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FULL PAPER

Effect of LED lighting on phytochemical content of lettuce plants (green coral and red coral) grown in plant factory condition

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Abstract

Light is one of the most important environmental factors that affect plant growth. Light-emitting diodes (LEDs) are alternative lighting that has been shown to have the potential for plant growth and development under controlled conditions in plant factories. Plant factory production technology can increase the productivity and nutritional content of the plant. The phytochemical content of lettuce plants (Green Coral and Red Coral) grown under plant factory conditions with different spectrum LED lights (combination of red and blue LED and red, blue and green LED) were studied. The lettuce was harvested from day 20 until 35 after transplanting with an interval of five days. The result showed that the combination of red, blue and green LED lights is positive in terms of phytochemical content in both lettuce plants. Harvesting at days 30 to 35 after transplanting was effective in presenting a higher value in total chlorophyll, percentage of DPPH inhibition and reduction of total phenolic content in green Coral lettuce compared to early harvesting as it showed a highly significant difference. Besides, the chlorogenic acid content in green and red Coral presented significantly increased when harvesting was done on day 35. The combination of red, blue and green LED greatly affected chlorogenic acid content in both lettuces' plants. Therefore, the result of the combination of LED and harvesting day might provide new information to improve the nutritional quality of lettuce plants after harvesting.

1. Introduction

Lettuce (*Lactuca sativa*) belongs to the family Asteraceae. Lettuce leaves are widely used in salads, sandwiches, wraps, and similar food items. Since lettuce has high market demand, it encounters several potentials in its production and nutritional content. Lettuce is rich in minerals, vitamins, fibre and health-related secondary metabolites, such as flavonoids, lutein, and carotenoids (Romani *et al.,* 2002; Kimura *et al.,* 2003). Similar findings in Caldwell (2003) and Zhao *et al.* (2007) highlighted that the high antioxidant capacity of phenolics has shown that chlorogenic acid content and quercetin glycosides were predominant in lettuce which eventually enhanced the potential health benefits.

Increasing population size directly affects the demand for food resources. Furthermore, food supply shortages due to environmental factors such as climatic changes, limited cultivated lands and pest and disease outbreaks are forcing people to be involved in indoor and urban plant production (Yeh and Chung, 2009). With a demanding world of high plant quality, urban culture systems and vertical farming has become more valuable and economical in creating high productivity and efficiency of vegetables (Sabzalian *et al.,* 2014).

A plant factory is an idea for an indoor vertical farming system with artificial lighting for efficient quality of food production (Kozai *et al.,* 2019). This system can control all environmental factors and plant requirements such as light conditions, temperature, ventilation, relative humidity, carbon dioxide and fertilization (Kozai *et al.,* 2019). According to Zhong *et al.* (2015), the light conditions can be a critical environmental factor in regulating phytochemicals such as polyphenols in plants. Solid-state lighting technology, based on light-emitting diodes (LEDs), has great potential as a supplemental or sole-source lighting system for plant production in horticulture (Massa *et al.,*

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2008). Besides, LEDs may affect beneficial phytochemicals, such as vitamins, soluble sugars, soluble proteins and secondary antioxidants that may produce high concentrations in vegetables (Zhong *et al.,* 2015; Zhong *et al.,* 2018). A similar finding in Johkan *et al.* (2010) also claimed that light quality strongly influences plant development, which refers to the colour or wavelength reaching a plant's surface. They found that red and blue lights have the most significant impact on plant growth because they are the primary energy sources for photosynthetic CO_2 assimilation in plants. Combinations of red, blue and far-red LED light wavelengths are controlled environment efficiency for microgreen (Kopsell *et al.,* 2013), lettuce, spinach and komatsuna (Koneko-Ohashi *et al.,* 2007). Moreover, continuous red and blue LED light for 24 hours with green light exposure significantly decreases nitrate content and enhances lettuce quality; increases phenolic compound concentrations and enhances free-radical scavenging activity (Zhong *et al.,* 2018).

