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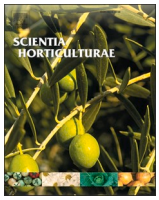

Research Paper

End-of-production light treatments as a tool for controlling chemical composition of herbs and lettuce

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ABSTRACT

Growing plants in indoor environments ("Vertical farms"/"Plant factories") provides good opportunities of controlling all climatic factors, including light intensity, daily light integral, and light quality. Thus, it is possible to control the quality of the produce to a great extent. The present study examines the possibilities to use "end-of-production" treatments with narrow band light to control the quality of leafy vegetables. Two different experiments were performed, comprising different types of leafy vegetables which were grown in sole multi-wavelength LED light, or in a greenhouse with high-pressure sodium light, for the first weeks of the production cycle. At the end of the production cycle, plants were subjected to narrow-band light at different peak wavelengths for the last four days before harvest. Four different narrow-band light treatments (peak wavelengths 456, 520, 596, and 663 nm) were included in the study. The leafy vegetables were analysed with respect to their content of chlorophylls, carotenoids, and total phenolic content, in addition to biometric data (fresh- and dry weight). The results indicated the potential of end-of-production treatments to modify the concentration of secondary metabolites in leafy vegetables, with especially blue and yellow light treatments having the potential of increasing the concentration of phenolic compounds and lutein/ϭ-carotene. However, for some of the treatments and cultivars, fresh- and/or dry weight was significantly reduced by the end-of-production treatment.

1. Introduction

Vertical farms ("plant factories") have received a lot of attention in recent years and several commercial operations of various sizes, from small in-shop systems to full-scale operations have been established in many countries. The fast development within lighting technology with the introduction of LED-based light sources has been the main driving force for this development (Butturini and Marcelis, 2020). This development has coincided with a generally increased interest among consumers in buying locally produced and pesticide-free vegetables, which has created the conditions for the establishment of vertical farms. Producing leafy vegetables in a fully controlled environment extends possibilities to control quality of the produce. Factors, such as temperature, fertigation, and light environment can be used to modulate appearance, taste, and nutritional value of plants. Especially modulation of plant quality and composition by varying light environment seems feasible in vertical farms. LEDs emit narrow-band light with many different peak wavelengths available within the light spectrum, from ultraviolet light via blue, green, red and far-red light. Using light in specific wavelengths or spectra has been proposed by several authors as a means of controlling the quality (taste, chemical composition) of herbs and leafy vegetables produced in greenhouses or vertical farms.

Light spectrum modulates production of primary as well as secondary metabolism of plants. Chlorophylls are primary pigments in photosynthesis. Chlorophylls existing in plants are classified in *a* and *b* chlorophylls, which together form light harvesting protein complex (Kume et al., 2018). Absorption spectrums of chlorophylls *a* and *b* differ, allowing broader capture of light energy (Tanaka and Tanaka, 2000). While the chlorophylls efficiently absorb blue and red light, the green and yellow wavelengths are assimilated by carotenoids (Pérez et al., 2006). Carotenoids act as light harvesting or photoprotective *secondary* metabolites. Occurrences of different carotenoid pigments varies between the plants. Typical carotenoids existing in vegetables are for example ϭ -carotene, lutein, zeaxanthin and lutein (Sathasivam et al., 2021). Chlorophylls and carotenoids have impacts on color development of leafy vegetables. The health implications of chlorophylls and carotenoids are associated to their antioxidative properties (Ebert, 2022) and particular carotenoids (especially *α*- and ϭ -carotene) are known as a

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pre-cursors of vitamin A (Gómez-Sagasti et al., 2023). Light quantity and quality are the key factors controlling chlorophyll and carotenoid concentrations in plants, since the plants regulate chlorophyll to carotenoid ratio to maintain photosystem (Sathasivam et al., 2021).

Phenolic compounds primarily consisting phenolic acids and polyphenols are defensive compounds in plants against abiotic stress (Fayezizadeh et al., 2024). The phenolic compounds are strong antioxidants, but simultaneously, as a high concentration connected to undesirable flavors, such as sourness, astringency and bitterness (Ebert, 2022). Son and Oh (2013) produced lettuce with a combination of red and blue LEDs at different ratios. They concluded that in general, there was an increase in the content of phenolic compounds and antioxidants in the leaves in plants that were grown with high proportions of blue light. However, plants grown with a high proportion of blue light had lower fresh weight at the time of harvest. Increased proportion of blue light was also generally associated with more pigments and a bitter taste in leafy vegetables (Kelly and Runkle, 2020). Also, Bantis et al. (2016) found higher contents of phenolic compounds in basil produced with LED light, rich in blue, as compared to plants grown under fluorescent tubes. Thongtip et al. (2024) demonstrated the impact of cultivation time, species and specific light spectrum responses on total phenolic compound contents of Peppermint, Thai basil, Cumin, Lemon balm, and Green holy basil. On DAS 22 plants grown under blue spectrum had highest total phenolic contents, whereas white and red light alterations increased the total phenolic contents in general. In red and green leaf Pak-choi with supplemented UV-A (400 nm) or blue LED light (430 nm) increased concentration of total phenolic compounds in comparison to control treatment without LED lighting (Mao et al., 2021). Supplementation of white light spectrum with blue LED light was found to increase total chlorophyll and carotenoid concentrations of Pak choi in comparison to red + white or only white spectrum treatments (Frede et al., 2023). In the study of Mao et al. (2021) UV-A (380 and 400 nm) and blue LED (430 nm) supplementations enhanced chlorophyll formation, whereas UV-A (400 nm) and blue led spectrums (430 and 460 nm) boosted carotenoid production in red-leaf Pak choi. Supplementing the visible spectrum with UV-A light was demonstrated to increase the contents of vitamin A, proteins and sugar in lettuce grown in a plant factory (He et al., 2021). Increasing the light intensity and/or daily light integral (DLI) will generally decrease nitrate content and increase content of vitamin C, sugars, proteins and anthocyanin in leafy vegetables grown with only artificial light (Zhang et al., 2018). Also yellow light has been suggested to trigger production of phenols (Brazaitytė et al., 2016b).

