

LED Lights Enhance Metabolites and Antioxidants in Chinese Cabbage and Kale

Min Ki Lee¹, MariadhasValanArasu^{2*}, SuhyoungPark³, Dong HaeByeon⁴, Sun-Ok Chung⁵, Sang UnPark⁶, Yong-PyoLim⁷, Sun-JuKim^{1*}

¹Department of Bio-Environmental Chemistry, Chungnam National University, Republic of Korea; ²Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saud University, Saudi Arabia; ³Department of Horticultural Crop Research, National Institute of Horticultural and Herbal Science (NIHHS), Republic of Korea; ⁴Chinese Cabbage & Breeding, Republic of Korea; ⁵Department of Biosystem Machinery Engineering; ⁶Department of Crop Science, ⁷Department of Horticultural Science, Chungnam National University, Republic of Korea.

ABSTRACT

Light emitting diode (LED) lights play an important role in the plant physiology and alter the metabolites in a significant manner. Glucosinolates (GSLs), polyphenols, flavonoids and antioxidant properties of Chinese cabbage and kale cultivated in varying LED lights were investigated. Analysis revealed 7 aliphatic, 3 indolyl and 1 aromatic GSLs in Chinese cabbage and kale. The total GSL content ranged from 1.5-19.08 and 1.85-24.87 $\mu\text{mol/g DW}$, and glucobrassicinapin was the predominant GSL (3) in Chinese cabbage, whereas; sinigrin (3.49 $\mu\text{mol/g DW}$) was in kale. Blue and red LED lights produced significantly higher amount of GSLs in Chinese cabbage and kale respectively. Results revealed higher amount of total polyphenol (3.845 $\mu\text{g/mL}$) and total flavanoids (3.939 $\mu\text{g/mL}$) in Chinese cabbage. Chinese cabbage and kale showed significant antioxidant activities when compare with positive control, and the antioxidant assays were slightly correlated with total GSLs, polyphenols and flavanoids contents. The influence of LED lights on glucobrassicin in Chinese cabbage and kale should be studied extensively, because GSL is the precursor of indole-3-carbinol, a potent anticancer isothiocyanate.

Key words: Chinese cabbage, Kale, Glucosinolates, Polyphenols, Flavanoids, Antioxidant activity

*Authors for correspondence: mvalanarasu@gmail.com; kimsunju@cnu.ac.kr

INTRODUCTION

Brassica vegetables such as Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*), kale (*B. oleracea* var. *acephala*), broccoli (*B. oleracea* var. *italica*), cauliflower (*B. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), and brussels sprouts (*B. oleracea* var. *gemmifera*) are considered to possess beneficial and nutritive effects for human health (Verkerk et al. 2009; Lee et al. 2015). These vegetables are important for animal and human diets because of the presence of proteins, minerals (iron, potassium, sodium, and zinc), vitamins, amino acids, health-promoting phytochemicals such as GSLs, phenolic compounds, carotenoids, anthocyanins, and vitamins (Dekker et al. 2002). The nutrient components of one hundred grams of edible portion of Chinese cabbage contains (carbohydrate, 3.23 g; protein, 1.2 g; fat, 0.2 g; folate, 79 µg; niacin, 400 µg; pantothenic acid, 105 µg; vitamin C, 27 mg; sodium, 8 mg; calcium, 77 mg; and carotenoids, 250 µg (National Nutrient Database, USA). Chinese cabbage belongs to the *Brassica* family is the most important ingredient in many foods in Korea, China, and Japan. It has been consumed as Kimchi, Kimchi stew, and fresh leaves with garlic and sesame in Korea. Especially, Kimchi is a traditional fermented food in Korea in which Chinese cabbage is a major ingredient. Kale was consumed as their leaves from 3 months after transplanting until almost 1 year afterward. At this stage, green flower buds are used for human consumption, being eaten as a boiled vegetable (Velasco et al. 2007). It also contains a high concentration of phytochemicals including GSLs, phenolic compounds, carotenoids, vitamins, and minerals. Glucosinolates (GSLs) and phenolic compounds are nutrient rich secondary metabolites present in plants, especially *Brassica* vegetables (Clarke 2010). GSLs have both beneficial and harmful effects. GSLs have various biological activities with being hydrolyzed by myrosinase (thioglucosidase) (Yang and Quiros 2010). The hydrolyzed products of GSLs have different structure and bioactivity depending on their parent GSLs (Bellostas et al. 2007). The hydrolyzed products of GSLs (sulforaphane and other isothiocyanates) contributed to have a great potential role in anti-carcinogenic effects (Commane et al. 2005). In the recent studies, individual GSL profiles are observed in different accessions of Chinese cabbage (Lee et al. 2014).

Total and individual GSLs are significantly affected by genotype and plant growth environment such as light, temperature, fertilization, biotic, and abiotic damage (Park et al. 2014a; 2014b). The objectives of this study were to investigate the total and individual GSL levels in Chinese cabbage and kale cultivated in different culture media and cultivated under various types of light emitting diodes (LEDs) at different development stages. Furthermore, Chinese cabbage and kale have been also investigated for their antioxidant activities.