Basically, maturity selection will be taken into account to ensure the quality of the lettuce. The suitability of the maturity can be seen in terms of appearance, size, leaf colour, texture, durability and storage life. The optimum maturity of lettuce contributes as an important factor in producing a better product and meeting the consumer's demand for vegetables with high nutritional value. Conversely, there is little information about the scheduled timing of harvesting of leafy vegetables in a controlled environment. According to Couceiro *et al.* (2006), the concentrations of major bioactive compounds (hyperforin, pseudohypericin and hypericin) in the shoots of St. John's wort reached a peak on day 52 compared to harvesting on days 21, 42, 62, and 72. In addition, harvesting on days 35 to 40 after transplanting for Butterhead lettuce under a controlled environment showed good quality, freshness and are marketable until days 14-17 after storage at 4±1°C (Nur Syafini *et al*., 2020).

Therefore, the objectives were aimed to compare the effect of the different LED light spectrums besides determining the optimum harvesting time on the quality and accumulation of phytochemical content in green and red coral lettuce.

2. Materials and methods

Coral lettuces were grown under artificial light with a combination of light-emitting diode (LED) lighting; (A) red: blue and (B) red: blue: green with a photoperiod of 12/12 hrs of light/dark. Measurement of photosynthetically active radiation (PAR) for red: blue LED light was 318 $\mu mol/m^2/s$ and red: blue: green LED light (327 $\mu mol/m^2/s$). Lettuce was planted using a multilayer hydroponic system in a plant factory. The study was conducted in a controlled environment with a temperature of 28±1°C, air humidity of 65-75% and 550 ppm CO_2 concentration. Lettuce was harvested on different days after transplanting; (1) Day 20, (2) Day 25, (3) Day 30 and (4) Day 35. They were selected in uniform size and free of physical damage. Then, samples were immediately analysed to determine the changes in quality.

2.1 Determination of postharvest quality

The postharvest quality evaluation included chemical and phytochemicals (soluble solids concentration (SSC), ascorbic acid content**,** DPPH radical-scavenging activity, total phenolic content, total chlorophyll content and chlorogenic acid content) characteristics. The samples for these analyses were blended using a kitchen blender. SSC was determined with a digital refractometer (Model DBX-55, Atago Co., Ltd, Japan). The results were recorded in °Brix. The ascorbic acid was determined by extracting 10 g of blended sample in 100 mL of 3% metaphosphoric acid (HPO_3), and the extract was filtered through a Whatman No. 1 filter paper. A 10 mL volume from the filtered solution was determined volumetrically with the 2 – 6 dichlorophenol-indophenol (DCPIP) reagent until a slightly pink colouration was observed and persisted for 15 s (Ranganna 1977). The results were expressed in mg/100 g FW sample using the following equation: Titre (mL) × DCPIP standard factor × dilution (100/5) × 100%.

Antioxidant activity for lettuce was studied by evaluating the free radical scavenging effect on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (Yen and Hsieh, 1997). A 0.5 mL sample of the extract was added to 1 mL methanolic solution of DPPH radical (0.2 mM). The mixture was shaken vigorously and left for 30 min. The absorbance was then measured at 517 nm. The antioxidant activity was reported as the percentage of radical scavenging as follows: % radical scavenging = (1 -$A_{sample}/A_{control}$) x 100 where As_{ample} is the absorbance of the mixture of the sample extract and DPPH while $A_{control}$ is the absorbance of the mixture of DPPH and acidified methanol.

Total phenolic content was determined using the Folin-Ciocalteu method based on a colourimetric oxidation and reduction reaction with some modification (Sunita and Dhananjay, 2010). Samples of 0.3 mL were introduced into test tubes, followed by 1.5 mL of Folin-Ciocalteu's reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were vortexes; covered with parafilm and allowed to stand for 30 mins. Absorption at 765 nm was measured. Total phenolic contents were expressed in Gallic Acid

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Equivalents (mg per 100 g fresh weight).

The total chlorophyll content was determined by the extraction of 80% acetone with 5 g blended fresh samples. The absorbance of r supernatant was recorded at 664 and 647 nm using a microplate reader (Model Thermo Scientific Multiskan Go). Total chlorophyll content was calculated by Lichtenthaler's formula (1987). The determination of chlorogenic acid content was made using the method proposed by Llorach *et al.* (2004). The separation of the fraction containing phenolic compounds was conducted by the high-performance liquid chromatography (HPLC) method.

2.2 Statistical analysis

The experimental design was a completely randomized design with four replications. Statistical analysis was made using Statistical Analysis System (SAS) Version 9.4. The means were separated by Duncan Multiple Range Test ($p \leq 0.05$) to evaluate significant differences among the treatments.