However, applying specific spectra designed to modify the chemical composition of the plants during the whole production cycle might lead to reduced biomass production and increased energy use as these light qualities might be less efficient for photosynthesis, increased pigmentation shading the photosynthetic pigments. Also, LEDs producing short-waveband have generally lower energy conversion rate than LEDs producing red light. Therefore, the concept "End of production lighting" (EOP) was developed, where plants are subjected to a specific wavelength or spectrum during the last days of production, in order to modify the quality of the produce with respect to taste, nutritional value or appearance like pigmentation. The objective of the present study was to evaluate the usefulness of EOP treatments as a means of controlling the quality of herbs and leafy vegetables grown in vertical farms with sole artificial lighting. Analysis of chlorophyll *a* and chlorophyll *b*, total carotenoids or lutein and *ϭ* -carotene, and total phenolic compounds were carried out after EOP treatments. Our hypotheses were that i) narrow-band light in short wavelengths (blue, green and yellow light) will increase the contents of secondary metabolites in the leaves, and ii) low light intensities of narrow band light are sufficient to induce a significant shift in the chemical composition of the leaves.

2. Materials and methods

2.1. Experiment 1

2.1.1. Plant material

Three different plant species were used in the experiment; *Brassica rapa* cv. Joi Choi F_1 (pak choi), *Ocimum basilicum* cv. Edwina (sweet basil), and *Beta vulgaris* cv. Perpetual Spinach (chard). Pak choi and chard seeds were sown in plug trays with a peat-based growing medium (Hasselfors S-soil, Hasselfors Garden, Örebro, Sweden). At 11 days after sowing (DAS), the plants were rooted through the growing medium in the plugs and the first true leave started to emerge, and thus plants were transplanted to 12 cm pots. The 12 cm pots were filled with a peat-based growing medium (Hasselfors K-soil, Hasselfors Garden, Örebro, Sweden), and supplemented with 5 g l^{-1} of controlled-release fertilizers (Basacote 3 M, N-P-K 16–8–12, COMPO GmbH & Co KG, Münster, Germany). The basil was sown directly in 11 cm pots with the same growing medium as for pak choi and chard.

2.1.2. Experimental conditions

The experiment took place from mid-October to mid-December 2022 at Alnarp research center, Sweden (55°39 N, 13°5 E). The plants were placed in a greenhouse chamber with heating temperature set to 17°C nighttime and 18°C daytime, with vents opening when the temperature exceeded 20°C. The climate was controlled and monitored by a climate computer (Priva Connext, Priva, de Lier, The Netherlands). The actual temperature in the greenhouse chamber during the growth cycle was 19.9 ± 2.4°C, and the relative humidity was 65.4 ± 8.7 %. Supplementary light (high pressure sodium, Philips Green Power 400 W, Philips, Eindhoven, the Netherlands) was given for 12 h day^{-1} at an intensity of 62 ± 9 μmol m^{-2} s^{-1}, corresponding to a DLI of 2.7 mol m^{-2} day^{-1} of supplementary light. The outside natural irradiation was declining from 5900 J cm^{-2} $week^{-1}$ at the start of the experiment to 600 J cm^{-2} $week^{-1}$ at the end of the experiment. The plants were irrigated manually with tap water.

At 41 DAS (pak choi), 48 DAS (chard), and 54 DAS (basil), the plants were transferred to a climate chamber without daylight for end-of-production (EOP) treatment. The temperature in the climate chamber was set to 22°C. Four different EOP treatments with narrow-band light was applied; blue light (peak λ 456 nm), green light (peak λ 520 nm), yellow light (peak λ 596 nm), and red light (peak λ 663 nm). The narrow-band light was supplied with 90 W custom-made LED arrays (Trädgårdsteknik AB, Ängelholm, Sweden). The narrow-band light was supplied for 16 h day^{-1} at an intensity of 50 μmol m^{-2} s^{-1} for four days (in total 64 h, corresponding to a DLI of 2.8 mol m^{-2} day^{-1}). Plants not subjected to EOP treatment but kept in the greenhouse under the same conditions as during the main growth phase were used as controls.