MATERIAL AND METHODS

Chemicals

Aryl sulfatase (type H-1, EC 3.1.6.1), sinigrin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Folin & Ciocalteu's phenol reagent were obtained from Sigma-Aldrich (St Louis, MO, USA). DEAE Sephadex A-25 was provided obtained from GE Healthcare Bio-Sciences AB (Uppsala, Sweden).

Plant materials

Seeds of Chinese cabbage ('CR Ha Gwang') and kale ('Kale TBC', 'Man Choo Collard') were purchased from Asia seed Co., Ltd. (Seoul, Korea). Chinese cabbage ('Chun Gwang') was obtained from the Sakata Korea Seed Co., Ltd (Seoul, Korea).

Cultivation under different light emitting diode (LED) lights

Chinese cabbage ('Chun Gwang', 'CR Ha Gwang') and kale ('Kale TBC', 'Man Choo Collard') were cultivated in 72 holes-trays with 'High' bed soil (Punong Co., Ltd., Gyeongju, Republic of Korea) on 25 April, 2012 in the greenhouse of Chungnam National University (latitude, 127°35'E; longitude, 36°36'N). After 23 days of sowing (DAS), seedlings were transplanted to plastic pots (7 × 12 cm) using the same bed soil under different LED light sources (Red, Blue, and Red+Blue (RB)) in the growth chamber (25°C, 10%, Red, 45; Blue, 86; RB, 52 µmol/m²s). After 30 days after sowing (DAS), Chinese cabbage and kale were harvested every week (30, 37, 44, and 51 DAS). The each sprout was packed in the aluminum foil, immediately frozen at -70°C, and freeze-dried. The frozen dried samples were powdered and used for extraction of the phytochemicals.

Extraction of glucosinolates

Crude GSLs were extracted according to the procedure of Kim et al. (2007) and ISO 9167-1 (1992). Briefly, 100 mg of the lyophilized sample was extracted with boiling 70% (v/v) methanol for 5 min and centrifuged at 12,000 rpm at 4°C for 10 min. The residue was further re-extracted twice to complete extraction of GSLs. The crude GSLs were loaded on a DEAE Sephadex A-25 anion exchange mini-column (ca. 78 mg as dry matrix). After washing the mini-column with ultra-pure water to remove neutrals and cations, the desulfation reaction of crude GSLs was carried out with 75 μ L of aryl sulfatase (E.C.3.1.6.1) overnight (16–18 h) at room temperature. The desulfated (DS) GSLs were eluted with 0.5 mL (\times 3 times) of ultra-pure water. The eluates were filtered through 0.45 μ m Teflon PTFE syringe filter and used the chemical analysis. Separately, 5 mL of sinigrin (5 mg/ 50 mL) was used as an external standard according to the same procedure mentioned above (Fig. 1A).

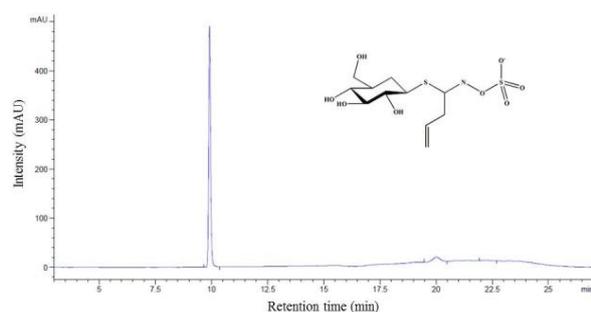


Figure 1A - HPLC chromatogram and chemical structure of sinigrin.

LC-ESI-MS for the identification of glucosinolates

GSLs were identified by API 4000 Q TRAP tandem mass spectrometer (Applied Biosystems, Foster City, CA). Electrospray ionization tandem mass spectrometry (ESI-MS/MS) source was used in positive ion mode ($[M+H]^+$) with an Agilent 1200 series HPLC system. The MS operating conditions were as follows: ion spray voltage, 5.5 kV; curtain gas (20 psi), nebulizing gas (50 psi) and heating gas (50 psi), high purity nitrogen (N_2); heating gas temperature, 550°C; declustering potential (100 V); entrance potential (10 V); spectra scanning range, m/z 100–800 (scan time 1.0 s).

HPLC analysis of glucosinolates

DS-GSLs were analyzed by 1200 series HPLC system. The mobile phase compositions were used as follows: 0 min (B) 0%, kept constant at (B) 0% by 2 min, constantly increased (B) 10% until 7 min, gradually increased (B) 31% by 16 min, kept constant at (B) 31% by 19 min, further (B) 0% at 21 min, and then kept constant at (B) 0% for 6 min (total 27 min). The individual GSLs were quantified with HPLC peak area of DS-sinigrin and multiplied with relative response factors from ISO 9167-1 (1992).

Total polyphenol analysis

Total polyphenols were estimated by following the method of Folin-Ciocalteu (Lin and Tang 2007).

Total flavonoid analysis

Lyophilized powder (10 mg) was dissolved in 1 mL of methanol. The mixture was mixed with 2 mL of ultra-pure water and stored at room temperature for 5 min. 150 μ L of 5% $NaNO_2$ and 10 % $AlCl_3$ solution were added to the mixture, respectively. After 6 min later, 1 mL of 1 M NaOH and 1.2 mL of ultra-pure water was added (total 5 mL). Total flavonoids were calculated as quercetin equivalent from the calibration curve of quercetin.