3. Results and discussion

Harvesting day and LED lighting affected all the parameters except ascorbic acid content for both coral lettuce. Red: blue LED is not favourable for most of the parameters measured compared to red: blue: green LED. Supplementary green LED lighting affects the phytochemicals of green coral lettuce (Table 1). Red: blue: green LED enhanced the value of most of the parameters measured except for the ascorbic acid content which showed no significant difference for both LED treatments. Similarly, in Giedre *et al.* (2012), supplemental red LED lighting had no positive effect on ascorbic acid content within Romaine 'Thumper' and curly 'Multibaby' lettuce compared to natural solar radiation (as a control treatment).

In contrast, according to Bliznikas *et al.* (2012), there was a significant increase in vitamin C content in spinach, dill, mustard, rocket, and onion leaves under supplemental red-LED treatment. For green coral lettuce, it was observed that the highest chlorophyll content and total antioxidant activity was reached for harvesting on day 30 after being exposed to red: blue: green LED lighting (Table 1). Moreover, late harvesting, which was on day 35, showed a higher value in SSC, total phenolic content, and chlorogenic acid content than on other days. Therefore, harvesting green coral lettuce on day 35 accumulated more phenolic contents in comparison with red coral lettuce (Table 1 and Table 2). Green LED lighting resulted higher in total phenolic content, antioxidant activity, and chlorogenic acid content. A similar finding in Shimomura *et al.* (2020) also found increasing chlorogenic acid concentration in lettuce because of continuous lighting and elevated CO_2 observed under fluorescent light and blue LED. Besides, Li and Kubota (2009) reported that supplemental red light increased phenolic concentration in baby leaf lettuce. Wu and co-authors (2007) also noticed that the antioxidant capacity of pea seedlings after 96 hrs radiation by various LED lights was significantly enhanced by red light radiation. However, red: blue LED lighting had a lower impact on the phytochemical content accumulation in coral lettuce than red: blue: green LED (Table 1 and Table 2). Moreover, based on these results, there were interactions between harvesting

Table 1. The effect of different LED lighting and harvesting day on phytochemical content (soluble solids concentration (SSC), ascorbic acid content, total chlorophyll, total antioxidant activity, total phenolic content and chlorogenic acid content of Green Coral Lettuce.

Factors	SSC (% Brix)	Ascorbic Acid Content (mg/100 g FW)	Total Chlorophyll (mg/mL)	% DPPH Inhibition	Total phenolic GAE (mg/100 g FW)	Chlorogenic acid content (mg/mL)
LED Lighting (T)						
Red:Blue	4.71±0.38^{b}	3.76±0.51	207.35±18.82^{b}	44.29±14.89^{b}	0.033±0.003^{b}	0.139±0.02^{b}
Red:Blue:Green	4.64±0.64^{a}	3.75±0.49	222.38±11.76^{a}	50.07±13.47^{a}	0.035±0.003^{a}	0.165±0.005^{a}
F-Test significant	*	ns	*	*	*	**
Harvesting day (D)						
20	3.78±0.23^{c}	3.74±0.41	207.45±12.92^{bc}	29.54±5.93^{d}	0.030±0.002^{c}	0.132±0.009^{c}
25	4.22±0.33^{b}	4.04±0.45	219.48±10.37^{ab}	42.13±3.17^{c}	0.034±0.002^{b}	0.122±0.007^{c}
30	4.70±0.53^{a}	3.87±0.48	231.01±6.91^{a}	64.25±4.43^{a}	0.035±0.001^{ab}	0.158±0.01^{b}
35	4.92±0.35^{a}	3.34±0.41	201.50±20.33^{c}	52.80±8.40^{b}	0.036±0.002^{a}	0.197±0.05^{a}
F-Test significant	**	ns	*	**	**	**
Interaction T*D	*	ns	ns	ns	*	**

Values are presented as mean±standard deviation. Values with different superscript within the columns and main effect are significantly different by DMRT test at $p \leq 0.05$.

ns, non-signidicant, *significant at $p \leq 0.05$, **highly significant at $p \leq 0.05$.

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Table 2. The effect of different LED lighting and harvesting day on phytochemical content (soluble solids concentration (SSC), ascorbic acid content, total chlorophyll, total antioxidant activity, total phenolic content and chlorogenic acid content of Red Coral Lettuce.