2.1.3. Measurements and analyses

The light intensity was measured using a quantum sensor (Skye PAR Quantum sensor, Skye Instruments Ltd, Llandrindod Wells, UK). The spectral output from the LED arrays was measured using a spectroradiometer (Licor LI-1800, LI-COR, Lincoln, USA). The phytochrome photostationary state (PSS) for the different EOP light qualities was calculated as described by Sager et al. (1988).The climatic conditions during the experiment were logged using data loggers (HOBO U12, Onset Computer Corp., Bourne, MA USA). The relative chlorophyll content (Chlorophyll Content Index, CCI) in the leaves was measured at the end of the experiment (Apogee MC-100, Apogee Instruments Inc, North Logan UT USA.

After the EOP treatment, the plants were harvested, and fresh weight was measured. The plants were then lyophilized, and prior to extraction of chemical compounds ground to a powder.

2.1.4. Extraction

Extractions of chlorophylls, carotenoids, and phenolic compounds

with ethanol (96 %) were carried out with accelerated solvent extraction (DIONEX™ ASE 350™ Thermo Fisher Scientific Inc, USA). The samples (0.5 g) were mixed with diatomaceous earth (Dionex™ ASE™ Prep DE, Thermo Scientific Inc, USA), and extracted in 22 mL extraction cells with glass fiber filter (Thermo Scientific Inc, USA) at 110°C and 1500 psi with a three 10 min extraction cycles. The extracts were transferred from collection vials to volumetric flasks, and sample volumes were adjusted to 50 mL. Selection of extraction parameters and solvent was based on earlier study of Repajić et al. (2020).

2.1.5. Analysis

Samples were analyzed spectrophotometrically (UV-1800 UV/VIS Spectrophotometer, Shimadzu Corporation, Kyoto, Japan), using wavelengths 665 and 649 nm for chlorophylls and 470 nm for carotenoids. The concentrations of total chlorophyll, chlorophyll *a* and *b* and total carotenoids were calculated with equations described earlier (Gao et al., 2021).

The total phenolic content was quantified using Folin Ciocalteu's method using gallic acid (GA) (Acros Organics, Belgium) as a standard. The GA was first diluted in distilled H_2O (1 mg mL^{-1}), which after standard curve was prepared with concentrations of 5, 10, 20, 30, 40, 100 and 200 mg l^{-1}. For the analysis, 200 μL of each standard or sample were combined with 1 mL of Folin-Ciocalteu's phenol reagent (Merck, Germany) (diluted in H_2O, 1:10), and 0.8 mL of 7.5 % Na_2CO_3 solution. The samples were incubated for 30 min in room temperature and analyzed with spectrophotometer (UV-1800 UV/VIS Spectrophotometer, Shimadzu, Japan) using wavelength 765 nm. The results were expressed as gallic acid equivalents (GAE) per mg GAE g^{-1} (dry weight).

2.1.6. Statistics and treatment of data

There were five independent replicates per treatment. The data retrieved from the experiment was subjected to one-way analysis of variance (ANOVA) with Tukey's multiple comparison test to examine differences between treatments. The analysis was performed with a significance level of $p < 0.05$ considered as significant (Minitab 21, Minitab, Inc., State College, PA, USA).

2.2. Experiment 2

2.2.1. Plant material

Four different plant species/cultivars were used in the experiment: *Lactuca sativa* cv. Freelou, *Lactuca sativa* cv. Javelo (lettuce), *Ocimum basilicum* cv. Aromatico (sweet basil), *Coriandrum sativum* cv. Calypso (cilantro). The specific varieties were selected as they are commercially used in plant factories. The plants were grown in 11 cm pots with Hasselfors K-soil (Hasselfors Garden, Örebro, Sweden) with added controlled-release fertilizers (Basacote 3 M, N-P-K 16–8–12, COMPO GmbH & Co KG, Münster, Germany).

2.2.2. Experimental conditions

The plants were grown a growth chamber with no access to natural light. Light was supplied using LED lamps, Valoya B150 with spectrum AP673L (Valoya OY, Helsinki, Finland) at an intensity of 300 ± 30 μmol m^{-2} s^{-1} for 16 h day^{-1}, resulting in a DLI of 17.3 mol m^{-2} day^{-1}. The temperature set point was kept constant at 20°C. The plants were irrigated manually with tap water. The measured temperature in the climate chamber was on average 20.4 ± 0.4°C and the relative humidity was 53.1 ± 8.3 %.

At 28 DAS, plants were transferred to separate chambers and subjected to EOP treatments as described for experiment 1. Control plants were kept in the original conditions.

2.2.3. Measurements and analyses

Biometric measurements as well as chlorophylls and total phenolic compounds were analysed as described in experiment 1.

Lutein and ℓ-carotene were analyzed with high performance liquid chromatography (UHPLC, Shimadzu Nexera XR LC-40, Japan) connected with PDA detector (SPD-M40, Shimadzu, Japan) and using YMC carotenoid C30 column (3 μm, 250 × 4.6 mm) and YMC Guard Cartridge (3 μm, 10 × 4.0 mm) (YMC Europe GmbH). The analysis was performed as described in the column manufacturer's instructions with minor modifications. Elution solvents A and B in analysis were MeOH:MTBE: H2O (81:15:4 vol), and MeOH (10:90 vol). The eluent gradients in UHPLC analysis were following; 100 % eluent A 0–44 min; 51 % A and 49 % B 44–46 min; 100 % B at 46–53 min. The flow rate was 1 mL min^{-1} and injection volume 20 μl. Prior to analysis, the EtOH extracts were filtered (Pall Acrodisc Syringe Filters, 0.45 μm). For quantification and identification of lutein and ℓ-carotene were based to retention times (rt 13.2 min and 33.7 min) in standard mix with seven concentrations of both carotenoids (Lutein; Supelco, >98 % and ℓ -carotene; Supelco, >95 %) was prepared. Additionally, neoxanthin was identified (Rt 8.3 min) but not quantified using analytical standard (9′-cis-neoxanthin, DHI). Three replicates were analysed.

