

# Light Intensity and Light Quality from Sole-source Light-emitting Diodes Impact Phytochemical Concentrations within *Brassica* Microgreens

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**ABSTRACT.** Multilayer vertical production systems using sole-source (SS) light-emitting diodes (LEDs) can be an alternative to more traditional methods of microgreens production. One significant benefit of using LEDs is the ability to select light qualities that have beneficial impacts on plant morphology and the synthesis of health-promoting phytochemicals. Therefore, the objective of this study was to quantify the impacts of SS LEDs of different light qualities and intensities on the phytochemical content of brassica (*Brassica* sp.) microgreens. Specifically, phytochemical measurements included 1) total anthocyanins, 2) total and individual carotenoids, 3) total and individual chlorophylls, and 4) total phenolics. Kohlrabi (*Brassica oleracea* var. *gongylodes*), mustard (*Brassica juncea* ‘Garnet Giant’), and mizuna (*Brassica rapa* var. *japonica*) were grown in hydroponic tray systems placed on multilayer shelves in a walk-in growth chamber. A daily light integral (DLI) of 6, 12, or 18 mol·m<sup>-2</sup>·d<sup>-1</sup> was achieved from SS LED arrays with light ratios (percent) of red:blue 87:13 (R<sub>87</sub>:B<sub>13</sub>), red:far-red:blue 84:7:9 (R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>), or red:green:blue 74:18:8 (R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub>) with a total photon flux from 400 to 800 nm of 105, 210, or 315 μmol·m<sup>-2</sup>·s<sup>-1</sup> for 16 hours, respectively. Phytochemical measurements were collected using spectrophotometry and high-performance liquid chromatography (HPLC). Regardless of light quality, total carotenoids were significantly lower under increasing light intensities for mizuna and mustard microgreens. In addition, light quality affected total integrated chlorophyll with higher values observed under the light ratio of R<sub>87</sub>:B<sub>13</sub> compared with R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub> and R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub> for kohlrabi and mustard microgreens, respectively. For kohlrabi, with increasing light intensities, the total concentration of anthocyanins was greater compared with those grown under lower light intensities. In addition, for kohlrabi, the light ratios of R<sub>87</sub>:B<sub>13</sub> or R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub> produced significantly higher anthocyanin concentrations compared with the light ratio of R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub> under a light intensity of 315 μmol·m<sup>-2</sup>·s<sup>-1</sup>. Light quality also influenced the total phenolic concentration of kohlrabi microgreens, with significantly greater levels for the light ratio of R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub> compared with R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub> under a light intensity of 105 μmol·m<sup>-2</sup>·s<sup>-1</sup>. However, the impact of light intensity on total phenolic concentration of kohlrabi was not significant. The results from this study provide further insight into the selection of light qualities and intensities using SS LEDs to achieve preferred phytochemical content of brassica microgreens.

Microgreens are specialty horticulture crops consisting of vegetables and herbs harvested and consumed during the fully-expanded cotyledon or first true leaf developmental stages (Brazaitytė et al., 2015; Resh, 2013; Xiao et al., 2012). Microgreens have become increasingly popular in high-end culinary markets due to their ability to enhance the flavor, color, and texture of various products and dishes (Treadwell et al., 2010; Xiao et al., 2012). The genus *Brassica* has become a popular choice for microgreen production given their short production time, simplistic germination requirements, and plethora of desired morphological and sensory traits (Xiao et al., 2012).

Although a common means of production involves a form of hydroponics using closed irrigation systems and capillary mats, many novel methods for lighting and other environmental controls are being investigated (Resh, 2013).

Plant factories have been a prospective means of producing various vegetables and herbs in areas such as Japan since the 1980s. The allure of these systems has typically been directed toward their ability to produce highly uniform crops year-round, while also allowing for the manipulation of taste and morphology based on consumer preferences (Goto, 2012). The development of LED technology has further progressed this form of production due to the higher electrical efficiency, lower output of radiant heat, and spectral control this lighting strategy provides. In addition, the development of these more efficient lighting methods has benefited the introduction of multilayer production on shelving units using SS lighting (Gerovac et al., 2016; Goto, 2012). With the introduction of these technologies, the manipulation of plant products for desired morphological traits and phytochemical composition is becoming an increasingly promising prospect (Morrow, 2008).

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An increased emphasis on human health has been prevalent in recent years, and specialty crops have become a major focus due to the variety of health benefits and phytochemicals they provide (Brazaitytė et al., 2015; Kopsell and Kopsell, 2006). Microgreens specifically have become a target of great interest as they contain significantly higher concentrations of various phytochemicals and vitamins than that of a mature crop (Xiao et al., 2012). Brassica vegetables, in particular, provide an excellent source of fiber, vitamins, and minerals, and many of the health-promoting attributes linked to their consumption are related to their antioxidant capacity (Brazaitytė et al., 2015; Podsedek, 2007). Although the content and composition of these antioxidants varies based on species, phenolic compounds, vitamins C and E, glucosinolates, and carotenoids have generally been linked to high levels of antioxidant and anticarcinogenic activity (Podsedek, 2007; Stoewsand, 1995). Light, amongst various other environmental factors, is one of the most important variables in influencing phytochemical concentrations within the plant (Samuolienė et al., 2012). The potential of LED technologies for microgreen production has been further realized given that phytochemical concentration and overall antioxidant capacity of leaf tissue has been found to increase under blue and ultraviolet wavelengths of light (Goto, 2012).

Carotenoids are lipid-soluble plant pigments that, in nature, serve roles in photoprotection and as accessory pigments in photosynthesis (Hirschberg, 2001; Hughes, 1999; Kopsell and Kopsell, 2006). Although there are over 600 naturally occurring carotenoids, only an estimated 24–40 of these are regularly consumed in the human diet (Bendich, 1993; Hughes, 1999). In plants, the primary carotenoids synthesized include lutein (LUT), zeaxanthin (ZEA), antheraxanthin (ANT), violaxanthin (VIO), neoxanthin (NEO), and  $\beta$ -carotene (BC) (Sandmann, 2001). Health benefits linked to carotenoid consumption are typically associated with roles such as vitamin A precursors, free-radical scavengers, and a variety of antioxidant activities (Bendich, 1993; Brazaitytė et al., 2015; Kopsell and Kopsell, 2006). Similar to carotenoids, anthocyanins may also have health benefits including increased visual acuity, reduction of coronary heart disease, and antioxidant and anticancer properties (Giusti and Wrolstad, 2001). Additional health benefits are gleaned from plant phenolics, which serve as excellent free-radical scavengers (Ainsworth and Gillespie, 2007). Therefore, there is an increasing interest in the potential health benefits provided by brassica microgreens.

