Effects of pre- and postharvest LEDs treatments on physio-biochemical properties of roselle (*Hibiscus sabdariffa* L.) microgreens: a preliminary study

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Abstract

Artificial light supplied by light-emitting diodes (LEDs) has been used to promote the bioactive compounds and antioxidants of horticultural crops. Therefore, this study aimed to investigate the effect of pre- and postharvest LED lights treatments on physio-biochemical properties of roselle microgreens (*Hibiscus sabdariffa* L.). The microgreens were grown under white fluorescent (control), blue, and blue+red LEDs (100 µmol m^{-2} s^{-1}). The harvested microgreens grown from white florescence were then exposed to blue and blue+red (100 µmol m^{-2} s^{-1}) for 8 h day^{-1} for 6 days at 10°C. The controls were kept in the dark. For preharvest treatments, controls showed the highest chlorophyll contents (0.3 g kg^{-1} FW). However, microgreens grown under blue and combination blue+red LEDs exhibited significantly higher ascorbic acid content (31-34%), DPPH radical scavenging activity (34%) and total phenolic content (21%) than control ones. For postharvest, continuous exposure to postharvest LEDs treatments maintained the chlorophyll content of the microgreens during storage. Additionally, microgreens illuminated with both LEDs treatments showed pronounced increment in ascorbic acid content (25-37% higher than controls). The microgreens illuminated with the combination blue+red LEDs (2.16 g kg^{-1} FW GAE) exhibited significantly higher DPPH radical scavenging activity than unilluminated microgreens (1.76 g kg^{-1} FW GAE), during 6 days of storage. In conclusion, pre- and postharvest LEDs treatments have potential to promote the phytonutrients in microgreens.

Keywords: antioxidants, light-emitting diode, miniature green salad, phytonutrients, storage

INTRODUCTION

Microgreens or often known as "vegetable confetti" are new class of specialty crop described as tender immature greens produced from seeds of vegetables, herbs or grains, having two fully developed cotyledon leaves with or without the emergence of a rudimentary pair of first true leaves (Kyriacou et al., 2016; Xiao et al., 2012). Popularity of the microgreens as functional foods has been accelerated worldwide ascribed to high content of micronutrients (Ca, Mg, Fe, Zn and Se), health promoting phytochemicals (polyphenols, β-carotene, α-tocopherol and glucosinolates) and lower nitrate content then their mature-leaf counterparts (Xiao et al., 2016).

Thailand which is home to an abundance of herbs and medicinal plants with some Thai herbs being major agricultural products used traditionally for medical treatment and as essential ingredients in several Thai dishes, cosmetics and animal feeds (Phianphak et al., 2007). Moreover, the demands of herbal and traditional products as supplementary foods accelerated with the expansion rate approximately 28% in 2004 and the market value increased markedly from 47 million USD in 2004 to 37 billion USD in 2016 (Phianphak et al., 2007; Sahawat, 2017). Therefore, the exploitation of these local herbs as microgreen would

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definitely add up to herbals market value not only as fresh consumption of main dishes but also as food ingredients to various recipes as well as food supplements. Roselle (*Hibiscus sabdariffa* L.) belongs to *Malvaceae* and is extensively cultivated locally. The herb extract also has been used in combatting high blood pressure, diabetes, cancer, cardiovascular diseases and bacterial infection (Martín et al., 2017; Sukkhaeng et al., 2018). Yet, there is still limited research available on microgreen production of these under-utilized Thai herb microgreens.

Light conditions are highly influential on the morpho-physiological of microgreens, and the biosynthesis and the accumulation of phytochemicals, especially in controlled growth environments (Delian et al., 2015). Advance light-emitting diode (LED) technology has become increasingly feasible for providing optimal management of light conditions: high photon flux (intensity) and spectral quality (wavelength) that elicit selective activation of photoreceptors and increase of phytochemical contents in vegetables, including microgreens (Bian et al., 2015; Kyriacou et al., 2016). Besides using light during plant growth to increase the secondary metabolites, the light treatment can also be used in the postharvest stage. Postharvest exposure to light is common in retail display and the usage of different colored lights have shown to improve the nutritional traits of postharvest produce such as flavor quality, enriched nutrition and shelf-life extension. Wilawan et al. (2019) and Lee et al. (2014), for example, used blue LED light to promote the biosynthesis and accumulation of phenolic compounds in okra and cabbage, respectively.