2.2.4. Statistics and data management

The experiment was designed as a split-plot design with two blocks with each 10 individual replicates. Statistical analysis from biometric and chemical analyses between treatments and within species were subjected to one-way ANOVA with Tukey's multiple comparison test, where $p < 0.05$ was considered as significant. Number of replicates in statistical analysis was ten (Freelou', Basil, Cilantro) or five (Lettuce 'Javelo') for biometric parameters and five ($N = 5$) for chemical composition ($N = 5$). Three replicates ($N = 3$) were used in comparison of lutein + β-carotene contents.

3. Results

3.1. Results from experiment 1

The PSS-values were calculated to 0.885 for the red (663 nm) treatment, 0.508 for the blue (456 nm) treatment, 0.825 for the green (520 nm) treatment and 0.915 for the yellow (596 nm) treatment. The plants subjected to the EOP-treatment were not significantly different from control plants with regard to fresh biomass for any of the plant species (Table 1). However, pak choi plants in the control treatments and in the green (520 nm) treatment had significantly lower dry weight than the other treatments (Table 1).

In experiment 1, the concentrations of phenolic compounds in pak choi were higher (4.87–5.46 mg g^{-1} DW, $p < 0.05$) under blue (456 nm), green (520 nm), yellow (596 nm), and control treatments ($p < 0.05$) than in the treatment with red (663 nm) light (4.44 mg g^{-1} DW) (Table 2). Differences in total phenolic contents of chard and basil grown in different light conditions were not found. EOP treatments did not have influence for total chlorophyll concentrations of plant species. However, under the yellow light (596 nm), the chlorophyll *b* concentration in pak choi was higher ($p < 0.05$) than in treatments with blue (465 nm) and red (663 nm), whereas differences in chlorophyll *a* or *b* concentrations with chard or basil were not found. Total carotenoid concentrations were the same in all EOP treatments. In general, amounts of carotenoids were higher in basil than in pak choi and chard.

3.2. Results from experiment 2

The chlorophyll content (measured photometrically) only differed significantly between treatments for one of the plant species included in the experiment, lettuce 'Freelou', where the control treatment displayed the highest chlorophyll concentration, while all other treatments had lower chlorophyll concentration (Table 3). For fresh weight, only the lettuce 'Javelo' showed significant differences, with significantly lower fresh weight for plants treated with blue (456) nm as end-of-production treatment, compared with all other treatments. For dry weight, however, there were significant differences for all plant species included in

Table 1
Biometric parameters from experiment 1. Three plant species were grown in a greenhouse environment and subjected to a 64-h end-of-production treatment with narrow-band light (50 μmol m-2 s-1) at four different wavelengths (WL); 456, 520, 596, or 663 nanometer (nm). Plants left in the greenhouse environment without specific end-of-production treatment served as control. FW = Fresh weight (g), DW = Dry weight (g). Figures within columns which do not share the same letter are significantly different at $p < 0.05$ (One-way ANOVA + Tukey's multiple comparison test), $N = 5$.

Plant species	Pak Choi		Chard		Basil	
WL (nm)	FW (g)	DW (g)	FW (g)	DW (g)	FW (g)	DW (g)
456	46.61 ± 5.81 a	1.93 ± 0.26 ab	19.30 ± 1.73 a	1.00 ± 0.15 a	26.26 ± 4.40 a	1.70 ± 0.25 a
520	38.80 ± 5.62 a	1.71 ± 0.26 b	21.34 ± 8.53 a	1.05 ± 0.36 a	26.52 ± 2.27 a	1.71 ± 0.16 a
596	48.16 ± 6.08 a	2.19 ± 0.29 a	25.36 ± 4.10 a	1.42 ± 0.26 a	32.10 ± 5.13 a	2.12 ± 0.36 a
663	46.61 ± 5.86 a	2.25 ± 0.10 a	24.37 ± 2.39 a	1.45 ± 0.33 a	34.87 ± 7.10 a	2.37 ± 0.50 a
Control	41.28 ± 7.07 a	1.68 ± 0.28 b	18.39 ± 6.18 a	1.03 ± 0.45 a	30.73 ± 6.22 a	1.95 ± 0.42 a
p-value	0.096	0.03	0.187	0.088	0.079	0.038

Table 2
Concentration of chlorophyll a and b (Chl a and b. mg g-1), total carotenoids (Carot) (mg g-1, DW) and total phenolic contents (Phen tot) (mg GAE g-1, DW) in the green tissue of three species of herbs and vegetables cultivated in a greenhouse environment and subjected to end-of-production treatments with narrow-band light at four different wavelengths (WL); 456, 520, 596 and 663 (nm) at and intensity of 50 μmol m-2 s-1 for four days before harvest. The control treatment was not subjected to narrow-band light but kept in the greenhouse until harvest. Figures within columns which do not share a letter are significantly different at $p < 0.05$ (One-way ANOVA + Tukey's multiple comparison test), $N = 5$.