The ability to impact the phytochemical content of brassica microgreens has been recently investigated using SS LEDs. In terms of light intensity, Samuolienė et al. (2013) grew four species of brassica microgreens in a growth chamber under LED arrays providing a photosynthetic photon flux (*PPF*) of 110, 220, 330, 440, or 545  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with a light ratio (percent) of red:far-red:blue 91:1:8 ( $R_{91}:FR_1:B_8$ ). These authors found that chlorophyll and carotenoid concentration generally increased with increasing *PPF* levels. In addition, significantly higher anthocyanin accumulation was found to occur at a *PPF* of 330 or 440  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  compared with those grown under a *PPF* of 220  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Similarly, Lefsrud et al. (2008) found that irradiance was a major factor for pigment accumulation in kale (*B. oleracea* var. *acephala*) leaves. However, the pigment accumulation did not always respond linearly for increasing irradiance, leading to the possibility that wavelength selection may also play a significant

role in secondary metabolite production (Lefsrud et al., 2008). In a separate study, Li and Kubota (2009) evaluated the impact of light quality on the phytochemical content of baby leaf lettuce (*Lactuca sativa* 'Red Cross'). These authors reported that baby leaf lettuce grown under cool white fluorescent lamps (white light) with supplemental blue or ultraviolet-A light had significantly higher anthocyanin concentrations compared with those grown under white light alone. In addition, they reported an increase in carotenoid concentration with the addition of blue light and an increase in phenolics with the addition of red light. Kopsell and Sams (2013) also found that blue light exposure led to increased plant tissue pigments, such as carotenoids, in broccoli (*B. oleracea* var. *italica*) microgreens. In these previous studies, SS LEDs impacted carotenoid metabolism in specialty crop shoot tissues. An important carotenoid, ZEA is a direct quencher of chlorophyll-excited states and can prevent photo-oxidative stress and lipid peroxidation. ZEA can also replace VIO and LUT in the light harvesting antennae of photosystem II (PSII) and photosystem I. Under high light stress VIO, and sometimes BC, are quickly converted to ZEA to maintain the integrity of the D1 protein of PSII (Depka et al., 1998; Havaux et al., 2007; Polle et al., 2001). It may be possible that changes in light quality from SS LEDs can impact ZEA cycling in ways similar to increases in light intensity.

Thus, it is apparent that various light intensities and qualities can have a significant impact on the phytochemical content of many plant species. However, few studies have evaluated how the interaction between light quality and intensity may further affect phytochemical concentrations within the plant. Therefore, the objective of this study was to quantify the impacts of SS LEDs of different light qualities and intensities on the phytochemical concentrations of: 1) total anthocyanins; 2) total and individual carotenoids; 3) total and individual chlorophylls; and 4) total phenolics within brassica microgreens.

## Materials and Methods

**PLANT MATERIAL AND CULTURE.** Microgreens were produced using a hydroponic tray system with polyethylene terephthalate fiber pads (50.8 × 24.7 × 0.89 cm; Sure to Grow®, Beachwood, OH) placed in trays (52 × 26 × 6 cm) without drainage holes. A  $\text{CaCl}_2$  and deionized (DI) water solution was used initially to hydrate the pads and provide 100  $\text{mg}\cdot\text{L}^{-1}$  Ca to assist in uniform germination. For each species, nine tray systems were established by sowing seeds evenly onto each hydrated pad in the amounts of 25 g for purple kohlrabi, 15 g for mizuna, and 15 g for mustard (cv. Garnet Giant) (Johnny's Selected Seeds, Winslow, ME). The  $\text{CaCl}_2$  solution was further used to stimulate seedling growth by adding 100 mL daily for the first 5 d after sowing. Once cotyledons were fully reflexed,  $\approx 300$  mL of a 25% Hoagland's no. 1 nutrient solution (Hoagland and Arnon, 1950) was added to each tray daily until harvest to provide ( $\text{mg}\cdot\text{L}^{-1}$ ): 53 N, 8 P, 59 K, 50 Ca, 12 Mg, 0.5 Fe, 0.13 Mn, 0.01 Zn, 0.005 Cu, 0.13 B, and 0.002 Mo.

**GROWTH CHAMBER ENVIRONMENT.** A walk-in growth chamber (C5 Control System; Environmental Growth Chambers, Chagrin Falls, OH) was used to produce the microgreens. Trays were placed in a dark germination environment on 28 July, 18 Aug., or 11 Sept. 2014 under a constant average daily temperature of  $21 \pm 0.1$  °C, a relative humidity (RH) of 80%  $\pm$

0.5%, and a CO<sub>2</sub> concentration of 500 ± 21 μmol·mol<sup>-1</sup> (mean ± SD). After 3 d for germination, the air temperature set point was changed to 21 °C/17 °C day/night [D/N (16 h/8 h)], the RH set point was changed to 55%/65% D/N, and the CO<sub>2</sub> concentration was maintained at 500 μmol·mol<sup>-1</sup>. A data logger (DL1 Datalogger; Environmental Growth Chambers) was used to record average D/N air temperatures, D/N RHs, and CO<sub>2</sub> concentrations every 15 min, with the mean ± SD of the combined experimental repetitions measuring 21.0 ± 0.1/17.0 ± 0.1 °C (D/N), 55.9% ± 1.0%/65.5% ± 0.6% (D/N), and 504.5 ± 47.8 μmol·mol<sup>-1</sup>, respectively.

**SS LED LIGHTING.** Light ratios of red:blue 87:13 (R<sub>87</sub>:B<sub>13</sub>), red:far-red:blue 84:7:9 (R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>), or red:green:blue 74:18:8 (R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub>) were provided by LED arrays (Philips GreenPower LED production modules; Koninklijke Philips Electronics, Amsterdam, The Netherlands), which were mounted to nine stainless steel shelves (123 cm long and 61 cm wide). Light pollution between treatments was prevented using nonreflective blackout cloth. To achieve an average total photon flux density (TPFD), from 400 to 800 nm, of 105, 210, or 315 μmol·m<sup>-2</sup>·s<sup>-1</sup> within the lighting treatments, two, four, or six arrays, spaced 20.3, 12.2, or 8.6 cm apart, respectively, were mounted 38 cm above the crop canopy. Therefore, a DLI of 6, 12, or 18 mol·m<sup>-2</sup>·d<sup>-1</sup> was established by providing a 16-h (0600 to 2200 HR) photoperiod. Light quality and TPFD were measured at the beginning and confirmed at the end of each experimental repetition by taking nine individual spectral scans per treatment using a miniature spectrometer (BLUE-Wave; StellarNet, Tampa, FL). Representative spectral scans for each lighting treatment are presented in Fig. 1, and the average TPFD and DLI are reported in Table 1. Kohlrabi was placed under light treatments on 31 July and 21 Aug. 2014. Mizuna and mustard were placed under light treatments on 31 July and 14 Sept. 2014.

