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# Accumulation of carotenoids in green and red Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) in response to light-emitting diodes

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Light-emitting diode (LED) technology provides an opportunity to investigate the relationships between plant metabolites and different light sources. In this study, we aimed to determine the effects of different LEDs on carotenoid accumulation in green and red Chinese cabbage. LED light irradiation had positive effects on the accumulation of carotenoids under different light conditions. Seven different carotenoids were detected in green and red Chinese cabbage. Among the different LED lights, white-colored irradiation proved to be the most effective in terms of accumulation of the highest amounts of total carotenoids and also for the highest accumulation of four different carotenoids, i.e., lutein, 13-*cis*-β-carotene, β-carotene, and 9-*cis*-β-carotene in red Chinese cabbage. White LED light also promoted the highest accumulation of β-cryptoxanthin and α-carotene in green Chinese cabbage. Among the carotenoids, accumulated levels of lutein were considerably higher than those of any of the other carotenoids in both green and red cabbage. In response to irradiation of different LED light, the lutein content in green cabbage ranged from 633.74 to 769.02 μg/g, whereas that in red cabbage ranged from 747.15 to 897.51 μg/g. Similar to lutein, the highest levels of 13-*cis*-β-carotene,β-carotene, and 9-*cis*-β-carotene in red cabbage were obtained in response to irradiation with white LED light. In green cabbage, the highest accumulation of both of α-carotene and β-cryptoxanthin was observed in response to irradiation with white LED light. In both green and red cabbage, the highest level of β-cryptoxanthin was almost the same, being slightly higher in green cabbage, and in both cases white LED light promoted the highest level of β-cryptoxanthin. Accumulation of zeaxanthin was markedly enhanced by blue + red LED light, and was considerably higher in green cabbage than in red cabbage. These results demonstrate that white LED light is effective for the high-level accumulation of carotenoids in both types of Chinese cabbage, and particularly in red cabbage.

**Keywords: Green and red cultivars of *Brassica rapa* ssp. *pekinensis*, carotenoid, LED light**

## INTRODUCTION

Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) is one of the most widely cultivated vegetables in Asia. Given the popularity of this vegetable, it has been the subject of extensive research to evaluate its nutrient compounds (Artemyeva and Solovyeva, 2006; Krumbein et al. 2005).

Carotenoids are naturally occurring compounds that are derived from a terpenoid precursor. These compounds correspond to various group of pigments that give rise to the red, orange, and yellow colors in plants (Cunningham and Gantt, 1998). Carotenoids also contribute to a variety of

critical processes in plants, such as light harvesting in photosynthetic membranes and in protection of the photosystems from photo-oxidation (Havaux, 1998). Carotenoids are of significance not only to the plant in which they are synthesized but also play vital roles in animals and humans. Carotenoids have long functioned as essential nutrients in human diets, particularly as precursors of vitamin A (Giovannucci, 1999; Krinsky et al., 2003). Numerous studies have also indicated that intake of an appropriate amount of carotenoids can reduce the risk of cancer, macular eye disease, and cardiovascular problems (Giovannucci, 1999; Mayne, 1996).

Temperature, photoperiod, rainfall, soil, and illumination by light emitting diodes (LEDs), have been shown to modify the nature and concentration of the therapeutic compounds found in medicinal plants. Light exposure, for example, has been shown to significantly influence the accumulation of secondary metabolite compounds in plants (Lefsrud et al., 2006; Kopsell et al*.,* 2012), and irradiance levels have been shown to influence the production and concentration of both carotenoid pigments and glucosinolates (Charron and Sams, 2004; Lefsrud et al., 2006). However, LED light has been demonstrated to be the most effective strategy for promoting plant growth in controlled environments, owing to its adjustable high energy-conversion efficiency, long lifetime, light intensity and quality, and low thermal energy output (Okamoto et al. 1996; Schuerger et al. 1997). Furthermore, LED technology is one of the most advanced applications for high-valued intensive care plants in controlled environments (Martineau et al. 2012).

No studies to date have evaluated the influence of LED wavelengths on carotenoid accumulation in Chinese cabbage. Therefore, we investigated the influence of different LED treatments on the accumulation of different types of carotenoid in green and red Chinese cabbage.