Antioxidant assays

DPPH radical scavenging assay

DPPH free radical scavenging assay was determined by the modified method of Xu et al. (2011).

Superoxide dismutase

Superoxide dismutase (SOD) activity was determined by the modified method of Marklund and Marklund (1974).

Statistical analysis

Mean values were compared by Tukey's multiple range test at $P \leq 0.05$, using SPSS statistical software.

RESULTS AND DISCUSSION

Identification of glucosinolates

LC-ESI-MS together with HPLC methods guided to identify and quantify eleven GSLs such as seven aliphatic GSLs (progoitrin, glucoraphanin, sinigrin, glucoalyssin, gluconapoleiferin, gluconapin, glucobrassicinapin), three indolyl

GSLs (glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin) and one aromatic GSLs (gluconasturtiin) (Table 1A, Fig. 1B, Fig. 1C).

Each fragment and ion of GSLs were matched with previous literature (Kim et al. 2010).

Table 1. Glucosinolates identified in Chinese cabbage and kale.

No. ^{a)}	RT ^{b)}	Semisystematic names of R -groups	Trivial names	Compound groups	[M+H] ⁺ (<i>m/z</i>)	Response factor ^{c)}
1	8.98	(2 <i>R</i>)-2-Hydroxy-3-butenyl	Progoitrin	Aliphatic	310	1.09
2	9.69	4-Methylsulfinylbutyl	Glucoraphanin	Aliphatic	358	1.07
3	9.89	2-Propenyl	Sinigrin	Aliphatic	280	1.00
4	11.03	5-Methylsulfinylpentyl	Glucosylsin	Aliphatic	372	1.07
5	11.21	2-Hydroxy-pent-4-enyl	Glucosylsin	Aliphatic	324	1.00
6	12.51	3-Butenyl	Glucosylsin	Aliphatic	294	1.11
7	14.90	Pent-4-enyl	Glucobrassicinapin	Aliphatic	308	1.15
8	16.07	3-Indolylmethyl	Glucobrassicin	Indolyl	369	0.29
9	17.12	4-Methoxy-3-indolylmethyl	4-Methoxyglucobrassicin	Indolyl	399	0.25
10	17.43	2-Phenethyl	Gluconasturtiin	Aromatic	344	0.95
11	19.31	<i>N</i> -Methoxy-3-indolylmethyl	Neoglucobrassicin	Indolyl	399	0.20

^{a)}No., the elution order of glucosinolates from HPLC chromatograms.

^{b)}RT, retention time (min).

^{c)}International Organization for Standardization (ISO 9167-1, 1992).

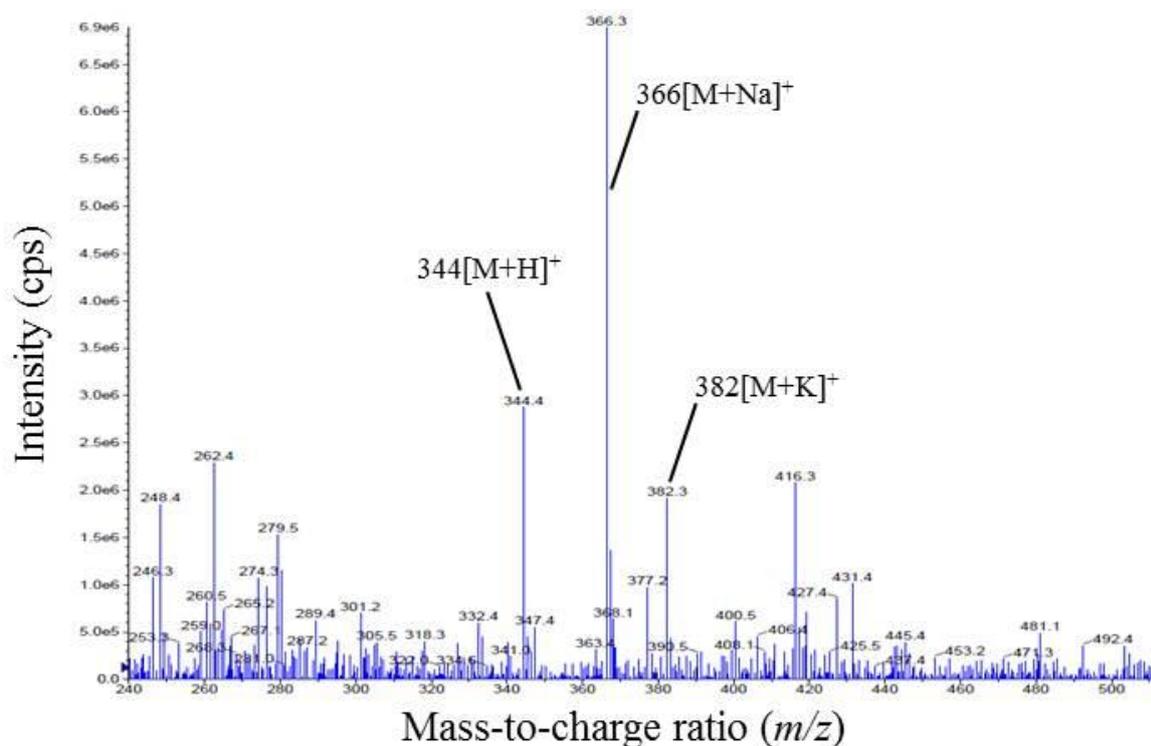


Figure 1B- Mass spectra of aromatic glucosinolate (gluconasturtiin)

