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Research article

Pre-harvest illumination with different spectral compositions improves quality of green oak lettuce in hydroponic system

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Abstract

Importance of the work: Pre-harvest illumination has an impact on the nutritional value of vegetables by improving antioxidants and regulating nitrate concentration.

Objectives: To focus on the effect of pre-harvest illumination with different spectral compositions and photoperiods on the nitrate content, total phenolic content (TPC) and antioxidant capacity of hydroponic green oak lettuce.

Materials & Methods: This experiment was arranged in a completely randomized design with three replications. The 10 treatments comprised no treated light (control) and pre-harvest-treatments with different spectral compositions of blue 460 nm-to-red 630 nm-to-red 660 nm as BRR 1:1:1 and BRR 2:1:1, respectively, and fluorescence light (FL) for 1 d, 2 d or 3 d (1D, 2D or 3D, respectively).

Results: The hydroponic green oak lettuce under pre-harvest lighting of 1D-BRR 1:1:1 contained the significantly lowest nitrate content of 14.36 mg/g which was a 67% reduction from the control. The TPC was the significantly highest in 3D-BRR 2:1:1 at 78.29 µg gallic acid equivalent (GAE)/g dry weight (dw), which was 59% greater than for the control. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay resulted in the significantly highest scavenging activities in the plant treated with 2D-BRR 2:1:1 (265.49 µg GAE/ g dw), which was 53% greater than the control. However, the results for both the TPC and DPPH were not significantly different from that of 1D-BRR 1:1:1.

Main finding: Among the pre-harvest illuminations treatments investigated with different spectral compositions, 1D-BRR 1:1:1 produced the lowest nitrate content, whereas the TPC and scavenging activity of hydroponic green oak lettuce were not significantly different for the 2D-BRR 2:1:1 and 3D-BRR 2:1:1 treatments. Therefore, 1D-BRR 1:1:1 pre-harvest illumination can be recommended for green oak lettuce production in a hydroponic system.

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Introduction

Lettuce is a well-known salad of green vegetables that is eaten worldwide; it has a low content of calories and zero fat and cholesterol, while being full of fiber and many other antioxidant compounds that provide several health benefits for human beings (Kim et al., 2016). Unfortunately, the health benefits of lettuce are not always accessible, especially those from hydroponic farming, where nitrate concentrations can rise to high levels (Chen et al., 2014) and may have a negative impact on consumers (Du et al., 2007; Hord et al., 2009). Daily consumption of nitrate in humans is approximately 75–100 mg and nearly 80% of total nitrate consumption is from vegetables (Du et al., 2007; Hord et al., 2009; Wu et al., 2013; Petpiamsiri et al., 2018). Nitrate itself is not harmful to human health but other nitrate-related compounds and excessive nitrate intake by humans may raise the incidence of bladder cancer (Abdel Mohsen et al., 1999), prostate cancer (Wu et al., 2013) and gastric cancer (Eroğlu et al., 1999). In the human body, nitrate reductase is active in saliva and gastric fluid, where it can convert a high nitrate concentration to a high nitrite concentration (Santamaria, 2006; Du et al., 2007). Nitrite can be reacted with amino acids, amides and amines to form N-nitroso compounds that are well-known as carcinogenic and can cause a variety of cancers (Santamaria, 2006; Du et al., 2007; Petpiamsiri et al., 2018). In vegetables or other plants, nitrate is a form of nitrogen that is a source of important nutrients for plant growth and as a key component of genetic material, protein and chlorophyll (Htwe and Ruangrak, 2021). Nitrate can be taken up easily through root hair, xylem and mesophyll cells. The nitrate is able to pass through cell walls via transporters and is reduced to nitrite and ammonium. Subsequently, the ammonium is assimilated into glutamine acid and other amino acids (Stitt, 1999). The assimilation or decrease of nitrates is influenced by a wide range of variables. One key factor in reducing the nitrate content of hydroponic lettuce is fertilizer management (Liu et al., 2011). Wang and Shen (2011) recommended the proper ammonium-to-nitrate ratio to lower the nitrate content of lettuce. Furthermore, it has been observed that the use of foliar fertilizers in lettuce, such as the proper proportions of urea, molybdenum and benzylidene, is effective in lowering nitrate levels (Wojciechowska and Kowalska, 2011). In hydroponic floatation systems growing lettuce, escarole and curly endive plants, nutrient solutions containing Mo (0.5, 1.5 and 3.0 mol/L) considerably decreased the nitrate accumulation (Moncada et al., 2018). However, the light factor can stimulate the expression of NR-related genes (NR), enhance NR activity and makes accessible surfactants that are the main inducer of nitrate degradation (Iglesias-Bartolome et al., 2004). In addition, increased light intensity significant impacts on the induction of the enzyme activity involved in nitrate metabolism (Demšar et al., 2004). For example, when the light intensity of red and blue (4:1) light emitting diode (LED) light increased from 60 µmol/m^2/s to 210 µmol/m^2/s, there was a substantial reduction in the nitrate concentration in lettuce (*Lactuca sativa* var. butterhead) leaves (Fu et al., 2017). Similarly, the nitrate concentration in brassica microgreens reduced from 37.7% to 84.5% when the light intensity of mixed LEDs (455, 638, 665 and 731 nm) increased from 110 µmol/m^2/s to 545 µmol/m^2/s (Samuolienė et al., 2013). Pérez-López et al. (2015) reported that the nitrate concentration in green-leaf and red-leaf lettuce significantly decreased by 25% and 86%, respectively, when the light intensity increased from 400 µmol/m^2/s to 700 µmol/m^2/s. Photoperiod is another important environmental component that controls nitrate assimilation. The nitrate contents in hydroponic lettuce were substantially lowered when continuously treated with a combination of red and blue (4:1) LEDs with a photosynthetic photon flux density of 200 µmol/m^2/s for 48 hr (Zhou et al., 2012). Similarly, a reduction in nitrate is impacted by the light spectral component. For example, the nitrate concentration in Boston lettuce (var. capitate) decreased by 29 mg/kg (dw) when cultivated under white light (400–700 nm) mixed with red (660 nm) and blue (454 nm) light, with a light intensity of 210 µmol/m^2/s (16 hr light/8 hr dark) (Lin et al., 2013). Green light also had beneficial effects on nitrate reduction when combined with other light spectra or sunlight. For example, the supplementation of high-pressure sodium lamps (170 µmol/m^2/s) with green light (30 µmol/m^2/s) significantly reduced the nitrate content while simultaneously increasing the carbohydrate accumulation in lettuce cultivated in greenhouses (Samuoliene et al., 2012). Additionally, UV-A light (315–400 nm) was effective in lowering the nitrate level in hydroponic lettuce (Chang and Chang, 2014). Furthermore, red light had an impact on the regulation of NR activity (Sakuraba and Yanagisawa, 2018). Bliznikas et al. (2012) reported that the nitrate content in spinach, parsley and dill reduced by 206 mg/kg, 566 mg/kg and 1,811 mg/kg, respectively, when these plants were exposed to red light (638 nm) plus HLP and nature light with a light intensity of 300 mol/m^2/s for 3 d prior to harvest. The blue light spectrum is another factor that influences nitrate reduction in plants. According to Zheng et al. (2018), growing pak choi (*Brassica rapa*) under a high light

intensity (150 µmol/m^2/s) reduced nitrate. The appropriate wavelength of light indirectly promotes plants to produce the necessary energy for the activation of enzymes, such as nitrate reductase, nitrite reductase, glutamine synthetase and glutamine 2-oxoglutarate aminotransferase, which play vital roles in transforming nitrate into amino acids, proteins and other organic nitrogen compounds (Htwe and Ruangrak, 2021). Therefore, light is a key factor in promoting the reduction of nitrate and the subsequent assimilation of nitrogen in plants.