## MATERIALS AND METHODS

### Plant materials and growth conditions

Seeds of green and red Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) were purchased from Asia Seed Co., Ltd. (Seoul, Korea). The seeds of both cultivars were immersed in sterilized water for 24 h and then placed in plastic pots containing vermiculite. The sterilized seeds were placed in a growth chamber for seedling establishment at a temperature of 25°C under standard cool white fluorescent tubes with a flux rate of 35 $\mu mol \cdot s^{-1} \cdot m^{-2}$, and were allowed to grow for 1 month.

The 1-month-old plantlets were subsequently placed in a growth chamber at 25°C and exposed to high-intensity irradiation from white (380 nm), blue (470 nm), red (660 nm), or blue + red (470 nm and 660 nm) LEDs. The flux rate was set at 50 $\mu mol \cdot s^{-1} \cdot m^{-2}$undera 16-h photoperiod and the plantlets were exposed to these LED treatments for a period of 3 weeks. Following the 3-week exposure period, plant samples were harvested, frozen in liquid nitrogen, and maintained at -80°C until carotenoid analysis using high-performance liquid chromatography (HPLC). The different sets of experiments were replicated three times, and mixtures of samples from the three independent replicates were used for the HPLC analysis of carotenoid accumulation.

### Extraction and analysis of carotenoids

The extraction method used in this study for carotenoid analysis followed that previously described by Howe and Tanumihardjo (2006).For the extraction of carotenoids from both red and green cabbage,1 g of each sample was added to 30 mL of ethanol containing 0.1% ascorbic acid (w/v). This mixture was vortexed for a period of 20 s, and then incubated in a water bath at 85°C for 5 min. To saponify any potentially interfering oils, 120 μL of potassium hydroxide (80% w/v) was added. The samples were placed on ice, and 1.5 mL of cold deionized water and 0.05 mL of β-apo-8′-carotenal (12.5 $\mu g \cdot mL^{-1}$), an internal standard, were added after vortexing and incubating at 85°C for 10 min. Thereafter, the carotenoids were extracted twice with 1.5 mL of hexane and centrifuged at 1200 × *g* each time to separate the layers. The extracts were then freeze-dried under a stream of nitrogen gas and re-suspended in 50:50 (v/v) dichloromethane/methanol.

The carotenoids were separated from the extracts using an Agilent 1100 HPLC system incorporating a $C_{30}$ YMC column (250×4.6 mm, 3 m; Waters Corporation, Milford, MA) and detected with a photodiode array (PDA) detector at 450 nm for HPLC analysis. Solvent A consisted of methanol/water (92:8 v/v) with 10 mM ammonium acetate. Solvent B consisted of 100% methyl *tert*-butyl ether (MTBE). The flow rate was 1 $mL \cdot min^{-1}$, and the samples were eluted using the following gradient: 0 min, 83% A/17% B; 23 min, 70% A/30% B; 29 min, 59% A/41% B; 35 min, 30% A/70% B; 40 min, 30% A/70% B; 44 min, 83% A/17% B; and 55 min, 83% A/17% B. Identification and peak assignment of carotenoids were

primarily based on the comparison of their retention time and UV-visible spectrum data with that of standards and with guidelines previously presented (Fraser et al., 2000; Howe and Tanumihardjo, 2006)