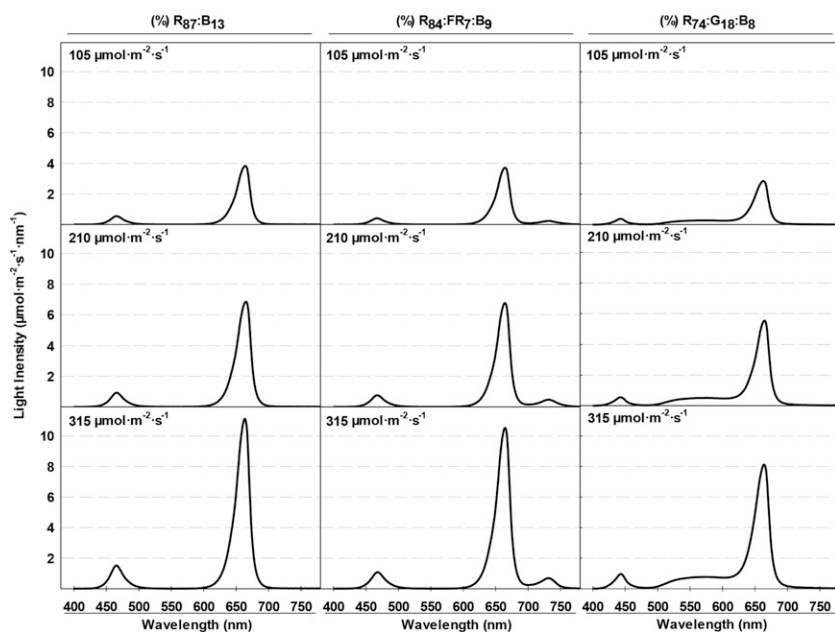


Fig. 1. Spectral quality delivered from sole-source light-emitting diode arrays with light ratios (percent) of red:blue 87:13 (R<sub>87</sub>:B<sub>13</sub>), red:far-red:blue 84:7:9 (R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>), or red:green:blue 74:18:8 (R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub>) at a total photon flux density from 400 to 800 nm of 105, 210, or 315 μmol·m<sup>-2</sup>·s<sup>-1</sup> at canopy level.

**PHYTOCHEMICAL MEASUREMENTS.** Three collective 5-g fresh-mass tissue samples were randomly collected from trays under each lighting treatment for each species and used to determine phytochemical content. Samples were placed in 50-mL centrifuge tubes, flash frozen in liquid nitrogen, and freeze-dried using a lyophilizer (FreeZone12; Labconco Corp., Kansas City, MO). Collective tissue samples were ground into a fine powder and homogenized using an ice-cold mortar and pestle. The freeze-dried samples were stored at -80 °C before extraction.

**TOTAL PHENOLIC CONCENTRATION.** Dried kohlrabi residues were removed from -80 °C storage and resolubilized in 2 mL of (% v/v) 98:2 (formic acid: DI water) before analysis. Total phenolic concentration was determined using the Folin-Ciocalteu (F-C) method described by Ainsworth and Gillespie (2007). For this method, homogenized samples were centrifuged at room temperature for 5 min at 13,000 g<sub>n</sub> and the supernatant collected. A sample of 100 μL was collected from each supernatant in 2-mL microtubes, and 200 μL of 10% (v/v) F-C reagent was added. Each sample was vortexed thoroughly, and 800 μL 700 mM Na<sub>2</sub>CO<sub>3</sub> was then added to each sample and the assay tubes incubated at room temperature for 2 h. Absorbance was measured at 765 nm with a spectrophotometer (SpectraMax 190 Microplate Reader; Molecular Devices, Sunnyvale, CA) against water. Total phenolic concentration was calculated using gallic acid as a standard, and was reported as gallic acid equivalents.

**TOTAL ANTHOCYANINS.** Resolubilized, concentrated extracts of kohlrabi underwent solid-phase extraction (SPE) using 18C SPE cartridges (Oasis HLB cartridge; Waters Corp., Milford, MA) according to Wang et al. (2013) to purify anthocyanins before measurement. Total anthocyanins were determined using the pH-differential method where two dilutions of each sample, one with 0.025 M KCl buffer (pH 1.0) and the other with 0.4 M C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub> buffer (pH 4.5), are prepared using a previously determined dilution factor (Giusti and Wrolstad, 2001). The absorption values of each dilution were measured with a spectrophotometer at 520 and 700 nm to correct for haze. Total anthocyanins were expressed as cyanidin 3-glucoside equivalents using a molar absorptivity (ε) of 26,900 M<sup>-1</sup>·cm<sup>-1</sup> and a molecular mass of 449.2 g·mol<sup>-1</sup>.

**CAROTENOID TISSUE EXTRACTION.** Plant pigments were extracted and analyzed from freeze-dried tissues of all three microgreens species according to Kopsell et al. (2007). A 0.1-g subsample was rehydrated with 0.8 mL of ultrapure H<sub>2</sub>O at 40 °C for 20 min. After incubation, 0.8 mL of the internal standard ethyl-β-8'-apo-carotenoate (Sigma-Aldrich, St. Louis, MO) was added to determine extraction efficiency. The addition of 2.5 mL of tetrahydrofuran (THF) was performed after sample hydration. The sample was then homogenized in a Potter-Elvehjem tissue grinding tube (Kontes, Vineland, NJ) using ≈25 insertions with a pestle attached to a drill press set at 540 rpm. During homogenization, the tube was immersed in ice to dissipate heat. The tube was then placed into a clinical

Table 1. Average total photon flux density (TPFD) from 400 to 800 nm delivered from sole-source light-emitting diodes with light ratios (percent) of red:blue 87:13 (R<sub>87</sub>:B<sub>13</sub>), red:far-red:blue 84:7:9 (R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>), or red:green:blue 74:18:8 (R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub>) to achieve target light intensities of 105, 210, and 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Daily light integral (DLI) targets from 400 to 800 nm were 6, 12, or 18  $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  under a 16-h photoperiod (0600 to 2200 HR). Mean values reported are the average of nine spectral scans across three repetitions.

Light intensity treatment ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Light quality treatment (%)	TPFD [mean $\pm$ SD ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )]	Avg DLI [mean $\pm$ SD ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ )]
105	R <sub>87</sub> :B <sub>13</sub>	110.8 $\pm$ 25.2	6.4 $\pm$ 1.4
	R <sub>84</sub> :FR <sub>7</sub> :B <sub>9</sub>	108.4 $\pm$ 22.8	6.2 $\pm$ 1.3
	R <sub>74</sub> :G <sub>18</sub> :B <sub>8</sub>	108.2 $\pm$ 23.7	6.2 $\pm$ 1.4
210	R <sub>87</sub> :B <sub>13</sub>	210.2 $\pm$ 31.4	12.1 $\pm$ 1.8
	R <sub>84</sub> :FR <sub>7</sub> :B <sub>9</sub>	207.6 $\pm$ 33.2	12.0 $\pm$ 1.9
	R <sub>74</sub> :G <sub>18</sub> :B <sub>8</sub>	215.1 $\pm$ 33.6	12.4 $\pm$ 1.9
315	R <sub>87</sub> :B <sub>13</sub>	313.2 $\pm$ 53.9	18.0 $\pm$ 3.1
	R <sub>84</sub> :FR <sub>7</sub> :B <sub>9</sub>	310.6 $\pm$ 50.9	17.9 $\pm$ 2.9
	R <sub>74</sub> :G <sub>18</sub> :B <sub>8</sub>	312.9 $\pm$ 54.4	18.0 $\pm$ 3.1

centrifuge for 3 min at 500  $g_n$ . The supernatant was removed and the sample pellet was resuspended in 2 mL THF and homogenized again with the same extraction technique. The procedure was repeated for a total of four extractions to obtain a colorless supernatant. The combined supernatants were reduced to 0.5 mL under a stream of nitrogen gas (N-EVAP 111; Organomation, Berlin, MA), and brought up to a final volume of 5 mL with methanol. A 2-mL aliquot was filtered through a 0.2- $\mu\text{m}$  polytetrafluoroethylene (PTFE) filter (model Econofilter PTFE 25/20; Agilent Technologies, Wilmington, DE) using a 5-mL syringe (Becton, Dickinson and Co., Franklin Lakes, NJ) before HPLC analysis.

