IMPROVING QUALITY OF BUTTERHEAD LETTUCE BY MANIPULATION OF LIGHT-EMITTING DIODES (LEDS) APPLICATION IN PLANT FACTORY

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ABSTRACT

Nowadays, crop production is increasingly threatened by unusual weather, water shortages, management challenge in pest and diseases and insufficient available land. Due to limited natural resources and for food security, plant factory is an idea for indoor vertical farming system with artificial lighting for efficient quality for food production. In plant factory, optimal control system to obtain higher yield and better quality of plant is essential. The control system in plant factory included light condition, temperature and relative humidity. Light condition (light quality, light intensity and photoperiod) is one of the most important environmental factor in regulating vegetable growth, development and phytochemical accumulation, particularly for selected vegetables produced in controlled environments. Studies on postharvest quality maintenance of lettuce var. Butterhead was carried out on different maturity harvesting and different timing of LED light application. Result showed that the harvesting time for lettuce is at day 35 to 40 after transplanting. The quality appearance showed a good quality, leaf is fresh and still marketable until day 14-17 after storage at 4°C. Besides that, study on time exposure to LED light was significantly ($p<0.05$) affect the physico-chemical content of lettuce after harvest. Lettuce exposed for 6 hours before harvesting were showed high optimum physico-chemical content such as ascorbic acids, total phenolic compound and total chlorophyll content. Additionally, it also higher in antioxidant activity after exposed to 12 hours LED light. Therefore, maturity harvesting, quality of storage life and exposure time of LED lighting have been identified to determine the optimum harvesting time to make them high nutritious and health benefit.

Key words: antioxidant activity, LED light, plant factory, total chlorophyll, total phenolic compound

INTRODUCTION

Lettuce is one of the world’s most popular leafy green vegetables. Since lettuce has high market demand, it encounters several potential in their production and nutritional content. Lettuce and other leafy greens are generally characterized as very perishable commodities, with high respiration and water loss rates (Cantwell and Kasmire, 2002). Leafy vegetables obtained from leaves reach their best quality attributes at various stages of growth and development of the leaves (Gil et. al, 2012). According to Shewfelt (2009), maturity at harvest is one of the main factors determining quality and the rate of quality changes during postharvest handling and shelf life. Consequently, it is recommended to harvest leafy vegetables at optimal maturity stage, not only because of the economic benefits for producers but the physiological response of plants during refrigerated storage allows optimal quality maintenance with respect to other plants harvested earlier or later than the optimal maturity stage (Barg et. al, 2008). Maturity at harvest can significantly impact product composition and the nutritive value of the crop. For example, small cabbage heads were found to be higher in ascorbic acid concentration than large heads (Weston and Barth, 1997). Harvest maturity indicators have been set to describe the right time to harvest for better quality and shelf life. In addition, lettuce was grown under control condition and might provide new technology to improve the nutritional quality of lettuces. In plant factory, optimal control system to obtain higher yield and better quality of plant is essential. The control system in plant factory included light condition, temperature and relative humidity. According to Zhong et. al (2014), light condition (light quality, light intensity and photoperiod) is one of the most important environmental variables in regulating vegetable growth, development and physiochemical accumulation, produced in controlled environments. Besides that, the effect of LEDs can produce high concentrations of beneficial phytochemicals, such as vitamins, soluble sugars, soluble proteins and secondary antioxidants (Zhong et al., 2015; 2018). Therefore, in this study, the exposure time of LED lighting have been identified to determine the optimum harvesting time of Butterhead lettuce that produce high nutritious and health benefit.

MATERIALS AND METHODS

Determination of maturity harvesting

Butterhead lettuces were grown under daylight using red: blue light-emitting diode (LED) lighting; with 12-h light exposure. Lettuce was planted using multilayer hydroponic system in plant factory. The environment in the plant factory was set up as follow; temperature (28^{o}C), humidity (65-75%), CO_2 (400-500 ppm) and air flow (2-5 m/s). Lettuce was harvested with different day after transplanting; (1) Day 25, (2) Day 30, (3) Day 35, (4) Day 40 and compared with conventional sample (5). They were selected in uniform size and free of physical damage. Then, samples were packed in PVC clamshell container. The samples were stored at 4°C for 21 days. The quality evaluations were carried out every 3-4 days basis. Postharvest quality evaluation included physical (appearance and leaf colour) and chemical (soluble solids content (SSC), total titratable acidity (TTA), sugar/acid ratio, ascorbic acid content (AAC) and total chlorophyll content) characteristics.