## RESULTS

The accumulation of carotenoids in the green and red Chinese cabbage in response to different LED lights were determined by HPLC (Table 1). Accumulation of carotenoids responded positively to light irradiation and varied considerably under the different light conditions. The following seven different carotenoids were detected in both green and red Chinese cabbage: lutein, β-cryptoxanthin, zeaxanthin, 9-*cis*-β-carotene, 13-*cis*-β-carotene, α-carotene, and β-carotene (Table 1). In red Chinese cabbage, white light irradiation was found to be the most effective in terms of accumulation of the highest amount of total carotenoids and also for the highest accumulation of lutein, 9-*cis*-β-carotene, 13-*cis*-β-carotene, and β-carotene (Table 1).White LED light irradiation also proved to be the most effective for the accumulation of β-cryptoxanthin and α-carotene in green Chinese cabbage (Table 1). In contrast, the highest amount of zeaxanthin was accumulated in green cabbage in response to blue + red light irradiation. The accumulation of zeaxanthin in green cabbage was considerably higher than that in red cabbage, although among the light treatments, blue + red LED light promoted the highest accumulation of zeaxanthin in the latter. In response to irradiation with different LED lights, zeaxanthin accumulation in red cabbage ranged from3.06 to 5.26μg/g, whereas that in green cabbage ranged from2.65 to 9.42μg/g. The highest zeaxanthin content observed in green cabbage under blue + red light irradiation was3.10, 2.44, 1.84, and 1.79 times higher than the zeaxanthin content in red cabbage exposed to blue, white, red, and blue + red light, respectively. In green cabbage, the amount of zeaxanthin in plants exposed to blue + red light was 3.55, 2.0, and 1.49 times higher than that in plants treated with red, white, and blue light, respectively. Among the carotenoids, the level of lutein accumulation was considerably higher than that of any of the other carotenoids in both green and red cabbage. Under exposure to different LED light, the lutein content in green cabbage ranged from 633.74 to 769.02 μg/g, whereas that in red cabbage ranged from 747.15 to 897.51 μg/g. For all the light conditions examined, lutein content was higher in the red cabbage than in green cabbage. The highest lutein content was observed in the red cabbage under white light irradiation, which was 1.42, 1.27, 1.21, and 1.17 times higher than the lutein accumulation under red, blue, white, and blue + red light, respectively, in green cabbage. The variation in lutein content in red cabbage under different LED light conditions was less pronounced than in green cabbage. Although lutein content in red cabbage did not differ substantially under the different light treatments, white LED light promoted the highest lutein content, which was 1.20, 1.17, and 1.13 times higher than that under blue + red, red, and blue light, respectively. Compared with lutein, the carotenoid with the next highest accumulation was β-carotene, in both green and red cabbage. Under the different LED treatments, the β-carotene content in green cabbage ranged from 258.46 to 352.19 μg/g, whereas that in red cabbage ranged from 282.77 to 373.15 μg/g. The highest β-carotene content was observed in the red cabbage exposed to white light irradiation, which 44%, 15%, 15%, and 6% higher than the β-carotene content in green cabbage treated with red, blue, white, and blue +red light, respectively. In red cabbage, the amount of β-carotene under white light conditions was 32%, 25%, and 24% higher than that in plants exposed to blue+ red, red, and blue light, respectively. Moreover the variation in the amount of β-carotene in red cabbage was considerably higher among the different light treatments than it was in green cabbage.

Similar to the accumulation patterns of lutein and β-carotene, the highest levels of both 9-*cis*-β-carotene and 13-*cis*-β-carotene in red cabbage were obtained with white LED light irradiation. Under the different LED light treatments, the content of 13-*cis*-β-carotene in green cabbage ranged from 17.69 to 27.54μg/g, and in red cabbage ranged from23.65 to 32.43μg/g. The highest level of 13-*cis*-β-carotene was observed in red cabbage exposed to white light irradiation, which was 1.83, 1.68, 1.20, and 1.18 times higher than that in green cabbage plants exposed to blue + red, red, white, and blue light, respectively. In red cabbage, the variation in this carotenoid under the different light conditions was less pronounced than that in green cabbage. The amount of 13-*cis*-β-carotene in red cabbage in response to white LED irradiation was 1.37, 1.23, and 1.09 times higher than that in plants exposed to red, blue + red, and blue light, respectively.

