

Microgreen leaf vegetable production by different wavelengths

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SUMMARY

*Microgreens are becoming more popular in gastronomy, especially as a salad ingredient. In this study, two plant species belonging to the cabbage family were grown as microgreens, namely red cabbage and broccoli. Three different light-emitting diodes (LEDs) were used in the experiment, blue, red, and combined (blue:red) lighting. The experiment was carried out by 118 $\mu\text{mol}^{-2} \text{s}^{-1}$ total Photosynthetic Photon Flux (PPF), LED lighting was applied for 16 hours a day. Blue light primarily stimulates leaf growth, while red light promotes flowering. In our experiment, blue and combined lighting favorably affected plant development, yield ($\sim 3000 \text{ g m}^{-2}$), chlorophyll-*a* ($\sim 8.0 \text{ mg g}^{-1}$), and carotenoid content (9.0 mg g^{-1}). However, the red light resulted in reduced harvest yields ($\sim 2200 \text{ g m}^{-2}$), chlorophyll-*a* ($\sim 6.0 \text{ mg g}^{-1}$), and carotenoid content ($\sim 7.0 \text{ mg g}^{-1}$). The development of red cabbage was favorably influenced by the blue spectrum, while the combined spectrum favorably influenced the development of broccoli.*

Keywords: microgreens, LEDs, carotenoids, chlorophyll-*a*, chlorophyll-*b*

INTRODUCTION

In the last few years, microgreens, young plants of edible herbs and vegetables, have become popular as a new culinary trend. These plants are becoming more popular in gastronomy due to their intense colors and taste, making them an increasingly widespread salad ingredient (Le et al., 2020; Renna et al., 2016; Turner et al., 2020; Xiao et al., 2012). However, due to the rapid deterioration of the product, its shelf life is typically short (Mir et al., 2017).

Depending on the species produced, the microgreen can be harvested 7–21 days after sowing, when the cotyledons are entirely developed, and the first true leaves have appeared. In addition, microgreens can be cultivated indoors in soil or other soilless growing media, such as rock wool and cocopeat (Paradiso et al., 2018a; Widiurjani et al., 2020).

Several species and varieties of many botanical families can be used for microgreen cultivation. The *Brassicaceae* family is one of the most consumed vegetables globally, and its seedlings frequently have good flavor and increased nutritional value (Palmitessa et al., 2020). Microgreens contain higher amounts of health-promoting phytochemicals compared to their mature ones. Carotenoids function as photosynthetic pigments in plants, some of which are precursors to vitamin A, and it is essential for vision or as an antioxidant that can prevent cancer and other chronic diseases (Ying et al., 2021). There are different chlorophylls; in nature, chlorophyll-*a* and chlorophyll-*b* are the most common types. They are found in all plants that perform photosynthesis. In general, chlorophyll-*a* and chlorophyll-*b* are present in a 3:1 ratio in the higher plants. The amount of these pigments determines the color intensity of the plants. The amount of chlorophyll pigment in plants is affected by weather, habitat, and anthropogenic influences (Zielewicz et al., 2020).

In addition, not only the chlorophyll content of plants is important for human health but also the appearance of the product (Bulgari et al., 2016).

Photosynthetically active radiation (PAR) is between 400 and 700 nm, which is utilized by plants during photosynthesis. The Photosynthetic Photon Flux (PPF) is the number of photons emitted per second by the light source in the PAR range (Cope et al., 2014).

In addition to light quality (wavelength), light intensity (irradiation) and photoperiod (day/night) also play an essential role in the morphogenetic and photosynthetic reactions of plants (Vaštakaitė and Viršilė, 2015; Su et al., 2013; Li et al., 2017).

Light quality is a significant factor in the plant environment, and different wavelengths of light directly affect the physiological and chemical processes of plants. In recent years, LED technology has been an alternative light source. In addition to energy-saving, short response time, small size, lightweight, and low heat output, one of the most outstanding advantages of LEDs is the ability to customize the light spectrum (Brazaitytė et al., 2016). Brazaitytė et al. (2021) reported that blue (B) and red (R) LEDs for crop production have the highest photon efficiency. It is important to know that such lights are better absorbed by chlorophylls than the light of other wavelengths in the visible spectrum. In addition, recently, the spectral effects of blue and red light have been studied on microgreen species belonging to various families, e.g., *Brassicaceae*, *Chenopodiaceae*, and *Lamiaceae*. However, such a few information is available on the secondary metabolite of the plant and how these bioactive compounds respond to the wavelength quality of LED (Toscano et al., 2021).

