Chapter 18
Effect of Light Quality on Secondary Metabolite Production in Leafy Greens and Seedlings

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Abstract It has been experimentally found that light stimulation significantly enhances the production of specific biomolecules in plants. The biosynthetic products mentioned herein are secondary metabolites, which are organic compounds that do not participate directly in cell growth or division; these include antioxidants, vitamins, sugars, and pigments. Several studies have investigated antioxidants in a variety of plants such as leaf lettuce, microgreen, sprout, and cherry tomatoes, and they have reported that the biosynthesis of chlorogenic acid, caffeic acid, chicory acid, flavonoids, rosmarinic acid, ascorbic acid, and carotenoids, along with antioxidant activity and DPPH radical scavenging capacity, is promoted by stimulating with light of a specific wavelength. It has been also reported that ascorbic acid, carotenoids, α-tocopherol, and ergosterol for antioxidants; sucrose, fructose, and glucose for sugars; and anthocyanin and chlorophyll for pigments are enhanced by light stimulus. Although the mechanism underlying the increase in the synthesis of these molecules by light stimulus remains unclear, the use of this technique is possible if reproducibility is ensured.

Keywords Antioxidant ability • Color development • Eating quality improvement • Light-emitting diode • Plant factory • Vitamins

18.1 Introduction

It is common knowledge that plant growth can be greatly influenced by environmental conditions. Light is a fundamental environmental factor affecting the growth of plants via processes including light morphogenesis and photosynthesis. While it is difficult to effectively control the light environment in greenhouses, as

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we cannot regulate solar light, plant factories with artificial light (PFAL) are able to control the entire suite of environmental factors, including the light environment.

A major purpose of PFAL has been year-round, pesticide-free production of vegetables. Recently, research has also focused on the production of vegetables containing certain useful compounds that cannot be cultivated in the open field. In addition, rather than edible vegetables, raw materials for the production of pharmaceutical products have been put to practical use. Plant secondary metabolites in plants are often a substance having a physiological activity such as chlorogenic acid with anticarcinogenic effect, and flavonoids increase the hormonal activity, and they have attracted attention as an effective component of many functional vegetables. In this article, recent research on the effects of the light environment on plant secondary metabolites will be introduced.

18.2 Antioxidant Ability

Total phenolic compounds are functional components that exhibit antioxidant activity. Many reports on the effects of light quality and intensity on the total phenolic compounds in plants have been published. These include several studies on the effects of blue and red lights, as provided by light-emitting diodes (LEDs), on functional compounds, with fewer studies examining the effects of green and yellow lights. To date, this research has examined the sprout, baby leaf, microgreen, and leafy lettuce of plants.

In a study of baby leaf, red leaf "Multired 4," green leaf "Multigreen 3," and light green leaf "Multiblond 2," baby leaf lettuce were grown under solar light and high-pressure sodium lamps (HPS) with supplementary LEDs (Samuoliene et al. 2012). Supplementary LEDs provided 30 μmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) at 455 nm (blue), 470 nm (blue), 505 nm (green), and 530 nm (green), with 170 μmol m^{-2} s^{-1} PPFD provided by HPS. In total, HPS and LEDs provided 200 μmol m^{-2} s^{-1} with a 16-h day^{-1} light: 8-h day^{-1} dark photoperiod. PPFD of the reference control plot was also set at 200 μmol m^{-2} s^{-1}, provided solely by HPS lamps.

The antioxidant activity of baby leaf lettuce was dependent on the variety of lettuce and the light quality (Table 18.1). Total phenol concentration in red leaf "Multired 4" and green leaf "Multigreen 3" baby leaf lettuce significantly decreased under 590-nm and 470-nm LED light, respectively, whereas total phenol concentration increased in light green leaf "Multiblond 2" baby leaf lettuce under 590 nm. Although 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging capacity was higher in all varieties of baby leaf lettuce than in control conditions, different wavelengths of LED light caused different effects per variety. The DPPH free-radical scavenging capacity significantly increased in red leaf baby lettuce with supplement of 470 nm LED light and in green and light green baby lettuce under supplementary 455 nm and 505 nm.

Table 18.1 Variation in antioxidant system under supplementary blue and green LED light

Treatment	Total phenols (mg g^{-1}, FM)	DPPH (μmol g^{-1}, FM)	Total anthocyanins (mg g^{-1}, FM)
Red leaf "Multired 4"			
HPS	1.23*	3.67**	31.9
HPS + 455 nm	1.14	4.06	42.6*
HPS + 470 nm	1.27*	5.43*	65.1*
HPS + 505 nm	1.15	4.31	37.4
HPS + 590 nm	0.99**	4.54	33.9
Green leaf "Multigreen 3"			
HPS	0.86**	7.78**	27.84*
HPS + 455 nm	1.16*	10.41*	7.21*
HPS + 470 nm	0.93**	9.05	12.27*
HPS + 505 nm	1.15*	9.90*	3.07**
HPS + 590 nm	1.00	9.29	1.83**
Light green leaf "Multiblond 2"			
HPS	0.63**	9.14**	ND
HPS + 455 nm	0.66	9.94*	ND
HPS + 470 nm	0.60**	9.19**	ND
HPS + 505 nm	0.62**	9.41*	ND
HPS + 590 nm	0.85[a]	8.80**	ND

Modified from Samuoliene et al. (2012)
FM fresh mass, *ND* not detected
Significant differences are denoted by asterisk – * under $P \leq 0.05$, ** above $P \leq 0.05$

The concentration of anthocyanin was significantly promoted with supplementary 455-nm and 470-nm LED light in red baby leaf lettuce, though it decreased with 505-nm and 550-nm LED supplementary lighting.

Effects of the spectrum of light on compounds produced by plants have been studied in the red leaf lettuce "Banchu Red Fire." The effect of raising seedlings under different light quality treatments was determined by Johkan et al. (2010). After transplant, seedlings were cultivated under solar light with supplemental fluorescent lamps (FL), and the influence of light quality on the raising stage of the harvest plant was studied. Lettuce seeds were germinated and grown under FL for 10 days. At 10 days after sowing (DAS), seedlings were transplanted into either

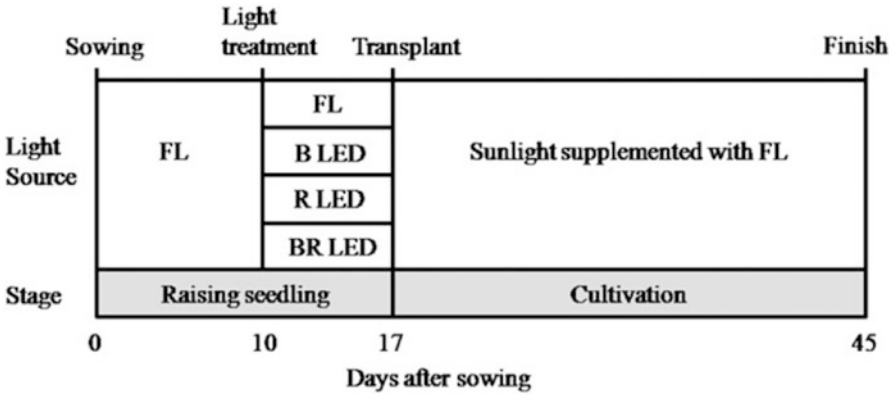

Fig. 18.1 Schematic of light treatment applied. *FL* fluorescent lamp, *B* blue, *R* red, *BR* blue + red (Adapted from Johkan et al. 2010)

conditions of FL, blue (468 nm), red (660 nm), and blue plus red light (467 and 655 nm) for the nursery stage. At 17 DAS, seedlings were transplanted to a greenhouse under solar light with supplementary FL from 1600 to 1900 h (Fig. 18.1).

Significant differences in total polyphenols, chlorogenic acid, and TAS (total antioxidant system) were apparent in relation to light quality at 17 DAS, the final day of the nursery stage under variable light quality treatments (Table 18.2). However, differences were not maintained post seedling stage.