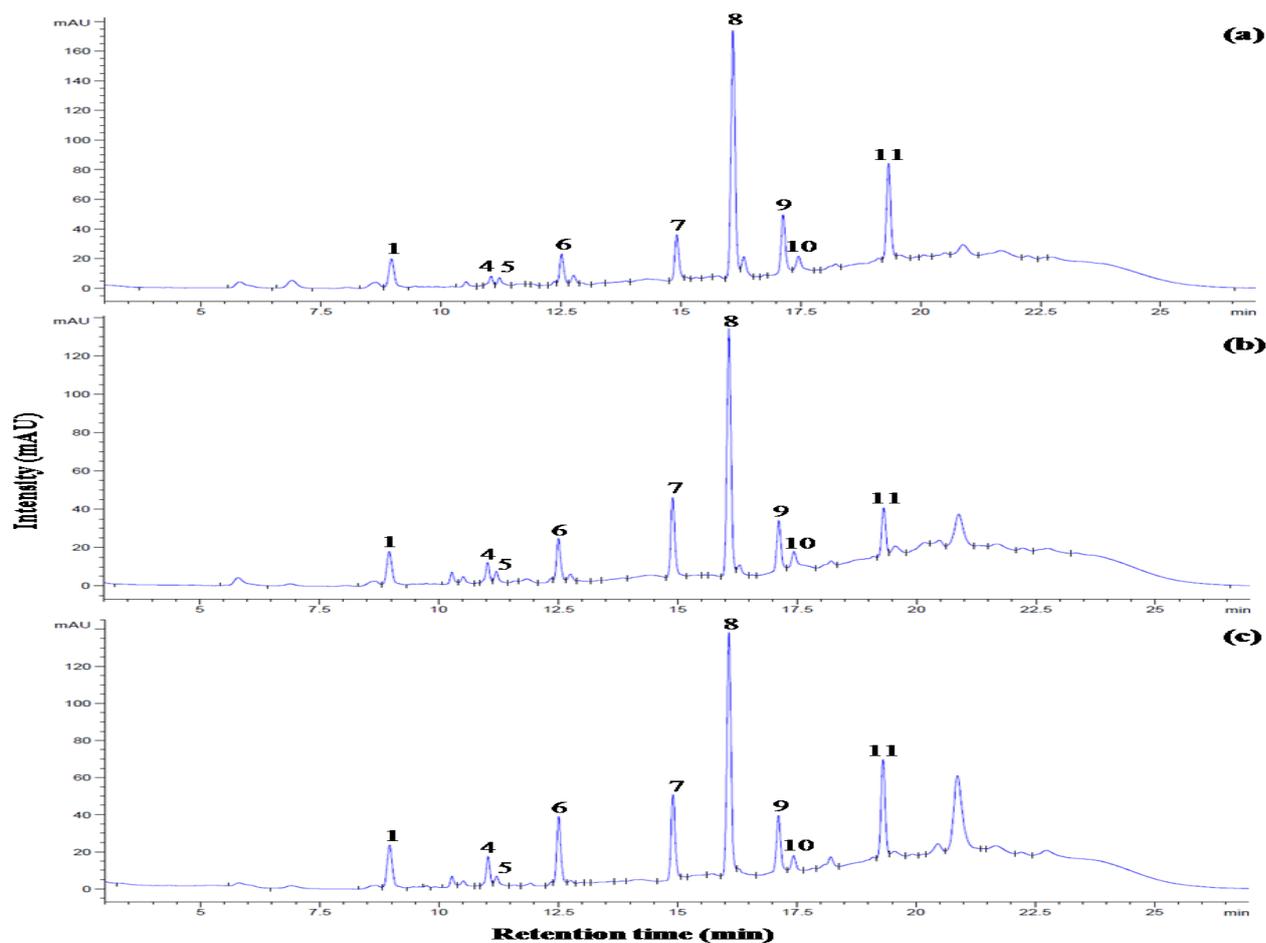


Figure 1C- HPLC chromatograms of glucosinolates at 42 DAS in Chinese cabbage ('CR Ha Gwang') with different culture media. (a), Red LED light; (b), Blue LED light; (c), Red + Blue LED lights.

Glucosinolates under different LED lights

Total GSLs in Chinese cabbage under all kinds of LED lights steadily increased until 44 DAS and decreased at 51 DAS. Total GSLs were ranged from 0.62 to 5.93 $\mu\text{mol/g DW}$ in Chinese cabbage (Table 2A). Brown et al. (2002) indicated that aliphatic and indolyl GSLs were affected by the genotype, environment, and genotype \times

environment (Brown et al. 2002). Plants generally respond to environmental stresses by inducing health-promoting phytochemicals as plant defense responses. Light condition can induce the levels of GSL in broccoli sprouts compared to the dark condition (Perez-Balibrea et al. 2008).

