The Effect of LED light combination on the anthocyanin expression of lettuce

Gyeong Y. Baek*, Myeong H. Kim*, Chi H. Kim*, Eun G. Choi*, Byeong O. Jin*, Jung E. Son, Hyeon T. Kim****

** Department of Bioindustrial Machinery Engineering Gyeongsang National University (Institute of Agriculture and Life Science), Jinju, Korea, (Tel: +82-70-4320-3636; e-mail: Qorruddbs@nate.com).*
*** Depatment of Plant Science (Institute of Agriculture Sciences and development Seoul national), Seoul, Korea.*
**** Department of Bioindustrial Machinery Engineering Gyeongsang National University (Institute of Agriculture and Life Science), Jinju, Korea, (e-mail: bioani@gnu.ac.kr).*

Abstract: This study conducted twice the growing of lettuce in DFT system and aeroponics method with different light combinations.

(1) Deep flow technique system(DFT) : The lettuce was grown in DFT system and taken image. Then image analysis and absorbance was analyzed to know the correlation.

(2) Aeroponics system : From the image of grown lettuce and HPLC, the contents of the major functional elements of anthocyanin - cyanidin-3-glucoside(C3G), peonidin-3-glucoside(P3G), and delphinidin-3-glucoside(D3G) – were measured. As a result, it turned out that in the light combination of red 53: blue 47, red 58: blue 42, the content of D3G was the highest.

Through the twice experiments, the content of anthocyanin may be estimated based on the imaging data to some extent. This study shows that blue light has significant effects on the development of anthocyanin.

*Keywords***:**. Anthocyanin, Image processing, LED light, Lettuce

1. INTRODUCTION

As climate changes and natural disasters have resulted in food shortages around the world, a number of researches have been conducted to search for a solution to solve this problem (Lee, 2003).

A plant factory is one of the methods to solve the problems stated below. A number of researches on this, here in Korea and abroad, have been conducted(Bourget, C. M. 2008), and the studies being domestically conducted include the development of nutriculture technology, automated systems, improvement of growth and functionality of crops in utilization of light sources, etc(Seo, 2009). Warrington and Mitchell (1976) was to investigate the growth of sorghum under blue and red light, the blue light as compared to the red light in the building of leaf length is approximately twice as high and a 46% increase in the leaf area was reported. Also ~~is~~ considered a major stress as a cause of the growth environment light environments (Perez-Balibrea, etc., 2008). Functional substances in plants that contain Phyto Chemicals due to stress generated in the course of the defense mechanisms, as the chemicals in the plant itself (Oh et al, 2009). Functional elements include anti-oxidant substances such as anthocyanin and vitamins. Leaf vegetables as well contain functional substances (Hogewoning et al., 2007). Anthocyanin, an antioxidant substance, goes through the coloration as chlorophyll is destroyed in fallen leaves. An active study on this in the food industry has been conducted for anti-aging and eye sight enhancement (Baroli et al, 2008, Swatz et al., 2001). But contents determination of anthocyanins is time-consuming and uncomfortable. So anthocyanins would be to estimate through the image processing.

In this study, lettuce, which is consumed more than any other leaf vegetables ~~,~~ domestically (Jang et al, 2007) and has the shortage growth span, is grown in a deep flow technique (DFT) system and aeroponics system with different light combinations in a plant factory. Anthocyanins estimates through image analysis were the same in the experiment of DFT and aeroponics. Anthocyanin of the grown lettuce in DFT system estimate total anthocyanin content by absorbance spectrometry. The anthocyanin of the grown lettuce is measured by means of the imaging method, and then the major elements of anthocyanin - cyanidin-3-glucoside(C3G), peonidin-3-glucoside(P3G), and delphinidin-3-glucoside(D3G) – were analyzed by means of HPLC in order to verify the correlation between the effect of light combination on the contents of anthocyanin and the figures from the imaging analysis and chemical analysis.

2. MATERIALS AND METHODS

2.1 Lettuce growth

The lettuce(seedlings: hongyeomjeokchukmyeon and aram seeds) was grown in area of the plant factory 4,420 mm × 3,060 mm × 2,170 mm as (Fig. 1). For LED light combination used only blue (445nm) and red (665nm) lights source in the experiment as shown in Table 1. Experiments were conducted a total of 2 times, the first experiment was

with using the ~~a~~ DFT system, the second experiment was with using the aeroponics system .