The effects of different blue/red light ratios across the entire growth period on morphological changes, growth characteristics, and accumulation of antioxidant phenolic compounds in two lettuce cultivars have been reported (Son and Oh 2013). Seeds of "Sunmang" (red leaf) and "Grand Rapid TBR" (green leaf) were germinated and grown under a combination of FL and HPS for 18 days. Seedlings were transplanted into six light quality treatments with varying proportions of blue and red LEDs (0B/100R, 13B/87R, 26B/74R, 35B/65R, 47B/53R, and 59B/41R) at 171-μmol m^{-2} s^{-1} PPFD, and grown for 4 weeks before measurement of antioxidant activity.

Total phenolic concentration significantly increased in "Sunmang" lettuce under 47B/53R conditions to levels 1.4 and 2.4 times those under control conditions (0B/100R). The total phenolic concentration in "Grand Rapid TBR" under 26B and 59B was 2.2- and 2.7-fold that observed in the control treatment, respectively (Fig. 18.2). Thus, the LED ratio of blue/red light was found to significantly alter the total phenolic concentration in these cultivars, with enhanced phenol accumulation apparent with increases in blue wavelengths of light.

The response of antioxidant activity to light quality treatments was comparable to that of total phenol concentrations. In "Sunmang" lettuce, antioxidant production was promoted by an increase in the ratio of blue/red LEDs and became maximal under 47B/53R. "Grand Rapid TBR" also showed a high antioxidant capacity under blue-rich light environments. However, no significant difference was observed in

Table 18.2 Effects of LED light quality treatments on phenols, chlorogenic acid, and TAS concentrations in red leaf lettuce

DAS[z]	Spectrum[y]	Total phenols[x] (nmol mg^{-1} DMW)	Chlorogenic acid (nmol mg^{-1} DMW)	TAS[w] (nmol mg^{-1} DMW)
10	FL[v]	55.4	2.5	278
17	FL	75.7[c]	6.2[b]	332[c]
	Blue (470 nm)	118.7[b]	15.0[a]	547[b]
	Red (660 nm)	47.0[d]	1.9[c]	194[d]
	Blue + red (470 + 660 nm)	161.6[a]	14.7[a]	749[a]
45	FL	138.8[a]	8.1[a]	376[a]
	Blue (470 nm)	126.8[a]	6.7[a]	424[a]
	Red (660 nm)	139.9[a]	8.7[a]	421[a]
	Blue + red (470 + 660 nm)	138.3[a]	9.1[a]	481[a]

Modified from Johkan et al. (2010)
Different letters indicate significant difference
$P < 0.05$; Tukey's multiple range test, $n = 6$
[z]Days after sowing. 17 DAS = raising seedlings; 45 DAS = final cultivation
[y]Photosynthetic photon flux 100 ± 10 μmol m^{-2} s^{-1}
[x]Chlorogenic acid equivalent
[w]Total antioxidant system, Trolox equivalent
[v]White fluorescent lamp

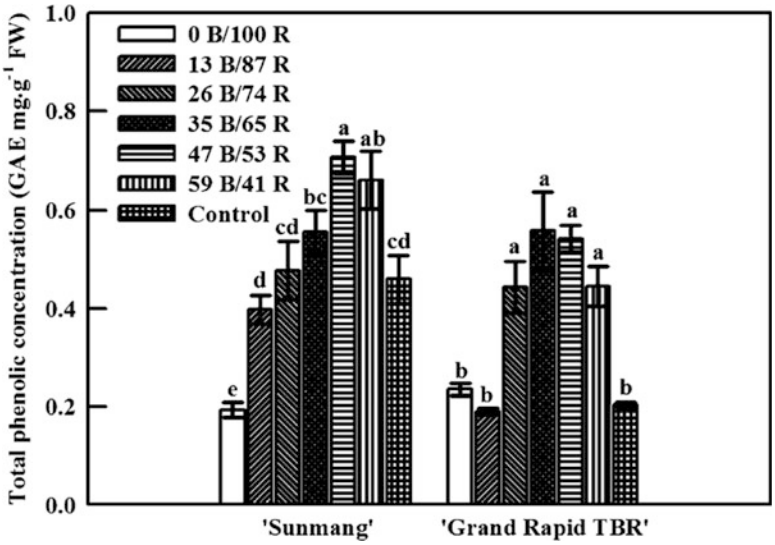

Fig. 18.2 Total phenolic concentration of lettuce grown under various combinations of blue and red LED light treatments after 4 weeks of growth (Adapted from Son and Oh 2013)

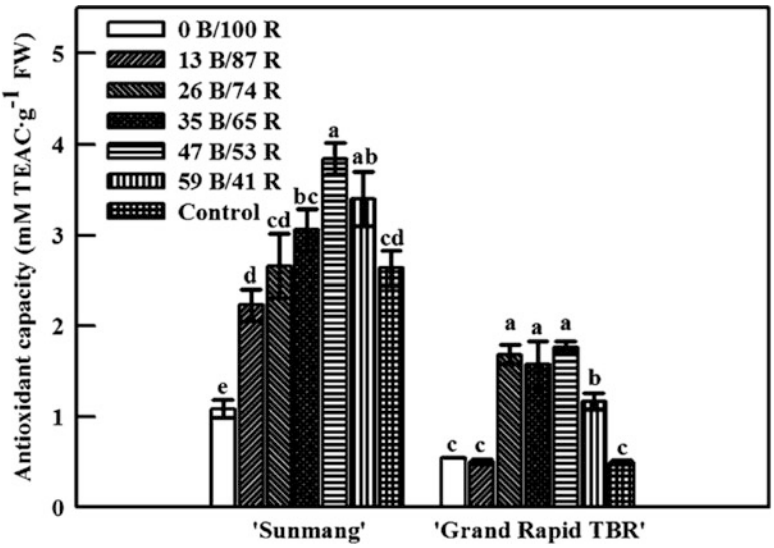

Fig. 18.3 Antioxidant capacity of lettuce grown under various combinations of blue and red LED light treatments after 4 weeks of growth (Adapted from Son and Oh 2013)

the antioxidant capacity of "Grand Rapid TBR" grown under light quality treatments with greater than 26 % blue light (Fig. 18.3). Comparing the two lettuce cultivars, "Sunmang" lettuce had greater antioxidant concentrations than "Grand Rapid TBR" under all light quality treatments, indicating species-specific responses.

In addition to stimulation by blue and red wavelengths, there are also reports of stimulation by yellow wavelengths (596 nm). For example, Urbonaviciute et al. (2009) investigated the total synthesis of an antioxidant in a sprout grown under a combination of HPS and flashing yellow LEDs (596 nm). Yellow LEDs pulsed at a frequency of 2.9 Hz (250-ms "on"; 100-ms "off") at a PPFD of 35 μmol m^{-2} s^{-1}. The intensity of the pulsed light was approximately 50 % of the total PPFD provided, with an 18-h photoperiod (Urbonaviciute et al. 2009).

Radical-binding activity increased approximately 1.5-fold due to the effect of flashing yellow LEDs on radish sprouts (Fig. 18.4). Similar trends were also apparent for the phenol component. Urbonaviciute et al. (2009) assumed such reactions were the result of photoinduced stress, i.e., the stimulation of the antioxidant activity of natural defense mechanisms mainly against damage by light oxidation. In radish sprouts, an approximate 30 % increase in phenolic compounds was observed under conditions of supplemental flashing yellow light. It is most likely that the formation of new pigments in the photosynthetic apparatus of young leaves, and the rapid adaptive response, including the synthesis of phenolic compounds, occurred as a reaction to the light quality treatment conditions (Fig. 18.5).