Table 2A. Glucosinolate contents ($\mu\text{mol/g DW}$) in Chinese cabbage ('Chun Gwang', 'CR Ha Gwang') under different LED lights ($n=2$)

No. ^{a)}	Red				Blue				Red+Blue			
	30 DAS ^{b)}	37 DAS	44 DAS	51 DAS	30 DAS	37 DAS	44 DAS	51 DAS	30 DAS	37 DAS	44 DAS	51 DAS
'Chun Gwang'												
1	0.11a	0.12±0.02a	0.13±0.03a	ND ^{c)}	0.08±0.02b	ND	0.19±0.01a	ND	ND	0.08±0.00a	0.11±0.02a	ND
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	0.05±0.00b	ND	0.07±0.00a	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	0.04±0.00a	ND	ND	ND	ND	ND
6	ND	0.19±0.00b	0.44±0.02a	0.16±0.00b	ND	0.08±0.00b	0.37±0.01a	0.06±0.03b	ND	0.11±0.01b	0.26±0.01a	0.11±0.01b
7	0.11±0.05c	0.52±0.03b	1.47±0.13a	0.46±0.06b	0.12±0.00b	0.16±0.00b	0.90±0.01a	0.14±0.03b	0.15±0.00c	0.37±0.02b	0.76±0.00a	0.40±0.05b
8	0.04±0.00c	0.27±0.00b	0.43±0.03a	0.19±0.06b	0.12±0.02c	0.21±0.00b	0.52±0.00a	0.20±0.00b	0.09±0.00b	0.30±0.01a	0.32±0.01a	0.33±0.08a
9	0.16±0.01b	0.54±0.02ab	0.93±0.03a	0.99±0.25a	0.17±0.02d	0.69±0.01c	1.36±0.02a	1.02±0.08b	0.29±0.00b	0.62±0.01b	1.21±0.02a	1.11±0.16a
10	0.13±0.04a	0.16±0.00a	0.12±0.02a	0.12a	0.17±0.03ab	0.13±0.05b	0.27±0.02a	ND	0.09±0.00a	0.11±0.00a	0.11±0.05a	ND
11	0.13±0.06a	0.96±0.24a	0.80±0.28a	0.27±0.24a	0.41±0.15a	0.24±0.00a	0.27±0.00a	0.22±0.05a	0.15±0.00b	1.00±0.04a	0.64±0.04ab	0.28±0.31b
Total	0.62±0.08c	2.76±0.24ab	4.33±0.54a	2.14±0.71bc	1.12±0.17c	1.51±0.05b	4.00±0.03a	1.63±0.03b	0.77±0.01b	2.59±0.03a	3.42±0.06a	2.27±0.65a
'CR Ha Gwang'												
1	0.18±0.00a	0.07±0.01b	0.08±0.01b	ND	0.09a	ND	0.07a	ND	0.09±0.02a	0.06a	0.09±0.02a	ND
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	0.05±0.00a	ND	0.06±0.01a	ND	ND	ND	ND	ND	ND	ND	0.06±0.00a	ND
6	0.08±0.00b	ND	0.06±0.01c	0.18±0.00a	ND	ND	ND	ND	ND	ND	0.06±0.00a	ND
7	0.61±0.01a	0.09±0.02c	0.29±0.00b	0.12±0.02c	ND	ND	0.06a	ND	0.12±0.08ab	0.05±0.01b	0.27±0.00a	ND
8	0.44±0.00b	0.48±0.03b	1.40±0.00a	0.47±0.05b	0.28±0.12b	0.12±0.01	1.08±0.19a	0.66±0.03ab	0.44±0.04c	0.57±0.02b	2.11±0.00a	0.39±0.01c
9	0.22±0.00b	0.16±0.01b	0.70±0.00a	0.63±0.04a	0.15±0.06c	0.25±0.01bc	0.61±0.16ab	0.64±0.03a	0.35±0.04b	0.24±0.00b	0.92±0.04a	0.27±0.04b
10	0.28±0.01a	0.12±0.01c	0.16±0.00b	0.06±0.00d	0.25±0.16a	0.11±0.00a	0.23±0.03a	0.08±0.01a	0.21±0.03a	0.17±0.02a	0.20±0.06a	0.13a
11	1.38±0.00a	0.71±0.06b	1.00±0.10b	0.48±0.10c	0.41±0.43a	0.20±0.02a	0.70±0.33a	0.31±0.16a	0.47±0.22b	0.39±0.12b	2.22±0.52a	0.49±0.27b
Total	3.25±0.01a	1.63±0.04b	3.75±0.12a	1.93±0.21b	1.13±0.48ab	0.68±0.04b	2.68±0.80a	1.69±0.10ab	1.68±0.24b	1.44±0.21b	5.93±0.57a	1.21±0.31b

^{a)}No., the elution order of glucosinolates from HPLC chromatograms. ^{b)}DAS, days after sowing. ^{c)}ND, not detected.

Mean values ($n=2$) indicated by the same letters in a line each LED lights do not significantly different at 5% level using Tukey's multiple range test.

GSLs : 1, Progoitrin; 2, glucoraphanin; 3, sinigrin; 4, glucoalyssin; 5, gluconapoleiferin; 6, gluconapin; 7, glucobrassicin; 8, glucobrassicin;

9, 4-methoxyglucobrassicin; 10, gluconasturtiin; 11, neoglucobrassicin.