However, limited studies for postharvest LED lights exposure for local Thai's microgreens during low storage temperature in maintaining or enhancing the phytochemicals and antioxidants contents. Therefore, the aim of this study was to investigate the influence of pre- and postharvest LED treatments on physio-biochemical properties of roselle microgreens.

MATERIALS AND METHODS

Plant materials and treatments

Roselle (*Hibiscus sabdariffa* L.) seeds were procured from a local farm in Thailand. Then, the defect-free seeds were selected, weighed, and divided into three main groups. The seeds of each group were rinsed with running tap water to remove dirt and debris. The rinsed seeds were immersed in tap water at a ratio of 1:10 (*g*/*v*) for 5 h at 24±1°C prior to seed decontamination treatment using 5% hydrogen peroxide containing 0.005% Tween 20 for 15 min at 24±1°C in a continuous agitation.

The decontaminated seeds weighed at 5 g were put in a box measuring 6×6×9 cm with a moist sponge inside, and sprouted for 2 d in the dark. The seeds were then transferred to plant factory and exposed to white (as control), blue and combination of blue+red LED lights (18 W, Philips, Shanghai, China) for 5 d at 25±1°C and 70-80% RH. The light intensity was maintained at 100 mol m^{-2} s^{-1} for 12 h and carbon dioxide concentration was kept at 800±100 mg L^{-1} (CO_2 Meter Inc., Ormond Beach, FL, USA). The microgreens were manually sprayed with water twice daily (morning and evening), excess water was discarded before spraying, and there was no watering on the harvest day. The harvested microgreens were then used for postharvest LED treatments during shelf-life and also for physio-biochemical analysis.

For postharvest LED treatments, the microgreens from control group were harvested, weighed (20±1 g) and packed in clear transparent clamshell box and stored at 10±1°C and 70-80% RH. In the cold storage, the packed microgreens were exposed to blue and combination blue+red LED lights at 100 mol m^{-2} s^{-1} for 8 h for 6 d. The controls were kept in the dark.

Weight loss, color and total chlorophyll content

The microgreens were weighed before and after storage. The percentage of weight loss was calculated based on equation: %Weight loss = [(initial weight – final weight)/initial weight]×100. For color, the cotyledons surface color (L*, a* and b* values) was measured using a colorimeter (Chromameter Model CR-400, Minolta Corp.). The total chlorophyll content was determined using *N,N*-dimethylformamide (Moran, 1982). The freshly chopped cotyledons weighed 1 g were mixed with 20 mL of *N,N*- dimethylformamide and incubated in the dark for

24 h at 4°C. The chlorophyll content was measured by spectrophotometer and the result was expressed as fresh weight (FW) basis in g kg^{-1}.

Total antioxidant activity, flavonoid and phenolic contents

Cold extraction for total antioxidant activity (TAA) and total phenolic content (TPC) was performed by homogenizing 1.5 g of frozen cotyledons with 10 mL of 80% (v/v) ethanol. The homogenate was centrifuged at 10,000×*g* for 20 min at 4°C. The crude extract was used to estimate TAA and TPC. Measurement of TAA was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity according to Rajurkar and Hande (2011) with some modifications. The extract was mixed with 200 µL ethanolic DPPH (0.8 mM) and 80% (v/v) ethanol. The mixture was incubated for 30 min in the dark at room temperature. Absorbance was read at 515 nm and the antioxidant activity was expressed on FW basis as g kg^{-1} trolox equivalent (TE).

TPC was estimated according to the method of Toor and Savage (2005) with modifications. The crude extract was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of 7.5% (w/v) sodium carbonate. Absorbance was read at 765 nm after 1 h incubation in the dark at 30°C and the results were compared with the standard curve of gallic acid. The results were expressed on a FW basis as g kg^{-1} gallic acid equivalent (GAE).

For total flavonoid content (TFC), sample weighed 1 g was homogenized with 14.5 mL methanol. Then, the homogenate was incubated at 30°C for 1 h and centrifuged at 10,000×*g* for 20 min. The TFC was determined using the method of Lin and Tang (2007) and expressed on a FW basis as g kg^{-1} quercetin equivalent (QE).