**Table 1. Carotenoids (μg/g) in 'green cabbage' and 'red cabbage' under different LED light**

| Cabbage | Light type | lutein | zeaxanthin | β-cryptoxanthin | 13-*cis*-β-carotene | α-carotene | β-carotene | 9-*cis*-β-carotene | Total |
|---|---|---|---|---|---|---|---|---|---|
| Green Cabbage | White | 739.52± 39.43 | 4.71 ± 0.23 | 2.17 ± 0.10 | 27.13 ± 0.48 | 4.71 ± 0.38 | 325.44 ± 26.66 | 32.39 ± 2.71 | 1136.07±42.33 |
| | Red | 633.74±165.88 | 2.65 ± 1.62 | 1.64 ± 0.80 | 19.31 ± 7.23 | 3.19 ± 1.23 | 258.46 ± 115.17 | 25.62 ± 8.47 | 944.61±152.63 |
| | Blue | 709.32± 21.70 | 6.31 ± 0.16 | 1.77 ± 0.06 | 27.54 ± 1.40 | 1.83 ± 0.13 | 325.36 ± 29.95 | 32.03 ± 2.53 | 1104.16±23.20 |
| | blue+red | 769.02± 58.58 | 9.42 ± 1.39 | 1.83 ± 0.28 | 17.69 ± 2.48 | 4.22 ± 1.29 | 352.19 ± 36.38 | 33.27 ± 4.96 | 1187.64±56.61 |
| Red Cabbage | White | 897.51± 50.62 | 3.86 ± 0.19 | 2.15 ± 0.13 | 32.43 ± 5.41 | 3.47 ± 1.54 | 373.15 ± 14.71 | 42.60 ± 1.86 | 1355.17±52.70 |
| | Red | 768.85± 85.69 | 5.13 ± 1.89 | 1.95 ± 0.17 | 23.65 ± 4.96 | 3.51 ± 1.06 | 297.42 ± 32.22 | 30.69 ± 4.07 | 1131.2±84.67 |
| | Blue | 794.67 ± 75.68 | 3.06 ± 0.27 | 1.85 ± 0.22 | 29.65 ± 5.38 | 2.65 ± 1.42 | 300.07 ± 37.49 | 33.21 ± 4.36 | 1165.16±77.53 |
| | blue+red | 747.15 ± 111.99 | 5.26 ± 0.67 | 1.85 ± 0.06 | 26.36 ± 5.99 | 3.80 ± 0.53 | 282.77 ± 53.02 | 30.41 ± 6.23 | 1097.6±109.70 |

Under exposure to the different LED lights, the 9-cis-β-carotene contents in green cabbage ranged from25.62 to 33.27μg/g, and in the red cabbage ranged from30.41 to 42.60μg/g. The highest level of this carotenoid was observed in the red cabbage exposed to white light irradiation, which was 1.60, 1.33, 1.32, and 1.28 times higher than that in green cabbage exposed to red, blue, white, and blue + red light, respectively. The variation in the content of this carotenoid in the red cabbage under different LED light conditions was less pronounced than that in green cabbage. White LED light promoted the highest level of this carotenoid in red cabbage, which was1.41, 1.39, and 1.28 times higher than the 9-cis-β-carotene in plants exposed to blue + red, red, and blue light, respectively.

A slightly different scenario was observed with regards to the synthesis of β-cryptoxanthin and α-carotene carotenoids. The highest levels of β-cryptoxanthin and α-carotene were promoted by white LED light irradiation in green cabbage. Under exposure to the different LED light treatments, the β-cryptoxanthin content in red cabbage ranged from1.85 to 2.15μg/g, whereas that in green cabbage ranged from 1.64 to 2.17μg/g. The highest level of β-cryptoxanthin was approximately the same in green and red cabbage, although slightly higher in the former, and in both cases white LED irradiation promoted the highest level of β-cryptoxanthin. The highest β-cryptoxanthin content was observed in the green cabbage exposed to white light irradiation, which was17%, 17%, 11%, and 1% higher than the β-cryptoxanthin content in red cabbage exposed to blue + red, blue, red, and white light, respectively. The level of β-cryptoxanthin in green cabbage exposed to white light was 32%, 23%, and 19% higher than that in plants exposed to red, blue, and blue + red light, respectively.

Under exposure to the different LED lights, the α-carotene content in red cabbage ranged from2.65 to 3.80μg/g, whereas that in green cabbage ranged from1.83 to 4.71μg/g. The highest α-carotene content was observed in the green cabbage treatment with white light irradiation, which was1.78, 1.36, 1.34, and 1.24 times higher than the α-carotene content in red cabbage treated with blue, white, red, and blue + red light, respectively. In green cabbage, the level of α-carotene was 2.57, 1.47, and 1.12 times higher under white light conditions than under blue, red, and blue+ red light conditions, respectively.

