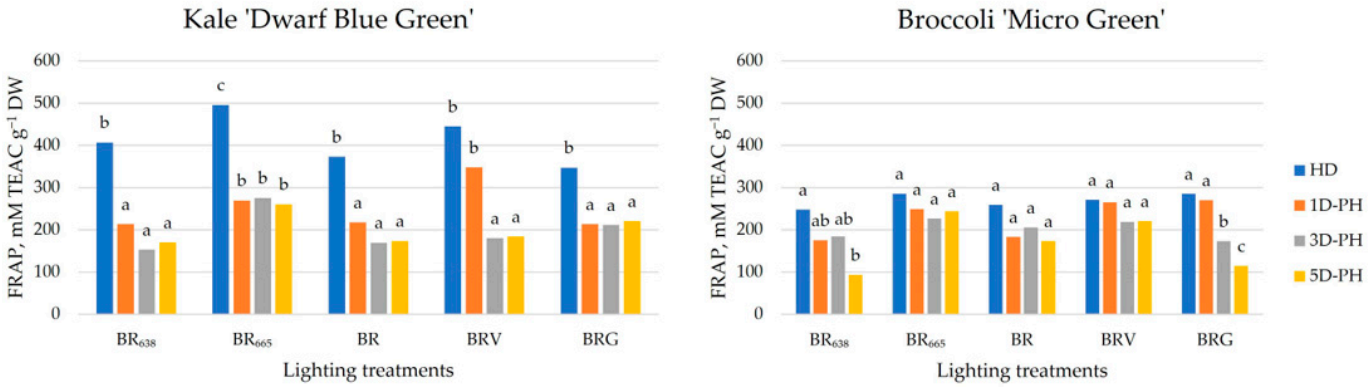

Figure 4. The ferric-reducing antioxidant power (FRAP) in microgreens under different lighting treatments on harvest day and during post-harvest storage. BR_{638}—blue (B, peak = 447 nm), red (R_{638}, peak = 638 nm); BR_{665}—blue and red (R_{665}, peak = 665 nm); BR—blue and red (R, peaks = 638 and 665 nm); BRV—blue, red, and violet (V, peak = 405 nm); BRG—blue, red, and green (G, peak = 520 nm); HD—harvest day; 1D-PH—one day after the harvest; 3D-PH—three days after the harvest; 5D-PH—five days after the harvest; DW—dry weight. Means with different letters significantly differ from the average at $p < 0.05$.

The highest total protein content (TP) on HD was found in the kale under the BR_{665} treatment and in the broccoli microgreens under the BR treatment (Figure 5). On 1D-PH, the TP decreased by 38%, 45%, and 42% in the kale under the BR_{638}, BR_{665}, and BR lighting treatments, respectively. On the contrary, there were no significant changes in the TP in the broccoli microgreens on 1D-PH compared to HD. On 3D- and 5D-PH, the TP decreased in the kale regardless of the lighting treatment. A significant TP decrement was observed in the broccoli under the BR_{638} and BR treatments on 3D- and 5D-PH compared to HD. No significant changes in the TP were found in the broccoli microgreens under the BR_{665}, BRV, and BRG treatments over the post-harvest storage period (1D- to 5D-PH) compared to HD.

The kale microgreens grown under the BRV treatment accumulated the highest total chlorophyll (CHL) and carotenoid (CAR) contents (Table 2). However, they decreased throughout 1D- to 5D-PH. Compared to HD, the CHL content was 65% lower, and the CAR content was 74% lower on 5D-PH. In addition, on 5D-PH, the CHL and CAR contents were significantly lower in the kale microgreens regardless of the lighting treatment.

In broccoli, the highest CHL content was seen under the BR_{638} treatment, and the highest CAR content was seen under the BR treatment. There were no significant changes in the CHL and CAR contents on 1D-PH; however, they started to decrease on 3D-PH in the broccoli under the BR treatment. In addition, in the microgreens under the BR_{638} and BRG treatments, a significantly lower CHL content was determined on 5D-PH. Compared to HD, there were no significant changes throughout post-harvest storage in the CHL and CAR contents in the broccoli under the BR_{665} and BRV lighting treatments.