**CAROTENOID AND CHLOROPHYLL HPLC ANALYSIS.** An HPLC unit with a photodiode array detector (1200 series; Agilent Technologies, Palo Alto, CA) was used for pigment separation. Chromatographic separations were achieved using an analytical scale (4.6 mm i.d.  $\times$  250 mm) 5  $\mu\text{m}$ , 200  $\text{\AA}$  polymeric C<sub>30</sub> reverse-phase column (ProntoSIL; MAC-MOD Analytical, Chadds Ford, PA), which allowed for effective separation of chemically similar carotenoid compounds. The column was equipped with a guard cartridge (4.0 mm i.d.  $\times$  10 mm) and holder (ProntoSIL), and was maintained at 30  $^{\circ}\text{C}$  using a thermostatted column compartment. All separations were achieved isocratically using a binary mobile phase of 11% methyl tert-butyl ether, 88.9% methanol, and 0.1% triethylamine (v/v). The flow rate was 1.0  $\text{mL}\cdot\text{min}^{-1}$ , with a run time of 53 min, followed by a 2-min equilibration before the next injection. Eluted compounds from a 10- $\mu\text{L}$  injection were detected at 453 (carotenoids and internal standard), 652 [chlorophyll *a* (Chl *a*)], and 665 [chlorophyll *b* (Chl *b*)] nm and data were collected, recorded, and integrated using ChemStation software (Agilent Technologies). Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards [ANT, BC, Chl *a*, Chl *b*, LUT, NEO, VIO, ZEA (ChromaDex, Irvine, CA)].

**EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS.** The experiment was laid out in a randomized block design in a factorial arrangement with light intensity (three levels) and light quality (three levels) as factors. The experiment was performed twice over time for each species and data were pooled across repetitions. The effect of light intensity was compared within light qualities for carotenoids and chlorophylls, while the

effects of light intensity, light quality, and their interaction were compared for anthocyanins and total phenolics by analysis of variance (ANOVA) using SAS (version 9.3; SAS Institute, Cary, NC) PROC MIXED, with an additional program (A.M. Saxton, unpublished data) that provided pairwise comparisons between treatments using Tukey's honestly significant difference test at  $P \leq 0.05$ . Results from the full ANOVA analysis conducted for the carotenoid and chlorophyll data can be found in Table 2.

## Results and Discussion

**CAROTENOID CONCENTRATION.** Total carotenoid concentration was 27% lower as the light intensity increased from 105 to 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for mizuna under the light ratio of R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>, and 18% and 33% lower for mustard under the light ratios of R<sub>87</sub>:B<sub>13</sub> and R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>, respectively (Table 3). In addition, mustard microgreens displayed a 21% lower total carotenoid concentration under the light ratio of R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub> as the light intensity increased from 105 to 210  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Table 3). This trend was also observed when many of the individual carotenoid concentrations were examined. As light intensity increased, the concentrations of BC, LUT, VIO, and NEO were generally lower in mustard and mizuna microgreens (Table 3). For example, as the light intensity increased from 105 to 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , the concentration of BC in mustard microgreens was 25% and 38% lower under the light ratios of R<sub>87</sub>:B<sub>13</sub> and R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>, respectively (Table 3). In addition, the concentration of LUT in mustard was 20% and 30% lower under the light ratios of R<sub>87</sub>:B<sub>13</sub> and R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>, respectively, as the light intensity increased from 105 to 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Table 3). Other research has found similar results of increasing light intensities leading to overall lower carotenoid concentrations. For example, Brazaitytė et al. (2015) found that carotenoid concentration of 'Red Lion' mustard was greatest at an irradiance of 110  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  compared with those grown at 220, 330, 440, or 545  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Similar results were found by Kopsell et al. (2012), where carotenoid concentrations were lower in 'Florida Broadleaf' mustard microgreens as the light intensity increased from 275 to 463  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Makus and Lester (2002) also found that 'Tenderleaf' and 'Florida Broadleaf' mustard microgreens grown in a reduced light environment displayed 46% higher carotenoid concentrations

Table 2. Analysis of variance for the effects of light intensity (LI), light quality (LQ), or LI × LQ from sole-source light-emitting diodes on chlorophyll and carotenoid concentrations of kohlrabi, mizuna, and mustard microgreens.

Carotenoids and chlorophylls	Kohlrabi			Mizuna			Mustard		
	LQ	LI	LQ × LI	LQ	LI	LQ × LI	LQ	LI	LQ × LI
AC	NS	NS	NS	*	NS	NS	**	*	NS
BC	**	NS	NS	**	**	**	*	***	NS
LUT	NS	NS	NS	NS	NS	*	NS	***	NS
ZEA	NS	NS	*	NS	*	NS	NS	NS	NS
ANT	*	*	NS	NS	NS	NS	NS	**	NS
VIO	NS	NS	NS	**	***	NS	NS	***	*
NEO	*	NS	NS	**	NS	NS	**	*	*
ZA/ZAV	*	NS	NS	NS	*	NS	**	**	NS
Total Car	NS	NS	NS	NS	*	NS	NS	***	NS
Chl <i>a</i>	*	NS	NS	NS	NS	NS	**	*	NS
Chl <i>b</i>	**	*	NS	NS	**	NS	NS	***	NS
Total Chl	*	NS	NS	NS	NS	NS	*	**	NS
Chl <i>a/b</i>	***	NS	NS	NS	NS	NS	**	*	NS

AC =  $\alpha$ -carotene, BC =  $\beta$ -carotene, LUT = lutein, ZEA = zeaxanthin, ANT = antheraxanthin, VIO = violaxanthin, NEO = neoxanthin, ZA/ZAV = ZEA + ANT/ZEA + ANT + VIO, Total Car = total 478 integrated carotenoid pigments, Chl *a* = chlorophyll *a*, Chl *b* = chlorophyll *b*, Total Chl = total chlorophyll, Chl *a/b* = chlorophyll *a/b* ratio.

NS, \*, \*\*, \*\*\*Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

compared with those grown in an ambient lighting environment, and Tarakanov and Wang (2009) found that tuberous rooted mustard (*Brassica napiformis*) and mizuna produced  $\approx 1.5$  times greater carotenoid concentrations under an unfavorable DLI of  $9.3 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  compared with those produced under  $13.9 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ .

