LIGHT - EMITTING DIODES (LEDs) FOR HIGHER NUTRITIONAL QUALITY OF *BRASSICACEAE* MICROGREENS

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Abstract
The aim of this study was to investigate the effect of industrially designed light-emitting diode (LED) lamp lighting on the nutritional quality of *Brassicaceae* microgreens. Red pak choi (*Brassica rapa* var. *chinensis* 'Rubi F_1'), tatsoi (*Brassica rapa* var. *rosularis*) and mustard (*Brassica juncea* L. 'Red Lion') were grown in a greenhouse (20±2/18±2 °C) during winter season, and the solar daily integral (DLI) was ~3.46±1.16 mol m^{-2} d^{-1}. The light spectra of lamp consist of 8 violet (420-430), 16 blue (460-470 nm), 8 orange (610-615 nm), 3 red (620-630 nm), 56 red (660-670 nm), 8 white (contain blue (400-500 nm), green (500-600 nm) and red (600-700 nm)) LEDs. The treatments of ~150 and ~250 µmol m^{-2} s^{-1} LED irradiance levels (LED 150 and LED 250) for 16 h d^{-1} in comparison with high pressure sodium (HPS) lamps (~150 µmol m^{-2} s^{-1}) as a control were performed. Photophysiological response to the artificial light varied among *Brassicaceae* species. Microgreens treated with LED 150 and LED 250 were significantly ($P \leq 0.05$) shorter and formed smaller hypocotyls. The photooxidative changes were evoked by both lighting treatments and led to higher phytochemical (phenols, ascorbic acid, flavonols, anthocyanins) and mineral element (Ca, K, Mg, Na, P, Fe, Zn) contents, and the DPPH and ABTS free radicals scavenging activities in all microgreens. Significantly lower content of nitrate was obtained with LED 150 treatment. Finally, LED lamps have the potential to be used as the main light source for growing high nutritional quality microgreens in greenhouses.
Key words: antioxidant, *Brassica,* greenhouse**,** light – emitting diode, microgreen, mineral element.

Introduction

Light is one of essential environmental factors influencing photophysiological responses of all higher plants (Ma et al., 2014). Plants are enabled to sense very broad light spectrum from UV to far red (280-750 nm) by photoreceptors. Photoreception mechanism is involved in response of photoreceptors to red-far red light (phytochromes), blue-UVA light (cryptochromes, phototropins, 'Zeitlupes') and UVB (UVR8) light (Galvão and Fankhauser, 2015; Casal et al., 2014; Pierik, de Wit, 2014). Besides light quality (wavelength), light intensity (irradiation) and photoperiod (duration of day/ night) also play a significant role in plant morphogenetic and photosynthetic responses (Björkman et al., 2011).

Greenhouse is an artificial growing system and requires the application of supplemental light sources to ensure plant growth. Light emitting diodes (LEDs), as the main or supplemental lighting source, despite having intensity, spectral and energy advances, can be used for target manipulation of metabolic responses in order to achieve high plant productivity and quality (Olle and Viršilė, 2013; Darko et al., 2014; Carvalho and Folta, 2014; Duchovskis et al., 2015). LEDs represent a promising light source in greenhouses for lettuce (Ouzounis et al., 2015; Sirtautas et al., 2014; Muneer et al., 2014; Samuolienė et al., 2013a; Samuolienė et al., 2011), red and green leaf microgreens (Samuolienė et al., 2012a; Brazaitytė et al., 2013), cucumber seedlings (Hernández and Kubota, 2014; Novičkovas et al., 2012), tomato (Lu et al., 2012; Brazaitytė et al., 2009), sweet pepper (Samuolienė et al., 2012b; Brown et al., 1995). Light combination with other climatic conditions (temperature, air humidity) and agronomic practices (water availability, soil/ substrate conditions, fertilizers) influence plant reaction to the environment. Plants have the adaptive mechanisms to thrive under environmental conditions which are typically found in greenhouses. Many antioxidants play a key role in plant adaptation to biotic stress. Antioxidants produced by plants in response to stress are secondary metabolites such as phenolic compounds or ascorbic acid (Oh et al., 2009), and are associated with the reduction of human chronic diseases like cancer or cardiovascular disease (Pinto et al., 2015). Biosynthesis of phenols is directly linked to blue light receptors (cryptochromes and phototropins) (Kang et al., 2008). Phytochromes participate in regulating processes of anthocyanin formation and accumulation (Iwai et al., 2010). Besides, biosynthesis of anthocyanins requires enzymes whose expression is regulated by light (Yamazaki et al., 1999). Ascorbic acid is also involved in anthocyanin biosynthesis (Iwai et al., 2010). Ascorbic acid metabolic pathway interacts with photosynthetic and respiratory electron transport chains, and the accumulation of ascorbic acid depends on quantity and quality of light (Bartoli et al., 2006). Minerals are also known to have protective benefits against oxidative stress related diseases, and found in plants as ions involved in secondary metabolite biosynthesis, or inorganic and organic salts and compounds (Mihaljev et al., 2014; Konieczynski et al., 2015). However, vegetables can also accumulate nitrate (NO_3^-) due to growing in closed environment, like greenhouse. Nitrate accumulation in plants mostly depends on nitrate reductase (NR) activity, which can be stimulated by red light (Lillo and Appenroth, 2001). Nitrate reduced to metabolites like toxic nitrite