Studies on the biosynthesis of antioxidants in relation to light quality in plants other than leafy vegetables have also been reported. For example, the seedlings of

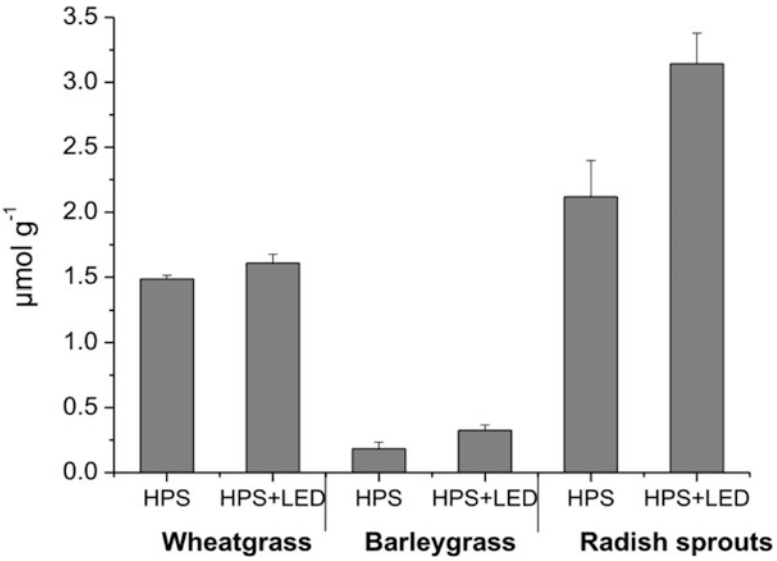

Fig. 18.4 Antioxidant activity of the extracts, as the ability to bind DPPH free radicals, in green sprouts grown solely under HPS lamps and with supplementation by flashing amber LED light (Adapted from Urbonaviciute et al. 2009)

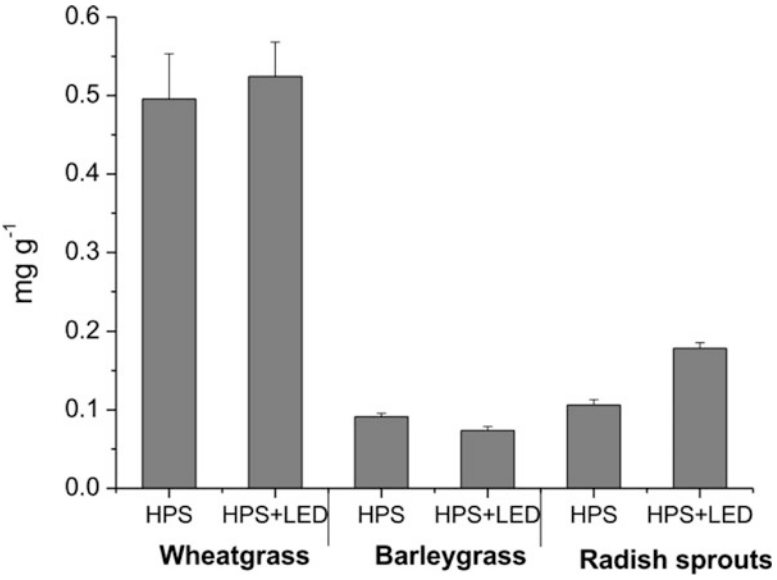

Fig. 18.5 Concentration of phenolic compounds in the fresh matter of green sprouts grown solely under HPS lamps and with supplementation by flashing amber LED light (Adapted from Urbonaviciute et al. 2009)

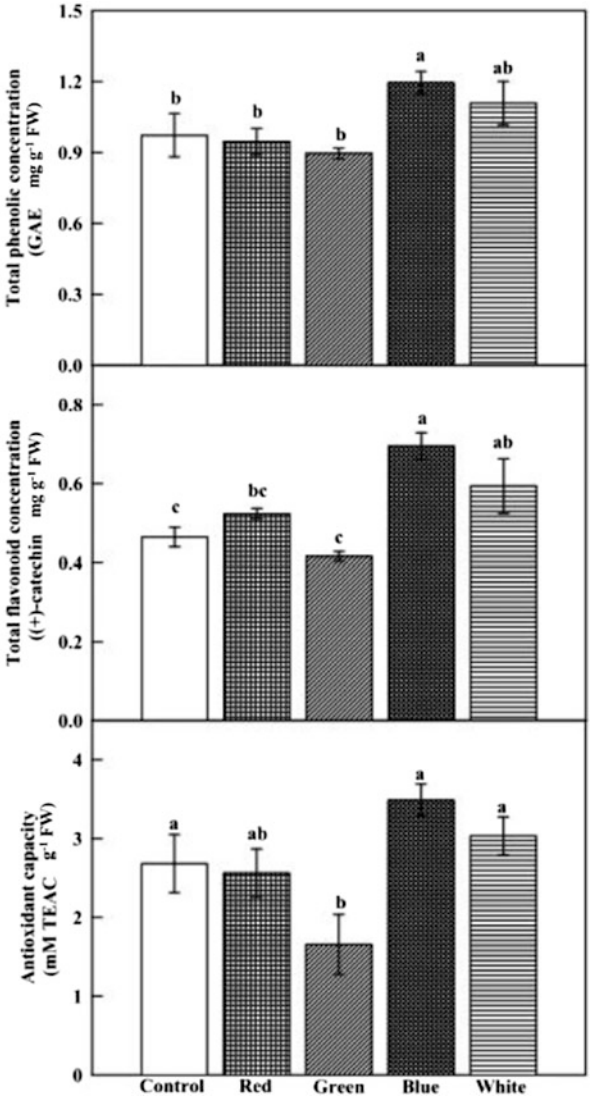

Fig. 18.6 Total phenolic concentration, total flavonoid concentration, and antioxidant capacity of cherry tomato seedlings (Adapted from Kim et al. 2014)

cherry tomatoes were grown under red, green, blue, and white LEDs and a fluorescent lamp, and the antioxidant phenolic compounds were analyzed (Kim et al. 2014). Total phenol concentration, total flavonoid concentration, and antioxidant capacity in seedlings grown under blue LEDs were significantly higher compared with those grown under other light quality treatments (red, green, and FL). In addition, red LEDs induced increases in these parameters in seedlings as compared to those grown under green LEDs and control conditions (FL) (Fig. 18.6).

In addition, Kim et al. (2013) analyzed the activity of the total phenolic concentration and antioxidant enzymes to examine the effect of light quality (white,

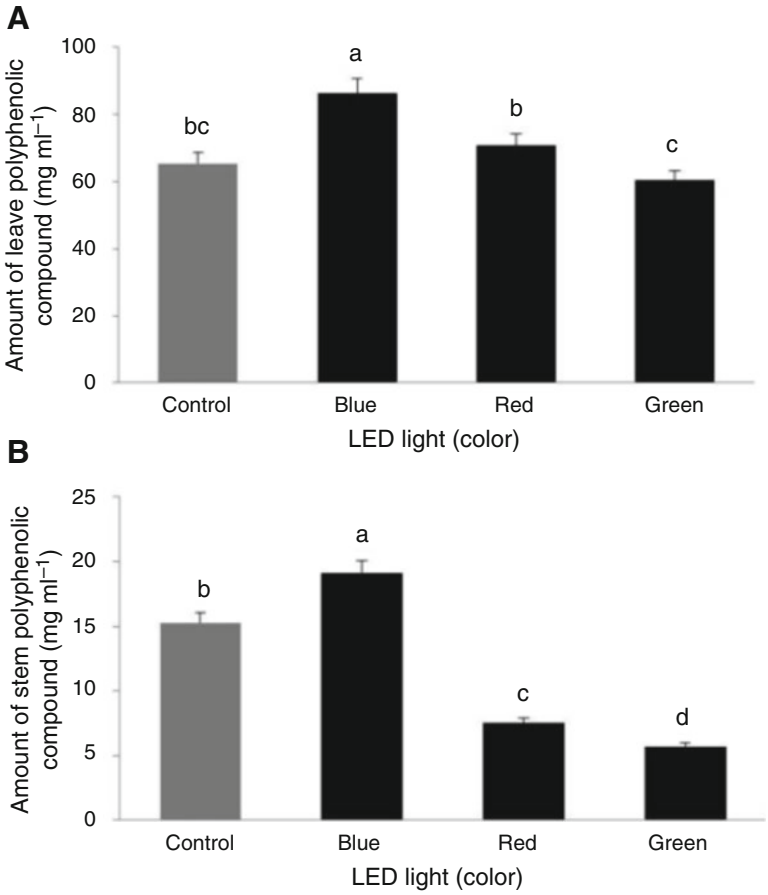

Fig. 18.7 The concentration of total phenolic compounds in the leaves and stems of tomatoes grown for 21 days under LED light treatments (Adapted from Kim et al. 2013)

blue, red, and green LEDs) on tomatoes (cv. Toy-mini tomato). When tomato seedlings were grown under blue LEDs, the concentration of total phenolic compounds was significantly in the leaves (1.3-fold) and the stem (1.2-fold) than in those of tomatoes grown under white LEDs (Fig. 18.7). In contrast, the concentration of total phenolic compounds in the leaves of tomatoes grown under red and green LEDs showed no difference to those grown under white LEDs. These findings are in line with the results of Kim et al. (2014).