The aim of our experiment was to increase the efficiency of microgreen production using LED lamps technology and give recommendations on which wavelength of light is most favorable to produce broccoli and red cabbage in microgreens.

MATERIALS AND METHODS

Growing Conditions

The study was conducted at the glasshouse of the Institute of Horticultural Science, Faculty of Agricultural and Food Sciences and Environmental Management, Debrecen, Hungary. For the experiment, special organic quality seeds were used for microgreen production. Red cabbage (*Brassica oleracea* convar. *capitata* var. *rubra*) and broccoli (*Brassica oleracea* convar. *botrytis* var. *italica*) were selected from the

Brassicaceae family for the experiment in the glasshouse. After sowing, these plants were grown in a germination chamber of type CSK 7.56 / 2018 until germination rate 90%. During the cultivation, we applied LED lamp treatment of different wavelengths. The experiment was repeated three times, from 05 May to 29 June.

Table 1 shows the temperature, the irradiance, and the humidity, where the temperature and irradiation increased steadily during the growing seasons.

Table 1: Environmental factors in the three growing periods

Growing period	Temperature °C	Irradiance (J cm ⁻²)	Humidity (%)
1 st growing period	19.90 ± 2.33	1182.09 ± 353.71	65.54 ± 10.82
2 nd growing period	22.88 ± 1.28	1497.17 ± 225.21	59.22 ± 8.30
3 rd growing period	28.17 ± 2.24	1550.45 ± 111.91	65.78 ± 9.48

Rock wool cubes were used as the growth medium, and the cubes were covered with vermiculite (phyllosilicate mineral). Bio Nova Veganics Grow (N:P:K – 3:2:4) organic liquid was applied as a nutrient supplement in a dose of 0.05% (5 ml of nutrient solution in 10 liters of water) during the growing season. In the first experiment 40 ml, in the second 25 ml, and in the third 20 ml of nutrients (N:P:K) were added.

Lighting system – LED light

A Research Toplight 4-channel LED lamp was used to light the plants in all three growing periods. During the lighting, three types of settings were used: blue – PPF 58 μmol⁻² s⁻¹, red – PPF 58 μmol⁻² s⁻¹, and combined (blue:red) light treatment – PPF 48:49 μmol⁻² s⁻¹. The total PPF (Photosynthetic Photon Flux) per lamp was 118 μmol⁻² s⁻¹. The LED lighting interval was used for 16 hours (from 5 a.m. till 9 p.m.).

Plant sampling (shoot and cotyledon) was taken at 26 days from the first experiment, 15 days from the second, and 13 days from the third growing season.

Determination of photosynthetic pigments in microgreens

Chlorophyll-*a*, *b*, and carotenoid contents were determined with a UV-1600PC spectrophotometer based on Moran and Porath (1980). Fresh leaf samples (50 mg) were dissolved in *N, N*-dimethylformamide (5 ml) at 4 °C for 72 hours. Subsequently, the amount of chlorophyll-*a* was measured at 664 nm, chlorophyll-*b* at 647 nm, and carotenoid content at 480 nm. Photosynthetic pigments were given in mg per g for fresh weight. For the calculation, the following formula was used:

$$\text{Chlorophyll-}a = 11.65*(A_{664}) - 2.69*(B_{647})$$

$$\text{Chlorophyll-}b = 20.81*(B_{647}) - 4.53*(A_{664})$$

$$\text{Carotenoids} = 1000*(Car_{480}) - 1.28*(A_{664}) - 56.7*(B_{647})$$

Absorbance values were measured in the wavelength range A₆₆₄, B₆₄₇, and Car₄₈₀.

Determination of dry matter content

The homogenized plant samples (5 g) were dried at 105 °C in a drying oven (VWR DRY-Line DL 53) to constant weight for about 4 hours. The measured data refer to the dry matter content.

Determination of microgreens yield

The yield was given based on the fresh weight of the plant.

Statistical analysis

Statistical analyses were performed using SPSS software (version 25). The data were submitted for analysis of variance (ANOVA), and significant differences among means (n=3) were determined using Tukey's post hoc test probability levels of 0.05 and 0.5.