	Pak Choi			
Treatment (WL)	chl a mg g^{-1}	chl b mg g^{-1}	Carot mg g^{-1}	Phen tot mg GAE g^{-1}
456	7.20 ± 0.29 a	2.43 ± 0.09 b	0.85 ± 0.06 a	5.12 ± 0.41 a
520	7.47 ± 0.44 a	2.66 ± 0.18 ab	0.92 ± 0.05 a	4.96 ± 0.49 ab
596	8.05 ± 1.06 a	2.96 ± 0.44 a	0.85 ± 0.10 a	4.87 ± 0.34 ab
663	7.38 ± 0.33 a	2.38 ± 0.13 b	0.85 ± 0.03 a	4.44 ± 0.25 b
Control	7.79 ± 0.37 a	2.72 ± 0.10 ab	0.93 ± 0.05 a	5.46 ± 0.10 a
p-value	0.176	0.005	0.131	0.003
	Chard			
	chl a mg g^{-1}	chl b mg g^{-1}	Carot mg g^{-1}	Phen tot mg GAE g^{-1}
456	6.62 ± 0.49 a	2.2 ± 0.15 a	0.95 ± 0.05 a	5.78 ± 1.05 a
520	6.43 ± 0.06 a	2.26 ± 0.15 a	0.92 ± 0.06 a	5.98 ± 0.73 a
596	5.79 ± 0.48 a	2.04 ± 0.14 a	0.84 ± 0.08 a	5.77 ± 0.83 a
663	5.75 ± 0.47 a	1.98 ± 0.14 a	0.86 ± 0.09 a	6.82 ± 0.59 a
Control	6.29 ± 0.97 a	2.18 ± 0.29 a	0.95 ± 0.14 a	5.42 ± 0.84 a
p-value	0.095	0.139	0.190	0.133
	Basil			
	chl a mg g^{-1}	chl b mg g^{-1}	Carot mg g^{-1}	Phen tot mg GAE g^{-1}
456	9.65 ± 0.50 a	3.4 ± 0.18 a	1.17 ± 0.09 a	12.98 ± 0.76 a
520	9.56 ± 0.68 a	3.63 ± 0.20 a	1.05 ± 0.10 a	11.75 ± 0.48 a
596	9.78 ± 0.80 a	3.74 ± 0.37 a	1.1 ± 0.06 a	11.81 ± 0.44 a
663	9.47 ± 0.33 a	3.56 ± 0.16 a	1.06 ± 0.05 a	12.54 ± 0.66 a
Control	9.64 ± 0.64 a	3.64 ± 0.16 a	1.15 ± 0.11 a	12.46 ± 0.86 a
p-value	0.949	0.239	0.145	0.037

the experiment, with a general trend of higher dry weights for plants in the control treatment, as compared with all treatments treated with narrow-band light as end-of-production treatments (Table 3).

For the secondary metabolites, the concentration of total phenolic compounds was higher ($p < 0.05$) in lettuce, cultivar Freelou, for plants treated with yellow (596 nm) light (Table 4) than in EOP treatments with green (520 nm) or red (663 nm) light or in the control treatment (Table 4). For basil, total phenolic concentrations were comparable in EOP treatments (456, 520 and 596 nm) subjected plants, whereas only treatment with yellow light led to the significantly higher total phenolic content than obtained in control treatment (Table 4). Light conditions did not have impact on concentrations of phenolic contents of cilantro or lettuce 'Javelo'.

In overall, the amounts of the main carotenoid pigments (lutein + 6 -carotene) (Fig. 1) and chlorophylls (Table 4) were higher in basil than in lettuces Freelou and Javelo or in cilantro. Lutein was the main carotenoid pigment in all studied plants. Also, *6-carotene and neoxanthin* existed in all samples. The incongruous results were obtained impact of EOP treatments in the carotenoid synthesis of the plants studied. The highest concentrations of analyzed carotenoids (lutein + 6 -carotene) were found in cilantro plants treated with red or blue light ($p < 0.05$), whereas in 'Javelo' higher carotenoid concentrations were obtained under blue than red EOP treatment ($p < 0.05$). Light did not have statistically significant effect on carotenoid concentrations of lettuce 'Freelou' and basil.

Lower concentrations of chlorophyll *a* as well as chlorophyll *b* were obtained in basil and cilantro in the control than in other treatments (<0.05), except in blue light treated basil, which chlorophyl concentrations were comparable with control (Table 4).

4. Discussion

In the present study, the potential of modifying the biochemical properties of leafy vegetables and herbs by applying narrow-band light in the form of end-of-production treatments was demonstrated. The hypotheses that narrow-band light at moderate intensities would have a significant effect on the biochemical composition of the plants was partly proven correct, but with inconsistencies between plant species.

For the fresh weight, differences between treatments were surprisingly small, given that the plants subjected to EOP-treatment received a lower total light integral during the production cycle, as compared to the control treatment. For the dry weight, differences were more pronounced, for the experiments performed in growth chamber. Increased water content due to reduced transpiration during the EOP phase could be one possible explanation for this phenomenon.