anion (NO_2^-) or nitric oxide (NO) can lead to human disorders, especially in children (Santamaria, 2006). Availability to reduce nitrate in vegetables before human consumption is very advisable (Pinto et al., 2015).

Horticultural *Brassicaceae* plants are excellent source of fibres, vitamins, minerals and antioxidants (Brazaitytė et al., 2015a; Vale et al., 2014; Björkman et al., 2011). In addition, it can be cultivated under different growth conditions and growth as microgreens from a wide range of seeds all the year round. Microgreens have a central stem with two fully developed cotyledon leaves and mostly one pair of small true leaves. In recent years, microgreens are gaining popularity as a 'functional' food due to high nutritional quality and as a culinary ingredient due to intense flavour, colour and tender texture (Pinto et al., 2015; Brazaitytė et al., 2015b). Unlike sprouts, microgreens are produced under the light.

The aim of this study was to investigate the effect of industrially designed light-emitting diode (LED) lamp lighting on the nutritional quality of *Brassicaceae* microgreens.

Materials and Methods

Experiments were performed at the Institute of Horticulture, Lithuanian Research Centre of Agriculture and Forestry. Microgreen species of a *Brassicaceae* family were selected for their known ability to grow as nutrient rich vegetables under artificial LED light. Red pak choi (*Brassica rapa* var. *Chinensis* 'Rubi F_1'), tatsoi (*Brassica rapa* var. *rosularis*) and mustard (*Brassica juncea* L. 'Red Lion') microgreens were grown from seed to harvest time for 8 days in greenhouse during winter season. Day/ night temperatures of 20±2/ 18±2 °C were maintained and the relative air humidity was 55±5%. 1g of seeds (CN Seeds, Ltd., UK) were sown in 18 x 11 x 6 cm plastic pots filled with pea substrate (N 100-120, P_2O_5 30-80, K_2O 120-200 mg L^{-1}; microelements Fe, Mn, Cu, S, Mo, Zn; pH 5.5-6.5) (Profi 1, Durpeta, Lithuania). Four pots of each microgreen species for each treatment were used. The seeded pots were sprayed daily with tap water as needed. The industrially designed lamps (HL8000, HORTILED, Lithuania) used in the present study were 49,5 x 28 x 8 cm in area and built with 8 violet (420-430 nm), 16 blue (460-470 nm), 8 orange (610-615 nm), 3 red (620-630 nm), 56 red (660-670 nm), 8 white (contain blue (400-500 nm), green (500-600 nm) and red (600 - 700 nm)) LEDs (280 W). Three light treatments were imposed: (1) high pressure sodium lamps (SON – T Agro, Philips, UK) at ~150 µmol m^{-2} s^{-1} (HPS, as a control); (2) LED light intensity at ~150 µmol m^{-2} s^{-1} (LED 150); (3) LED light intensity at ~250 µmol m^{-2} s^{-1} (LED 250). The 16/ 8 light/ dark photoperiod of artificial light was maintained. Photosynthetic photon flux density (PPFD) was measured daily by photometer-radiometer RF 100 (Sonopan, Poland). The natural light was limited due to internal shading from installed lamps. The solar daily light integral (DLI) was measured at ~3.46±1.16 mol m^{-2} d^{-1} inside a greenhouse.