RESULTS AND DISCUSSION

In microgreen production, yield is a rather important parameter. Therefore, it is crucial to use the suitable cultivation technology method, with which we get higher yields and thus higher revenue. In the first period, we detected high yields (4222 g m⁻²) for the red cabbage, which is due to the favorable temperature (Table 2). In the second growing period, nearly similar yields (~2722 g m⁻² and 2889 g m⁻²) were measured for blue and combined light for each plant species. In our experiment, we detected that the blue and combined lighting were more favorable for broccoli and red cabbage microgreen production than red light. Di Gioia et al. (2019) measured a lower yield of 1786 g m⁻² for red cabbage microgreen grown in a high tunnel covered with polyethylene film in soilless cultivations. Paradiso et al. (2018b) also found lower yields for broccoli (600 and 1500 g m⁻²) on a mixture of peat for a 12 h photoperiod where the light irradiance of 200

$\mu\text{mol m}^{-2} \text{s}^{-1}$. Based on these, it can be stated that LED lighting can produce higher yields by favorable environmental factors (temperature: $19.90 \pm 2.33 \text{ }^\circ\text{C}$, humidity: $65.54 \pm 10.82\%$).

Microgreens are young, herbaceous plants that wither rapidly, so it is important to select species that

have a higher dry matter content. In addition, it is essential to use a favorable light spectrum that is appropriate to the species, which increases the dry matter content of the seedlings.

Table 2: Yield (g m^{-2}) of microgreens at different LED lighting

Species / LED treatment		Yield (g m^{-2})			
		1. cultivation period	2. cultivation period	3. cultivation period	the mean of the growing period
Red cabbage	Blue	4222d	2778b	2444ab	3148.00 ± 944.98
	Red	2889c	2222a	2000a	2370.33 ± 462.69
	Combined	2333b	2667b	2333ab	2444.33 ± 192.83
Broccoli	Blue	1444a	2889a	2444ab	2259.00 ± 740.05
	Red	2111b	2222a	2000a	2111.00 ± 111.00
	Combined	1444a	2889b	2556b	2296.33 ± 756.69

Notes: Data were subjected to ANOVA. Different letters in the same column indicate significant difference at $P \leq 0.05$ level between the treatments (Tukey's post-hoc test). Values are means \pm standard errors

The first growing season had the highest dry matter content of the plants (Table 3). Presumably, this is due to the more favorable temperature values, as these are cold-tolerant plants, and the temperature during this period was cooler ($19.90 \pm 2.33 \text{ }^\circ\text{C}$) than in the other two periods ($22.88 \pm 1.28 \text{ }^\circ\text{C}$; $28.17 \pm 2.24 \text{ }^\circ\text{C}$). The dry matter content of the plants was similar in the other two periods.

In all three growing seasons, blue light has more favorably increased the dry matter content (~15%) of red cabbage than red or combined LED treatment. The combined light significantly raised the dry matter content of the broccoli in the different growing periods.

During the three growing seasons, the amount of dry matter of the microgreens developed almost similarly due to the more favorable light spectrum for the plants. Thus, red cabbage dry matter content was $15.35 \pm 0.77\%$ by the blue light, and broccoli was $15.24 \pm 0.74\%$ by the combined light in the 3-growing period.

Overall, blue light for red cabbage and combined light for broccoli are favorable for this parameter. In addition, the first growing season was more beneficial for both plant species, presumably due to the more favorable cooler temperatures for the microgreens.

Table 3: Dry matter content (%) by different LED lighting

Species / LED treatment		Dry matter content (%)			
		1. growing period	2. growing period	3. growing period	the mean of the growing period
Red cabbage	Blue	$15.88 \pm 1.01\text{ab}$	$14.46 \pm 0.44\text{a}$	$15.70 \pm 0.42\text{b}$	15.35 ± 0.77
	Red	$15.58 \pm 0.26\text{a}$	$13.77 \pm 0.56\text{a}$	$14.97 \pm 0.28\text{b}$	14.77 ± 0.92
	Combined	$14.58 \pm 0.83\text{a}$	$12.97 \pm 0.28\text{a}$	$12.60 \pm 0.28\text{a}$	13.38 ± 1.05
Broccoli	Blue	$18.08 \pm 0.12\text{b}$	$13.60 \pm 0.28\text{a}$	$13.17 \pm 0.00\text{a}$	14.95 ± 2.72
	Red	$16.88 \pm 0.12\text{ab}$	$12.87 \pm 1.27\text{a}$	$12.87 \pm 0.14\text{a}$	14.21 ± 2.32
	Combined	$16.08 \pm 0.73\text{ab}$	$14.67 \pm 1.03\text{a}$	$14.97 \pm 0.28\text{b}$	15.24 ± 0.74