Scientific evidence related to carotenoid concentrations under various light intensities for brassicas is somewhat contradictory. For example, in the same study conducted by Brazaitytė et al. (2015), these authors found that red pak choi (*B. rapa* var. *chinensis* 'Rubi F<sub>1</sub>') and tatsoi (*B. rapa* var. *rosularis*) microgreens produced the highest concentrations of carotenoids under the light intensities of 330 and 440  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Similar results of higher concentrations of carotenoids under increasing irradiance levels for brassicas have been found in related studies (Samuolienė et al., 2012). Therefore, it has been concluded by many authors that the response of carotenoid concentration to light may likely be species specific and subject to genetic variation (Brazaitytė et al., 2015; Kopsell and Kopsell, 2006). Carotenoid concentrations tend to increase under higher light intensities due to their role in photoprotection of higher plants (Demmig-Adams et al., 1996). However, Lefsrud et al. (2006) suggested that high light intensities may lead to the photodegradation of pigment molecules. In addition, it was suggested that a dilution effect with higher light intensities leading to increases in water content and mass may lead to decreased pigment concentrations as well (Lefsrud et al., 2006). Thus, variables such as genetic composition and increases in fresh and dry mass (DM) may have led to some of the differences observed between light treatments and species observed in these studies. However, from the results found in the present study, it seems that lower light intensities will lead to a final microgreens product that contains higher carotenoid concentrations.

In the current study, it was found that pigments involved in the xanthophyll cycle were affected by light intensity. The xanthophyll cycle is involved in excess light energy

dissipation within the plant, with the specific pigments involved in this cycle including ZEA, ANT, and VIO (Kopsell et al., 2012). Under high light intensities, VIO will actively convert to ZEA to dissipate thermal energy, while the reverse reaction will occur under low light intensities (Kopsell et al., 2012; Latowski et al., 2004). For both of these reactions, the intermediate product through which this conversion takes place is ANT (Yamamoto et al., 1962). Although data were not always significant, it was generally found that an increased flux toward ZEA occurred in both mizuna and mustard microgreens under increasing light intensities (Table 3). This flux toward ZEA is displayed through the ratio of ZEA + ANT/ZEA + ANT + VIO (ZA/ZAV), with higher values showing a higher degree of de-epoxydation toward ZEA in the xanthophyll cycle (Latowski et al., 2004). For example, ZA/ZAV in mustard increased from 0.54 to 0.64 under the light ratio of R<sub>87</sub>:B<sub>13</sub>, as the light intensity increased from 105 to 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively. Similar results were found by Kopsell et al. (2012) as they reported an increase in ZA/ZAV from 0.21 to 0.35 for 'Florida Broadleaf' mustard microgreens as the light intensity increased from 275 to 463  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Thus, it is possible that the higher light intensities were imposing stress upon the microgreens in the present study due to the higher degree of de-epoxydation toward ZEA in the xanthophyll cycle and often higher values of ZEA observed under these intensities. However, it is important to consider that these results were also dependent on the light qualities and species evaluated.

For light quality, results related to carotenoid concentrations were rarely found to be significant (Table 2). Thus, the emphasis of the statistical analysis was placed on the evaluation of light intensity. However, light quality did occasionally affect the concentration of carotenoids (Table 2). For example, it was found that  $\alpha$ -carotene (AC) concentrations increased from 0.02 to 0.04  $\text{mg}\cdot\text{g}^{-1}$  DM (50% higher) for mizuna microgreens and from 0.02 to 0.03  $\text{mg}\cdot\text{g}^{-1}$  DM (33% higher) for mustard microgreens under the light ratios of R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub> and R<sub>87</sub>:B<sub>13</sub>, respectively, regardless of light intensity. In addition, AC increased from 0.02 to 0.04  $\text{mg}\cdot\text{g}^{-1}$  DM (50% higher) for mizuna microgreens and from 0.02 to 0.03  $\text{mg}\cdot\text{g}^{-1}$  DM (33% higher) for mustard microgreens under the light ratios of R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub> and R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub>, respectively, regardless of light intensity. The light ratios of R<sub>87</sub>:B<sub>13</sub> and R<sub>74</sub>:G<sub>18</sub>:B<sub>8</sub> seemed to produce higher concentrations of carotenoids than microgreens grown under R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub>. This may have been due to the higher percent DM often observed in mizuna and mustard microgreens under the light ratio of R<sub>84</sub>:FR<sub>7</sub>:B<sub>9</sub> in previous studies (Gerovac et al., 2016). As discussed before, this may have led to a dilution effect resulting in lower concentrations of pigments in the tissue (Lefsrud et al., 2006).

Previous research findings have been mixed regarding the impacts of light quality on the synthesis and accumulation of carotenoids in microgreens tissues. For example, Kopsell et al.

Table 3. Carotenoid pigment concentrations [ $\text{mg}\cdot\text{g}^{-1}$  dry mass (DM)] of kohlrabi, mizuna, and mustard microgreens placed under light intensities (LIs) of 105, 210, or 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  delivered from sole-source light-emitting diodes with light ratios (percent) of red:blue 87:13 ( $\text{R}_{87}:\text{B}_{13}$ ), red:far-red:blue 84:7:9 ( $\text{R}_{84}:\text{FR}_7:\text{B}_9$ ), or red:green:blue 74:18:8 ( $\text{R}_{74}:\text{G}_{18}:\text{B}_8$ ).