The edible biomass (cotyledons with stems) of microgreens (8 days old) was harvested. From each light treatment ten randomly selected plants were used for biometric measurements. The conjugated biological samples of fresh weight (FW) of randomly selected microgreens (0.5 – 1.0 per sample) were used for phytochemical analysis. Three analytical replications were performed for each phytochemical measurement. The leaf area (cm^2) was measured by an automatic leaf area meter (AT Delta-T Devices, UK). Ascorbic acid was determined spectrophotometrically according to the method published by Janghel et al. (2007). In this assay ascorbic acid reduces methyl viologen to form blue coloured free radical ion. The absorbance of the radical ion was measured by UV/ Vis spectrophotometer at 600 nm (M501, Spectronic Camspec Ltd., UK). The total phenolic content in the extract was determined according to the Folin-Ciocalteu method as outlined by Ragaee et al. (2006). Frozen in liquid nitrogen microgreen FW samples were extracted with 80 % methanol (1:10). Absorbance was measured at 765 nm (M501, Spectronic Camspec Ltd., UK). The results were expressed as gallic acid equivalents. Non-destructive measurements of the flavonol index of the microgreen leaf were performed using Dualex meter (Force-A, France). The 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was evaluated as described by Ragaee et al. (2006). The sample extracts were the same as the ones used in the total phenols assay. The absorbance was measured at 515 nm at 16 min of the reaction (M501, Spectronic Camspec Ltd., UK). The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity was determined according to Kadri et al. (2013). The methanolic (80%) extracts were diluted with $ABTS^+$ solution (A=0.7±0.2). The decrease of absorbance was measured after 15 min at 734 nm (M501, Spectronic Camspec Ltd., UK). The total anthocyanin content was estimated using pH-differential spectrophotometric method according to Stanciu et al. (2009). The absorption values of sample extracts were measured at 420, 520, 700 nm (Genesys 6, Thermospectronic, USA). Anthocyanins were expressed as cyanidin 3-glucoside equivalents, using a molar extinction coefficient of 25.740 mol^{-1} cm^{-1} and a molecular weight of 485 g mol^{-1}. In order to prepare the plant material for the determination of nitrates, plant tissue samples were dried in a drying oven (Venticell, MBT, Czech Republic) at 70 °C for 48 h.

Table 1

Growth parameters of microgreens cultivated under different light treatments

Light treatment	Hypocotyls length, cm	Plant height, cm	Leaf area, cm^2
Tatsoi			
HPS	4.59±0.46	5.63±0.36	0.73±0.15
LED 150	2.46±0.23[b]	3.52±0.27[b]	0.57±0.09
LED 250	2.81±0.33[b]	3.88±0.31[b]	0.57±0.07
LSD_{05}	0.53	0.49	0.20
Red pak choi 'Rubi F_1'			
HPS	3.66±0.33	4.86±0.51	0.89±0.03
LED 150	2.79±0.25[b]	3.98±0.30[b]	0.90±0.10
LED 250	2.59±0.35[b]	3.96±0.30[b]	1.00±0.10
LSD_{05}	0.30	0.46	0.37
Mustard 'Red Lion'			
HPS	3.39±0.22	4.71±0.24	0.91±0.10
LED 150	2.56±0.11[b]	3.84±0.15[b]	0.81±0.07
LED 250	2.05±0.06[b]	3.47±0.10[b]	0.85±0.13
LSD_{05}	0.22	0.24	0.27

Values are expressed as mean ± SD (n=10). LSD_{05} - Fisher's protected least significant difference (P≤0.05); b - the value is significantly lower than control (HPS). FW - fresh weight.

Nitrate concentration in microgreens was measured by a potentiometric method (Geniatakis et al., 2003) using ion meter (Oakton, USA) and combined nitrate ion selective electrode HI4113 (HANNA instruments, USA). The mineral elements (Ca (II), K (I), Mg (II), Na (I), P (I), Fe (II) and Zn (I)) contents in microgreens were determined by microwave digestion technique combined with inductively coupled plasma optical emission spectrometry (Marin et al., 2011). A complete digestion of dry microgreen material (0.5 g) was achieved with 65% HNO_3 and 30% H_2O_2 (5:3) using microwave digestion system Multiwave GO (Anton Paar GmbH, Austria). The mineral element profile was analysed by ICP – OES spectrometer (Spectro Genesis, SPECTRO Analytical Instruments, Germany).