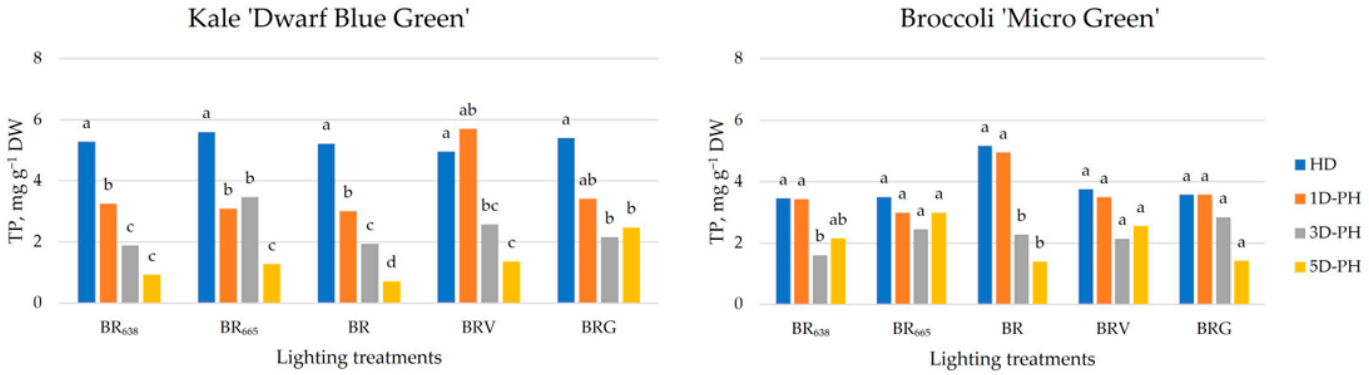

Figure 5. The total protein content (TP) in microgreens under different lighting treatments on harvest day and during post-harvest storage. BR_{638}—blue (B, peak = 447 nm), red (R_{638}, peak = 638 nm); BR_{665}—blue and red (R_{665}, peak = 665 nm); BR—blue and red (R, peaks = 638 and 665 nm); BRV—blue, red, and violet (V, peak = 405 nm); BRG—blue, red, and green (G, peak = 520 nm); HD—harvest day; 1D-PH—one day after the harvest; 3D-PH—three days after the harvest; 5D-PH—five days after the harvest; DW—dry weight. Means with different letters significantly differ from the control (HD) at $p < 0.05$.

Table 2. The chlorophyll and carotenoid contents in microgreens under different lighting treatments on harvest day and during post-harvest storage.

Total Chlorophyll and Carotenoid Contents, mg g^{-1} DW			
Kale 'Dwarf Blue Green'			
Treatments		**CHL**	**CAR**
BR_{638}	HD	26.49 a	2.43 a
	1D-PH	29.08 a	3.36 a
	3D-PH	24.42 a	1.00 b
	5D-PH	16.84 b	1.37 b
	p (95%)	0.002	0.0003
BR_{665}	HD	24.46 ab	3.97 a
	1D-PH	28.65 a	3.40 a
	3D-PH	22.07 b	3.76 a
	5D-PH	23.25 b	1.55 b
	p (95%)	0.021	0.002
BR	HD	25.99 a	2.58 a
	1D-PH	28.36 a	2.02 a
	3D-PH	24.71 a	2.28 a
	5D-PH	16.57 b	2.00 a
	p (95%)	0.0001	0.682
BRV	HD	47.59 a	7.14 a
	1D-PH	27.36 b	2.57 b
	3D-PH	21.72 c	2.01 b
	5D-PH	16.68 c	1.87 b
	p (95%)	<0.0001	<0.0001
BRG	HD	28.28 a	3.40 a
	1D-PH	23.99 b	2.05 b
	3D-PH	23.69 b	1.85 b
	5D-PH	19.59 c	1.99 b
	p (95%)	<0.0001	0.016