Against our hypothesis, the end-of-production treatments with narrow-band light in short wavelengths (blue and green light) had little effect on the concentrations of phenolic compounds, chlorophyll and total carotenoids or 6-carotene and lutein. Yellow and blue lights boosted the phenolic compounds production of lettuce Freelou and basil in comparison to control, but not other species studied. However, in pak choi, the lower concentration of total phenolic compounds was obtained under red light in comparison to control. The increased concentrations of phenolic compounds as a result of treatment with yellow light was anticipated, as yellow light is shown to trigger production of phenolic compounds in sweet basil (Loughrin and Kasperbauer, 2001). Similar results were also found by Brazaitytė et al. (2016a).

Earlier studies have shown that influence of light spectrum for chemical profile of plants is species specific, and the chemical composition shifts during the cultivation time. Detailed analysis of phenolic acids of lettuce (*Lactuca sativa* var. Lollo Rossa) and basil (*Ocimum*

Table 3
Biometric parameters from experiment 2, measured for four different plant types subjected to end-of-production treatments for four days with narrow band LED light at 456, 520, 596, or 663 nanometer (nm) wavelength. Plants grown under multi-spectral light were used as control., FW = Fresh weight (g), DW = Dry weight (g), Chl CCI = Chlorophyll Concentration Index. Figures within columns which do not share a letter are significantly different at $p < 0.05$ (One-way ANOVA + Tukey's multiple comparison test), $N = 10$ (Lettuce 'Freelou', Basil, Cilantro) $N = 5$ (Lettuce 'Javelo').

	Lettuce 'Freelou'			Lettuce 'Javelo'		
WL (nm)	Chl (CCI)	FW (g)	DW (g)	Chl (CCI)	FW (g)	DW (g)
456	3.54 ± 0.42 b	28.41 ± 7.11 a	1.35 ± 0.41 ab	4.38 ± 1.52 a	29.71 ± 7.42 b	1.24 ± 0.31 c
520	3.22 ± 0.79 bc	22.95 ± 7.43 a	0.97 ± 0.35 b	3.40 ± 1.30 a	37.18 ± 4.9 ab	1.50 ± 0.21 bc
596	2.64 ± 0.43 c	28.68 ± 10.51 a	1.22 ± 0.47 b	3.72 ± 0.90 a	39.26 ± 2.20 a	1.72 ± 0.11 bc
663	2.85 ± 0.55 bc	28.00 ± 7.76 a	1.18 ± 0.44 b	2.22 ± 0.31 a	42.37 ± 3.56 a	1.82 ± 0.17 b
Control	4.79 ± 0.94 a	28.87 ± 11.28 a	1.86 ± 0.74 a	4.36 ± 1.50 a	44.13 ± 4.70 a	2.48 ± 0.39 a
p-value	0.000	0.548	0.004	0.048	0.001	0.000
	Basil			Cilantro		
	Chl (CCI)	FW (g)	DW (g)	Chl (CCI)	FW (g)	DW (g)
456	10.06 ± 1.91 a	47.77 ± 5.73 a	3.17 ± 0.54 b	10.91 ± 3.25 a	42.48 ± 4.95 a	3.44 ± 0.41 b
520	10.57 ± 2.24 a	48.17 ± 8.69 a	3.21 ± 0.36 b	12.81 ± 3.01 a	41.34 ± 5.62 a	3.59 ± 0.56 b
596	8.91 ± 1.47 a	44.94 ± 54.77 a	3.04 ± 0.37 b	11.89 ± 2.52 a	37.06 ± 6.25 a	3.22 ± 0.69 b
663	10.22 ± 1.31 a	54.77 ± 3.13 a	3.52 ± 0.32 b	11.60 ± 4.66 a	38.36 ± 6.71 a	3.53 ± 0.46 b
Control	11.41 ± 3.56 a	49.45 ± 7.73 a	4.74 ± 0.67 a	13.01 ± 3.90 a	40.76 ± 5.65 a	4.90 ± 0.39 a
p-value	0.220	0.230	0.000	0.663	0.235	0.000

Table 4
Concentration of secondary metabolites in the green tissue of four different species of herbs grown in a climate chamber and subjected to four-day end-of-production treatments with narrow-band light at four (Freelou, Javelo and cilantro) or three (basil) different wavelengths (WL); 456, 520, 596 and 663 nanometer (nm), with multispectral light as control treatment. Chl = chlorophyll content (mg g-1), Phen tot = total phenolic content (mg GAE g-1). Figures within columns which do not share a letter are significantly different at $p < 0.05$ (One-way ANOVA + Tukey's multiple comparison test). For Chl a and b, and Phen tot $N = 5$. For Lutein + β-carotene $N = 3$.