All data are expressed on a fresh weight basis and presented as mean values ± standard deviation. All measurements were evaluated for significance by an analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the P≤0.05 level.

Results and Discussion

The results of biometric measurements of microgreens influenced by different light treatments are shown in Table 1. Differences of growth responses of three *Brassicaceae* species were clearly observed after providing LED 150 and LED 250 treatments.

The height of all microgreens of the LED 150 and LED 250 treatments was significantly lower (~1.4 – 1.6 times) compared with those of HPS treatment (Table 1). The same tendency was observed measuring

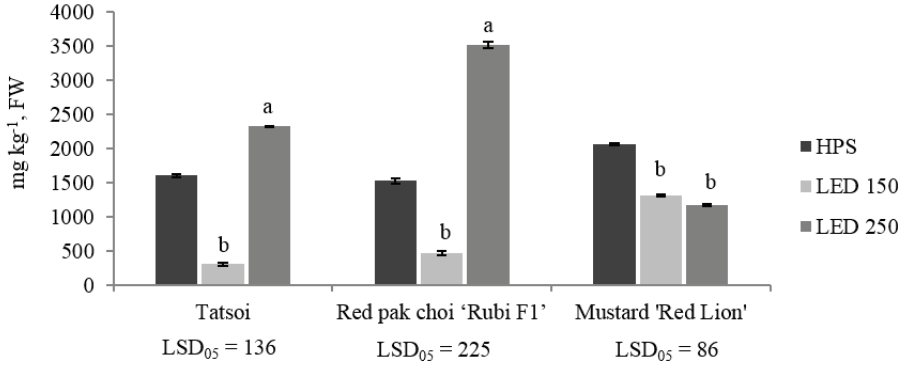

Figure 1. Nitrate contents in *Brassicaceae* microgreens cultivated under different light treatments
Values are expressed as mean ± SD (n=3). LSD_{05} –Fisher's protected least significant difference (P≤0.05); a – the value is significantly higher than control (HPS), b – the value is significantly lower than control (HPS). FW – fresh weight.

hypocotyl length. LED light prevented from an undesirable microgreen elongation. Increasing LED irradiation resulted in decreased hypocotyl length of red pak choi, mustard and tatsoi. It is known that hypocotyl elongation could be prevented by adding at least 15 µmol m^{-2} s^{-1} of blue light (Darko et al., 2014; Hoenecke et al., 1992). In our experiments, blue (460-470 nm) contains ~16% of common spectra of LED lamps used in LED 150 and LED 250 treatments (~24 and ~40 µmol m^{-2} s^{-1}, respectively). Otherwise, tatsoi treated under LED 150 had lower height and formed shorter hypocotyls in comparison with LED 250. No significant differences of leaf area were determined. However, microgreens cultivated under LED light looked smaller in comparison with plants grown under HPS.

Figure 1 shows the effect of LED light on the content of nitrate in microgreens. Nitrate content was greatly affected by LED 150 treatment – the significantly lower content of nitrate was determined in tatsoi and red pak choi (~5.2 and 3.3 times, respectively). Otherwise, higher LED irradiation (LED 250) led to an increased nitrate content in these microgreens. Nitrate content significantly decreased during LED 150 and LED 250 treatments in mustard (~1.5 and ~1.8, respectively).

As shown in Table 2, the artificial light treatments differentially affected the metabolic system of the investigated microgreens. According to Samuolienė et al. (2013b), a higher ascorbic acid content in red pak choi and tatsoi microgreens resulted in 110 µmol m^{-2} s^{-1} blue (455 nm), red (638 nm), red (665 nm) and far red (731) LEDs irradiation, while no significant differences between irradiation levels (110-545 µmol m^{-2} s^{-1}) were determined in mustard. In the present study, the increased biosynthesis of ascorbic acid was determined under LED 150 and LED 250 treatments in red pak choi (both ~1.2 times) and mustard (~1.1 and ~1.5 times, respectively), and under LED 250 in tatsoi (~1.8 times). It can be assumed that the metabolic pathway of ascorbic acid depended more on spectral quality than the irradiance level of LED light.