Ascorbic acid content

The method of Roe et al. (1948) was modified to measure the total ascorbic acid content. Using 10 mL of cold, 5% metaphosphoric acid, a sample weighing 2 g was homogenized before being filtered using Whatman no. 1 filter paper. For the reaction to begin, 0.4 mL of the filtrate was combined with 0.2 mL of 0.02% di-indophenol and let to sit at room temperature for 3 min. After that, 0.4 mL of 2% thiourea and 0.2 mL of 1% dinitrophenol hydrazine were added to the mixture, and it was then incubated at 50°C for 1 h. The reaction was stopped by adding 85% sulfuric acid to the mixture. The result of the measurement of absorbance at 540 nm was expressed as FW basis of g kg^{-1}.

Statistical analysis

The experiments were arranged in a completely randomized design where all the data were presented as means ± standard error (SE) with three replications. The data were analyzed using analysis of variance (ANOVA). The obtained means were compared using least significant difference test (LSD) when F-test was significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Preharvest treatment

In this preliminary study, the roselle microgreens were illuminated with white LED as control, blue and combination blue+red LEDs lights during growth (preharvest). For color, we found that the combined blue+red LEDs resulted in lowest a* value blue light (Figure 1A). Despite that, during growth period for 5 d, the microgreens exhibited significantly lower total chlorophyll content when exposure to blue and combination blue+red LEDs than the ones with white LEDs (16% lower than control; Figure 1B). A study has reported that continuous exposure to white LED lighting increased greenness and total chlorophyll content of lettuce (Kasim and Kasim, 2017).

In contrast, the microgreens grown under blue and combination LEDs exhibited significant elevation in ascorbic acid content (Figure 2). Specifically, the microgreens showed 31 and 34% higher ascorbic acid content than control ones when exposed to blue and combination blue+red LEDs, respectively, during 5 d of growth. Similarly, the microgreens exposed to blue and blue+red recorded 34% higher TAA than control microgreens (Figure 2B).

Previous research has also reported that the combination blue+red LED lighting at 120 mol m^{-2} s^{-1} for 12 h significantly improved free radical scavenging activity in acyanic and cyanic basil (*Ocimum basilicum* L.) microgreens as compared to the ones grown under white LED light (Lobiuc et al., 2017).

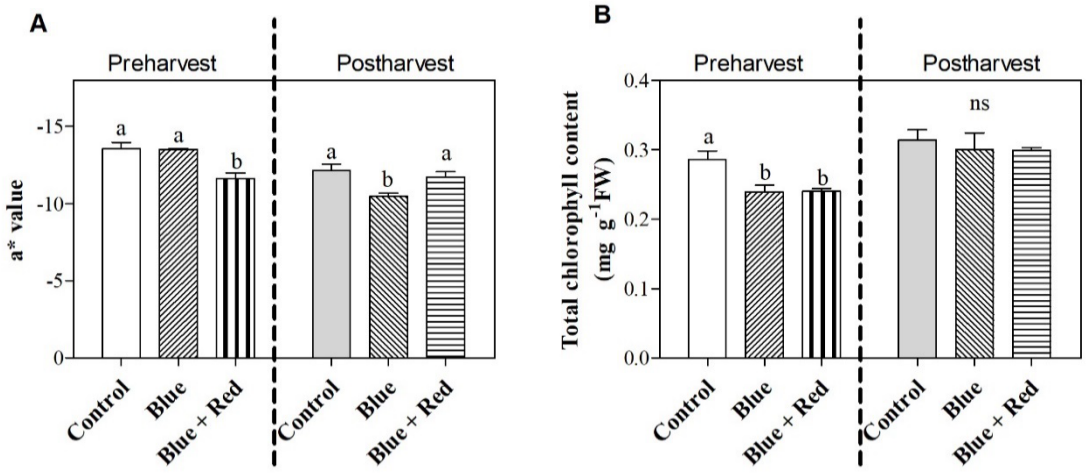

Figure 1. The effects of preharvest (5 days after growing) and postharvest (6 days after storage) LED light treatments on (A) a* value and (B) total chlorophyll content of roselle microgreens. Data are means of triplicates ± SE. Means within treatments (pre- or postharvest) with different letter are significantly different at P≤0.05 by LSD test. ns = not significantly different.