Phenolic compounds are secondary metabolites of plants that have positive health effects for humans, including anti-inflammatory, anti-cancer, coronary heart disease and diabetes prevention, as well as cardiovascular protection (Chen et al., 2018; Zhang et al., 2019). The primary dietary sources of phenolic compounds for humans are fruits and vegetables (Lin et al., 2016). Light is one of the most significant environmental factors controlling the biosynthesis of phenolic compounds in plants (Zoratti et al., 2014). Several photoreceptors, including phytochromes, cryptochromes, phototropins and UV response locus 8 (UVR8), play important functions in the regulation of the biosynthesis of phenolic compounds (Jiao et al., 2007; Rizzini et al., 2011). For example, buckwheat sprouts had higher phenolic compounds when exposed to white and blue light than to red light (Thwe et al., 2014). The TPC also increased in response to the green light (524 nm) (Battistoni et al., 2021). Furthermore, the total phenolic compounds in rice leaves were higher under different light spectra in the following order: blue > white > red > green > dark (Lakshmanan et al., 2015).

Antioxidants help to increase the nutrient value in food and maintain human health (Chen et al. 2018). Multiple environmental factors, including salinity, drought, metal toxicity, extremely high or low temperatures, air pollutants, ultraviolet-B (UV-B) radiation, pesticides and pathogen infection, cause enhanced antioxidant capacity in plants (Xie et al., 2019). Light can also increase antioxidant capacity. For example, the antioxidant capacity increased in the presence of white light, blue light and UV-A when there was a higher phenolic content (Bendary et al., 2013). Baby spinach leaves (*Spinacia oleracea* L.) exposed to blue light (B: 468 nm) had phenolic contents and antioxidant capacity that were noticeably higher compared to leaves of plants exposed to white light (Battistoni et al., 2021). In addition, the largest antioxidant capability was reported by water convolvulus, red holy basil, dill and lemon basil microgreens while exposed to irradiation at 330 mol/m^2/s photosynthetic photon flux density (Harakotr et al., 2019). Furthermore, lettuce (*Lactuca sativa* L. cv. Rome), cherry radish (*Raphanus sativas* L. cv. Hongxin) and cherry tomato (*Lycopersicon esculentum* M. cv. Mosite) had higher antioxidant activity when exposed to high-intensity blue light in red/blue/green (RBG) and red/blue/white (RBW) light than W light (Tang et al., 2020). Similarly, common buckwheat sprouts treated with B light had increased antioxidant activity (Nam et al., 2018).

Pre-harvesting processes can reduce the nitrate content in vegetables and improve the quality of the vegetables. There are many advantages to pre-harvesting, such as reducing health risks from the accumulation of chemical residue and increasing essential nutrients in vegetables for human health. For example, substituting the fertilizer solution with tap water for different durations before harvesting has been reported to have a positive impact by accumulating soluble sugars while simultaneously reducing the nitrate contents in lettuce (Ruangrak et al., 2023). However, the chemical composition and bioactive compounds of tomatoes, garlic and lettuce were affected by pre-harvest illumination (Martins et al., 2016). According to Zukauskas et al. (2011), pre-harvesting using a R LED supplement on spinach, parsley, dill, mustard, rocket and onion leaves for 3 d improved the antioxidant and nutritional qualities of those vegetables. Similarly, pre-harvesting using continuous lighting (R:B = 3:1) and short-term nitrogen limitation for 3 d improved the quantities of soluble sugar, soluble protein, anthocyanins and phenolic compounds and reduced the contents of nitrate and sesquiterpene lactones in lettuce (Yang et al., 2022). In addition, Zhou et al. (2012) found that after pre-harvesting lettuce plants with 48 hr of continuous light, the nitrate concentrations greatly decreased, while the contents of soluble sugars and vitamin C significantly increased. However, more research is needed to explore the pre-harvest application of light sources on each type of lettuce vegetable and the different spectral compositions of light. Therefore, the current study focused on the effect of pre-harvest illumination of different spectral compositions of light and their duration of exposure on the nitrate content, TPC and antioxidant capacity in hydroponic green oak lettuce.

Materials and Methods

Plant materials and cultivation conditions

Green oak lettuce (*Lactuca sativa* L.) seeds were planted in a covered box and allowed to germinate on a wet paper

towel for 3 d (Fig. 1A). Then, the seedlings were transplanted into a polyfoam cube (2.5 cm × 2.5 cm × 3.0 cm), as shown in Fig. 1B and placed for 15 d in a hydroponic dynamic root floating technique system under a 50% aluminum net shade with a plastic tunnel (Figs. 1C–1E). Then, the seedlings were transplanted into a hydroponic system using the nutrient film technique (NFT) for 28 d under a 50% aluminum net shade (Figs. 1F and 1G). The nutrient solution was applied (electrical conductivity = 1.5–2.0 mS/cm, pH = 5.5–6.5) as mentioned in Table 1 (Sirinupong, 2017).

Light conditions and treatments

To investigate light quality effects on the nitrate content, TPC and antioxidant capacity in hydroponic green oak lettuce, plants aged 6 wk were grown using NFT hydroponics under a 50% aluminum net shade (Fig. 1G) and placed in a chamber with different light spectral compositions (Fig. 1H). The chamber had three shelves (each 240 cm length × 40 cm width × 170 height cm), each with three cells (80 cm length × 40 cm width × 30 cm height). More details can be found in Etae et al. (2020). This chamber was set up with three different spectral compositions of light: 1) combined B and R LED light using at 1:1:1 of blue 460 nm-to-red 630 nm-red 660 nm light (BRR 1:1:1 LED); 2) combined B and R LED light at 2:1:1 of blue 460 nm-red 630 nm-red 660 nm light (BRR 2:1:1 LED); and 3) fluorescence light (FL) with a spectral wavelength of 400–700 nm. To avoid light contamination, non-reflective whiteboards were placed between neighboring treatments. This experiment was arranged in a completely randomized design with three replications. The 10 treatments comprised: 1) no treated light (control); 2) 1 d of light exposure with BRR 2:1:1 (1D-BRR 2:1:1); 3) 1 d of light exposure with BRR 1:1:1 (1D-BRR 1:1:1); 4) 1 d of light exposure with FL (D-FL); 5) 2 d of light exposure with BRR 2:1:1 (2D-BRR 2:1:1); 6) 2 d of light exposure with BRR 1:1:1 (2D-BRR 1:1:1); 7) 2 d of light exposure with FL (2D-FL); 8) 3 d of light exposure with BRR 2:1:1 (3D-BRR 2:1:1); 9) 3 d of light exposure with