## DISCUSSION

Recent pertinent studies have focused on identifying the genetic and environmental factors that influence the accumulation of higher levels of important secondary metabolites compounds in plants the enrichment of which could improve plant nutritional quality. In this regard, light quality, irrigation, temperature fluctuation, and fertilization are considered as the influential environmental factors (Borevitz et al., 2000). Irradiation with different wavelengths of light using LEDs has been widely applied to various crops, including lettuce (Johkan et al., 2010), chrysanthemum (Jeong et al., 2012), and buckwheat (Hossen, 2007; Thwe et al. 2014), in order to achieve maximum output. In controlled environments, the use of LEDs could enable the application of different wavelengths and light intensities, thereby allowing us to easily analyze the effects of light on the accumulation of phenolic compounds. Irradiation with different types of light, either individually or in combination, has been observed to promote positive responses in different crop species with respect to the accumulation of specific phenolic compounds. Blue light irradiation has been shown to bethe most effective in lettuce for increasing the content of polyphenols (Johkan et al., 2010) and also higher amounts of rutin and cyanidin 3-*O*-rutinoside in buckwheat (Thwe et al., 2014). In contrast, blue light showed lower efficiency in chrysanthemum compared with red light for the accumulation of polyphenol contents (Jeong et al., 2012). Although supplemental red light irradiation has been demonstrated to promote an increase in the phenolic content of baby leaf lettuce (Li and Kubota, 2009), irradiation by red, green, and blue light in combination was found to be more effective for the accumulation of rutin in buckwheat sprouts than either all blue, or red, blue, and far-red combinations (Hossen, 2007). The few research findings mentioned above indicate that irradiation using LED lights can be an effective method for inducing the production of phenolic compounds and that such irradiation has species-specific effects with regards to the accumulation of different secondary compounds. Kim et al., (2015) proclaimed that LED light enhanced phenylpropanoid biosynthesis and the distribution of phenylpropanoids in the organs of Chinese cabbage. These authors found that plants responded well to blue light in terms of a higher accumulation of phenolic compounds, including p-hydroxybenzoic acid, ferulic acid, quercetin, and kaempferol, at 12 days after irradiation (DAI). It was also mentioned that red

light promoted the accumulation of ferulic acid at its highest level at 12 DAI. Furthermore, white light induced the highest accumulation of kaempferol at 9 DAI. Analysis of the phenylpropanoid content in different organs revealed organ-specific accumulation of p-hydroxybenzoic acid, quercetin, and kaempferol. These results suggest that blue light is effective for increasing phenylpropanoid biosynthesis in Chinese cabbage, with leaves and flowers representing the most suitable organs for the production of specific phenylpropanoids (Kim et al., 2015). The amounts of carotenoids have been shown to be considerably higher in the skins of kohlrabi than in their flesh, and pale green kohlrabi contain more carotenoids in both skin and flesh than purple kohlrabi (Park et al., 2012). Only a few studies have documented the isolation and quantification of carotenoids in Brassica spp, particularly in broccoli (Granado et al., 2006; Ibrahim and Juvik, 2009) and *Brassica oleracea* (Kurilich et al., 1999).

## CONCLUSION

The aim of this study was to investigate the effects of different LEDs on the accumulation of carotenoids in Chinese cabbage, and also to determine the species-specific distribution of carotenoids. Our results demonstrate that white light is the most effective light source for promoting higher amounts of carotenoid accumulation. We found that for most of the carotenoids examined, LED light promotes a higher accumulation in red Chinese cabbage than in green Chinese cabbage. Our results are expected to provide useful information for the enhancement of secondary metabolites, particularly for carotenoid biosynthesis, in different crops.

## CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

All authors contributed to the research and manuscript preparation. Jae Kwang Kim: Performed the experiments and analyzed the data. Sung Un Park: Designed the experiments and wrote the manuscript. All authors read and approved the final version.



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