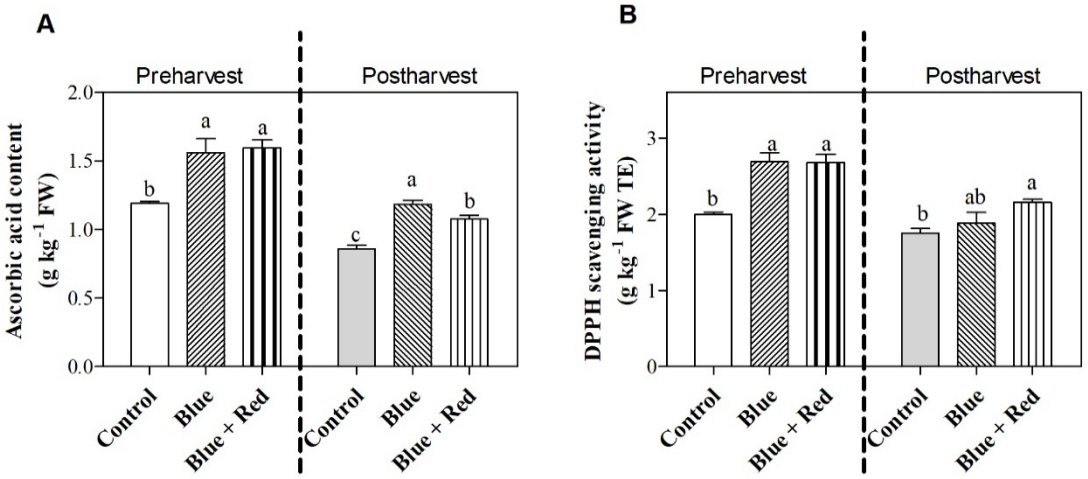

Figure 2. The effects of preharvest (5 days after growing) and postharvest (6 days after storage) on (A) ascorbic acid content and (B) DPPH radical scavenging activity of roselle microgreens. Data are means of triplicates ± SE. Means within treatment (pre- or postharvest) with different letter are significantly different at P≤0.05 by LSD test. ns = not significantly different.

Blue (470 nm) LED light at 30 mmol s^{-1} m^{-2} was effective in reducing the bitter-tasting, undesirable gluconapin content in shoots of seven-day old Chinese kale sprouts while enhancing the levels of total phenolics, anthocyanins and antioxidant capacity (Qian et al., 2016). In this study, the microgreen grown under blue and combination blue+red LEDs

registered about 21% higher TPC than control ones (Figure 3A). The combination blue+red LED lighting at 120 mol m^{-2} s^{-1} for 12 h significantly showed pronounced elevation of TPC in basil (*Ocimum basilicum* L.) microgreens, which recorded 2-fold higher content than the ones grown under white LED light (Lobiuc et al., 2017). From the results the increase of antioxidative compounds and antioxidant activity in microgreens might be due to theirs protect role as anti-stress agents.

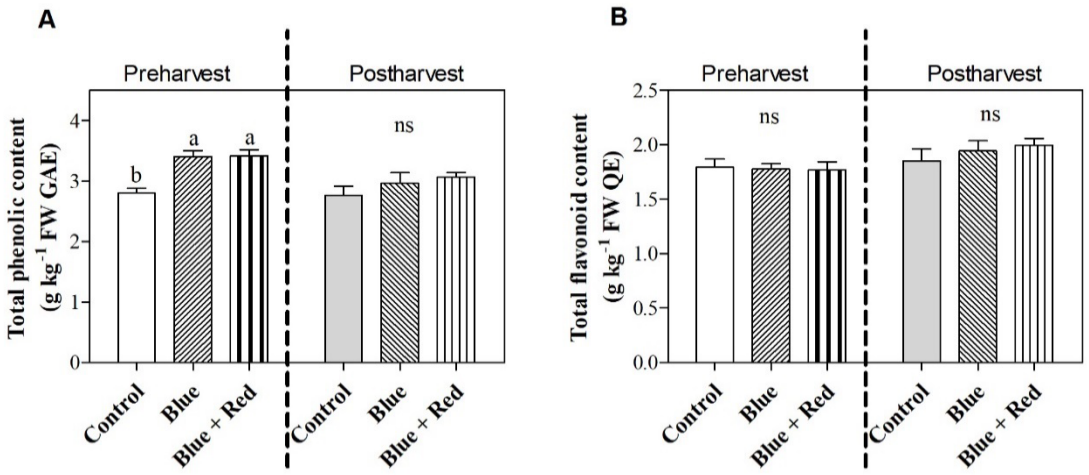

Figure 3. The effects of preharvest (5 days after growing) and postharvest (6 days after storage) on (A) total phenolic content and (B) total flavonoid content of roselle microgreens. Data are means of triplicates ± SE. Means within treatment (pre- or postharvest) with different letter are significantly different at P≤0.05 by LSD test. ns = not significantly different.