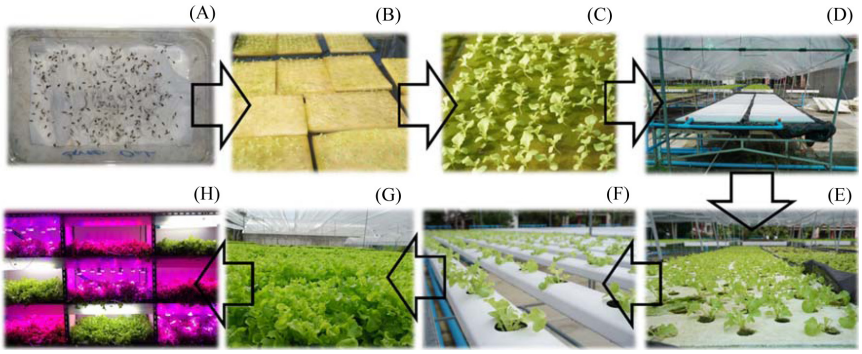

Fig. 1 Germination to pre-harvesting stages of hydroponic green oak lettuce plant: (A) tissue paper germination in covered box; (B) transplanting to polyfoam cube; (C, D, E) seedlings growing using dynamic root floating technique; (F, G) plants after transplanting into nutrient film technique; (H) plants in chamber for pre-harvesting with different light spectral compositions

Table 1 Ingredient of stock A and B nutrient solutions

Fertilizer	Elemental nutrient (%)	Amount (g/10 L of water)
Solution A		
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	Mg (9.35), S (13)	500
Potassium nitrate (KNO_3)	K (38.2), N (13)	780
Mono ammonium phosphate ($NH_4H_2PO_4$)	P (26.18), N (12)	130
Mono potassium phosphate (KH_2PO_4)	P (22.69), K (28.23)	100
Manganese EDTA (MnEDTA)	Mn (13)	8
Micro element (Boron EDTA, MnEDTA, MgO, CuEDTA, MoEDTA and FeEDTA)	B (2), Mn (2), Mg (4.5), Cu (1.9), Mo (0.023), Fe (1.8)	10
Solution B		
Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$)	Ca (18.8), N (15.5)	1000
Iron chelate (FeEDTA)	Fe (13.2)	10

EDTA = ethylenediaminetetraacetic acid

BRR 1:1:1 (3D-BRR 1:1:1); and 10) 3 d of light exposure with FL (3D-FL). For all treatments, the photoperiod was set at 12 hr light/12 hr dark, the temperature was maintained in the range 28–30°C, and the relative humidity was maintained in the range 75–85%. Nutrient solutions were substituted with drinking water during the 3 d of the pre-harvest illumination treatments to minimize the nutrient effect on plant growth. The experiment was carried out at the Laboratory of Urban Agriculture Technology, Division of Agricultural Technology, Department of Agricultural and Fishery Science, Faculty of Science and Technology, Prince of Songkla University, Pattani campus, Thailand.

Light quality of the three different spectral compositions

The light parameters of the three different spectral compositions of light—photosynthetically active radiation (PAR), photosynthetic photon flux density (PPFD), yield photon flux density (YPFD) and the intensities of ultra violet (UV) B, G, R and far-red light were measured using a plant light spectrum analyzer (OHSP-350P; Hangzhou HOPOO Optical Color Technology Co., Ltd; China).

Determination of nitrate content

The nitrate content was determined using the method described by Lastra (2003). One fresh lettuce leaves (one selected from each of 11 uniform plants) were washed three times in distilled water after being washed with running tap water. These leaves were oven-dried at 65°C for 24 hr, ground and blended until all samples passed through a 40-mesh sieve. Then, 0.1 g of ground tissue samples was suspended in 10 mL of distilled water, kept at 45°C for 1 hr and filtered through No. 40 Whatman paper. In a freshly made concentrated sulfuric acid (H_2SO_4) solution, 0.1 mL of the extraction solution was added to a 50 mL tube along with 0.4 mL of 5% (weight per volume, w/v) salicylic acid (Ajax Finechem; Australia). After 20 min at room temperature, 9.5 mL of 2M sodium hydroxide (NaOH) solution was slowly added to raise the pH. The extracted samples were immediately determined using a spectrometer (Libra S12; Biochrom; UK) at an absorbance of 412 nm.

Determination of total phenolic content

The TPC was determined following the method mentioned in Sulaiman et al. (2011). Briefly, 5 g of ground tissue sample were suspended in 10 mL of 70% (volume per volume, v/v) ethanol, shaken for 1 hr, filtered and then kept at 20°C in the dark before use. Folin-Ciocalteu's reagent (25 mL) was added to 50 ml of extract (500 mg/mL), allowed to stand for 6 min at room temperature and then added with 500 ml of 7% (w/v) sodium carbonate. After incubation at room temperature for 20 min, the TPC was measured using a spectrophotometer (Libra S12; Biochrom; UK) at 760 nm.

Determinations of antioxidant capacity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay described by Sulaiman et al. (2011) was used to determine the free radical scavenging activity of the plant samples. A stock radical solution of DPPH (Merck; USA) was prepared by dissolving 0.02 mM DPPH in methanol. The reaction mixtures were kept at room temperature for 12 hr in the dark before use. The extracted sample (450 µL) suspended in 70 % (v/v) ethanol was added to 900 µL of methanolic DPPH for the DPPH assay. Before measuring, the mixture was blended well and kept in the dark at room temperature for 30 min. Then, the absorbance of the reaction mixture was measured for DPPH using a spectrophotometer (Libra S12; Biochrom; UK) at 515 nm.

Statistical analysis

Data analysis was performed using a one-way analysis of variance. The Excel software (version 7.0; Microsoft Corp.; USA) was used for data processing. The means were tested based on the least significant difference at a confidence level of 95%.

Results

Light quality of three different spectral compositions

The light parameters of the BRR 1:1:1-treatment, consisting of PAR, PPFD, YPFD and the intensities of UV, B, G, R and far-red lights, had significantly higher intensities than BRR 2:1:1 and FL; however, parameters of UV and G light for the BRR 1:1:1-treatment were not significantly different from the FL treatment (Table 2). These different light parameters affected the quality of hydroponic green oak lettuce. The results showed that the PAR value of BRR 1:1:1 was the highest (42.87 ± 2.39 mW/cm^2), followed by BRR 2:1:1 (5.60 ± 0.24 mW/cm^2) and FL (1.26 ± 0.27 mW/cm^2), as shown in Table 2.

Table 2 Light quality parameters in three different spectral compositions (blue 460 nm-to-red 630 nm-red 660 nm light (BRR 2:1:1 and BRR 1:1:1) and FL), photosynthetically active radiation (PAR), photosynthetic photon flux density (PPFD), PPFD-UV (ultraviolet), PPFD-B (blue), PPFD-G (green), PPFD-R (red), PPFD-FR (far-red) and yield photon flux density (YPFD)

Light parameter	BRR 2:1:1	BRR 1:1:1	FL
PAR (mW/cm^2)	5.60±0.24^b	42.87±2.39^a	1.26±0.27^c
PPFD (µmol/m^2/s)	256.32±11.56^b	2005.05±53.62^a	51.64±11.20^c
UV (µmol/m^2/s)	0.35±0.08^b	1.93±0.30^a	1.78±0.43^a
Blue (µmol/m^2/s)	105.64± 7.49^b	748.60±159.40^a	17.14±3.78^b
Green (µmol/m^2/s)	3.27±0.22^b	22.44±4.16^a	23.89±5.17^a
Red(µmol/m^2/s)	147.41±11.30^b	1234.01±149.61^a	10.60±2.28^c
Far Red (µmol/m^2/s)	2.36±0.44^b	15.97±1.95^a	2.56±0.43^b
YPFD (µmol/m^2/s)	219.71±10.49^b	1712.69±31.43^a	44.45±9.65^c

Mean ± SD (n = 3) in each row superscripted with different lowercase letters are significantly (p < 0.05) different.