Table 2. *Cont.*

Total Chlorophyll and Carotenoid Contents, mg g^{-1} DW			
Broccoli 'Micro Green'			
Treatments		**CHL**	**CAR**
BR_{638}	HD	34.04 a	2.55 ab
	1D-PH	34.97 a	3.37 a
	3D-PH	33.86 a	2.06 b
	5D-PH	26.49 b	2.05 b
	p (95%)	0.002	0.020
BR_{665}	HD	29.58 a	2.91 a
	1D-PH	30.26 a	3.36 a
	3D-PH	27.92 a	3.54 a
	5D-PH	29.76 a	2.78 a
	p (95%)	0.978	0.701
BR	HD	30.32 a	3.53 a
	1D-PH	30.68 a	3.44 a
	3D-PH	26.02 b	0.73 b
	5D-PH	24.25 b	1.40 b
	p (95%)	0.001	0.0002
BRV	HD	24.94 a	2.90 a
	1D-PH	33.95 a	3.83 a
	3D-PH	29.09 a	2.24 a
	5D-PH	27.55 a	2.96 a
	p (95%)	0.615	0.558
BRG	HD	32.36 a	2.58 a
	1D-PH	30.08 a	2.97 a
	3D-PH	29.54 a	2.64 a
	5D-PH	24.09 b	2.63 a
	p (95%)	0.007	0.911

BR_{638}—blue (B, peak = 447 nm) and red (R_{638}, peak = 638 nm); BR_{665}—blue and red (R_{665}, peak = 665 nm); BR—blue and red (R, peaks = 638 and 665 nm); BRV—blue, red, and violet (V, peak = 405 nm); BRG—blue, red, and green (G, peak = 520 nm); HD—harvest day; 1D-PH—one day after the harvest; 3D-PH—three days after the harvest; 5D-PH—five days after the harvest; DW—dry weight. Means with different letters significantly differ from the average at $p < 0.05$.

The principal component analysis (PCA) results for the factor loadings, scores, and eigenvalues for the first two principal components (F1 and F2) are presented in Table S3. The PCA showed that the antioxidant capacity according to the FRAP, the ABTS and DPPH free radical activities, and the TP in kale (Figure 6, A Biplot) was associated with the BR_{665}, BR, and BRG lighting treatments on HD and with the BRV lighting treatment at 1D-PH. Also, the TPC, CHL, and CAR were associated with the BRV treatment at HD, with the BR_{638} and BR_{665} treatments at 1D-PH, and with the BR_{665} lighting treatment at 3D-PH. In broccoli, the antioxidant capacity was associated with the BR_{665}, BRV, and BRG treatments at HD and with the BR_{665} lighting treatment at 3D-PH (Figure 6, B Biplot). The contents of the total protein, phenolic compounds, and photosynthetic pigments (CHL and CAR) were associated with the BR_{638} and BR treatments at HD, the BR_{665}, BR, BRV, and BRG treatments at 1D-PH, and the BR_{665} lighting treatment at 5D-PH.