Table 18.3 Variation in vitamin C under supplementary blue and green LED light quality treatments

Treatment	Vitamin C (mg g^{-1}, FM)
Red leaf "Multired 4"	
HPS	2.91*
HPS + 455 nm	1.45**
HPS + 470 nm	1.31**
HPS + 505 nm	1.83
HPS + 590 nm	1.95
Green leaf "Multigreen 3"	
HPS	0.29*
HPS + 455 nm	0.27
HPS + 470 nm	0.23**
HPS + 505 nm	0.29*
HPS + 590 nm	0.29*
Light green leaf "Multiblond 2"	
HPS	0.23
HPS + 455 nm	0.22
HPS + 470 nm	0.23
HPS + 505 nm	0.24
HPS + 590 nm	0.24

Modified from Samuoliene et al. (2012)
FM fresh mass, *ND* not detected
Significant differences are denoted by asterisk – * under $P \leq 0.05$; ** above $P \leq 0.05$

18.3 Vitamins

With respect to vitamins, the effects of light quality have been reported for ascorbic acid, carotenoids, α-tocopherol, and ergosterol. Experiments on baby leaf lettuce (red leaf "Multired 4," green leaf "Multigreen 3," light green leaf "Multiblond 2" baby leaf lettuce) were performed by Samuoliene et al. (2012), using supplementary LEDs (455, 470, 505, and 530 nm, 30-μmol m^{-2} s^{-1} PPFD) and HPS (170-μmol m^{-2} s^{-1} PPFD). The ascorbic acid concentration of red leaf lettuce was reduced under blue and green supplementary LED treatments approximately 2-fold and 1.5-fold, respectively (Table 18.3).

The effects of light stimulation on vitamins in microgreens have also been investigated (Samuoliene et al. 2013). Microgreen lettuces (kohlrabi [*Brassica oleracea* var. *gongylodes*, "Delicacy Purple"], mustard [*Brassica juncea* L., "Red Lion"], red pak choi [*Brassica chinensis*, "Rubi F1"], and tatsoi [*Brassica rapa* var. *rosularis*]) were grown under five light intensities (545-, 440-, 330-, 220-, and 110-μmol m^{-2} s^{-1} PPFD) with a mixture of light qualities using four different LEDs (455 nm [blue], 638 nm [red], 660 nm [red], and 735 nm [far red]). Ascorbic acid in the red pak choi and tatsoi showed high values under the low PPFD of 110-μmol m^{-2} s^{-1} PPFD, which was 3.8 and 3.5 times those under normal 220-μmol m^{-2} s^{-1} PPFD, respectively (Table 18.4). Given that the experiments

Table 18.4 Combinations of LED photosynthetic photon flux density

%	Total PPFD (μmol m^{-2} s^{-1})	Blue 445 nm	Red 638 nm	Red 665 nm	Far red 735 nm
100	545	41	225	275	4
80	440	34	181	221.5	3.5
60	330	25	136	166	3
40	220	17	90	111	2
20	110	8	45	55	1

Modified from Samuoliene et al. (2013)

with baby leaf used LEDs as a supplemental light additional to HPS, it is not possible to compare the results directly; the higher investigated PPFD level had uneven effect on ascorbic acid accumulation. Lower PPFD promoted the biosynthesis of ascorbic acid during this experiment.

In addition, α-tocopherol concentration was varied by PPFD, and significant accumulation of α-tocopherol was observed under minimum PPFD in mustard, red pak choi, and kohlrabi, and significant increase was occurred in tatsoi under 220- and 110-μmol m^{-2} s^{-1} PPFD. The accumulation of α-tocopherol and ascorbic acid under high PPFD was not consistent. In mustard plants grown under 545-μmol m^{-2} s^{-1} PPFD, α-tocopherol concentration increased to 1.6 times values observed in plants grown under 220-μmol m^{-2} s^{-1} PPFD. In contrast, α-tocopherol concentration in the red pak choi and tatsoi grown under 545-μmol m^{-2} s^{-1} PPFD was significantly lower as compared to plants grown under normal PPFD of 220 μmol m^{-2} s^{-1} (Fig. 18.8).

Li and Kubota (2009) examined the influence of supplementary UV-A, blue, green, red, and far-red light, with FL as the main light source, on phytochemicals (total anthocyanins, carotenoids, chlorophyll, total phenolic compounds, and ascorbic acid) and growth (biomass, the stem length varies the leaves under the amount of auxiliary light, leaf length, and leaf width) in leafy lettuce (*Lactuca sativa* L. "Red Cross") (Table 18.5).

The concentrations of carotenoids in lettuce leaves were affected by light quality, with xanthophylls and β-carotene increasing by 6–8 % in blue auxiliary light, though they reduced by 12–16 % under far-red light (Table 18.6). Xanthophylls have an absorption peak at 446 nm in the visible region and are produced in order to protect plants growing under high-energy blue wavelengths of light. At present, however, the mechanism of β-carotene increase remains unknown. Carotenoid and chlorophyll concentrations reduced by 12–16 % with supplementary far-red light.

Jang et al. (2013) investigated the effect of light quality (darkness, FL, blue, green, yellow, and red LEDs) on growth of the mushroom (Bunashimeji). Results demonstrated an increase in ergosterol concentration to a maximum under blue LEDs, with a minimum under red LEDs, indicating that both PPFD and quality (spectrum) had an effect on the biosynthesis of vitamins (Fig. 18.9).

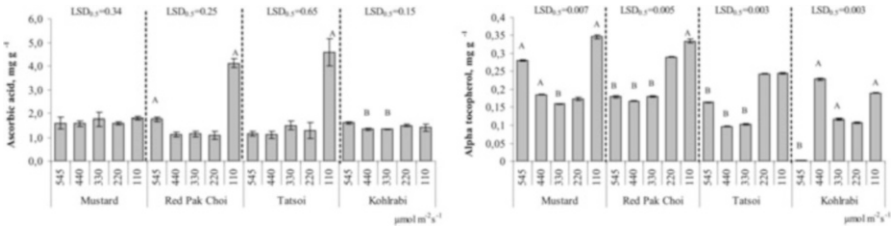

Fig. 18.8 Changes of ascorbic acid and α-tocopherol of microgreens grown under different light quality and intensity treatments (Modified from Samuoliene et al. 2013)

18.4 Eating Quality Improvement

Sugar concentration is closely related to the taste of vegetables, with several studies reporting that the light environment also influences sugar concentration. Nutritional values of herbs (white mustard [*Sinapis alba* "Yellow mustard"], spinach [*Spinacia oleracea* "Geant d'hiver"], rocket [*Eruca sativa* "Rucola"], dill [*Anethum graveolens* "Mammoth"], parsley [*Petroselinum crispum* "Plain Leaved"], green onion [*Allium cepa* "White Lisbon"]) grown using red LEDs as supplementary light were evaluated (Bliznikas and Zulauskas 2012). Natural light (average 300 μmol m^{-2} s^{-1} PPFD) was the main light source in this experiment. When natural PPFD was low, supplementary light was provided by HPS with a photoperiod of 12-h day^{-1} and intensity of 130-μmol m^{-2} s^{-1} PPFD. At the preharvest stage of 3 days, plants were provided with supplementary illumination by red (638 nm) LEDs from 0500 h to 1000 h and 1700 h to 0000 h.