Total GSL contents were ranged from 0.74 to 21.36 $\mu\text{mol/g DW}$ in kale (Table 2B). Total GSLs in 'Kale TBC' presented the highest levels at 44 DAS and then decreased at 51 DAS, but total GSLs in 'Man Choo Collard' constantly increased till 51 DAS, except for Red LED light. Sinigrin at all development stages was detected at Blue LED light (mean 2.00) > Red LED light (1.90) > RB

LED light (1.77 $\mu\text{mol/g DW}$) in 'Kale TBC'. In the previous study, sinigrin was detected at the highest level (1.15 $\mu\text{mol/g DW}$) under Red LED light, and it was not detected in Blue LED light of kale which grown under different LED lights (400–730 nm) (Lefsrud 2008).

Table 2A. Glucosinolate contents ($\mu\text{mol/g DW}$) in Chinese cabbage ('Chun Gwang', 'CR Ha Gwang') under different LED lights ($n=2$)

No. ^{a)}	Red				Blue				Red+Blue			
	30 DAS ^{b)}	37 DAS	44 DAS	51 DAS	30 DAS	37 DAS	44 DAS	51 DAS	30 DAS	37 DAS	44 DAS	51 DAS
'Chun Gwang'												
1	0.11a	0.12±0.02a	0.13±0.03a	ND ^{c)}	0.08±0.02b	ND	0.19±0.01a	ND	ND	0.08±0.00a	0.11±0.02a	ND
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	0.05±0.00b	ND	0.07±0.00a	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	0.04±0.00a	ND	ND	ND	ND	ND
6	ND	0.19±0.00b	0.44±0.02a	0.16±0.00b	ND	0.08±0.00b	0.37±0.01a	0.06±0.03b	ND	0.11±0.01b	0.26±0.01a	0.11±0.01b
7	0.11±0.05c	0.52±0.03b	1.47±0.13a	0.46±0.06b	0.12±0.00b	0.16±0.00b	0.90±0.01a	0.14±0.03b	0.15±0.00c	0.37±0.02b	0.76±0.00a	0.40±0.05b
8	0.04±0.00c	0.27±0.00b	0.43±0.03a	0.19±0.06b	0.12±0.02c	0.21±0.00b	0.52±0.00a	0.20±0.00b	0.09±0.00b	0.30±0.01a	0.32±0.01a	0.33±0.08a
9	0.16±0.01b	0.54±0.02ab	0.93±0.03a	0.99±0.25a	0.17±0.02d	0.69±0.01c	1.36±0.02a	1.02±0.08b	0.29±0.00b	0.62±0.01b	1.21±0.02a	1.11±0.16a
10	0.13±0.04a	0.16±0.00a	0.12±0.02a	0.12a	0.17±0.03ab	0.13±0.05b	0.27±0.02a	ND	0.09±0.00a	0.11±0.00a	0.11±0.05a	ND
11	0.13±0.06a	0.96±0.24a	0.80±0.28a	0.27±0.24a	0.41±0.15a	0.24±0.00a	0.27±0.00a	0.22±0.05a	0.15±0.00b	1.00±0.04a	0.64±0.04ab	0.28±0.31b
Total	0.62±0.08c	2.76±0.24ab	4.33±0.54a	2.14±0.71bc	1.12±0.17c	1.51±0.05b	4.00±0.03a	1.63±0.03b	0.77±0.01b	2.59±0.03a	3.42±0.06a	2.27±0.65a
'CR Ha Gwang'												
1	0.18±0.00a	0.07±0.01b	0.08±0.01b	ND	0.09a	ND	0.07a	ND	0.09±0.02a	0.06a	0.09±0.02a	ND
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	0.05±0.00a	ND	0.06±0.01a	ND	ND	ND	ND	ND	ND	ND	0.06±0.00a	ND
6	0.08±0.00b	ND	0.06±0.01c	0.18±0.00a	ND	ND	ND	ND	ND	ND	0.06±0.00a	ND
7	0.61±0.01a	0.09±0.02c	0.29±0.00b	0.12±0.02c	ND	ND	0.06a	ND	0.12±0.08ab	0.05±0.01b	0.27±0.00a	ND
8	0.44±0.00b	0.48±0.03b	1.40±0.00a	0.47±0.05b	0.28±0.12b	0.12±0.01	1.08±0.19a	0.66±0.03ab	0.44±0.04c	0.57±0.02b	2.11±0.00a	0.39±0.01c
9	0.22±0.00b	0.16±0.01b	0.70±0.00a	0.63±0.04a	0.15±0.06c	0.25±0.01bc	0.61±0.16ab	0.64±0.03a	0.35±0.04b	0.24±0.00b	0.92±0.04a	0.27±0.04b
10	0.28±0.01a	0.12±0.01c	0.16±0.00b	0.06±0.00d	0.25±0.16a	0.11±0.00a	0.23±0.03a	0.08±0.01a	0.21±0.03a	0.17±0.02a	0.20±0.06a	0.13a
11	1.38±0.00a	0.71±0.06b	1.00±0.10b	0.48±0.10c	0.41±0.43a	0.20±0.02a	0.70±0.33a	0.31±0.16a	0.47±0.22b	0.39±0.12b	2.22±0.52a	0.49±0.27b
Total	3.25±0.01a	1.63±0.04b	3.75±0.12a	1.93±0.21b	1.13±0.48ab	0.68±0.04b	2.68±0.80a	1.69±0.10ab	1.68±0.24b	1.44±0.21b	5.93±0.57a	1.21±0.31b

^{a)}No., the elution order of glucosinolates from HPLC chromatograms. ^{b)}DAS, days after sowing. ^{c)}ND, not detected.