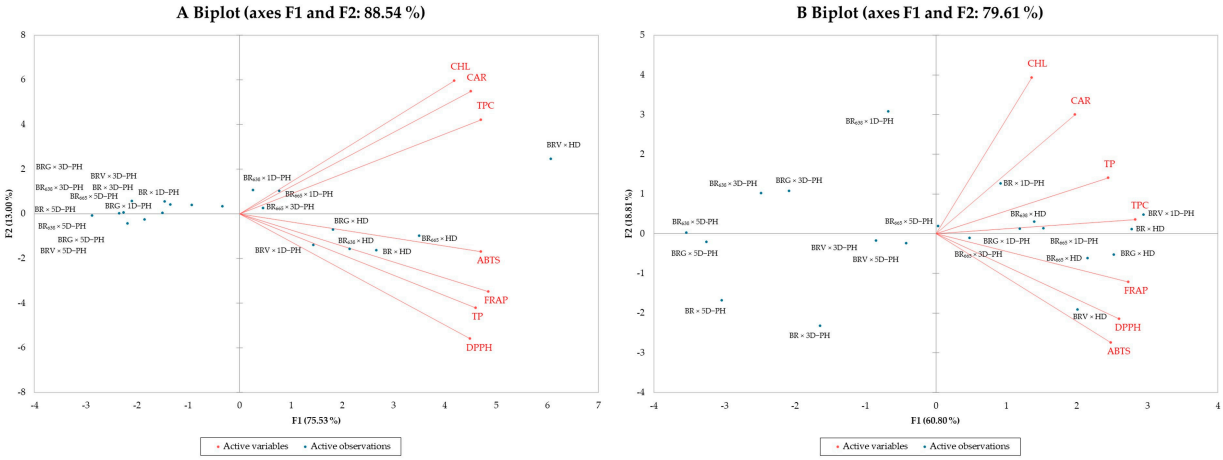

Figure 6. The principal component analysis (PCA) results of kale (**A Biplot**) and broccoli (**B Biplot**) microgreens under different lighting treatments on harvest day and during post-harvest storage. BR638—blue (B, peak = 447 nm) and red (R638, peak = 638 nm); BR665—blue and red (R665, peak = 665 nm); BR—blue and red (R, peaks = 638 and 665 nm); BRV—blue, red, and violet (V, peak = 405 nm); BRG—blue, red, and green (G, peak = 520 nm); HD—harvest day; 1D-PH—one day after the harvest; 3D-PH—three days after the harvest; 5D-PH—five days after the harvest; ABTS—ABTS free radical scavenging activity; DPPH—DPPH free radical scavenging activity; FRAP—ferric-reducing antioxidant power; TP—total protein content; TPC—total phenolic content; CHL—chlorophyll a and b content; CAR—total carotenoid content.

4. Discussion

The metabolic parameters of microgreens depend on the plant species and the type of light spectral composition used for cultivation. Most vegetables rapidly age and deteriorate in quality during post-harvest storage, showing characteristics such as increased respiration, softening, tissue destruction, lipid peroxidation, and water loss, and decreased visual scale, flavor, texture, aroma, and nutritional quality. The use of light-emitting diodes (LEDs) was proven to be an effective inhibitory treatment for the post-harvest senescence of fresh produce in recent years by extending the shelf life and maintaining the quality of fresh produce [18].

Several previously published studies demonstrated the potential of pre-harvest LEDs to enhance or delay the loss of phytochemicals during post-harvest storage. For example, when the Chinese kale 'Bailey' was exposed to red (R) light 24 h before harvest, the vitamin C level remarkably increased on the first and second days after the harvest compared to the control plants that were grown under white light. Also, in the same study, R-light-treated plants had a higher total phenolic content (TPC) and antioxidant activity [19]. These results agree with those published by Vaštakaitė-Kairienė et al. [20] in which the highest TPC, total anthocyanin content (TAC), and antioxidant activity was seen in pre-harvest supplemental R-light-treated baby leaf lettuce 'Rouxai' after seven days of storage. Furthermore, our results demonstrate that the kale microgreens under the blue, red, and violet (BRV) LEDs accumulated the highest TPC. In broccoli, it was higher under the blue, red, and green (BRG) light. However, in kale, the TPC significantly decreased throughout the post-harvest storage, leading to the highest TPC in the plants under the blue and red (BR_{665}) light treatment at the end of storage (5D-PH). In contrast, the TPC in the broccoli did not change over the post-harvest period.