Determination of optimum time exposure time to LED light

Lettuce was harvested at day 30 and 45 after transplanting. Lettuces were exposed to different time exposure of LED light namely 0 (control), 2, 4, 6, 8, 10 and 12-h using red: blue LEDs before harvested. Then, samples were immediately analyzed to determine the changes in quality. Postharvest quality evaluation included physical (colour) and chemical (SSC, TTA, sugar/acid ratio, ascorbic acid content**,** DPPH radical-scavenging activity, total phenol content and total chlorophyll content) characteristics. The postharvest quality of lettuce was judged visually and the criteria used were retention of original colour, freshness and severity. The colour of leaf was measured using a chromameter (Model CR-400 Minolta, Japan). Each colour value of lightness (L*), chroma (C*), and hue angle (h°) was expressed as the means of three measurements. Soluble solids content (SSC) was determined with a digital refractometer (Model DBX-55, Atago Co., Ltd, Japan). Titratable acidity (TTA) was determined by titrating 20 ml of extraction with 0.1 mol l^{-1}NaOH to pH 8.2 (Shaw et al. 1987). Ascorbic acid content was determined by extraction of 10 g of sample with the addition of 100 ml of 3% metaphosphoric acid. Then, 10 ml of extraction was titrated immediately with a standard dye solution to first permanent pink endpoint. Antioxidant activity for lettuce was studied through the evaluation of 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, $A_{control}$ is the absorbance of the mixture of DPPH and acidified methanol. Total phenolic content were determined using the Folin-Ciocalteu method and gallic acid was used as standard (Sunita and Dhananjay, 2010). The total chlorophyll content was determined by extraction of 80 % acetone with 5 g blended fresh samples. The absorbance of resulting supernatant was recorded at 664 and 647 nm using ELISA microplate reader.

Statistical Analysis

The experimental design was a completely randomized design with four replications. The obtained data was analyzed using analysis of variance (ANOVA) and mean comparison was conducted on the data collected using the Statistical Analysis System Version 9.4. The means was separated by Duncan Multiple Range at the 5% level of significant treatment effects within the analysis of variance. Unless otherwise specified, all significant differences in this paper were $p \leq 0.05$.

RESULTS AND DISCUSSION

Determination of maturity harvesting

During storage, Butterhead lettuce harvested at day 35 to 40 showed a better quality compared to other harvesting days, characterized by a good appearance as well as no or less symptom of brown stain at the cut surface. However, comparing between both harvesting days; 40 days was appeared to be the optimum one to maintain the freshness of lettuce. Harvesting at 40 days after planting enables the lettuce to be stored for up to 14 days at 4°C compared to only 7 days for lettuce harvested at day 25. Harvesting at day 35 could also prolong the storage life, but the quality dropped slightly with brown stain, though it was still marketable. According to Olfati et al. (2011), delaying harvest when the lettuce reaches its maximum yield decreases their quality. Similar finding in Kang et al. (2008), the decay rate was higher in immature lettuce than mature and over-matured ones. The browning rate was higher in over-matured lettuce than immature and matured ones.

Harvesting day significantly affected the colour parameters. Conventional sample showed light green with more saturated (higher L* and C*) and less green (hue value) compared to Butterhead plant factory harvested at day 30-40 that showed bright green colour (lower L* and higher hue value) (Table 1). The day 40-harvested lettuce had higher SSC value, TTA and sugar/acid ratio and gave significant difference among harvesting day compared to conventional samples that showed lower values (Table 1). According to Table 1, ascorbic acid content (AAC) was significantly high among the treatments. Lettuce harvested at day 40 showed high value of AAC (6.29 mg/100g), followed by day 35 (5.34 mg/100g), day 30 (5.20 mg/100g) and day 25 (3.81 mg/100g). According to Giedre et, al. (2011) there were significant increase of vitamin C after LED treatment was observed in red leaf lettuce 'Multired 4' (63.3%) after exposed to red 638-nm LEDs within a 16-h photoperiod for three days. Li and Kubota (2009) showed that nutritional value of baby leaf lettuce could be enhanced by supplemental light quality, with supplemental red light phenolic concentration increased by 6%. All extracts were evaluated for their total chlorophyll content is shown in Table 1. As observed, lettuce harvested at day 35 displayed the highest total chlorophyll content (313.93 mg/ml, respectively). There were significant differences in total chlorophyll content among harvesting days. Beside that, late harvesting of Butterhead lettuce grown in plant factory and conventional sample influenced significant lower of total chlorophyll content after harvested.

Table 1: The effect of different harvesting day on physio-chemical properties (leaf colour, soluble solids content (SSC), pH, total titratable acidity (TTA), sugar acid ratio (SCC/TTS), ascorbic acid content and total chlorophyll) of lettuce var. Butterhead.