Notes: Data were subjected to ANOVA. Different letters in the same column indicate significant difference at $P \leq 0.05$ level between the treatments (Tukey's post-hoc test). Values are means \pm standard errors

The chlorophyll content of plants is an indicating factor for photosynthetic activity. The two most common types of chlorophylls are chlorophyll-*a* and chlorophyll-*b*. In addition, the color of microgreens is one of the main characteristics that influence the choice of customers. Chlorophyll and carotenoid pigments

play an essential role in the color development of microgreens (Kowitcharoen et al., 2021).

The blue and combined light also had a positive effect on the chlorophyll-*a* content of red cabbage (Table 4) compared to the red light. For broccoli, blue light had a more favorable effect on chlorophyll-*a* content, except in the first period, where chlorophyll-*a*



content was remarkably high ($12.21 \pm 0.01 \text{ mg g}^{-1}$) by the combined light. This is probably due to the colder temperatures, which are favorable for broccoli. As the temperature in the first period was $19.90 \pm 2.33 \text{ }^\circ\text{C}$, however a temperature rise of $8 \text{ }^\circ\text{C}$ was observed for the last period, which may have affected this parameter. Ying et al. (2021) measured the chlorophyll-*a*,

chlorophyll-*b*, and carotenoid content of plants belonging to the *Brassicaceae* family and found that changing the percentage of blue light in LEDs giving $300 \mu\text{mol}^{-2} \text{ s}^{-1}$ PPFD was not affected the concentration of these pigments. The average total chlorophyll content for all six blue light treatments was about 0.6 mg g^{-1} fresh weight for cabbages.

Table 4: Chlorophyll-*a* content (mg g^{-1}) by different LED lighting

		Chlorophyll- <i>a</i> content (mg g^{-1})			
Species / LED treatment		1. growing period	2. growing period	3. growing period	the mean of the growing period
Red cabbage	Blue	$8.28 \pm 0.02\text{d}$	$7.71 \pm 0.74\text{c}$	$9.65 \pm 2.75\text{b}$	8.55 ± 1.00
	Red	$8.09 \pm 0.01\text{c}$	$7.48 \pm 1.35\text{bc}$	$7.20 \pm 1.29\text{ab}$	7.59 ± 0.46
	Combined	$4.89 \pm 0.01\text{a}$	$8.14 \pm 0.47\text{c}$	$8.73 \pm 1.22\text{ab}$	7.25 ± 2.07
Broccoli	Blue	$8.84 \pm 0.00\text{e}$	$5.96 \pm 2.15\text{abc}$	$7.40 \pm 0.82\text{ab}$	7.40 ± 1.44
	Red	$5.76 \pm 0.01\text{b}$	$4.36 \pm 1.98\text{ab}$	$5.41 \pm 0.10\text{a}$	5.18 ± 0.73
	Combined	$12.21 \pm 0.01\text{f}$	$4.13 \pm 2.19\text{a}$	$6.90 \pm 0.18\text{ab}$	7.75 ± 4.11

Notes: Data were subjected to ANOVA. Different letters in the same column indicate significant difference at $P \leq 0.05$ level between the treatments (Tukey's post-hoc test). Values are means \pm standard errors

In terms of different wavelengths, blue light had the most significant effect on the chlorophyll-*b* content (average $5.87 \pm 2.97 \text{ mg g}^{-1}$) of red cabbage in each growing season (Table 5). However, the combined light had the most considerable effect on the chlorophyll-*b*

content (average $6.06 \pm 3.08 \text{ mg g}^{-1}$) of broccoli during different growing seasons. In addition, in the second period for both plant species, we are detected high chlorophyll-*b* content ($8.99 \pm 2.96 \text{ mg g}^{-1}$ and $9.59 \pm 0.17 \text{ mg g}^{-1}$).