Pre-harvest illumination effect on nitrate content in hydroponic green oak lettuce

The spectral compositions of light have been identified as one of the major factors that influence the major nitrogen content in vegetables (Nájera and Urrestarazu, 2019). The current study compared the nitrate contents in hydroponic green oak lettuce following pre-harvest illumination using different spectral compositions of light (BRR 2:1:1, BRR 1:1:1 and FL) in combination with 1 d, 2 d or 3 d of light exposure and a control with no treated light. According to the findings, the nitrate contents of the hydroponic green oak lettuce under the 2D-FL and 3D-FL treatments (34.77 mg/g and 37.17 mg/g, respectively) did not differ significantly from the control treatment, while the other treatments did. The hydroponic green oak lettuce under 1D-BRR 1:1:1 had the lowest nitrate content (14.36 mg/g) but this was not significantly different from 1D-BRR 2:1:1 (29.07 mg/g), 1D-FL (18.85 mg/g), 2D-BRR 2:1:1 (14.84 mg/g), 2D-BRR 1:1:1 (17.93 mg/g), 3D-BRR 2:1:1 (24.96 mg/g) and 3D-BRR 1:1:1 (19.16 mg/g).

Pre-harvest illumination effect on total phenolic content of hydroponic green oak lettuce

Vegetables contain various kinds of antioxidants that help protect the human body from disease and oxidative damage when consumed regularly (Wootton-Beard et al., 2011). The TPC is an indicator of the number of polyphenols in vegetables that have antioxidant properties that can help to protect the human body against chronic diseases. The current study measured the TPC in hydroponic green oak lettuce pre-harvested under different spectral compositions of light treatments for 1 d, 2 d or 3 d (Fig. 3). The results showed that the TPC in hydroponic green oak lettuce was significantly affected in different ways by the varying treatments. The TPC was in the range 45.53–78.28 µg GAE/g dw and increased by 59% compared to the control treatment (49.24 µg GAE/ g dw). However, the TPC content in the 3D-BRR 2:1:1 treatment did not differ significantly from the treatments of 1D-BRR 1:1:1, 2D-BRR 2:1:1, 3D-BRR 1:1:1 and 3D-FL (Fig. 3).

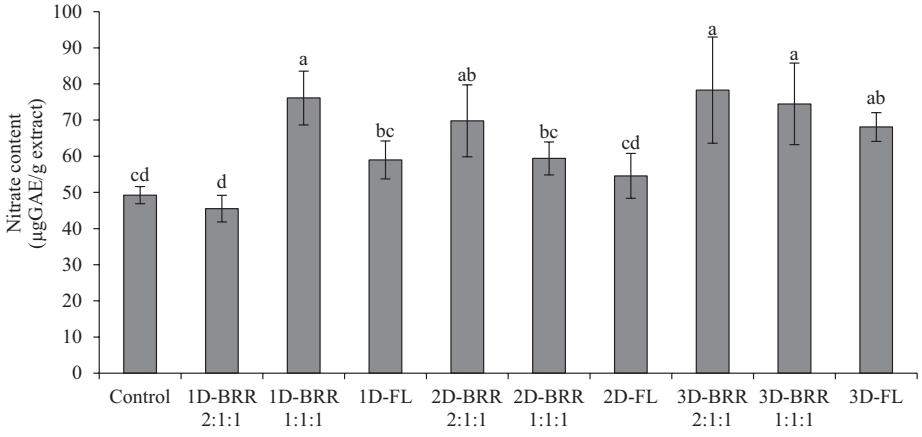

Fig. 2 Nitrate content in green oak lettuce leaves under non-treated light (control) and LED lighting at different ratios of blue 460 nm-to-red 630 nm-red 660 nm light (BRR 1:1:1) for 1 d, 2 d or 3 d (1D, 2D, 3D, respectively) = 1D-BRR 2:1:1, 2D-BRR 2:1:1, 3D-BRR 2:1:1, 1D-BRR 1:1:1, 2D-BRR 1:1:1, 3D-BRR 1:1:1 and fluorescent light (FL) for 1D-FL, 2D-FL and 3D-FL, where error bars show ± SD of three replications; Different lowercase letters above bars indicate significant (p < 0.05) difference.

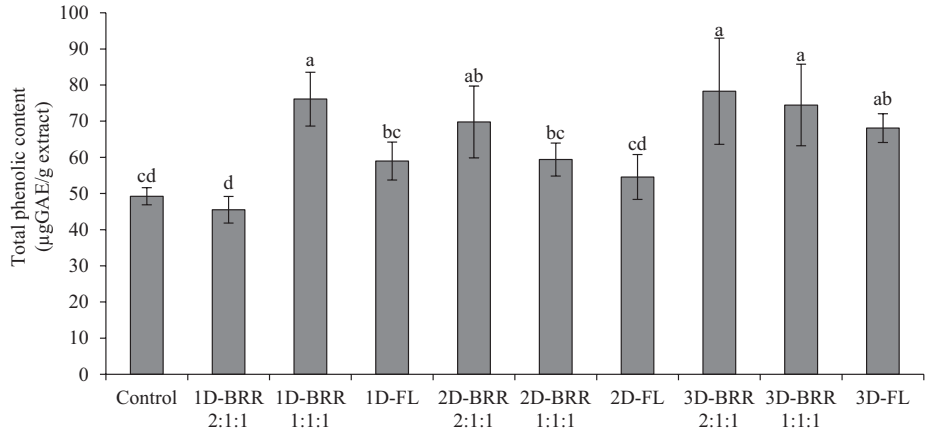

Fig. 3 Total phenolic content in green oak lettuce leaves under non-treated light under non-treated light (control) and LED lighting at different ratios of blue 460 nm-to-red 630 nm-red 660 nm light (BRR 1:1:1) for 1 d, 2 d or 3 d (1D, 2D, 3D, respectively) = 1D-BRR 2:1:1, 2D-BRR 2:1:1, 3D-BRR 2:1:1, 1D-BRR 1:1:1, 2D-BRR 1:1:1, 3D-BRR 1:1:1 and fluorescent light (FL) for 1D-FL, 2D-FL and 3D-FL, where GAE = gallic acid equivalent; error bars show ± SD of three replications; Different lowercase letters above bars indicate significant ($p < 0.05$) difference.