Factor (Harvesting Day)	**Leaf Colour**			**SSC (%)**	**TTA (% citric acid)**	**SSC/TTA ratio**	**Ascorbic Acid Content (mg/100g)**	**Total Chloro phyll (mg/ml)**
	L*	**C***	**h°**					
T1 – D30	48.34d	37.72b	123.67a	3.10d	0.09c	32.85bc	3.81b	266.58b
T2 – D35	49.46cd	38.78bc	122.31b	4.40c	0.12b	37.55ab	5.20ab	280.45b
T3 – D40	51.59b	33.02c	122.36b	5.15b	0.14a	37.27ab	5.34ab	313.93a
T4 – D45	50.35bc	32.53c	122.71ab	6.05a	0.15a	39.75a	6.29a	202.37c
T5 – D45 (Conventional)	56.20a	44.46a	119.35c	3.5d	0.12b	29.37c	4.17b	110.01d
F-Test significant	**	**	**	**	**	*	*	**

Determination of optimum time exposure time to LED light

According these result showed that, physico-chemical quality of Butterhead were greatly affected by the light quality and duration harvesting. 4 to 6 hours exposed to LED lights before harvesting gave optimum quality for phytochemical content; antioxidant capacity and total chlorophyll content. For leaf colour of Butterhead lettuce did not significantly affected among the treatments except value of lightness (L*), however during harvesting day at 35 and 40 were highly significant in L* value, respectively (Table 2). Percentage of SSC was significantly increased among all the treatments; harvesting day and time exposure (Table 2). The TTA, sugar/acid ratio, ascorbic acid content and total chlorophyll were found no significant differences among different harvesting day (Table 2). Besides that, there were significant difference in time exposure of LEDs after harvesting. Analyzing ascorbic acid content in 4 to 6 hours exposed showed higher value 5.44-5.69 mg/100g. However, Giedre at. al, (2012) found that supplemental short-term red LED lighting had no positive effect (decrease 64.3%) on ascorbic acid content within in Romaine 'Thumper' and curly 'Multibaby' lettuce compare to natural solar radiation (as a control). All extracts were evaluated for their DPPH-scavenging ability and total phenolic content is shown in Table 2. As observed, harvesting day at 35 to 40 and followed by time exposed to LED lights showed significantly increased in their DPPH-scavenging ability and total phenolic content after harvested. As reported by Giedre and co-workers (2012), we found that similar supplemental short-term of red LED lighting can produce baby leaf lettuce Romaine 'Thumper' and curly 'Multibaby' rich in total phenolic (28.5%) and antioxidant capacity (14.5%) compared to natural solar radiation. According to Spinardi et. al (2010), phenolic compound in vegetable have potential benefit on human health. Thus, time exposure of LED light can promote high phenolic compound in lettuce. Besides that, Table 2 shows time exposed during 4 to 6 hours of LED light influenced significant increase of total chlorophyll in lettuce after harvested.

CONCLUSION

Harvesting at 35 – 40 days after transplanting is the optimum harvesting day which could maintain quality and prolong the storage life of Butterhead lettuce. Henceforward, the combination of exposure time to LEDs and optimum harvesting resulted in many positive effects on appearance, promotes high nutritional value and edible quality of plant factory product.

Table 2: The effect of different time exposure of LED light on physiochemical properties (leaf colour, soluble solids content (SSC), pH, total titratable acidity (TTA), SCC/TTS, ascorbic acid content, percentage of DPPH inhibition, reduction of total phenol content (GAE) and total chlorophyll) in lettuce var. Butterhead after harvested.

Factors	**Leaf Colour**			**SSC (°BRIX)**	**TTA (% citric acid)**	**SSC/TTA ratio**	**Ascorbic Acid Content (mg/100g)**	**% DPPH inhibition**	**GAE (mg/100 g fresh fruit)**	**Total Chlorophyll (mg/ml)**
	L*	**C***	**h°**							
Harvesting day (D)										
T1 – Day 35	48.26b	34.07	122.90a	4.08b	0.12	36.24	5.01	71.20b	0.0450a	277.11
T2 – Day 40	50.59a	35.01	121.82b	4.55a	0.13	34.31	5.47	78.24a	0.040b	277.46
F-Test significant	**	NS	*	*	NS	NS	NS	*	*	NS
Hour (H)										
T1 – 0	48.42cde	35.44	122.48	3.47c	0.11c	32.82bc	5.38abc	69.21c	0.040bc	252.69c
T2 – 2	47.90de	35.99	121.86	3.52c	0.12bc	30.93c	6.02 a	70.03bc	0.039c	254.98c
T3 – 4	47.67e	33.47	122.92	4.23b	0.12bc	34.95abc	5.69ab	77.48a	0.044ab	300.77 a
T4 – 6	49.95bc	34.44	122.44	4.83a	0.13ab	37.72ab	5.44abc	76.64ab	0.042abc	299.02ab
T5 – 8	50.52ab	33.58	122.17	4.60ab	0.13ab	37.72ab	4.75bc	79.50a	0.043abc	268.47bc
T6 – 10	51.97a	34.69	121.99	4.83a	0.14a	34.58abc	4.39c	75.47abc	0.046a	269.83abc
T7 – 12	49.52bcd	34.18	122.68	4.83a	0.12bc	38.18a	5.02abc	74.73abc	0.045ab	295.2ab
F-Test significant	**	NS	NS	**	*	*	*	*	*	*
Interaction (DxH)	*	NS	NS	*	NS	*	*	**	NS	NS