Postharvest treatment

Postharvest exposure to light is common in retail display of fresh horticultural products including microgreens, and has increasingly come under investigation as a storage application with respect to its effect on sensorial quality, phytonutrient composition and on shelf-life at large (Kyriacou et al., 2016). For postharvest treatment, microgreens from control group were continued to expose to blue and combination blue+red LEDs lights during storage at 10°C, while controls were kept in the dark. The weight loss of the stored microgreens for 6 d was determined. The results showed that the exposure to LEDs light treatments did not influence the fresh weight loss of roselle microgreens during storage (Figure 4). This finding is in contrast with the research on illumination of LED to stored broccoli which shown higher weight loss compared to control (Jin et al., 2015).

As for color, during storage, blue light affected the a* value. The color changes after 6 d storage were not apparent nonetheless regardless of the treatment applied (data not shown for L and b* values). During postharvest storage, the illumination of both blue and combined blue+red did not affect total chlorophyll content of the stored roselle microgreens at 10°C as compared to control.

On the other hand, the continuous illumination with the blue and blue+red LED lights particularly resulted in 25-37% higher ascorbic content in roselle microgreens during storage at 10°C (Figure 2A). Likewise, the microgreens illuminated with combination blue+red showed 23% higher TAA than unilluminated microgreens (Figure 2B). Enhancement of ascorbic acid levels in radish microgreens by postharvest light exposure has been interpreted as derivative of ongoing photosynthetic activity and increase in the availability of soluble carbohydrates, especially of D-glucose which serves as a precursor for ascorbate synthesis (Glowacz et al., 2015; Kyriacou et al., 2016). Similar increase in ascorbate levels has been reported for fresh packaged spinach leaves under simulated retail conditions of continuous

low intensity fluorescent light, suggesting that this effect is independent of leaf maturity (Lester et al., 2010). Nonetheless, the LED lights did not affect the TPC and TFC of the stored roselle microgreens after 6 d storage at 10°C.

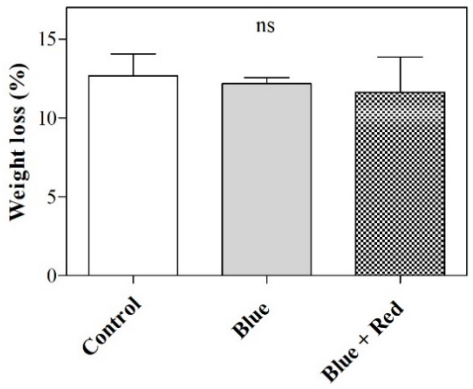

Figure 4. The effects of postharvest LED light treatments on weight loss of roselle microgreens stored for six days at 10±1°C. Data are means of triplicates ± SE. ns = not significantly different at P≤0.05 by LSD test.

CONCLUSIONS

In conclusion, the preliminary study results showed that illumination of blue and combined blue+red LED light (100 µmol m^{-2} s^{-1}) during growth for 5 d enhanced ascorbic acid content, TPC and total antioxidant activity (DPPH radical scavenging activity). For postharvest treatment, the LEDs light exposure to the harvested roselle microgreens during storage in 10°C for 8 h daily for 6 d significantly preserving the ascorbic acid content and total antioxidant activity (DPPH• scavenging activity). However, there is no changes observed for TPC and total flavonoid content in the treated samples compared to control. Another positive result is that the illumination with blue and combined blue+red LED light did not affect the fresh weight loss of the stored microgreens compared to control. Further investigation needs to carry out to improve the phytochemicals of microgreens such as comparing with fluorescence light, red and other LED lights. Additionally, more parameters to be analyzed for in-depth study of the effect of these LED lights on growth and functional properties of the microgreens.

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