LI	Carotenoids ( $\text{mg}\cdot\text{g}^{-1}$ DM)									Total Car
	AC	BC	LUT	ZEA	ANT	VIO	NEO	ZA/ZAV		
<i>Kohlrabi</i>										
$\text{R}_{87}:\text{B}_{13}$										
105	0.03 <sup>z</sup>	0.34	0.62	0.01 ab <sup>y</sup>	0.08	0.05	0.06	0.67		1.19
210	0.03	0.31	0.56	0.01 b	0.07	0.05	0.07	0.60		1.10
315	0.04	0.33	0.60	0.02 a	0.08	0.05	0.07	0.64		1.19
$\text{R}_{84}:\text{FR}_7:\text{B}_9$										
105	0.03	0.34	0.60	0.01	0.07	0.06	0.07	0.58		1.18
210	0.03	0.32	0.56	0.01	0.07	0.07	0.09	0.54		1.14
315	0.03	0.28	0.52	0.01	0.07	0.05	0.08	0.62		1.03
$\text{R}_{74}:\text{G}_{18}:\text{B}_8$										
105	0.03	0.33	0.63	0.01 ab	0.08	0.04	0.08	0.68		1.21
210	0.03	0.31	0.58	0.01 b	0.07	0.05	0.08	0.62		1.13
315	0.04	0.32	0.63	0.01 a	0.09	0.06	0.08	0.65		1.23
<i>Mizuna</i>										
$\text{R}_{87}:\text{B}_{13}$										
105	0.03	0.28	0.56	0.01	0.04	0.04 a	0.09	0.53		1.06
210	0.04	0.26	0.55	0.01	0.04	0.04 ab	0.08	0.57		1.02
315	0.03	0.24	0.49	0.01	0.03	0.03 b	0.08	0.58		0.91
$\text{R}_{84}:\text{FR}_7:\text{B}_9$										
105	0.02	0.35 a	0.64 a	0.01	0.03	0.04	0.12 a	0.51		1.21 a
210	0.02	0.27 b	0.52 b	0.01	0.04	0.03	0.09 b	0.59		0.99 b
315	0.03	0.22 b	0.46 b	0.01	0.03	0.03	0.10 ab	0.53		0.88 b
$\text{R}_{74}:\text{G}_{18}:\text{B}_8$										
105	0.04	0.22	0.49	0.01	0.05	0.06 a	0.07	0.49 b		0.94
210	0.04	0.22	0.52	0.01	0.05	0.04 b	0.09	0.57 a		0.96
315	0.03	0.23	0.53	0.01	0.04	0.04 b	0.09	0.56 a		0.96
<i>Mustard</i>										
$\text{R}_{87}:\text{B}_{13}$										
105	0.03	0.32 a	0.55 a	0.02	0.03	0.04 a	0.04	0.54 b		1.03 a
210	0.03	0.26 ab	0.46 ab	0.01	0.03	0.03 b	0.05	0.62 a		0.87 ab
315	0.03	0.24 b	0.44 b	0.02	0.04	0.03 ab	0.03	0.64 a		0.84 b
$\text{R}_{84}:\text{FR}_7:\text{B}_9$										
105	0.03	0.34 a	0.56 a	0.02	0.04	0.04 a	0.07 a	0.59		1.11 a
210	0.02	0.24 b	0.41 b	0.01	0.03	0.03 ab	0.06 a	0.57		0.81 b
315	0.02	0.21 b	0.39 b	0.02	0.04	0.02 b	0.03 b	0.74		0.74 b
$\text{R}_{74}:\text{G}_{18}:\text{B}_8$										
105	0.04	0.25	0.48 a	0.01	0.04 a	0.06 a	0.05 a	0.48		0.92 a
210	0.03	0.22	0.40 b	0.02	0.02 c	0.03 b	0.03 b	0.55		0.73 b
315	0.03	0.23	0.44 ab	0.01	0.03 b	0.03 b	0.03 b	0.56		0.80 ab

AC =  $\alpha$ -carotene, BC =  $\beta$ -carotene, LUT = lutein, ZEA = zeaxanthin, ANT = antheraxanthin, VIO = violaxanthin, NEO = neoxanthin, ZA/ZAV = ZEA + ANT/ZEA + ANT + VIO, Total Car = total 478 integrated carotenoid pigments.

<sup>z</sup>Mean values are based on three representative samples from each treatment across two experimental repetitions.

<sup>y</sup>Means sharing a letter are not statistically different by Tukey's honestly significant difference test at  $P \leq 0.05$ .

(2014) found that BC and LUT, the dominant carotenoids found in broccoli microgreens, did not differ in concentration after subjection to various red:green:blue ratios of LED light. Similarly, Lin et al. (2013) found that carotenoid concentration was not significantly different for lettuce (*L. sativa* var. *capitata*) under red:blue or red:blue:white LED light ratios.

However, Tuan et al. (2013) found that total carotenoids were higher in tartary buckwheat (*Fagopyrum tataricum*) sprouts when exposed to ultraviolet (380 nm) light vs. red or blue wavelengths. Furthermore, Brazaitytė et al. (2015) reported that the addition of green, yellow, or orange wavelengths in tandem with red and blue wavelengths led to higher total carotenoids for 'Red Lion' mustard microgreens, but lower total carotenoids for 'Rubi F<sub>1</sub>' red pak choi. In this same study, it was also found that supplemental yellow wavelengths led to higher total carotenoids for tatsoi microgreens, but that green and orange wavelengths added to the spectrum led to overall lower values (Brazaitytė et al., 2015). Li and Kubota (2009) found that supplemental blue wavelengths resulted in higher BC and xanthophyll concentrations in 'Red Cross' lettuce, whereas supplemental far-red light added to the spectrum lowered these concentrations. Similar to the observations regarding light intensity, it is apparent that the response of total carotenoid concentration to light quality is species dependent. In addition, it is possible that the light quality ratios used for the present study did not contain large enough differences in the wavelengths used to initiate significant changes in carotenoid pigment concentrations. Thus, further research is necessary to evaluate whether specific wavelengths of light may be consistently used to produce brassica microgreens with increased carotenoid concentrations.

**CHLOROPHYLL CONCENTRATION.** Generally, increased light intensities resulted in a lower total chlorophyll concentration for all three species (Table 4). For example, as the light intensity increased from 105 to 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , total chlorophyll concentration of mizuna was 32% lower under the light ratio of  $\text{R}_{84}:\text{FR}_7:\text{B}_9$  and total chlorophyll concentration of mustard was 28% and 34% lower under the light ratios of  $\text{R}_{87}:\text{B}_{13}$  and  $\text{R}_{84}:\text{FR}_7:\text{B}_9$ , respectively (Table 4). In addition, under the light ratio of  $\text{R}_{84}:\text{FR}_7:\text{B}_9$ , Chl *a* and Chl *b* were 31% and 34% lower, respectively, for mizuna and 33% and 38% lower, respectively, for mustard as the light intensity increased from 105 to 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Table 4). In contrast, Samuolienė et al. (2013) found that increasing PPF levels led to higher leaf chlorophyll

Table 4. Chlorophyll pigment concentrations [ $\text{mg}\cdot\text{g}^{-1}$  dry mass (DM)] of kohlrabi, mizuna, and mustard microgreens placed under light intensities (LIs) of 105, 210, or 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  delivered from sole-source light-emitting diodes with light ratios (percent) of red:blue 87:13 ( $\text{R}_{87}:\text{B}_{13}$ ), red:far-red:blue 84:7:9 ( $\text{R}_{84}:\text{FR}_{7}:\text{B}_9$ ), or red:green:blue 74:18:8 ( $\text{R}_{74}:\text{G}_{18}:\text{B}_8$ ).