The response to red LED light differed in each variety of plant. Marked increase in ascorbic acid concentration was observed in vegetables under red LED treatment. Although there was a significant accumulation of fructose and glucose with supplementary red LED treatment, the accumulation of sucrose showed a different effect. In dill and parsley grown under supplementary red light, a significant increase in monosaccharides (especially fructose and glucose) and a reduction of nitric acid concentration were observed (Table 18.7). No significant increase in carbohydrates was observed in vegetables, while nitrate concentrations either increased (mustard, rocket, and onion leaves) or remained unchanged (spinach).

Samuoliene et al. (2012) investigated the effects of PPFD on sucrose concentration in microgreens. A mixture of spectral irradiance was provided by LEDs with different peak wavelengths (455 [blue], 638 [red], 660 [red], and 735 nm [far-red]), and experiments were conducted using different light intensities (545-, 440-, 330-, 220-, and 110-μmol m^{-2} s^{-1} PPFD), as previously described.

Sucrose accumulation in microgreen leaves was shown to be related to PPFD, with a tendency for decreasing sucrose concentration with decreasing PPFD observed (Table 18.8). However, the PPFD resulting in maximal sucrose concentrations differed between microgreen varieties. Sucrose synthesis in mustard plants decreased with a decrease in PPFD, and the sucrose concentration of kohlrabi was

Table 18.5 Spectral qualities tested for the white (W), the white light with supplementary UV-A (WUA), the white light with supplementary blue (WB), the white light with supplementary green (WG), the white light with supplementary red (WR), and the white light with supplementary far-red (WFR) light

	Treatment					
Parameter	W	WUV	WB	WG	WR	WFR
Photon flux (μmol m^{-2} s^{-1})[a]						
UV-A (350–400 nm)	4.6 ± 0.3	20.9 ± 1.1	2.5 ± 0.4	2.1 ± 0.3	2.2 ± 0.3	4.4 ± 0.3
Blue (400–500 nm)	70.5 ± 3.0 (23.2 %)	70.3 ± 3.1 (23.1 %)	166.9 ± 16.0 (55.1 %)	48.6 ± 3.2 (16.3 %)	38.5 ± 3.5 (12.6 %)	69.5 ± 3.1 (22.8 %)
Green (500–600 nm)	159.1 ± 7.0 (52.3 %)	157.5 ± 6.8 (51.9 %)	95.2 ± 6.7 (31.4 %)	211.1 ± 13.0 (70.1 %)	89.5 ± 7.2 (29.5 %)	156.5 ± 7.1 (51.4 %)
Red (600–700 nm)	75.9 ± 5.6 (24.5 %)	76.8 ± 4.6 (24.9)	41.5 ± 3.7 (13.5 %)	41.5 ± 3.8 (13.6 %)	177.2 ± 12.8 (57.9 %)	79.7 ± 5.3 (25.8 %)
Far red (700–800 nm)	6.6 ± 0.4	6.7 ± 0.3	4.1 ± 0.2	3.9 ± 0.2	4.6 ± 0.3	160.4 ± 8.8
PPF (400–700 nm)	305.4 ± 10.0 (100 %)	304.6 ± 8.2 (100 %)	303.5 ± 7.4 (100 %)	301 ± 8.3 (100 %)	305.2 ± 8.8 (100 %)	305.7 ± 9.3 (100 %)
Ratios						
Red/far red	11.5	11.4	10.2	10.7	38.5	0.5
P_{fr}/P_{total}[b]	0.84	0.83	0.81	0.84	0.87	0.56

Modified from Li and Kubota (2009)

[a]Total photon flux of the background white fluorescent lamp and the supplemental LEDs

[b]P_{fr}/P_{total}: phytochrome photostationary state

Table 18.6 Xanthophyll and β-carotene and ascorbic acid concentration in lettuce under various light quality treatments

Treatment	Xanthophylls (mg g^{-1} DW)	β-Carotene (mg g^{-1} DW)	Ascorbic acid (mg g^{-1} DW)
Experiment 1			
W1	0.49 a	0.25 a	2.32 a
WUV	0.50 a	0.25 a	2.42 a
WR	0.47 ab	0.23 ab	2.36 a
WFR	0.43 b	0.21 b	2.27 a
Experiment 2			
W2	0.52 b	0.26 b	2.19 a
WB	0.55 a	0.28 a	2.34 a
WG	0.51 b	0.26 b	2.07 a

Modified from Li and Kubota (2009)
p-value from one-way ANOVA

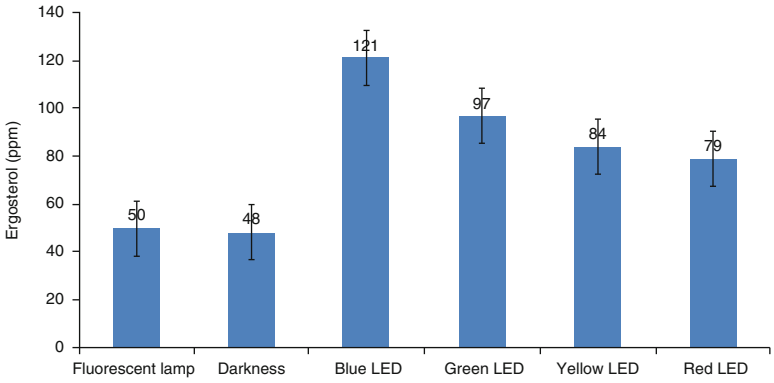

Fig. 18.9 Ergosterol concentration of *Hypsizygus marmoreus* under different LED lights (Adapted from Jang et al. 2013)

maximal at 545-μmol m^{-2} s^{-1} PPFD. In contrast, sucrose concentration in red pak choi peaked at 440-μmol m^{-2} s^{-1} PPFD and that in Tatsoi was maximal at 330-μmol m^{-2} s^{-1} PPFD.

In studies concerning the effects of light quality on growth and synthesis of important compounds by plants, several experiments with blue and red light, the spectra preferentially absorbed by chlorophyll and photoreceptors, have been conducted. An experiment conducted with yellow spectra and sprout species (green sprouts of wheatgrass [*Triticum aestivum* L. "Sirvinta"], barley grass [*Hordeum vulgare* L. "Luoke"], and leafy radish [*Raphanus sativus* L. "Tamina"]) has also been reported. Urbonaviciute et al. (2009) analyzed sugar concentrations of sprouts grown under yellow flashing light (596 nm, 2.9 Hz

Table 18.7 Nutritional properties of green vegetables after 3 days of red LED treatment before harvesting

	Vitamin C (mg g^{-1} FW)		Nitrate (mg kg^{-1} FW)	
	HPS	HPS + LED	HPS	HPS + LED
Mustard	0.46 ± 0.011	0.58 ± 0.057	3011 ± 160.6	5361 ± 363.6
Spinach	0.34 ± 0.017	0.41 ± 0.044	2989 ± 127.3	2783 ± 102.0
Rocket	0.35 ± 0.032	0.39 ± 0.007	4120 ± 188.8	4491 ± 80.4
Dill	0.52 ± 0.008	0.87 ± 0.016	4879 ± 80.9	4313 ± 154.5
Parsley	0.53 ± 0.021	0.43 ± 0.015	4486 ± 408.5	2675 ± 35.3
Onion leaves	0.33 ± 0.012	0.35 ± 0.017	2142 ± 26.1	2650 ± 33.2

	Carbohydrates					
	Fructose (mg g^{-1} FW)		Glucose (mg g^{-1} FW)		Sucrose (mg g^{-1} FW)	
	HPS	HPS + LED	HPS	HPS + LED	HPS	HPS + LED
Mustard	–	0.33 ± 0.027	–	0.22 ± 0.011	0.03 ± 0.07	0.58 ± 0.068
Spinach	–	0.92 ± 0.047	–	0.98 ± 0.003	0.06 ± 0.53	–
Rocket	0.26 ± 0.034	1.61 ± 0.316	0.61 ± 0.016	3.76 ± 0.084	–	0.95 ± 0.002
Dill	4.11 ± 0.360	10.37 ± 0.141	2.48 ± 0.610	13.82 ± 2.177	–	9.16 ± 0.092
Parsley	3.25 ± 0.103	3.32 ± 0.150	3.34 ± 0.375	7.30 ± 0.266	4.04 ± 0.113	16.12 ± 0.050
Onion leaves	1.65 ± 0.262	3.06 ± 0.608	1.59 ± 0.019	2.86 ± 0.101	2.51 ± 0.033	2.36 ± 0.236