Table 5: Chlorophyll-*b* content (mg g^{-1}) by different LED lighting

		Chlorophyll- <i>b</i> content (mg g^{-1})			
Species / LED treatment		1. growing period	2. growing period	3. growing period	the mean of the growing period
Red cabbage	Blue	$3.07 \pm 0.01\text{d}$	$8.99 \pm 2.96\text{a}$	$5.55 \pm 0.17\text{b}$	5.87 ± 2.97
	Red	$2.65 \pm 0.02\text{c}$	$8.36 \pm 0.90\text{a}$	$3.42 \pm 0.63\text{a}$	4.81 ± 3.10
	Combined	$1.81 \pm 0.01\text{a}$	$8.58 \pm 0.39\text{a}$	$3.88 \pm 0.28\text{ab}$	4.76 ± 3.47
Broccoli	Blue	$3.19 \pm 0.01\text{e}$	$7.88 \pm 1.71\text{a}$	$3.48 \pm 0.06\text{ab}$	4.85 ± 2.63
	Red	$1.91 \pm 0.01\text{b}$	$7.52 \pm 0.17\text{a}$	$3.87 \pm 1.09\text{ab}$	4.43 ± 2.85
	Combined	$4.64 \pm 0.01\text{f}$	$9.59 \pm 0.17\text{a}$	$3.95 \pm 0.03\text{ab}$	6.06 ± 3.08

Notes: Data were subjected to ANOVA. Different letters in the same column indicate significant difference at $P \leq 0.05$ level between the treatments (Tukey's post-hoc test). Values are means \pm standard errors

Comparing the three growing periods in the development of the carotenoid content (Table 6), we measured more favorable values for this parameter in the second cultivation. Presumably, this period had a more favorable effect on carotenoid synthesis.

The blue light was also more favorable for the red cabbage, while combined light for broccoli was more favorable for carotenoid content for several growing

periods. However, in the second cultivation times, the highest carotenoid content (10.47 ± 2.77 and $10.25 \pm 4.28 \text{ mg g}^{-1}$) was measured for both plant species. Kowitcharoen et al. (2021) cultivated microgreens belonging to the *Brassicaceae* family were grown soilless with white fluorescent tubes. In their experiment, higher carotenoid was measured in broccoli and lower content for red cabbage.

Table 6: Carotenoid (mg g⁻¹) of microgreens at different LED lighting treatment

Species / LED treatment		Carotenoid (mg g ⁻¹)			
		1. growing period	2. growing period	3. growing period	the mean of the growing period
Red cabbage	Blue	5.92 ± 0.02d	10.47 ± 2.77c	8.97 ± 2.10b	8.45 ± 2.32
	Red	5.70 ± 0.00c	9.92 ± 0.65cb	6.71 ± 0.86ab	7.44 ± 2.20
	Combined	4.77 ± 0.01b	10.55 ± 1.30c	7.35 ± 0.20ab	7.56 ± 2.90
Broccoli	Blue	6.80 ± 0.02e	8.63 ± 1.0ab	6.77 ± 0.18ab	7.40 ± 1.07
	Red	4.10 ± 0.01a	8.05 ± 0.20a	5.86 ± 0.66a	6.00 ± 1.98
	Combined	9.25 ± 0.01f	10.25 ± 4.28bc	7.72 ± 0.24ab	9.07 ± 1.27

Notes: Data were subjected to ANOVA. Different letters in the same column indicate significant difference at P ≤ 0.05 level between the treatments (Tukey's post-hoc test). Values are means ± standard errors

CONCLUSIONS

In the experiment, three different light intensities were used to grow red cabbage and broccoli. Overall, the development of red cabbage was favorably influenced by the blue wavelength range. As for the broccoli, the combined light was more favorable compared to the other two light sources. Finally, we can conclude that the proper condition of microgreen production (LED light, temperature, humidity) will be an excellent source for raw materials of different salads. The increased bioactive component (chlorophyll-*a,b*,

and carotenoid) has proved that in a closed system (glasshouse) it is possible to produce healthy plant material. In addition, the choice of a more favorable growing climate condition for the plant species is also an essential factor, as these parameters also influence the bioactive content (chlorophyll-*a,b*, and carotenoid).

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