LI	Chlorophylls ( $\text{mg}\cdot\text{g}^{-1}$ DM)			
	Chl <i>a</i>	Chl <i>b</i>	Total Chl	Chl <i>a/b</i>
<i>Kohlrabi</i>				
$\text{R}_{87}:\text{B}_{13}$				
105	5.84 <sup>z</sup>	2.63	8.47	2.22
210	5.47	2.30	7.77	2.39
315	5.16	2.49	7.65	2.05
$\text{R}_{84}:\text{FR}_{7}:\text{B}_9$				
105	5.05	2.36	7.41	2.15
210	4.84	2.20	7.04	2.18
315	4.54	2.01	6.54	2.30
$\text{R}_{74}:\text{G}_{18}:\text{B}_8$				
105	5.05 a <sup>y</sup>	2.72	7.77 a	1.88 ab
210	3.91 b	2.31	6.21 b	1.70 b
315	5.33 a	2.71	8.04 a	1.97 a
<i>Mizuna</i>				
$\text{R}_{87}:\text{B}_{13}$				
105	4.43	2.27 a	6.70	1.97
210	4.46	2.14 ab	6.60	2.13
315	4.35	1.77 b	6.12	2.33
$\text{R}_{84}:\text{FR}_{7}:\text{B}_9$				
105	5.47 a	2.42 a	7.89 a	2.14
210	4.27 ab	1.88 b	6.14 b	2.24
315	3.80 b	1.59 b	5.40 b	2.32
$\text{R}_{74}:\text{G}_{18}:\text{B}_8$				
105	3.90	2.30	6.20	1.79
210	3.69	2.14	5.82	1.83
315	4.61	2.07	6.68	2.24
<i>Mustard</i>				
$\text{R}_{87}:\text{B}_{13}$				
105	4.74	1.95 a	6.70 a	2.40
210	3.71	1.51 b	5.22 ab	2.48
315	3.40	1.44 b	4.84 b	2.34
$\text{R}_{84}:\text{FR}_{7}:\text{B}_9$				
105	4.64 a	2.04 a	6.68 a	2.32
210	3.47 ab	1.37 b	4.85 b	2.52
315	3.12 b	1.27 b	4.39 b	2.50
$\text{R}_{74}:\text{G}_{18}:\text{B}_8$				
105	2.75	1.95 a	4.71	1.41 b
210	3.46	1.37 b	4.83	2.51 a
315	2.89	1.48 b	4.37	1.96 ab

Chl *a* = chlorophyll *a*, Chl *b* = chlorophyll *b*, Total Chl = total chlorophyll, Chl *a/b* = chlorophyll *a/b* ratio.

<sup>z</sup>Mean values are based on three representative samples from each treatment across two experimental repetitions.

<sup>y</sup>Means sharing a letter are not statistically different by Tukey's honestly significant difference test at  $P \leq 0.05$ .

content. Specifically, these researchers found that irradiance levels of 545 and 440  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  led to significantly greater chlorophyll content for 'Red Lion' mustard and 'Rubi F<sub>1</sub>' red pak choi microgreens compared with those grown at 220  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . However, Kopsell et al. (2012) found that total

chlorophyll concentration was lower in 'Florida Broadleaf' mustard microgreens as the light intensity increased from 275 to 463  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and Makus and Lester (2002) reported that mustard green total leaf chlorophyll concentration was higher under a reduced light environment. In addition, Lefsrud et al. (2006) reported that maximum chlorophyll accumulation in kale (*B. oleracea* 'Winterbor') occurred at 335  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , whereas maximum chlorophyll accumulation of spinach (*Spinacia oleracea* 'Melody') occurred at 200  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , with irradiance treatments for the study ranging from 125 to 620  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Thus, it is apparent that optimal light intensities for chlorophyll accumulation differ among species and cultivars. In the present study, it was observed that total chlorophyll was highest under the lowest light intensity treatment. This result may have been due to the same photodegradation or dilution effect discussed previously regarding carotenoid concentrations (Lefsrud et al., 2006).

The main effect of light quality also impacted the chlorophyll concentration of microgreens (Table 2). However, these results were not as evident and the primary focus was placed on the effect of light intensity. Kohlrabi and mustard microgreens produced the highest total chlorophyll concentration when grown under the light ratio of  $\text{R}_{87}:\text{B}_{13}$ , regardless of light intensity. For example, total chlorophyll concentration of mustard microgreens grown under the light ratio of  $\text{R}_{87}:\text{B}_{13}$  (5.59  $\text{mg}\cdot\text{g}^{-1}$  DM) was 17% higher than those grown under  $\text{R}_{74}:\text{G}_{18}:\text{B}_8$  (4.64  $\text{mg}\cdot\text{g}^{-1}$  DM), regardless of light intensity. In addition, total chlorophyll concentration of kohlrabi microgreens grown under the light ratio of  $\text{R}_{87}:\text{B}_{13}$  (7.96  $\text{mg}\cdot\text{g}^{-1}$  DM) was 12% higher than those grown under  $\text{R}_{84}:\text{FR}_{7}:\text{B}_9$  (7.0  $\text{mg}\cdot\text{g}^{-1}$  DM), regardless of light intensity. These results are similar to those described by Kopsell et al. (2014) as they found that chlorophyll pigments in broccoli microgreens were of the highest concentration when grown under an increased percentage of blue light ( $\text{R}_{80}:\text{B}_{20}$ ). The present study agrees with the conclusion presented by Kopsell et al. (2014), that higher chlorophyll concentrations may be a result of increased percentages of blue light; although, further research is needed to confirm this conclusion.

The chlorophyll *a/b* ratio (Chl *a/b*) was also impacted by light quality for both kohlrabi and mustard microgreens (Table 2). For example, Chl *a/b* was significantly lower for kohlrabi microgreens grown under the light ratio of  $\text{R}_{74}:\text{G}_{18}:\text{B}_8$  (1.85) compared with those grown under  $\text{R}_{87}:\text{B}_{13}$  (2.22) or  $\text{R}_{84}:\text{FR}_{7}:\text{B}_9$  (2.21). Similarly, Chl *a/b* was significantly lower for mustard microgreens grown under the light ratio of  $\text{R}_{74}:\text{G}_{18}:\text{B}_8$  (1.96) compared with those grown under  $\text{R}_{87}:\text{B}_{13}$  (2.41) or  $\text{R}_{84}:\text{FR}_{7}:\text{B}_9$  (2.45). Reductions in Chl *a/b* have been previously observed under shade conditions. Specifically, Shao et al. (2014) proposed that a reduction in Chl *a/b* for *Anoectochilus roxburghii* under decreased irradiance may have been due to increases in Chl *b* from a reorganization of light harvesting and electron transport components. In terms of light quality, green light absorbed by cryptochrome has been found to elicit shade-like responses, although the specific mechanisms for these responses have yet to be thoroughly investigated (Wang and Folta, 2013; Zhang and Folta, 2012). Thus, it is possible that the addition of green light under the light ratio of  $\text{R}_{74}:\text{G}_{18}:\text{B}_8$  may have initiated a shade-induced decrease in Chl *a/b* in an attempt to increase light harvesting capabilities. However, further research is needed to more fully understand the mechanism behind this response.

**ANTHOCYANIN CONCENTRATION.** In the current study, the total anthocyanin concentration of kohlrabi microgreens grown under a light ratio of  $R_{87}:B_{13}$  was 17% and 18% higher under a light intensity of 210 or 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively, compared with those grown under a light intensity of 105  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Fig. 2A). In addition, total anthocyanin concentration of kohlrabi grown under a light ratio of  $R_{84}:FR_7:B_9$  was 31% and 24% higher under a light intensity of 210 or 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively, compared with those grown under a light intensity of 105  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Fig. 2A). Anthocyanin concentration of kohlrabi grown under a light ratio of  $R_{74}:G_{18}:B_8$  was 14% higher with a light intensity of 210  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  compared with those grown under a light intensity of 105  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , however, no further increase occurred in those grown under a light intensity of 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Fig. 2A). These results are similar to those by Samuolienė et al. (2013) as they found that brassica microgreens accumulated the highest anthocyanin concentrations under a *PPF* of 330 or 440  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  compared with those grown under a *PPF* of 220  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . One of the suggested roles of anthocyanin accumulation in the plant is to act in photoprotection (Logan et al., 2015; Ougham et al., 2008). Thus, under higher light intensities, it might be expected that kohlrabi microgreens in the present study accumulated greater

concentrations of anthocyanins in the leaf as a means of protection against these high light levels.