Pre-harvest illumination effect on antioxidant activities of hydroponic green oak lettuce

The scavenging activities were measured based on the total antioxidant capacity (DPPH) in green oak lettuce using assays and electron transfer according to Huang et al. (2005). There were significantly different results in the free radical scavenging activities, with a range of 173.69–265.49 µg GAE/g dw, representing an increase by 23–53% compared to the control (Fig. 4A). The hydroponic green oak lettuce in the pre-harvest treatment under 2D-BRR 2:1:1 had the highest free radical scavenging activity based on DPPH assay (265.49 g GAE/g dw), followed by 3D-BRR 2:1:1 (252.59 µg GAE/g dw), 1D-BRR 1:1:1 (247.10 µg GAE/g dw), 1D-FL (246.68 µg GAE/ g dw), 2D-FL (231.86 µg GAE/g dw), 3D-FL (231.15 µg GAE/ g dw), 3D-BRR 1:1:1 (222.44 µg GAE/ g dw), 1D-BRR 1:1:1 (217.83 µg GAE/ g dw), 1D-BRR 2:1:1 (212.93 µg GAE/ g dw) and the control treatment (173.96 µg GAE/ g dw), respectively. However, there was no significant difference among the treatments of 2D-BRR 2:1:1, 1D-FL, 2D-BRR 1:1:1, 2D-FL, 3D-BRR 2:1:1 and 3D-FL (Fig. 4A).

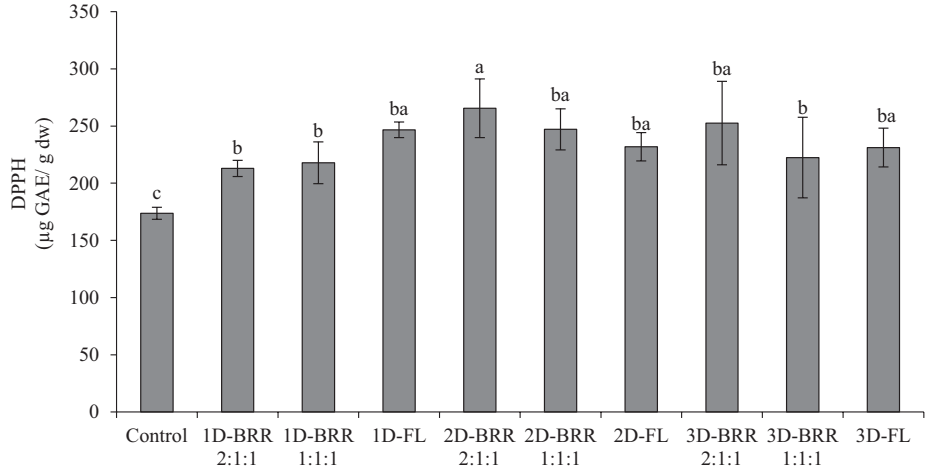

Fig. 4 Antioxidant activities of green oak lettuce leaves for 2,2-diphenyl-1-picrylhydrazyl (DDPH) under non-treated light (control) and LED lighting at different ratios of blue 460 nm-to-red 630 nm-red 660 nm light (BRR 1:1:1) for 1 d, 2 d or 3 d (1D, 2D, 3D, respectively) = 1D-BRR 2:1:1, 2D-BRR 2:1:1, 3D-BRR 2:1:1, 1D-BRR 1:1:1, 2D-BRR 1:1:1, 3D-BRR 1:1:1 and fluorescent light (FL) for 1D-FL, 2D-FL and 3D-FL, where GAE = gallic acid equivalent; error bars show ± SD of three replications; Different lowercase letters above bars indicate significant ($p < 0.05$) difference.

Discussion

The results of the nitrate concentration were compatible with the light quality of the three different spectral compositions (Fig. 2). PAR (400–700 nm) is important for the photosynthesis process as a high PAR value indicates high energy from the photosynthesis process. Therefore, pre-harvest illumination with BRR 1:1:1 produced a higher energy, because it provided higher PAR value than BRR 2:1:1 and FL. This energy activates the enzymes nitrate reductase, nitrite reductase, GS and GOGS to reduce nitrate to nitrite, ammonium, glutamate and glutamine, respectively. Far-red radiation (FR, 700–800 nm) plays an important role in promoting the mechanism of radiation capture and regulating morphological and developmental plants, especially their survival under shade (Park and Runkle, 2017). B light (400–500 nm) is vital for plant photosynthesis at high capacity because it is highly captured by chlorophyll a and b (Lanoue et al., 2019). G light (500–600 nm) activates a shade avoidance syndrome that is similar to far-red light (Zhang et al., 2022). R light (600–700 nm) has great potential for use as a light source to drive photosynthesis (Liu and van Iersel, 2021).

The effects of pre-harvest illumination on the nitrate content in hydroponic green oak lettuce in the current study were similar to the report of Ohashi et al. (2007), which showed a notable decrease in the nitrate content under combined R and B LED light compared to under FL. Similarly, in the Spanish plant, it was reported that a mixture of R and B LED light was more effective in the reduction of the nitrate content than white or yellow light (Qi et al., 2007). Furthermore, the nitrate content in hydroponic green oak lettuce pre-harvest illumination under 1D-BRR 1:1:1 was the lowest but not significantly different from that treated with 2 d and 3 d of illumination, indicating that 1 d of illumination is sufficient (Fig. 2). The comparison of BRR 1:1:1 and BRR 2:1:1 showed that the double amount of B light of the former might have been responsible for the increase in the nitrate content, because nitrate reduction had been observed under R light in radish plants, with the R light activating a higher capacity of the nitrate reductase enzyme than the B light (Maevskaya and Bukhov, 2005). In addition, modification of the R and B light ratio affected nitrate levels in plants as shown by the lowest nitrate content being observed in the treatment with an R-to-B ratio of 4:1 (Wanlai et al., 2013). It could be possible that the LED treatment increased the products from photosynthesis (adenosine triphosphate and nicotinamide adenine dinucleotide phosphate hydrogen), which play a vital role in enzyme regulation and nitrate reduction in leaves (Huner et al., 1998; Velez-Ramirez et al., 2011). It was also reported that the assimilation rate of nitrate was substantially enhanced under light, leading to a decrease in plant nitrate accumulation (Scaife and Schloemer, 1994).

An increased phenolic content promotes health benefits by producing antioxidant activity to prevent cell aging and various diseases (Matsumura et al., 2023). Plants produce phenolic compounds as secondary metabolites; these substances can be divided into a variety of major subclasses, including phenolic acids, phenylpropanoids, flavonols, flavanones, flavones and isoflavones (Ross and Kasum, 2002). Along with assisting in plant growth and development, they play a role in how plants interact with the environment (Pourcel et al., 2007; Nakabayashi and Saito, 2015). Light is one of the most important environmental factors. It is known that several photoreceptors, including phytochromes, cryptochromes, phototropins and UV response locus 8, sense light signals that control the biosynthesis of phenolic compounds in plants (Jiao et al., 2007; Rizzini et al., 2011; Nakabayashi et al., 2015). For example, buckwheat sprouts were reported to contain more phenolic compounds when exposed to W and B light compared to R light (Thwe et al., 2014). Furthermore, Lobiuc et al. (2017) and Samuolienė et al. (2012) found that R light or a combination of R and B ratios enhanced the TPC in young seedlings of lettuce and basil plants. In addition, the total phenolic compounds in rice leaves subjected to various light treatments were in the following order: B > W > R > G > darkness (Lakshmanan et al., 2015). For hydroponic green oak lettuce, the current results showed that all pre-harvest illumination treatments, except 1D-BRR 2:1:1, were more effective at increasing the TPC than the control treatment. It was reported that pre-harvest illumination with continuous light exposure effectively increased the phytochemical contents in lettuce plants (Bian et al., 2015). Furthermore, continuous lighting could lead to greater use of absorbed energy for generating more reactive oxygen species (ROS), which induces the production of TPC in plants (Huner et al., 1998; Cakmak and Kirkby, 2008).