Means separation within columns and main effect by DMRT test at p≤0.05.
L*= lightness, C*= chroma and h° = hue angle
NS, *, ** Non significant or significant or highly significant at p≤0.05, respectively

REFERENCE

Barg, M., Aguero, M.V., Yommi, A. and Roura, S.I. 2008. Evolution of plant water status indices during butterhead lettuce growth and its impact on post storage quality. Journal of the Science and Food of Agriculture 89:422–429.

Cantwell, M.I. and Kasmire, R.F. 2002. Postharvest handling systems: fruit vegetables. In: Postharvest technology of horticultural crops, Edited by Kader AA. University of California, Agriculture and Natural Resources, Publication 3311; pp. 407–421.

Giedre, S., Ausra, B., Ramunas, S., Algirdas, N. and Pavelas, D. 2011. Supplementary red-LED lighting affects phytochemicals and nitrate of baby leaf lettuce. Journal of Food, Agriculture and Environment, Vol.9 (3&4): 271-274.

Giedre, S., Ramunas, S., Ausra, B., Akvile, V. and Pavelas, D. (2012). Supplementary red-LED lighting and the changes in phytochemical content of two baby leaf lettuce varieties during three seasons. Journal of Food, Agriculture and Environment Vol.10 (3&4): 701-706.

Gil, M.I., Tudela, J.A., Martinez-Sanchez, A. and MC Luna, M.C. 2012. Harvest maturity indicators of leafy vegetables. Stewart Postharvest Review 1:1.Kader, A.A. 2002. Quality and safety factors: Definition and evaluation of fresh horticultural crops. In: Postharvest technology of horticultural crops, Edited by Kader AA. University of California, Agriculture and Natural Resources, Publication 3311; pp. 279–285.

Kang, Y.J., Choi, J.H., Jeong, M.C. and Kim, D. M. 2008. Effect of maturity at harvest on the quality of head lettuce during storage. Korean Journal of Horticultural Science and Technology 26:272–276.

Li, Q. and Kubota, C. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Journal of environmental and experimental botany 67: 59–64.Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K. and Kurata, K. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environmental Control in Biology 45(3):189–198.

Olfati, J.A., Saadatian. M., Peyvast, G., Malakouti, S.H., Kiani, A. and Poor- Abdollah, M. 2011. Effect of harvesting date on yield and quality of lettuce. Advances in Environmental Biology 5:1647–1650.

Shaw, D.V., Bringhurst, R.S. and Voth, V. (1987). Genetic variation for quality traits in an advanced-cycle breeding population of strawberries. J. Amer. Soc. Hort. Sci. 112(4): 699 – 702

Shewfelt, R.L. 2009. Measuring quality and maturity. Chapter 17 In: Postharvest handling (Second edition): A systems approach, Edited by Florkowski WJ, Shewfelt RL, Brueckner B and Prussia SE: 461–477.

Spinardi, A., Cocetta, G., Baldassarre, V., Ferrante, A. and Mignani, I. (2010). Quality Changes during Storage of Spinach and Lettuce Baby Leaf. Acta Hort. 877: 571-576.

Sunita, M. and Dhananjay, S. (2010) Quantitative analysis of total phenolic content in Adhatoda vasica Nees Extracts .Int.J. PharmTech Res,2 (4).

Weston, L.A. and Barth, M. M. 1997. Preharvest factors affecting postharvest quality of vegetables. HortScience 32:812–816.

Yen, G.C. and Hsieh, G.L. 1997. Antioxidant effects on dopamine and related compounds. Biosci. Biotech. Biochem. 61:1646-1649.

Zhong, H.B., Qi, C.Y. and Wen, K.L. 2014. Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. Journal of the Science of Food and Agriculture. 95(5): 869–877.

Zhong, H.B., Qi, C.Y. and Wen, K.L. 2015. Effect of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. Journal of Science Food Agriculture 95: 869-877.

Zhong, H.B., Ruifeng, C. Yu, W., Qichang, Y. and Chungui L. 2018. Effect of green light on nitrate reduction and edible quality of hydroponically grown lettuce (Lactuce sativa L.) under short-term continuous light from red and blue light–emitting diodes. Journal of environmental and experimental botany 153: 63-71.