Anthocyanin concentration of kohlrabi microgreens grown under LEDs of different light qualities were not significantly different from one another at light intensities of 105 or 210  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . However, kohlrabi microgreens grown under a light ratio of  $R_{74}:G_{18}:B_8$  and a light intensity of 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  had significantly reduced anthocyanin concentration compared with those grown under the other light ratios (Fig. 2A). Cryptochrome B is primarily responsible for anthocyanin accumulation in arabidopsis (*Arabidopsis thaliana*) seedlings (Bouly et al., 2007). Bouly et al. (2007) grew cryptochrome (*cry1*) deficient seedlings of arabidopsis under 25  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of white light supplemented with 20  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of blue light and reported anthocyanin accumulation was reduced  $\approx 90\%$  compared with wild type (WT) arabidopsis. In addition, the authors added an additional 50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of green light to these treatments and reported that anthocyanin accumulation of *cry1* deficient arabidopsis was not influenced, while the WT arabidopsis showed a reduction of  $\approx 25\%$  compared with those grown without green light (Bouly et al., 2007). These results indicate that green light may negatively influence blue light-induced anthocyanin accumulation.

A separate study was conducted by Zhang and Folta (2012) to confirm that green light can reverse blue light mediated anthocyanin accumulation. These authors grew ‘Red Sails’ lettuce in a growth chamber with light ratios of white ( $W_{100}$ ),  $B_{100}$ ,  $B_{50}:G_{50}$ , or  $G_{100}$  at a *PPF* of 90  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . They reported that anthocyanin accumulation of lettuce grown under the  $B_{100}$  light ratio was 79% higher compared with the  $W_{100}$ , whereas those grown under a light ratio of  $B_{50}:G_{50}$  had similar anthocyanin accumulation to the  $W_{100}$  (Zhang and Folta, 2012). For the present study, these findings indicate that the green light included in the light ratio of  $R_{74}:G_{18}:B_8$  may have resulted in the significant reduction of anthocyanin concentration for kohlrabi microgreens grown under the light intensity of 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  compared with those grown under the light ratios of  $R_{87}:B_{13}$  and  $R_{84}:FR_7:B_9$  (Fig. 2A). However, further studies need to be performed to confirm or deny these results, as it is uncertain how this green light response may be mediated at various light intensities.

**TOTAL PHENOLIC CONCENTRATION.** In the current study, the total phenolic concentration of kohlrabi microgreens grown under a light ratio  $R_{74}:G_{18}:B_8$  and a light intensity of 105  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  was 11% lower than those grown with a light ratio of  $R_{84}:FR_7:B_9$  at this same light intensity (Fig. 2B). In addition, the total phenolic concentration of kohlrabi microgreens grown under a light ratio  $R_{74}:G_{18}:B_8$  and a light intensity of 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  was 9% lower compared with those grown under a light ratio of  $R_{84}:FR_7:B_9$  (Fig. 2B). Our results indicate that light quality influenced total phenolic concentration of kohlrabi, while the impacts of light intensity were not significant (Fig. 2B). Li and Kubota (2009) reported the phenolic concentration of baby green lettuce was 6% higher when grown under white light supplemented with red LEDs compared with white light alone. Thus, it is suggested that the lower total phenolic concentrations observed under the ratio of  $R_{74}:G_{18}:B_8$  could be due to the decrease in red wavelengths observed in this treatment. However, although the influence of light quality was significant in both studies, the impacts observed were minimal and a trend in wavelengths directly affecting phenolic concentration is ultimately indeterminable.

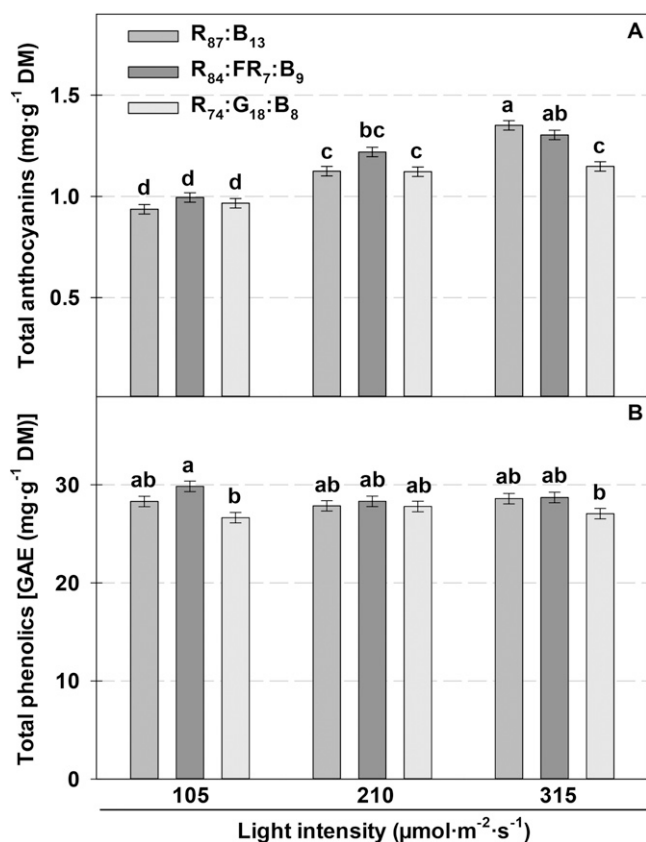


Fig. 2. Total anthocyanin [dry mass (DM)] and total phenolic concentration [gallic acid equivalents (GAEs)] of kohlrabi microgreens placed under light intensities of 105, 210, or 315  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  delivered from sole-source light-emitting diodes with light ratios (percent) of red:blue 87:13 ( $R_{87}:B_{13}$ ), red:far-red:blue 84:7:9 ( $R_{84}:FR_7:B_9$ ), or red:green:blue 74:18:8 ( $R_{74}:G_{18}:B_8$ ). Means sharing a letter are not statistically different by Tukey's honestly significant difference test at  $P \leq 0.05$ . Error bars indicate  $\pm$ SE.



## Conclusions

In conclusion, the present research has further demonstrated the ability to manipulate phytochemical concentrations within brassica microgreens through changes in light quality and intensity. However, additional research with larger differences in the light quality ratios is recommended to better understand the relationship between light intensity and light quality regarding phytochemical concentrations. These findings will also further serve researchers who continue to investigate the impacts of LED lighting on plant morphology and the synthesis of health-promoting phytochemicals in specialty crop production systems.

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