Dietary antioxidants, such as phenolic compounds, are extremely beneficial for boosting food value and preserving human health (Chen et al., 2018). The pre-harvest illumination effects on the hydroponic green oak lettuce, based on the DPPH assay, produced significantly higher levels of antioxidant activity than for the control treatment. Plants increase the activity of antioxidant enzymes and secondary metabolites,

such as phenolic compounds, under abiotic stress conditions, particularly light factors during photosynthesis system. Plants protect themselves from ROS-induced oxidative damage by activating phenolic compounds (Grassmann et al., 2002; Foyer, 2018). Zhang et al. (2019) studied the antioxidant activity in soybean microgreens grown under different light spectra. They found that soybean microgreens grown under UV-A had the highest antioxidant activity, followed by B and W lights, and was comparable to that of pea sprouts (Qian et al., 2016) and Chinese kale sprouts (Liu et al., 2016). In the current study using hydroponic green oak lettuce, many treatments with different light spectral components resulted in increased TPC levels and DPPH radical scavenging activity compared to the control treatment. Similar findings were made by Zhang et al. (2019), who found that soybean microgreens grown under a light spectrum had higher antioxidant capacity because of the higher transcript level of genes involved in the phenylpropanoid pathway.

In conclusion, hydroponic green oak lettuce under a pre-harvest light treatment of 1D-BRR 1:1:1 had the significantly lowest nitrate content. The TPC was the significantly highest in 3D-BRR 2:1:1. The DPPH assay results showed that the significantly highest scavenging activity was in 2D-BRR 2:1:1. However, the results for both TPC and DPPH were not significantly different from those of 1D-BRR 1:1:1. Among the pre-harvest illumination options investigated with different spectral compositions, 1D-BRR 1:1:1 resulted in the lowest nitrate, whereas the levels of TPC and scavenging activity of hydroponic green oak lettuce were not significantly different for 2D-BRR 2:1:1 and 3D-BRR 2:1:1. Therefore, 1D-BRR 1:1:1 pre-harvest illumination can be recommended for green oak lettuce production in a hydroponic system. Furthermore, the application of pre-harvest illumination can be beneficial for the production of phenolic compound-rich functional vegetables with higher antioxidant capacity and reduced nitrate content.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Abdel Mohsen, M.A., Hassan, A.A., El-Sewedy, S.M., Aboul-Azm, T., Magagnotti, C., Fanelli, R., Airoldi, L. 1999. Biomonitoring of n-nitroso compounds, nitrite and nitrate in the urine of Egyptian bladder cancer patients with or without *Schistosoma haematobium* infection. Int. J. Cancer 82: 789–794. doi.org/10.1002/(SICI)1097-0215(19990909)82:6<789::AID-IJC3>3.0.CO;2-C

Battistoni, B., Amorós, A., Tapia, M.L., Escalona, V. 2021. Effect of LED lights on the antioxidant properties of baby spinach leaves (*Spinacia oleracea* L.). Rev. FCA UNCuyo. 53: 98–108.

Bian, Z.H., Yang, Q.C., Liu, W.K. 2015. Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: A review. J. Sci. Food Agric. 95: 869–877. doi.org/10.1002/jsfa.6789

Bendary, E., Francis, R.R., Ali, H.M.G., Sarwat, M.I., Hady, S.E. 2013. Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. Ann. Agric. Sci. 58:173−181. doi: 10.1016/j.aoas.2013.07.002

Bliznikas, B., Zukauskas, A., Samuolienė, G., Viršilė, A., Brazaitytė, A., Jankauskienė, J., Duchovskis, A., Novickovas, P. 2012. Effect of supplementary pre-harvest LED lighting on the antioxidant and nutritional properties of green vegetables. Acta Hortic. 939: 85–91.

Cakmak, I., Kirkby, E.A. 2008. Role of magnesium in carbon partitioning and alleviating photooxidative damage. Physiol. Plant. 133: 692–704. doi.org/10.1111/j.1399-3054.2007.01042.x

Chang, C.-L., Chang, K.-P. 2014. The growth response of leaf lettuce at different stages to multiple wavelength-band light-emitting diode lighting. Sci. Hortic. 179: 78–84. doi.org/10.1016/j.scienta.2014.09.013

Chen, L., Teng, H., Xie, Z., Cao, H., Cheang, W.S., Skalicka-Woniak, K., Georgiev, M.I., Xiao, J. 2018. Modifications of dietary flavonoids towards improved bioactivity: An update on structure-activity relationship. Crit. Rev. Food Sci. Nutr. 58: 513−527. doi.org/10.1080/10408398.2016.1196334

Chen, X., Guo, W., Xue, X., Wang, L., Qiao, X. 2014. Growth and quality responses of 'Green Oak Leaf' lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). Sci. Hortic. 172: 168–175. doi.org/10.1016/j.scienta.2014.04.009

Demšar, J., Osvald, J., Vodnik, D. 2004. The effect of light-dependent application of nitrate on the growth of aeroponically grown lettuce (*Lactuca sativa* L.). J. Amer. Hort. Soc. Hortic. Sci. 129: 570–575.

Du, S., Zhang, Y., Lin, X. 2007. Accumulation of nitrate in vegetables and its possible implications to human health. Agr. Sci. China 6: 1246–1255. doi.org/10.1016/S1671-2927(07)60169-2

Eroğlu, A., Demirci, S., Ayyildiz, A., Kocaoğlu, H., Akbulut, H., Akgül, H., Elhan, H.A. 1999. Serum concentrations of vascular endothelial growth factor and nitrite as an estimate of *in vivo* nitric oxide in patients with gastric cancer. Br. J. Cancer 80: 1630–1634.

Etae, N., Wamae, Y., Khummueng, W., Utaipan, T., Ruangrak, E. 2020. Effects of artificial light sources on growth and phytochemicals content in green oak lettuce. Hortic. Bras. 38: 204–210. doi.org/10.1590/S0102-053620200213

Foyer, C.H. 2018. Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environ. Exp. Bot. 154: 134–142. doi.org/10.1016/j.envexpbot.2018.05.003

Fu, Y., Li, H., Yu, J., Liu, H., Cao, Z., Manukovsky, N., Liu, H. 2017. Interaction effects of light intensity and nitrogen concentration on growth, photosynthetic characteristics and quality of lettuce (*Lactuca sativa* L. var. *youmaicai*). Sci. Hortic. 214: 51–57. doi.org/10.1016/j.scienta.2016.11.020

Grassmann, J., Hippeli, S., Elstner, E.F. 2002. Plant's defence and its benefits for animals and medicine: Role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiol. Biochem. 40: 471–478. doi.org/10.1016/S0981-9428(02)01395-5

Harakotr, B., Srijunteuk, S., Rithichai, P., Tabunhan, S. 2019. Effects of light-emitting diode light irradiance levels on yield, antioxidants and antioxidant capacities of indigenous vegetable microgreens. Science & Technology Asia 24: 59–66.