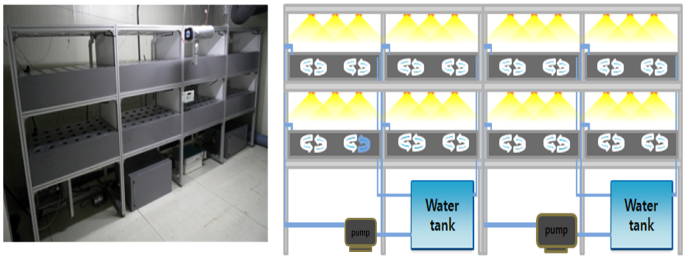

Fig.1 Inside and schematic of plant factory

Table 1. Light intensity and light combination of experiment

Tests	1	2	3	4	5	6	7	8
Light (665:445)	R : B	R : B	R : B	R : B	R : B	R : B	R : B	R : B
Light rate	53 : 47	89 : 11	84 : 16	78 : 22	58 : 42	76 : 24	88 : 12	69 : 31
Intensity (μmol/㎡·s)	94:82	162:21	107:21	142:39	107:77	123:39	145:20	125:56
	140 - 172	165 - 184	150 - 162	160 - 180	164 - 184	152 - 166	150 - 167	163 - 182

2.1.1 Deep flow technique system(DFT)

The first experiment was conducted from 2012.03.19 to 2012.04.12 with the DFT system to harvest time (about 26days) in the plant factory. The indoor air temperature was 20-25□, the water temperature was 18-22□, and the contents of CO2 were 400ppm respectively.

2.1.2 Aeroponics system

The second experiment was conducted using the aeroponic system from 2012.09.01 to 2012.10.08 for 38days. Growth of environmental conditions was the same as the NFT system, but the nutrients used was different. The aeroponics system was adopted with the nutrition supplied at the rate of 20s/min and stopped at the rate of 40s/min. The pump capacity (KOTEC R-1305) was 2-2.51 L/min. The EC was 2.1-2.15 and the pH was 5.5-6 respectively according to the standard table for Sonneveld lettuce nutriculture.

2. 2 Image processing

The first and second experiments were to conduct all the same imaging devices as (Fig. 2). CCD(POINTGRAY) color cameras were utilized to take pictures of the grown lettuce, and the coloration in images was analyzed by means of Microsoft visual c++(ver6.0).

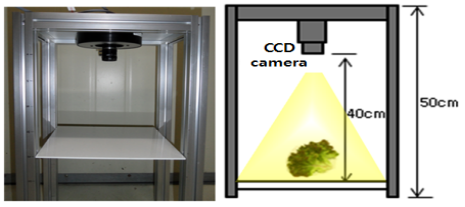

Fig. 2 Image device for taking the lettuce

2. 3 Anthocyanin contents analysis

Lettuce grown in the DFT system in order to obtain total anthocyanin absorbance analysis was conducted. In the second experiment (aeroponic system), grown lettuce was conducted ingredient contents by using High-performance liquid chromatography analysis (HPLC).

2. 3. 1 Absorbance analysis

Absorbing strength of the Lettuce grown in the experiment was analyzed. First, 20g of Lettuce was incased in 95% ethanol 40ml and was extracted by using sonication. The extracted material was concentrated and frozen by using freeze dryer in -80℃ for six hours. Next frozen samples dried in -50℃. The pH-differential method is based on coloured oxonium predomination (0.025 M potassium chloride buffer, pH 1) versus colourless hemiketal (0.4 M sodium acetate buffer, pH 4.5) reaction. The absorption values of the extracts were measured at 510 and 700 nm wavelengths. Then total anthoayanin was expressed by equation (1) Monomeric anthocyanin's calculation.

$$\text{Monomeric anthocyanin (mg/L)} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)} \quad (1)$$

$$A = (A_{\lambda\ vis\text{-}max} - A_{700\ nm})_{pH1.0} - (A_{\lambda\ vis\text{-}max} - A_{700\ nm})_{pH4.5}$$

$A_{\lambda\ vis\text{-}max}$: Absorbance at maximum(510nm)

$A_{700\ nm}$: Absorbance at 700nm

MW : 449.2(Molecular weight of cyanidin-3-3glucoside)

DF : 50 (Dilution ratio)2ml/40mg

ε : 26.9 (Molar extinction coefficient)

2. 3. 2 High-performance liquid chromatography analysis

The leaves and roots of the grown lettuce were separated for anthocyanin analysis, and the leaves were dried for three days at 40℃ by means of a dry oven. The dried lettuce was mixed with 5ml/0.1g of 99%MeOH and 1%HCL, extracted for 24 hours three times for three days in a cold and dark room, and left there. From the extract, three major elements of anthocyanin - cyanidin (C3G), peonidin(P3G), and delphinidin(D3G) – were measured by means of LC-20A(SHINADZU). As for the measuring condition of HPLC (ELSD model200), the mixing ratio was as follows: Stationary phase Ultra C18 5um 250*4.6mm, and Mobile phase DW(75%) : MeOH(20%) : formic acid(5%). The flow rate was 0.700 ml/min at 30℃ with the Detector set at as much as 530nm.

2. 4 Statistical analysis

According to the second experiment, the images of the grown lettuce and anthocyanin content analysis were conducted through the Bivariate correlation analysis by means of SPSS(ver6.0).