		Freelou				Javelo		
Treatment (WL)	Lutein + β-carotene mg g^{-1}	chl a mg g^{-1}	chl b mg g^{-1}	Phen tot mg GAE g^{-1}	Lutein + β-carotene mg g^{-1}	chl a mg g^{-1}	chl b mg g^{-1}	Phen tot mg GAE g^{-1}
456	0.16 ± 0.02 a	2.94 ± 0.19 a	1.11 ± 0.13 a	28.62 ± 2.70 ab	0.49 ± 0.043 a	3.61 ± 0.26 a	1.26 ± 0.9 ab	7.17 ± 0.98 a
520	0.15 ± 0.05 a	2.74 ± 0.33 a	1.12 ± 0.12 a	23.51 ± 2.99 b	0.36 ± 0.013 bc	3.55 ± 0.16 a	1.26 ± 0.46 a	6.75 ± 0.53 a
596	0.20 ± 0.02 a	2.55 ± 0.21 a	1.00 ± 0.12 a	31.07 ± 4.20 a	0.46 ± 0.074 ab	3.51 ± 0.36 a	1.17 ± 0.12 ab	6.52 ± 0.61 a
663	0.20 ± 0.07 a	2.73 ± 0.34 a	1.01 ± 0.13 a	23.13 ± 3.32 b	0.33 ± 0.019 c	3.24 ± 0.22 a	1.08 ± 0.10 b	6.68 ± 1.28 a
Control	0.19 ± 0.06 a	2.67 ± 0.30 a	0.90 ± 0.14 a	24.20 ± 2.77 b	0.40 ± 0.023 bc	3.33 ± 0.25 a	1.087 ± 0.07 b	7.18 ± 0.76 a
p-value	0.609	0.396	0.073	0.003	0.004	0.165	0.007	0.688
		Basil				Cilantro		
Treatment (WL)	Lutein + β-carotene mg g^{-1}	chl a mg g^{-1}	chl b mg g^{-1}	Phen tot mg GAE g^{-1}	Lutein + β-carotene mg g^{-1}	chl a mg g^{-1}	chl b mg g^{-1}	Phen tot mg GAE g^{-1}
456	1.25 ± 0.10 a	5.52 ± 0.47 ab	1.80 ± 0.17 ab	20.10 ± 2.42 ab	0.82 ± 0.09 ab	6.14 ± 0.08 a	1.95 ± 0.05 a	6.26 ± 0.91 a
520	1.11 ± 0.14 a	5.99 ± 0.49 a	2.10 ± 0.21 a	17.50 ± 2.88 ab	0.72 ± 0.38 b	5.64 ± 0.69 a	1.92 ± 0.29 a	6.25 ± 0.49 a
596	1.35 ± 0.10 a	5.93 ± 0.82 a	2.11 ± 0.30 a	20.41 ± 3.17 a	0.77 ± 0.87 b	6.07 ± 0.63 a	1.97 ± 0.19 a	7.76 ± 0.67 a
663	n/a	n/a	n/a	n/a	0.99 ± 0.04 a	5.42 ± 0.16 a	1.73 ± 0.98 a	7.19 ± 1.41 a
Control	1.06 ± 0.11 a	4.39 ± 0.66 b	1.48 ± 0.34 b	15.46 ± 1.21 b	0.73 ± 0.08 b	3.87 ± 0.39 b	1.34 ± 0.21 b	7.68 ± 0.60 a
p-value	0.056	0.003	0.004	0.026	0.006	0.000	0.000	0.021

basilicum cv. Genovese gigante) grown under red or blue weighed spectrum revealed increase of several phenolic acids under blue light in lettuce, whereas only minor differences were obtained in phenolic acids of basil grown under blue either red light (Taulavuori et al., 2016). However, Thongtip et al. (2024) showed variation in phenolic content of basil microgreens in different development stages of plants. Ouzounis et al. (2015) found increased concentrations of specific phenolic compounds in red-leaf lettuce under blue spectrum with light intensity of 45 μmol m^{-2} s^{-1}. However, Gómez and Jimenéz (2020) applying blue light as end-of-production treatment, but at much higher light intensity than in our study did not find any significant effects on the concentrations of phenolic compounds in lettuce.

In study of Bungala et al. (2024), the combination of blue and red spectrum resulted to higher total phenolic content in red pak choi baby leaves than treatment with white, red or blue spectrum alone. Instead, increased total phenolic content in green- and red-leave pak choi with supplemental blue light at 430 nm, but not with the blue spectrum at 460 nm, comparable to wavelength used in this study (456 nm), was shown by Mao et al. (2021). Enhanced phenolic compound production in cilantro has been reported under blue spectrum (Nguyen et al., 2020), which was not obtained in this study. According to Lin et al. (2022), high light intensity in combination with either dominant blue or red spectrum seems to improve production of phenolic compounds of cilantro. To our knowledge, the influence of light spectrum on production of phenolic compounds of chard is not earlier studied. In future studies should pay attention to concurrent impacts of light spectrum, light intensity and cultivation stage for production of total as well as specific phenolic compounds to create more comprehensive picture of opportunities to modify plant chemical quality in plant factories.