Hord, N.G., Tang, Y., Bryan, N.S. 2009. Food sources of nitrates and nitrites: The physiologic context for potential health benefits. Am. J. Clin. Nutr. 90: 1–10. doi.org/10.3945/ajcn.2008.27131

Htwe, N.M.P.S., Ruangrak, E. 2021. A review of sensing, uptake, and environmental factors influencing nitrate accumulation in crops. J. Plant Nutr. 44: 1054–1065. doi.org/10.1080/01904167.2021.1871757

Huang, D., Ou, B., Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53: 1841–1856. doi.org/10.1021/jf030723c

Huner, N.P.A., Öquist, G., Sarhan, F. 1998. Energy balance and acclimation to light and cold. Trends Plant Sci. 3: 224–230. doi.org/10.1016/S1360-1385(98)01248-5

Iglesias-Bartolome, R., Gonzalez, C., Kenis, J. 2004. Nitrate reductase dephosphorylation is induced by sugars and sugar-phosphates in corn leaf segments. Physiologia Plantarum. 122: 62–67. doi.org/10.1111/j.1399-3054.2004.00375.x

Jiao, Y., Lau, O.S., Deng, X.W. 2007. Light-regulated transcriptional networks in higher plants. Nat. Rev. Genet. 8: 217−230. doi: 10.1038/nrg2049

Kim, M.J., Moon, Y., Tou, J.C., Mou, B., Waterland, N.L. 2016. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). J. Food Compos. Anal. 49: 19–34. doi.org/10.1016/j.jfca.2016.03.004

Lakshmanan, M., Lim, S.-H., Mohanty, B., Kim, J.K., Ha, S.H., Lee, D.Y. 2015. Unraveling the light-specific metabolic and regulatory signatures of rice through combined *in silico* modeling and multiomics analysis. Plant Physiol. 169: 3002−3020. doi.org/10.1104/pp.15.01379

Lanoue, J., Leonardos, E., Grodzinski, B. 2019. Artificial lighting technologies for agricultural production. In: Moo-Young, M. (Ed.). Comprehensive Biotechnology, 3rd ed, Vol. 4. Elsevier. Pergamon, Turkey, pp. 818–832.

Lastra, O.C. 2003. Derivative spectrophotometric determination of nitrate in plant tissue. J. AOAC Int. 86: 1101–1105.

Lin, D., Xiao, M., Zhao, J., et al. 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21: 1374. doi.org/10.3390/molecules21101374

Lin, K.-H., Huang, M.-Y., Huang, W.-D., Hsu, M.-H., Yang, Z.-W., Yang, C.-M. 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. capitata). Sci. Hortic. 150: 86–91. doi.org/10.1016/j.scienta.2012.10.002

Liu, J., van Iersel, M.W. 2021. Photosynthetic physiology of blue, green, and red light: Light intensity effects and underlying mechanisms. Front. Plant Sci. 12: 1–14. doi.org/10.3389/fpls.2021.619987

Liu, H., Chen, Y., Hu, T., Zhang, S., Zhang, Y., Zhao, T., Yu, H., Kang, Y. 2016. The influence of light-emitting diodes on the phenolic compounds and antioxidant activities in pea sprouts. J. Funct. Foods. 25: 459−465. doi.org/10.3390/horticulturae6040077

Liu, W.K., Yang, Q.C., Du, L.F. 2011. Short-term treatment with hydroponic solutions containing osmotic ions and ammonium molybdate decreased nitrate concentration in lettuce. Acta Agric. Scand. B Soil Plant Sci. 61: 573–576. doi.org/10.1080/09064710.2010.521762

Lobiuc, A., Vasilache, V., Pintilie, O., Stoleru, T., Burducea, M., Oroian, M., Zamfirache, M.-M. 2017. Blue and red led illumination improves growth and bioactive compounds contents in acyanic and cyanic *Ocimum basilicum* L. microgreens. Molecules 22: 2111. doi.org/10.3390/molecules22122111

Maevskaya, S.N., Bukhov, N.G. 2005. Effect of light quality on nitrogen metabolism of radish plants. Russ. J. Plant Physiol. 52: 304–310. doi.org/10.1007/s11183-005-0046-1

Matsumura, Y., Kitabatake, M., Kayano, S., Ito, T. 2023. Dietary phenolic compounds: Their health benefits and association with the gut microbiota. Antioxidants 12: 1–41. doi.org/10.3390/antiox12040880

Martins, N., Petropoulos, S., Ferreira, I.C.F.R. 2016. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. Food Chem. 211: 41–50. doi.org/10.1016/j.foodchem.2016.05.029

Moncada, A., Miceli, A., Sabatino, L., Iapichino, G., D'Anna, F., Vetrano, F. 2018. Effect of molybdenum rate on yield and quality of lettuce, escarole, and curly endive grown in a floating system. Agronomy 8: 171. doi.org/10.3390/agronomy8090171

Nájera, C., Urrestarazu, M. 2019. Effect of the intensity and spectral quality of LED light on yield and nitrate accumulation in vegetables. HortScience 54: 1745–1750. doi.org/10.21273/HORTSCI14263-19

Nakabayashi, R., Saito, K. 2015. Integrated metabolomics for abiotic stress responses in plants. Curr. Opin. Plant Biol. 24: 10−16. doi.org/10.1016/j.pbi.2015.01.003

Nam, T.G., Kim, D.O., Eom, S.H. 2018. Effects of light sources on major flavonoids and antioxidant activity in common buckwheat sprouts. Food Sci. Biotechnol. 27: 169–176. doi.org/10.1007/s10068-017-0204-1

Ohashi, K., Takase, M., Kon, N., Fujiwara, K., Kurata, K. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environ. Control. Biol. 45: 189–198. doi.org/10.2525/ecb.45.189

Park, Y., Runkle, E.S. 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Exp. Bot. 136: 41–49. doi.org/10.1016/j.envexpbot.2016.12.013

Pérez-López, U., Miranda-Apodaca, J., Muñoz-Rueda, A., Mena-Petite, A. 2015. Interacting effects of high light and elevated CO_2 on the nutraceutical quality of two differently pigmented *Lactuca sativa* cultivars (Blonde of Paris Batavia and Oak Leaf). Sci. Hortic. 191: 38–48. doi.org/10.1016/j.scienta.2015.04.030

Petpiamsiri, C., Siritientong, T., Kangsadalampai, K., Tongyonk, L. 2018. The nitrate content in some green leafy vegetables with different cultivation methods in Thailand. Journal of Public Health 48: 385–397.

Pourcel, L., Routaboul, J.M., Cheynier, V., Lepiniec, L., Debeaujon, I. 2007. Flavonoid oxidation in plants: From biochemical properties to physiological functions. Trends Plant Sci. 12: 29−36. doi.org/10.1016/j.tplants.2006.11.006

Qi, L., Liu, S., Xu, L., Yu, W., Liang, Q., Hao, S. 2007. Effects of light qualities on accumulation of oxalate, tannin and nitrate in spinach. Transactions of the Chinese Society of Agricultural Engineering 23: 201–205.