3. RESULTS AND DISCUSSION

3. 1 Image processing analysis

3. 1. 1 Deep flow technique system(DFT)

One lettuce per tests was selected and the largest leaf was then separated. The leaf taken CCD camera was showed the pixels by using microsoft visual c++. The number of pixels and coloring ratio is shown in table 2. (58.42) test coloring was higher percentage, but impact wasn't known whether blue light in the result of image processing.

Table 2. Coloring ratio of lettuce in deep flow technique system

Treatment (R:B)	53:47	89:11	84:16	78:22	58:42	76:24	88:12	69:31
Green Pixels	91419	90553	77865	52974	82951	66129	76851	51839
Red Pixels	3283	5940	3794	4586	5099	5386	4290	4435
Coloring ratio(%)	3.47	6.16	4.65	7.97	5.80	7.53	5.29	7.88

3. 1. 2 Aeroponic system

Total area of grown lettuce in the aeroponics system was analysis image processing. Table 3. Image analysis values are shown as blue the higher the ratio, the entire tests ratio were higher. Test(53:47) and test(58:42) were mostly the coloring ratio. The results was the same as paper by Baroli et al, 2008, Swatz et al., 2001

Table 3. Coloring ratio of lettuce in deep flow technique system

Treatment (R:B)	**53:47**	**89:11**	**84:16**	**78:22**	**58:42**	**76:24**	**88:12**	**69:31**
Green Pixels	73740	131434	114022	132831	84384	111403	167567	126464
Red Pixels	7764	327	1288	709	5235	1411	177	722
Coloring ratio(%)	9.79	0.24	1.12	0.56	6.31	1.59	0.11	0.63

3. 2 Anthocanin contents analysis

3. 2. 1 Absorbance analysis

The lettuces after image processing had their absorbance analyzed using the Monomeric method. Table 4. was shown three-time result of total anthocyanin contents. Test(58:42) and test (69:31) were higher than another tests. But the contents were similar and the contents were less than all data.

Table 4. Content of total anthocyanin

Treatment (R:B)	**53:47**	**89:11**	**84:16**	**78:22**	**58:42**	**76:24**	**88:12**	**69:31**
First (mg/L)	0.03674	0.01753	0.09268	0.18118	0.3148	0.092	0.27553	0.643
Second (mg/L)	0.0359	0.01921	0.09351	0.17951	0.3131	0.092	0.27804	0.643
Third (mg/L)	0.03674	0.01586	0.09435	0.18035	0.3156	0.092	0.27887	0.642
Average (mg/L)	0.03646	0.01753	0.09351	0.18035	0.3145	0.092	0.27748	0.643

3. 2. 2 High-performance liquid chromatography analysis

This study aims to verify the effect of light combination on the development of anthocyanin. The (Fig.3) graph is standard of the major three elements of anthocyanin - D3G, C3G, and P3G – and was analyzed and expressed in graph. The peak time was 7min, 11min, and 22min respectively.

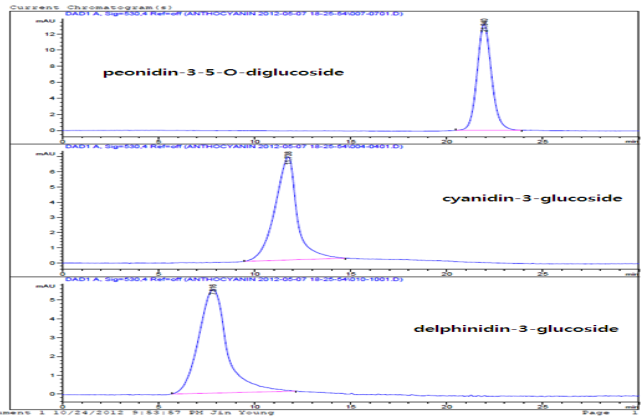

Fig. 3 Peonidin-3-glucoside(P3G), Cyanidin-3-glucoside(C3G), Delphinidin-3-glucoside(D3G)

The standard elements and specimen extracted from each experiment group were compared to estimate the contents of anthocyanin. As shown in Table 5, the content of P3G was higher than that of the other groups when the rate of blue light was high, which corresponds to the existing studies (Hogewoning et al., 2007). In contrast, the other standard elements - D3G and C3G from the extract showed no peak during the same period of time, and if any, then only some insignificant inclination was shown.