Mean values ($n=2$) indicated by the same letters in a line each LED lights do not significantly different at 5% level using Tukey's multiple range test.

GSLs : 1, Progoitrin; 2, glucoraphanin; 3, sinigrin; 4, glucoalyssin; 5, gluconapoleiferin; 6, gluconapin; 7, glucobrassicin; 8, glucobrassicin;

9, 4-methoxyglucobrassicin; 10, gluconasturtiin; 11, neoglucobrassicin.

Total polyphenols

The total polyphenols in 'CR Ha Gwang' cultivated under LED lights were followed by RB (3.889) > Red (3.817) > Blue LED light (3.776 $\mu\text{g}/\text{mL}$), and 'Kale TBC' is RB (3.738) > Red (3.772) = Blue (3.772) (Fig. 2A). RB LED light contributed to increase their total polyphenols in Chinese cabbage and kale. Polyphenols possess the ideal chemical structure with many biological activities such as antioxidant, anti-inflammatory, anti-carcinogenic, anti-thrombotic, anti-viral and vasodilatory (Manach 2005).

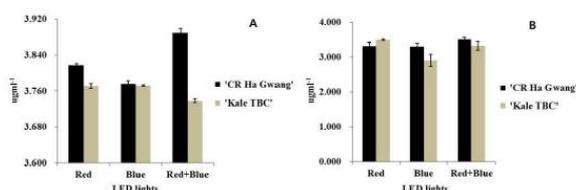


Figure 2 - Effect of different LED lights on the production of total polyphenols (A) and total flavonoids (B)

Total flavonoids

The mean value of the total flavonoids in 'CR Ha Gwang' (3.376) was higher than that of 'Kale TBC' (3.244 $\mu\text{g}/\text{mL}$) under LED lights (Fig. 2B). Total flavonoids in 'CR Ha Gwang' were the highest level in RB (3.503) followed by Red (3.315), Blue LED light (3.309 $\mu\text{g}/\text{mL}$), respectively. Total flavonoids in 'Kale TBC' are Red (3.497) > RB (3.327) > Blue LED light (2.909 $\mu\text{g}/\text{mL}$).

Antioxidant properties

DPPH is based on an electron-transfer reaction which considered hydrogen atom donating capacity. The key point of atom transfer reaction is a radical chain reaction and SOD protects against oxygen to expose cells in plants. Superoxide (O_2^-), as a reducing agent gives extra electron that reduce to become H_2O_2 (Halliwell 1974; Swatsitang and Wonginyoo 2008). The scavenging activity of Chinese cabbage cultivated in different LED lights in 'CR Ha Gwang' was as follows, Blue (89.8) > RB (86.2) > Red LED light (84.8%), and 'Kale TBC' as, Blue (86.0) > Red (84.4) > RB (83.0%) respectively (Fig. 3A, 3B). Results claimed that that the Blue LED light is more efficient to contribute their antioxidant activity in Chinese cabbage and kale (Table 3). The levels of polyphenols and the antioxidant capacity displayed by cabbage extracts studied were similar to those of the previous report.

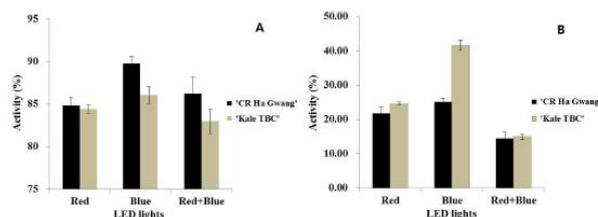


Figure 3 - DPPH radical scavenging activity (A) and Superoxide dismutase activity (B) of Chinese cabbage ('CR Há Gwang') and kale ('Kale TBC') with different LED lights.

Table 3. Correlation of glucosinolate and antioxidant activities between LED lights.

<i>Brassica</i> species	Correlation	Total GSLs	Total polyphenols	Total Flavanoids
'CR Ha Gwang'	DPPH	-0.213	-0.257	-0.108
	<i>P</i> value	0.583	0.505	0.783
	SOD	-0.551	-0.473	-0.2
	<i>P</i> value	0.124	0.199	0.606
'Kale TBC'	DPPH	-0.073	0.293	-0.082
	<i>P</i> value	0.851	0.447	0.835
	SOD	-0.11	0.338	-0.658
	<i>P</i> value	0.777	0.302	0.054

The data were analyzed using ANOVA with the means of three replicates ($P \leq 0.05$)

CONCLUSION

Red LED lights were suitable for the cultivation of Chinese cabbage and kale. Present results showed

that Red LED light was good for the GSLs production in Chinese cabbage and kale, whereas, combination of RB light was better for the production of polyphenols and flavonoids. These

overall reports clearly indicate that application of LED lamps might stimulate the production of metabolites in *Brassica* vegetables when compare to without LED treatments. Total polyphenols, flavonoids, and GSLs in Chinese cabbage and kale had no significant correlation with their antioxidant activities. The findings from the present study could serve as a foundation for developing cultivation methods for improving the nutritional compounds such as glucoraphanin, glucobrassicin, polyphenols and anthocyanins.