Qian, H., Liu, T., Deng, M., Miao, H., Cai, C., Shen, W., Wang, Q. 2016. Effects of light quality on main health-promoting compounds and antioxidant capacity of Chinese kale sprouts. Food Chem. 196: 1232−1238. doi.org/10.1016/j.foodchem.2015.10.055

Rizzini, L., Favory, J.J., Cloix, C., et al. 2011. Perception of UV-B by the Arabidopsis UVR8 protein. Science 332: 103−106. doi: 10.1126/science.1200660

Ross, J.A., Kasum, C.M. 2002. Dietary flavonoids: Bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 22: 19−34. doi.org/10.1146/annurev.nutr.22.111401.144957

Ruangrak, E., Salaemae, N., Sornnok, S., Htwe, N. 2023. Effect of pre-harvest periods by replacing nutrient solution with tap water on nitrate and quality in hydroponic lettuce. ASEAN J. Sci. Tech. Report. 26: 76–83.

Sakuraba, Y., Yanagisawa, S. 2018. Light signalling-induced regulation of nutrient acquisition and utilisation in plants. Semin. Cell Dev. Biol. 83: 123–132. doi.org/10.1016/j.semcdb.2017.12.014

Samuolienė, G., Brazaitytė, A., Jankauskienė, J., et al. 2013. LED irradiance level affects growth and nutritional quality of *Brassica* microgreens. Cent. Eur. J. Biol. 8: 1241–1249. doi.org/10.2478/s11535-013-0246-1

Samuoliene, G., Brazaitytė, A., Sirtautas, R., Novickovas, A., Duchovskis, P. 2012. The effect of supplementary LED lighting on the antioxidant and nutritional properties of lettuce. Acta Hortic. 952: 835–841. doi.org/10.17660/ActaHortic.2012.952.106

Santamaria, P. 2006. Nitrate in vegetables: Toxicity, content, intake and EC regulation. J. Sci. Food Agric. 86: 10–17. doi.org/10.1002/jsfa.2351

Scaife, A., Schloemer, S. 1994. The diurnal pattern of nitrate uptake and reduction by spinach (*Spinacia oleracea* L.). Ann. Bot. 73: 337–343. doi.org/10.1006/anbo.1994.1040

Sirinupong, M. 2017. Practical for Soilless Culture in Thailand, 4th ed. Flam-up Design Press. Bangkok, Thailand.

Stitt, M. 1999. Nitrate regulation of metabolism and growth. Curr. Opin. Plant Biol. 2: 178–186. doi.org/10.1016/S1369-5266(99)80033-8

Sulaiman, S.F., Sajak, A.A.B., Ooi, K.L., Supriatno, Seow, E.M. 2011. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. J. Food Compos. Anal. 24: 506–515. doi.org/10.1016/j.jfca.2011.01.020

Tang, Y., Mao, R., Guo, S. 2020. Effects of LED spectra on growth, gas exchange, antioxidant activity and nutritional quality of vegetable species. Life Sci. Space Res. 26: 77–84. doi.org/10.1016/j.lssr.2020.05.002

Thwe, A.A., Kim, Y.B., Li, X., Seo, J.M., Kim, S.-J., Suzuki, T., Chung, S.-O., Park, S.U. 2014. Effects of light-emitting diodes on expression of phenylpropanoid biosynthetic genes and accumulation of phenylpropanoids in *Fagopyrum tataricum* sprouts. J. Agric. Food Chem. 62: 4839−4845. doi.org/10.1021/jf501335q

Velez-Ramirez, A.I., van Ieperen, W., Vreugdenhil, D., Millenaar, F.F. 2011. Plants under continuous light. Trends Plant Sci. 16: 310–318. doi.org/10.1016/j.tplants.2011.02.003

Wang, B., Shen, Q. 2011. $NH4^+$ -N/NO_3^- -N ratios on growth and NO_3^- -N remobilization in root vacuoles and cytoplasm of lettuce genotypes. Can. J. Plant Sci. 91: 411–417. doi.org/10.4141/CJPS10044

Wanlai, Z., Wenke, L., Qichang, Y. 2013. Reducing nitrate content in lettuce by pre-harvest continuous light delivered by red and blue light-emitting diodes. J. Plant Nutr. 36: 481–490. doi.org/10.1080/01904167.2012.748069

Wojciechowska, R., Kowalska, I. 2011. The effect of foliar application of urea, Mo and BA on nitrate metabolism in lettuce leaves in the spring and summer-autumn seasons. Folia Hort. 23: 119–123. doi.org/10.2478/v10245-011-0018-1

Wootton-Beard, P.C., Moran, A., Ryan, L. 2011. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin–Ciocalteu methods. Food Res. Int. 44: 217–224. doi.org/10.1016/j.foodres.2010.10.033

Wu, T., Wang, Y., Ho, S.-M., Giovannucci, E. 2013. Plasma levels of nitrate and risk of prostate cancer: A prospective study. Cancer Epidemiol. Biomarkers Prev. 22: 1210–1218.

Xie, X., He, Z., Chen, N., Tang, Z., Wang, Q., Cai, Y. 2019. The roles of environmental factors in regulation of oxidative stress in plant. Biomed Res. Int. 2019: 9732325. doi: 10.1155/2019/9732325

Yang, X., Hu, J., Wang, Z., et al. 2022. Pre-harvest nitrogen limitation and continuous lighting improve the quality and flavor of lettuce (*Lactuca sativa* L.) under hydroponic conditions in greenhouse. J. Agric. Food Chem. 71: 710–720. doi.org/10.1021/acs.jafc.2c07420

Zhang, X., Bian, Z., Li, S., Chen, X., Lu, C. 2019. Comparative analysis of phenolic compound profiles, antioxidant capacities, and expressions of phenolic biosynthesis-related genes in soybean microgreens grown under different light spectra. J. Agric. Food Chem. 67: 13577−13588. doi.org/10.1021/acs.jafc.9b05594

Zhang, X., Heuvelink, E., Melegkou, M., Yuan, X., Jiang, W., Marcelis, L.F.M. 2022. Effects of green light on elongation do not interact with far-red, unless the phytochrome photostationary state (PSS) changes in tomato. Biology 11: 151. doi.org/10.3390/biology11010151

Zheng, Y.J., Zhang, Y.T., Liu, H.C., Li, Y.M., Liu, Y.L., Hao, Y.W., Lei, B.F. 2018. Supplemental blue light increases growth and quality of greenhouse pak choi depending on cultivar and supplemental light intensity. J. Integr. Agric. 17: 2245–2256. doi.org/10.1016/S2095-3119(18)62064-7

Zhou, W.L., Liu, W.K., Yang, Q.C. 2012. Quality changes in hydroponic lettuce grown under pre-harvest short-duration continuous light of different intensities. J. Hortic. Sci. Biotechnol. 87: 429–434. doi.org/10.1080/14620316.2012.11512890

Zukauskas, A., Bliznikas, Z., Breivė, K., et al. 2011. Effect of supplementary pre-harvest LED lighting on the antioxidant properties of lettuce cultivars. Acta Hortic. 907: 87–90.

Zoratti, L., Karppinen, K., Escobar, A.L., Haggman, H., Jaakola, L. 2014. Light-controlled flavonoid biosynthesis in fruits. Front. Plant Sci. 5: 534. doi.org/10.3389/fpls.2014.00534