Factors	SSC (% Brix)	Ascorbic Acid Content (mg/100 g FW)	Total Chlorophyll (mg/mL)	% DPPH Inhibition	Total phenolic GAE (mg/100 g FW)	Chlorogenic acid content (mg/mL)
LED Lighting (T)						
Red:Blue	4.87±0.68^{a}	3.21±0.30	288.00±32.02	75.99±4.72	0.057±0.008	0.259±0.11^{b}
Red:Blue:Green	4.52±0.26^{b}	3.23±0.33	292.38±32.72	77.31±4.00	0.059±0.006	0.327±0.19^{a}
F-Test significant	*	ns	ns	ns	ns	*
Harvesting day (D)						
20	4.68±0.21^{b}	3.16±0.36	278.87±23.19^{b}	82.35±1.45^{a}	0.051±0.003^{c}	0.136±0.004^{d}
25	4.30±0.61^{b}	3.34±0.35	293.15±15.15^{b}	75.68±1.85^{b}	0.054±0.005^{c}	0.205±0.01^{c}
30	4.58±0.29^{b}	3.11±0.21	332.71±9.86^{a}	75.25±1.06^{b}	0.067±0.001^{a}	0.342±0.02^{b}
35	5.20±0.56^{a}	3.28±0.31	256.04±6.06^{c}	73.33±4.10^{b}	0.060±0.005^{b}	0.490±0.014^{a}
F-Test significant	*	ns	**	**	**	**
Interaction T*D	ns	ns	ns	*	*	**

Values are presented as mean±standard deviation. Values with different superscript within the columns and main effect are significantly different by DMRT test at $p \leq 0.05$.
ns, non-signidicant, *significant at $p \leq 0.05$, **highly significant at $p \leq 0.05$.

day treatments and LED lighting on SSC, total phenolic content, and chlorogenic acid content for green coral lettuce (Table 1).

Early harvesting on day 20 also presented the highest value in SSC (4.70% Brix), whereas phytochemicals content; total chlorophyll, total antioxidant activity, and total phenolic and chlorogenic acid showed lower values compared to other days (Table 1). Besides, total antioxidant activity showed a higher value of 82.35% DPPH inhibition for red coral lettuce when harvesting on day 20. Total chlorophyll and total phenolic content for red coral lettuce on day 30 have the highest value; meanwhile, harvesting on day 35 showed the highest SSC and chlorogenic acid content (Table 2). Red leaf vegetables are known to be richer in total phenolic contents than green leafy vegetables because of the presence of anthocyanin (Llorach *et al.,* 2008). Kim *et al.* (2019) reported that the total phenolic content was highly accumulated in light intensity; photosynthetic photon flux density (PPFD) at 500 µmol/m/s, under which the colour of leaves was seen to be reddish. Perez-Lopez *et al.* (2018) also have reported that environmental stress such as high light intensity enhances phenolic contents and improves antioxidant capacity under ambient CO_2 conditions (400 µmol/mol).

In addition, chlorogenic acid content increased significantly from early harvesting to day 35 in both coral lettuce (Table 1 and Table 2). Red: blue: green LED lighting significantly enhanced chlorogenic acid content in red coral lettuce with 0.327 mg/mL compared to 0.259 mg/mL of chlorogenic acid content grown under red: blue LED lighting. Similarly, green coral lettuce showed a higher value in chlorogenic acid content when exposed to red: blue: green LED. On the contrary, according to Kim *et al.* (2019), anthocyanin contents, total phenolic contents, and free radical activity (DPPH) was decreased at 18 DAT of Indian lettuce ‘Sunhyang’ compared to harvesting at 12 DAT which resulted in the best for optimal growth, leaf colour, and functional components. Additionally, there was a significant interaction between harvesting day treatments and LED lighting on total antioxidant activity and total phenolic and chlorogenic acid content of red coral lettuce (Table 2).

4. Conclusion

Optimum harvesting at 30-35 days after transplanting and supplementary green LED light treatment were found to remarkably enhance phytochemicals content like antioxidant activity, total phenolic content and chlorogenic acid content of coral lettuce. Henceforward, a plant factory system fitted with artificial lighting is able to increase secondary metabolites that could become a useful technology for the production of highly valued fresh leafy vegetables.

Conflict of Interest

The authors declare no competing interests.

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