ACKNOWLEDGEMENT

This research was supported by Bio-industry Technology Development Program (311022-05-4-SB020), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Ministry of Oceans and Fisheries (MOF), Rural Development Administration (RDA) and Korea Forest Service (KFS).

REFERENCES

- Bellostas N, Kachlicki P, Sorensen JC, Soensen H. Glucosinolate profiling of seeds and sprouts of *B. oleracea* varieties used for food. *Scien Horticult*. 2007; 114: 234-242.
- Brown AF, Yousef GG, Jeffery EH, Klein BP, Wallig MA, Kushad MM, et al. Glucosinolate profiles in broccoli: Variation in levels and implications in breeding for cancer chemoprotection. *J Am Soc Horticult Sci*. 2002; 127: 807-813.
- Clarke DB. Glucosinolates, structures and analysis in food. *Anal Met*. 2010; 2: 310-325.
- Commane D, Hughes R, Shortt C, Rowland I. The potential mechanisms involved in the anti-carcinogenic action of probiotics. *Mutat Res*. 2005; 591 (1-2): 276-289.
- Dekker M, Verkerk R, Jongen WMF. Predictive modelling of health aspects in the food production chain: A case study on glucosinolates in cabbage. *Trend Food Sci Technol*. 2000; 11: 174-181.
- Halliwell B. Superoxide dismutase, catalase and glutathione peroxidase: Solutions to the problems of living with oxygen. *New Phytol*. 1974; 73: 1075-1086.
- International Standards Organisation (ISO). Rapeseed: Determination of glucosinolates content - Part 1: Method using High performance liquid chromatography, ISO 9167-1:1992 (E). Geneva, Switzerland. 1992; pp. 1-9.
- Kim JK, Chu SM, Kim SJ, Lee DJ, Lee SY, Lim SH, Ha SH, Kweon SJ, Cho HS.. Variation of glucosinolates in vegetable crops of *Brassica rapa* L. ssp. *pekinensis*. *Food Chem*. 2010; 119: 423-428.
- Kim SJ, Kawaharada C, Jin S, Hashimoto M, Ishii G, Yamauchi H. Structural elucidation of 4-(cystein-S-yl)butyl glucosinolate from the leaves of *Eruca sativa*. *Biosci Biotech Biochem*. 2007; 71:114-121.
- Lee M-K, Chun J-H, Byeon DH, Chung S-O, Park SU, Park S, Arasu MV, Al-Dhabi NA, Lim Y-P, Kim S-J. Variation of glucosinolates in 62 varieties of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) and their antioxidant activity. *LWT- Food Sci Technol*. 2014; 58: 93-101.
- Lee DS, Jeon DS, Park SG, Arasu MV, Al-Dhabi NA, Kim SC, et al. Effect of cold storage on the contents of glucosinolates in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *South Ind J Biol Sci*. 2015; 1(1): 38-42.
- Lefsrud MG. Irradiance from distinct wavelength light-emitting diodes affect secondary metabolites in kale. *Hort Sci*. 2008; 43: 2243-2244.
- Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem*. 2007; 101:140-147.

- Manach C, Mazur A, Scalbert A. Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipid*. 2005; 16: 77-84.
- Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Euro J Biochem*. 1974; 47(3): 469-474.
- Park S, Arasu MV, Lee M-K, Chun J-H, Seo JM, Al-Dhabi NA, et al. Analysis and metabolite profiling of glucosinolates, anthocyanins and free amino acids in inbred lines of green and red cabbage (*Brassica oleracea* L.). *LWT- Food Sci Technol*. 2014a; 58(1): 203-213.
- Park S, Arasu MV, Lee M-K, Chun J-H, Seo JM, Lee S-W, et al. Quantification of glucosinolates, anthocyanins, free amino acids, and vitamin C in inbred lines of cabbage (*Brassica oleracea* L.). *Food Chem*. 2014b; 145: 77-85.
- Perez-Balibrea S, Moreno DA, Garcia-Viguera C. Influence of light on health-promoting phytochemicals of broccoli sprouts. *J Sci Food Agricul*. 2008; 88: 904-910.
- Swatsitang P, Wonginyoo R. Antioxidant capacity of vegetable juices. *KKU Sci J*. 2008; 36: 83-94.
- Velasco P, Cartea ME, Gonzalez C, Vilar M, Ordas A. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* Group). *J Agricul Food Chem*. 2007; 55: 955-962.
- Verkerk R, Schreiner M, Krumbein A, Ciska E, Holst B, Rowland I, et al. Glucosinolates in *Brassica* vegetables: The influence of the food supply chain on intake, bioavailability and human health. *Mol Nutr Food Res*. 2009; 53: 219-265.
- Xu ML, Wang L, Wang MH. The antioxidant and anticancer effects of MeOH extract of *Liriodendron tulipifera*. *Korean J Plant Resour*. 2011; 24: 23-29.
- Yang B, Quiros CF. Survey of glucosinolate variation in leaves of *Brassica rapa* crops. *Genet Resour Crop Evol*. 2010; 57: 1079-1089.

Received: September 10, 2015;
Accepted: December 16, 2015.