In this study, EOP treatment with blue spectrum boosted carotenoid concentrations of lettuces, even the difference was significant only with 'Javelo'. Likewise, Ouzounis et al. (2015) demonstrated increased concentrations of lutein and 6-carotene in lettuce when supplying supplementary blue light at intensities of 45–80 μmol m^{-2} s^{-1} light. Also, Li and Kubota (2009) found that blue light increased the leaf carotenoid contents in lettuce at light intensity of 300 μmol m^{-2} s^{-1}. Similar results were

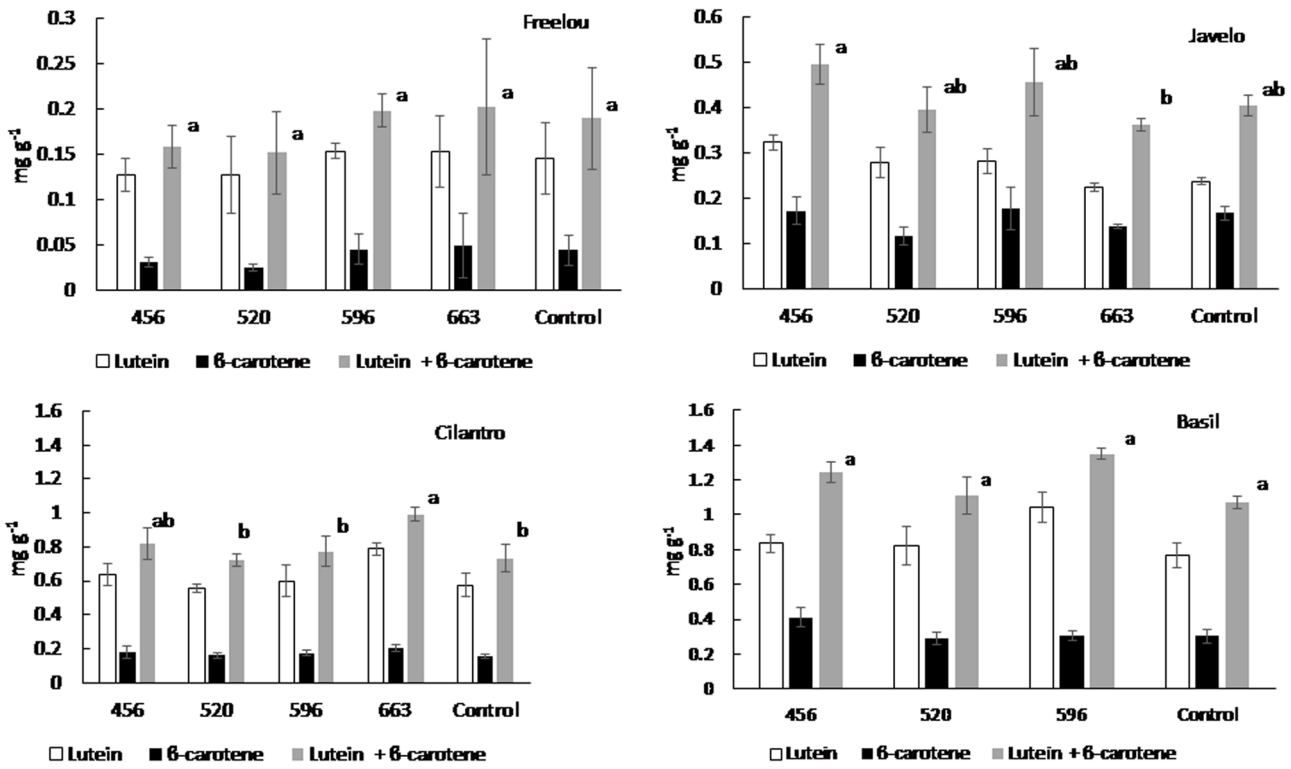

Fig. 1. Concentrations of lutein and ß -carotene and the sum of the lutein and ß -carotene (mg g^{-1}, DW) in the green tissue of four different species of herbs. Climate chamber grown plants were subjected to four-day end-of-production treatments with narrow-band light at four (freelou, javelo and cilantro) or three (basil) different wavelengths; 456, 520, 596 and 663 nm, with multispectral light as control treatment. DW = dry weight. Differences between sum of lutein and ß -carotene within species in different treatments were statistically analysed. Bars which do not share the same letter are significantly different at $p < 0.05$ (One way ANOVA + Tukey's multiple comparison test), $N = 3$.

found by Son and Oh (2013). Since the light intensities in this and previous studies varied, it can be assumed that blue spectrum is the prominent factor improving carotenoid production in lettuces.

In contrast to the present study showing increase of ß-carotene and lutein concentrations in EOP treatments with red and blue spectra, Gao *et al.* (2022), did not find any influence of red, blue or combinations of those for carotenoid contents of cilantro. Lin *et al.* (2022) demonstrated a trend of reduced carotenoid concentrations of cilantro subjected to blue or red spectrum with high light intensity as compared to lower intensities.

Carotenoid concentrations of basil were not significantly affected by EOP treatments in the present study. Similar results were indicated by Lobiuc et al. (2017). In the second experiment, chlorophyll concentrations of basil and cilantro increased, and biomass production decreased in EOP treated plants in comparison to control regardless of used spectrum. As reviewed by Ptushenko *et al.* (2020), plants accumulate chlorophyll more than necessary for photosynthesis. Since the specific narrow band lights alone do not support efficient photosynthesis, the excess chlorophyll was not able to allocate for growth.

As shown here and earlier, narrow band light can be used to modulate plants chemistry, which has direct impact on quality. According to the present stage of research, much is known regarding plant responses to light, but moving towards practice is more challenging. Plant factories are environments for precise planning of growth conditions, but the plant light biology is not simple. In future studies, attention should be paid to systematic research taking account plant age as well as both light intensity and spectrum as a variable.

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CRediT authorship contribution statement

Karl-Johan Bergstrand: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Vilhelmiina Harju:** Writing – review & editing, Methodology, Data curation. **Marika Tossavainen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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