Modified from Bliznikas et al. (2012)

Table 18.8 Sucrose concentration of microgreens grown under different irradiation levels

	Sucrose (mg g^{-1})			
PPFD (μmol m^{-2} s^{-1})	Mustard	Red Pak Choi	Tatsoi	Kohlrabi
545	0.83 ± 0.04	0.62 ± 0.05	0.92 ± 0.15	2.62 ± 0.33*
440	0.81 ± 0.41	3.47 ± 0.29*	0.91 ± 0.30	0.77 ± 0.07*
330	0.65 ± 0.07	0.94 ± 0.25*	5.71 ± 0.28*	0.35 ± 0.07
220	0.53 ± 0.19	0.58 ± 0.06	0.60 ± 0.17	0.23 ± 0.10
110	0.34 ± 0.09	0.61 ± 0.26	0.71 ± 0.11	0.19 ± 0.05

Modified from Samuoliene et al. (2013)
*Values are significantly ($P \leq 0.05$) higher than normal 220 μmol m^{-2} s^{-1} irradiance level

[250-ms "on" and 100-ms "off"]) supplementary to the main HPS light source. An 18-h photoperiod was employed, and the intensity of the pulsed light was approximately 50 % of the total PPFD, which was approximately 35-μmol m^{-2} s^{-1} PPFD.

Glucose concentrations in barley young leaves and radish sprouts were 2–2.5 times greater than concentrations recorded in control plots, and a slight decrease in the maltose (malt sugar) concentration in young barley leaves was observed. Although yellow light is said to inhibit the formation of chlorophyll and chloroplasts, given the monosaccharide concentration of sprouts recorded during this experiment, such an inhibitory effect was not apparent (Fig. 18.10).

The proline concentration of tomatoes (cv. Toy-mini tomato) grown under five different light sources (white LED [420–680 nm] as control, blue LED [460 nm], red LED [635 nm], and green LED [520 nm]) has been investigated. Light quality

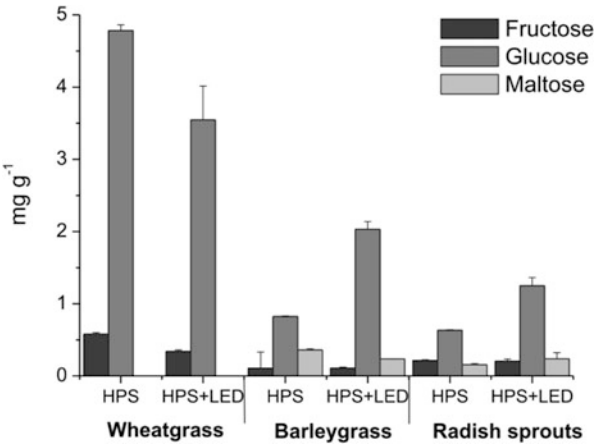

Fig. 18.10 Saccharide concentration in green sprouts grown under solely HPS lamps and with supplementation by flashing amber LED light (Adapted from Urbonaviciute et al. 2009)

had a significant effect on the proline concentration of tomato seedlings, with proline concentration 296 % in leaves and 127 % in stems grown under blue LEDs as compared to white LEDs (Kim et al. 2013).

Chicory acid related to eating quality since has a bitter taste. Ouzounis et al. (2015) investigated this secondary metabolite in two varieties of leafy lettuce ("Batavia" and "Lollo Rossa"). Lettuces were grown under supplementary blue light (45-μmol m^{-2} s^{-1} PPFD, with a variety of irradiation durations), with the main light source provided by a combination of natural light and HPS. Chicory acid concentration significantly increased with an irradiation regime of blue light from 0600 to 0800 h and 2100 to 0800 h, suggesting that an effective time period of irradiation may exist.

Sirtautas et al. (2014) examined changes in the saccharide concentrations of baby leaf lettuce "Multigreen 3." During experiments, the main light source was provided by a combination of approximately 80–120-μmol m^{-2} s^{-1} PPFD solar light and 90-μmol m^{-2} s^{-1} PPFD HPS, with approximately 15-μmol m^{-2} s^{-1} PPFD of supplementary light provided by blue (455 and 470 nm), cyan (505 nm), and green (535 nm) LEDs. The concentration of water-soluble monosaccharides in lettuce grown under blue light (455 nm) was higher than in other plots, with sucrose, glucose, mannose, and fructose concentrations 6, 2.9, 3.5, and 1.5 times those of control plots, respectively (Table 18.9). Despite 470 nm of blue wavelengths close to the 455 nm, the noticeable effect of 455 nm was not observed. The effects of sucrose, glucose, and mannose were 1.8, 2.3, and 3.3 times, respectively. In other words, a shift of the little peak wavelength in the same blue caused remarkable difference of the effect on the biosynthesis of sucrose, glucose, mannose, and fructose. Green light (535 nm) had no effect on sucrose biosynthesis. However, the concentration of glucose and mannose was higher under green (535 nm) than blue light (455 nm). In contrast, only a small effect of cyan light (505 nm) was apparent on any monosaccharide concentration.

Table 18.9 Soluble saccharide concentrations (mg g^{-1} fresh mass) in baby leaf lettuces grown under HPS and supplementary LED light quality treatments

	Soluble saccharides			
	Sucrose	Glucose	Mannose	Fructose
HPS	0.69 ± 0.03	2.49 ± 0.04	1.20 ± 0.03	0.23 ± 0.04
HPS + 455 nm	$4.18 \pm 0.04^{*}$	$7.32 \pm 0.02^{*}$	$4.33 \pm 0.04^{*}$	$0.36 \pm 0.03^{*}$
HPS + 470 nm	$1.22 \pm 0.05^{*}$	$5.70 \pm 0.03^{*}$	$3.96 \pm 0.03^{*}$	$0.28 \pm 0.01^{*}$
HPS + 505 nm	$1.08 \pm 0.04^{*}$	$3.47 \pm 0.04^{*}$	$2.93 \pm 0.05^{*}$	$0.28 \pm 0.03^{*}$
HPS + 535 nm	0.77 ± 0.03	$7.50 \pm 0.04^{*}$	$5.96 \pm 0.05^{*}$	$0.29 \pm 0.04^{*}$

Modified from Sirtautas et al. (2014)
*Values in each column are significantly different ($P \leq 0.05$) from HPS by Fisher's LSD test

18.5 Color Development

The color of vegetables is related to their nutritional value, and research has demonstrated that color is also controlled by light quality. Seeds of leafy lettuce were germinated and grown under FL for 10 days, after which seedlings were raised under different light qualities until 17 DAS. Following the raising stage, seedlings were transplanted to a greenhouse under solar light with supplementary FL and grown until 45 DAS (Johkan et al. 2010).

Anthocyanin concentration at 17 DAS in lettuce seedlings treated with blue light was higher than in seedlings at 10 DAS cultivated under FL (Table 18.10). Blue-containing LED lights significantly increased the anthocyanin concentration in lettuce seedlings, and maximal anthocyanin concentration was observed in seedlings raised under a combination of blue and red light. However, the anthocyanin concentration in lettuce at 45 DAS was lower than in seedlings at 17 DAS. The anthocyanin concentration at 17 DAS in lettuce seedlings grown under red light was significantly lower than in those raised under FL. However, the concentration at 45 DAS reduced by half. Overall, no significant difference was observed in the anthocyanin concentration of seedlings grown under any light quality treatment at 45 DAS relative to FL controls, indicating that the effects of light quality observed at 17 DAS were not retained.