Table 5. Content of peodinin-3-glucoside

Treatment (R:B)	53:47	89:11	84:16	78:22	58:42	76:24	88:12	69:31
standard area	151.9	151.9	151.9	151.9	151.9	151.9	151.9	151.9
sample area	398.72	362.05	144.74	116.9	68.982	47.014	23.377	16.77
sample concentration (ug/ml)	6.562	5.959	2.382	1.924	1.135	0.774	0.385	0.276

3. 3 Statistical analysis

3. 3. 1 Statistical analysis between coloring ratio and total anthocyanins content

The values of image analysis and Anthocyanin contents to know correlation were statistically analyzed by SPSS(ver. 6.0). The table 6 is shown P value and correlation coefficient between coloring ratio and total anthocyanin. Correlation of

coloring ratio and total anthocyanin was significant even though the P value was low through the correlation analysis.

Table 6. Correlation of coloring ratio and total anthocyanin

Target		Red ratio	Antho cyanin
Coloring ratio	Pearson Correlation coefficient	1	.728*
	P-value (both)		0.041
	N	48	8
Anthocyanin	Pearson Correlation coefficient	.728*	1
	P-value (both)	0.041	
	N	8	8

3. 3. 2 Statistical analysis between coloring ratio and content of peodinin-3-glucoside

The table 7 shows the correlation between image analysis and P3G content. The result of comparing the figures from the image and HPLC analysis with Pearson's bivariate correlation analysis was 0.404**, which is significant.

Table 7. Correlation of coloring ratio and P3G content

Target		Red ratio	Antho cyanin
P3G content	Pearson Correlation coefficient	1	.404**
	P-value (both)		0.000
	N	72	72
Coloring ratio	Pearson Correlation coefficient	.404**	1
	P-value (both)	0.000	
	N	72	72

In the twice experiment, statistical analysis, image analysis values and anthocyanin contents were correlated. So Anthocyanin estimation may be possible through image processing.

4. CONCLUDING REMARKS

This study was conducted growing of lettuce in DFT system and aeroponics method with different light combinations.

4.1 Deep flow technique system(DFT)

The lettuce was grown in DFT system and images were taken. Then image analysis and absorbance was analyzed to find out the correlation. According to the experimental results, it was higher than the anthocyanin content in the test of high blue ratio. There was a correlation image analysis and total anthocyanin content.

4.1 Aeroponics system

From the image of grown lettuce and HPLC, the contents of the major functional elements of anthocyanin - cyanidin-3-glucoside(C3G), peonidin-3-glucoside(P3G), and delphinidin-3-glucoside(D3G) – were measured. As a result, it turned out that in the light combination of red 53: blue 47, red 58: blue 42, the content of D3G was the highest. All experiment groups except Test(89:11) showed no contents of C3G and D3G, or if any, an extremely small amount of them might have been contained.

Through the twice experiments, the content of anthocyanin may be estimated based on the imaging data to some extent. This study shows that blue light has significant effects on the development of anthocyanin.

ACKNOLEDGEMENTS

This Study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea

REFERENCES

Delvin, R. 1975. *Plant Physiology 3nd Edition*. p.354

Fankhauser, C. and J. Chory. 1997. Light control of plant development. Annual Review of Cell. and Developmental *Biology 13*:203-229.

Giedre, Samuoliene, Ram_unas Sirtautas, Aušra Brazaityte, Pavelas Duchovskis (2012). LED lighting and seasonality effects antioxidant properties of baby leaf lettuce, *Food Chemistry*.

Giliberto, L., G. Perrotta, P. Pallara, J.L. Weller, P.D. Fraser, P.M. Bramley,A. Fiore, M. Tavazza, and G. Giuliano. 2005. Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. *Plant Physiol*. 137:199-208.

Henry, B.S., G.F.A. Hendry, and J.D. Hougton. 1992. Natural food color. Innational food colorants, blackie and son Ltd, *glasgo* p.39

Hogewoning, S.W., G. Trouwborst, G.J. Engbers, J. Harbinson, W. van Ieperen,J. Ruijsch, O. van Kooten, A.H.C.M. Schapendonk, and C.S. Pot. 2007. Plantphysiological acclimation to irradiation by light-emitting diodes (LEDS). *Acta Hort. 761*:183-191.

Meyer, B., D. Anderson, R. Bohning, and D. Eratianne. 1975. Introduction to plant phsiology. 2nd edition. p.282.

Ninu et al., 1999L. Ninu, M. Ahmad, C. Miarelli, A.R. Cashmore, G. GiulianoCryptochrome 1 controls tomato development in response to blue light Plant J., 18 (1999), pp. 551–556

Oh, M.M., E.C. Edward, and C.B. Rajashekar. 2009. Environmental stresses induce health promoting phytochemicals in lettuce. Plant physiol. *Biochem. 47*:578-583.

Swatz, T. E., Corchnoy, S. B., Christie, J. M., Lewis, J. W., Szundi, I., Brigs, W. R., et al. (2001). The photocycle of flavin-binding domain of the blue light photoreceptor

Warrington, I.J. and K.J. Mitchell. 1976. The influence of blue- and red-biased light spectra on the growth and development of plants. Agric. *Meteorol. 16*:247-262.