Senescence is an undesirable phenomenon in horticultural crops, instigating the deterioration of fresh produce, and thus resulting in a loss of nutritional and commercial value [21]. The senescence process's mechanism involves allocating nutrients from one part of the tissue to another and modifying the cell structure's physical characteristics and biochemical attributes [22,23]. It is well understood that plant senescence is primarily caused by the production of reactive oxygen species (ROS), which can cause oxidative

damage to plant lipids and proteins; thus, controlling ROS production is an important way to delay the senescence of fresh horticultural produce during post-harvest storage. [24]. According to the DPPH free radical scavenging activity, the highest antiradical activity was seen in the kale under the BR treatment and in the broccoli under the BRV light treatment. At the end of storage, the broccoli under the BRV light demonstrated higher antiradical activity; however, in the kale, the higher DPPH scavenging activity was seen under the BRG light. The lighting treatments of BR_{665} and BR led to the highest total protein content (TP) on harvest day (HD) in the kale and broccoli microgreens, respectively. However, in kale, the TP started to decrease on the first day after the harvest (1D-PH), and in broccoli, the TP started to decrease on the third day after the harvest (3D-PH). At the end of storage, the kale under the BRG light and the broccoli under the BR_{665} light demonstrated the highest values of TP.

The enhancement of chlorophyll (CHL) and carotenoid (CAR) biosynthesis in plants in response to visible light has long been investigated. The biosynthesis and accumulation of pigments in plants occur during cultivation as they are primarily photosynthetic pigments [25,26]. Moreover, upon light radiation, carotenoids possess the roles of ROS scavengers and photoprotectors to prevent damage to the plant's cellular and photosynthetic apparatus. For humans, carotenoids are well recognized for their roles in reducing the risks of age-related macular degeneration, cancer, and cardiovascular diseases [27]. In our study, BRV treatment led to the highest contents of total CHL and CAR in kale when, in broccoli, the highest CHL content was seen under the blue and red (BR_{638}) light treatment, and the highest CAR content was seen under the blue and red (BR) light treatment. The results agree with those of Brazaitytė et al. [8] that the effect of natural pigment regulation via altering the spectral quality was microgreens-species-dependent. During post-harvest storage, the CHL and CAR contents decreased in the kale microgreens regardless of the lighting treatment. In broccoli, there were no significant changes in the CHL and CAR contents under the BR_{665} and BRV lighting treatments during post-harvest storage. Natural pigments would naturally degrade upon plant senescence. Controlling post-harvest storage conditions (temperature, light, and gas composition) can delay senescence and hence prevent the degradation of carotenoids in vegetables [27,28].

Based on the principal component analysis (PCA), which summarized the effects of the different lighting treatments during short-term post-harvest storage, the common effects of the BR_{665} and BR treatments at harvest day on the FRAP, ABTS, and DPPH free radical scavenging activities were observed in both microgreens species. Also, the BR_{638} and BR treatments at HD, and the BR treatment at 1D-PH demonstrated the same influence on the total protein contents in kale and broccoli. However, only the BR_{665} treatment at 1D-PH had a similar effect on the total phenolic content and the chlorophyll and carotenoid contents for both microgreens species.

5. Conclusions

The influence of the pre-harvest LED spectrum on the total phenolic content, total protein content, accumulation of chlorophylls and carotenoids, and antiradical capacity varied among the microgreens species. The similar effects of BR light at harvest day on the FRAP, ABTS and DPPH free radical scavenging activities, and the total protein content were observed in the kale and broccoli microgreens. However, the contents of the phenolic compounds and photosynthetic pigments were influenced by the BR_{665} treatment after one day of storage in the dark. No common effects of the BR_{638}, BRG, and BRV light treatments on the phytochemical constituents and antioxidant capacity in both microgreens species after three or five days of storage were found.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13082188/s1, Table S1: Correlation matrixes of ABTS free radical scavenging activity, ferric reducing antioxidant power (FRAP) and DPPH free radical scavenging activity in kale 'Dwarf Blue Green' microgreens under different lighting treatments. Table S2: Correlation matrixes of ABTS free radical scavenging activity, ferric reducing antioxidant

power (FRAP) and DPPH free radical scavenging activity in broccoli 'Micro Green' microgreens under different lighting treatments. Table S3: Factor loadings, eigenvalue, variability (%), cumulative variability (%) and score for the first two principal (F1–F2) components for phytochemical constituents and antioxidant activity in microgreens under different lighting treatments during post-harvest storage in dark.

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