Li and Kubota (2009) examined the color responses of red leafy lettuce (*Lactuca sativa* L. cv. "Red Cross") grown with FL as the main light source and supplementary UV-A (373 nm), blue (476 nm), green (526 nm), red (658 nm), and far-red (734 nm) light provided by LEDs, across the entire period of growth. Anthocyanin concentration of lettuce grown with supplementary UV-A and blue light increased by 11 % and 13 %, respectively, though decreased by 40 % under far-red illumination. Carotenoids (xanthophylls and β-carotene) increased 6–8 % with supplementary blue light, but were reduced by 12–16 % under far-red light.

Chlorophyll concentration was reduced by 12 % in lettuce grown under supplementary far-red light, and phenol concentration increased by 6 % in the red light quality treatment compared to controls (Table 18.11). Previously, Ninu et al. (1999) demonstrated that light quality had a strong influence on anthocyanin concentration, with blue light indicated as one of the most effective wavelengths for

Table 18.10 Effects of LED light quality treatments on the concentration of anthocyanin in red leaf lettuce

DAS[z]	Spectrum	Anthocyanin (OD530 mg^{-1} DMW)
10	FL	0.07
17	FL	0.08 c
	Blue (470 nm)	0.15 b
	Red (660 nm)	0.06 d
	Blue + red (470 + 660 nm)	0.27 a
45	FL	0.11 a
	Blue (470 nm)	0.14 a
	Red (660 nm)	0.12 a
	Blue + red (470 + 660 nm)	0.13 a

Modified from Johkan et al. (2010)
Different letters indicate significant difference
$P \leq 0.05$; Tukey's multiple range test, $n = 6$
[z]Days after sowing. 17 DAS = rising seedlings, 45 DAS = final cultivation
[v]White fluorescent lamp
[y]Photosynthetic photon flux was 100 ± 10 μmol m^{-2} s^{-1} for all light treatments

Table 18.11 Total anthocyanin and chlorophyll concentration in red leaf lettuce (For light treatments in the table, see Table 18.5)

Treatment	Anthocyanins (mg g^{-1} DW)	Chlorophyll (mg g^{-1} DW)
Experiment 1		
W1	3.31b	0.51a
WUV	3.68a	0.53a
WR	3.47ab	0.47a
WFR	1.97c	0.45a
Experiment 2		
W2	3.20b	0.50a
WB	4.18a	0.53a
WG	2.95b	0.54a

Modified from Li and Kubota (2009)
p-value from one-way ANOVA

controlling anthocyanin biosynthesis in tomatoes due to the response of cryptochrome blue/UV light receptors.

In addition to research conducted with red leaf vegetables, studies have also performed experiments using green cabbages. The pigmentation in green and red varieties of cabbage (*Brassica oleracea* var. *capitata* L. "Kinshun" [green leaf type] and "Red Rookie" [red leaf]) was examined under different monochromic LEDs (blue [470 nm], blue green [500 nm], green [525 nm], and red [660 nm]) (Mizuno et al. 2011).

In Red Rookie, leaf anthocyanin concentration was greater than or equal to 0.6 g m^{-2} leaf in all light quality treatments, with the greatest concentrations observed in cabbage grown in the red light quality treatment (Fig. 18.11).

In general, chalcone synthase, a precursor of anthocyanin biosynthesis, was reduced by blue light or UV-B radiation. In contrast, there is little evidence to suggest that red light has the ability to promote anthocyanin production. Miura and Iwata (1981) reported that the anthocyanin concentration of *Polygonum hydropiper* was higher when grown in red light than when grown at other wavelengths. Phytochrome is a red light receptor that may have a role in the anthocyanin biosynthesis of Red Rookie cabbage.

In a study of fruit and vegetable seedlings, the chlorophyll concentration of the tomato hybrid "Magnus" F1, sweet pepper variety "Reda," and cucumber hybrid "Mirabelle" F1 was evaluated under supplementary LED light at 455, 470, 505, and 530 nm, with solar light and HPS as the main light source. PPFD in experimental plots was daylight plus 15-μmol m^{-2} s^{-1} PPFD LEDs and 90-μmol m^{-2} s^{-1} PPFD HPS, with a photoperiod of 18 h. The control plot was provided with daylight and HPS (110-μmol m^{-2} s^{-1} PPFD) (Samuoliene et al. 2012).

Chlorophyll, a concentration in the leaves of cucumber seedlings, was significantly increased under supplementary light of 470-nm and 530-nm wavelengths. In addition, although chlorophyll, a concentration, increased in all supplementary light quality treatments, supplementary LED light had no effect on chlorophyll a:b ratios. In the tomato seedling, chlorophyll a and chlorophyll b concentration increased significantly under the 470-nm light quality treatment, and 470-nm supplementary light significantly increased the photosynthetic pigment concentration in pepper leaves. In addition, chlorophyll a and carotenoid concentration significantly increased in the 455-nm light quality treatment (Table 18.12).

Similarly, the chlorophyll concentration in baby leaf "Multigreen 3" was investigated under solar light (~80–120-μmol m^{-2} s^{-1} PPFD) plus HPS (~90-μmol m^{-2} s^{-1} PPFD) as a main light source with supplemental LEDs (~15-μmol m^{-2} s^{-1} PPFD): blue (455 and 470 nm), cyan (505 nm), and green (535 nm) (Sirtautas et al. 2014). Generally, photosynthetic capacity enhances with the increase of the ratio of chlorophyll a to b. However, the chlorophyll a:b ratio was shown to decrease under blue light of 455-nm wavelength, whereby monosaccharide concentration was increased (Table 18.13). In contrast, the chlorophyll a:b ratio was significantly increased under blue light of 470-nm wavelength. However, this was due to a decrease in chlorophyll b concentration as opposed to an increase in chlorophyll a. Treatment with cyan light of 505-nm wavelength resulted in a significant effect on photosynthetic pigments, with chlorophylls a and b and carotenoid concentrations increasing up to 1.2 times relative to controls.

18.6 Concluding Summary

In this article, recent studies describing the effect of light environment on plant secondary metabolites are discussed. It is known that biosynthesis of a particular molecule is promoted by stimulating with light of a specific wavelength. Since the light stimuli used in these studies were different with respect to wavelength, PPFD,

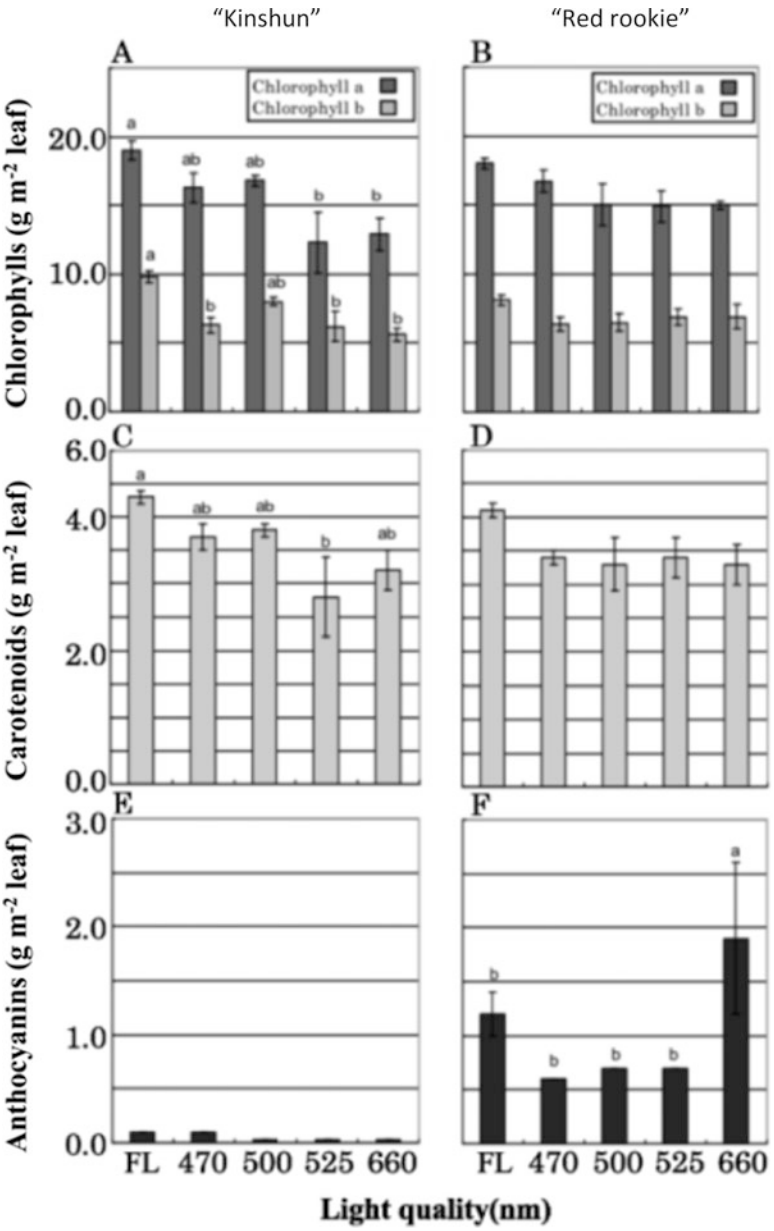

Fig. 18.11 Effects of light quality on the concentrations of chlorophylls, carotenoids, and anthocyanins in cabbage seedlings (Adapted from Mizuno et al. 2011)

Table 18.12 The pigment concentration and chlorophyll a to b ratio of vegetable transplants grown under different light quality treatments

	Chlorophyll a	Chlorophyll b	Carotenoids	Chlorophyll a and b ratio
	Cucumber "Mirabelle" F_1			
HPS	0.63 ± 0.038	0.23 ± 0.014	0.25 ± 0.015	2.73 ± 0.051
HPS + 455 nm	0.73 ± 0.029	0.27 ± 0.006*	0.26 ± 0.010	2.65 ± 0.074
HPS + 470 nm	0.74 ± 0.026*	0.27 ± 0.006*	0.27 ± 0.017	2.72 ± 0.081
HPS + 505 nm	0.86 ± 0.041**	0.32 ± 0.012**	0.30 ± 0.016**	2.69 ± 0.031
HPS + 535 nm	0.76 ± 0.031*	0.28 ± 0.011*	0.27 ± 0.012	2.77 ± 0.052
	Tomato "Magnus" F_1			
HPS	0.54 ± 0.053	0.18 ± 0.018	0.18 ± 0.019	3.07 ± 0.049
HPS + 455 nm	0.76 ± 0.047*	0.25 ± 0.019*	0.24 ± 0.014*	3.00 ± 0.085
HPS + 470 nm	0.79 ± 0.073*	0.27 ± 0.029*	0.25 ± 0.022	2.98 ± 0.050
HPS + 505 nm	0.77 ± 0.074*	0.26 ± 0.018*	0.26 ± 0.029	3.05 ± 0.085
HPS + 535 nm	0.60 ± 0.046	0.20 ± 0.017	0.19 ± 0.012	2.95 ± 0.059
	Sweet pepper "Reda"			
HPS	0.63 ± 0.031	0.25 ± 0.014	0.23 ± 0.009	2.49 ± 0.077
HPS + 455 nm	0.83 ± 0.041**	0.29 ± 0.011	0.30 ± 0.015*	2.89 ± 0.045**
HPS + 470 nm	1.00 ± 0.37**	0.34 ± 0.018*	0.35 ± 0.019**	2.99 ± 0.059**
HPS + 505 nm	0.79 ± 0.059	0.28 ± 0.022	0.28 ± 0.024	2.85 ± 0.031**
HPS + 535 nm	0.62 ± 0.036	0.21 ± 0.016	0.22 ± 0.012	2.88 ± 0.057**

Modified from Samuoliene et al. (2012)
Mean significantly (*$P < 0.05$; **$P < 0.01$) different from control (HPS) plants as determined by paired *t*-test

Table 18.13 The concentration of photosynthetic pigments in baby leaf lettuce under HPS and supplemental LED light quality treatments

	Photosynthetic pigments			
	Chlorophyll a	Chlorophyll b	Carotenoids	Chlorophyll a and b ratio
HPS	0.71 ± 0.01	0.27 ± 0.01	0.24 ± 0.02	2.63 ± 0.01
HPS + 455 nm	0.67 ± 0.04	0.29 ± 0.03	0.24 ± 0.03	2.31 ± 0.03
HPS + 470 nm	0.70 ± 0.02	0.24 ± 0.02	0.24 ± 0.01	2.91 ± 0.02*
HPS + 505 nm	0.88 ± 0.04*	0.32 ± 0.01*	0.29 ± 0.03*	2.75 ± 0.03
HPS + 535 nm	0.60 ± 0.03	0.24 ± 0.03	0.20 ± 0.03*	2.50 ± 0.03

Modified from Sirtautas et al. (2014)
Mean significantly (*$P < 0.05$) different from control (HPS) plants as determined by paired *t*-test

and photoperiod, the comparison cannot be straightforward. Even when the same stimulus is used, different responses are observed depending on the plant species. However, if reproducibility can be ensured, this can be utilized as a highly efficient technology. Future studies should elucidate the mechanisms underlying these differences and should demonstrate efficient and effective methods of providing light stimuli; after these improvements in methodology, high-value-added vegetables that cannot be grown in the open field can be produced in plant factories.

References

Bliznikas Z, Zulauskas A (2012) Effect of supplementary pre-harvest LED lighting on the antioxidant and nutritional properties of green vegetables. Acta Hortic 939:85–92

Bliznikas Z, Žukauskas A, Samuolienė G, Viršilė V, Brazaitytė A, Jankauskienė J (2012) Effect of supplementary pre-harvest LED lighting on the antioxidant and nutritional properties of green vegetables. Acta Hortic 939:85–91

Jang M, Lee Y, Ju Y et al (2013) Effect of color of light emitting diode on development of fruit body in *Hypsizygus marmoreus*. Mycobiology 41(1):63–66

Johkan M, Shoji K, Goto F et al (2010) Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. Hortscience 45 (12):1809–1814

Kim K, Kook H, Jang Y et al (2013) The effect of blue-light-emitting diodes on antioxidant properties and resistance of *Botrytis cinerea* in tomato. J Plant Pathol Microbiol 4:203. doi:10.4172/2157-7471.1000203

Kim E, Park S, Park B et al (2014) Growth and antioxidant phenolic compounds in cherry tomato seedlings grown under monochromic light-emitting diodes. Hortic Environ Biotechnol 55(6):506–513

Li Q, Kubota C (2009) Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ Exp Bot 67:59–64

Miura H, Iwata M (1981) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–383

Mizuno T, Amaki W, Watanabe H (2011) Effects of monochromatic light irradiation by LED on the growth and anthocyanin concentrations in leaves of cabbage seedlings. Acta Hortic 907:179–184

Ninu L, Ahmad M, Miarelli C et al (1999) Cryptochrome 1 controls tomato development in response to blue light. Plant J 18:551–556

Ouzounis T, Parjikolaei B R, Frette X, Rosenqvist E, Ottosen C (2015) Predawn and high intensity application of supplemental blue light decreases the quantum yield of PSII and enhances the amount of phenolic acids, flavonoids, and pigments in Lactuca sativa. Front. Plant Sci 26: http://dx.doi.org/10.3389/fpls.2015.00019

Samuoliene A, Brazaityte A, Sitrautas R, Novickovas A, Duchovskis (2012) The effect of supplementary LED lighting on the antioxidant and nutritional properties of lettuce. Acta Hortic 952:835–842

Samuoliene G, Brazaityte A, Jankauskiene J et al (2013) LED irradiance level affects growth and nutritional quality of Brassica microgreens. Cent Eur J Biol 8(12):1241–1249

Sirtautas R, Virsile A, Samulioene G et al (2014) Growing of leaf lettuce (*Lactuca sativa* L.) under high-pressure sodium lamps with supplemental blue, cyan and green LEDs. Zemdirbyste-Agriculture 101:75–78. doi:10.13080/z-a.2014.101.010

Son K, Oh M (2013) Leaf shape, growth and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. Hortscience 48(8):988–995

Urbonaviciute A, Samuoliene G, Sakalauskiene S et al (2009) Effect of flashing amber light in the nutritional quality of green sprouts. Agron Res 7:761–767