The greatest quantity of total phenols was found in the order: mustard> red pak choi> tatsoi (Table 2). Significantly higher content of phenols accumulated in these microgreens cultivated under LED 150 (~1.2 times, respectively to all species) and LED 250 treatments (~1.2, ~1.3 and ~1.5 times, respectively - tatsoi, red pak choi, mustard). A similar trend was also observed in flavonol index. The greatest flavonol index was determined in mustard, the lowest in tatsoi. Significantly higher flavonol index was in microgreens grown under both LEDs treatments. It can be noted that flavonol index changed slightly among LED 150 and LED 250 treatments. The significantly higher content of anthocyanins was determined only in microgreens grown under LED 150 treatment (~1.2 times, respectively to all species). An increased phenolic compound content in microgreens along LED lighting exposure influenced free radical scavenging activity. The methanol extracts of microgreens studied were analysed to determine their antioxidant activity against ABTS and DPPH radicals (Table 2). Microgreens grown under LED 150 and LED 250 treatments demonstrated significantly higher antioxidant activity evaluated using ABTS and DPPH methods.

Table 2

Antioxidant properties of *Brassicaceae* microgreens cultivated under different light treatments

Light treatment	Ascorbic acid, mg g^{-1}, FW	Total phenols, mg g^{-1}, FW	Total anthocyanins, mg g^{-1}, FW	Flavonols, index	DPPH•, µmol g^{-1}, FW	$ABTS^{+}$, µmol g^{-1}, FW
Tatsoi						
HPS	1.33±0.05	1.20±0.03	0.51±0.02	0.31±0,00	2.35±0.07	11.94±0.29
LED 150	1.43±0.08	1.42±0.04[a]	0.66±0.03[a]	0.50±0,03[a]	3.46±0.04[a]	16.69±0.05[a]
LED 250	1.88±0.08[a]	1.47±0.05[a]	0.56±0.06	0.49±0,05[a]	3.98±0.06[a]	17.43±0.15[a]
LSD_{05}	0.19	0.10	0.10	0.11	0.16	0.43
Red pak choi 'Rubi F_1'						
HPS	0.92±0.07	1.32±0.01	0.52±0.02	0.33±0,01	3.75±0.26	14.25±0.10
LED 150	1.08±0.05[a]	1.61±0.05[a]	0.63±0.03[a]	0.57±0,03[a]	4.28±0.28	20.86±0.24[a]
LED 250	1.12±0.05[a]	1.69±0.03[a]	0.53±0.01	0.59±0,04[a]	5.10±0.29[a]	21.86±0.15[a]
LSD_{05}	0.11	0.07	0.03	0.13	0.66	0.46
Mustard 'Red Lion'						
HPS	1.47±0.02	1.41±0.06	0.38±0.02	0.36±0,01	3.95±0.13	18.52±0.11
LED 150	1.58±0.03[a]	1.65±0.02[a]	0.44±0.03[a]	0.58±0,02[a]	4.19±0.12	23.28±0.08[a]
LED 250	2.26±0.06[a]	2.10±0.05[a]	0.43±0.02	0.58±0,02[a]	5.39±0.09[a]	28.07±0.21[a]
LSD_{05}	0.10	0.12	0.05	0.06	0.27	0.33

Values are expressed as mean ± SD (n=3). LSD_{05} - Fisher's protected least significant difference ($P \leq 0.05$); a - the value is significantly ($P \leq 0.05$) higher than control (HPS). FW - fresh weight.

Table 3

Mineral elements content in *Brassicaceae* microgreens cultivated under different light treatments

Light treatment	Ca (II)	K (I)	Mg (II)	Na (I)	P (I)	Fe (II)	Zn (I)
	mg g^{-1}, FW					µg g^{-1}, FW	
Tatsoi							
HPS	1.24±0.02	2.66±0.02	0.33±0.00	0.61±0.00	0.55±0.01	13.26±0.14	13.57±0.32
LED 150	1.23±0.02	3.06±0.04[a]	0.36±0.00[a]	0.80±0.00[a]	0.70±0.01[a]	21.55±0.24[a]	17.63±0.17[a]
LED 250	1.49±0.00[a]	3.04±0.01[a]	0.39±0.00[a]	0.76±0.00[a]	0.72±0.00[a]	16.79±0.07[a]	15.20±0.07[a]
LSD_{05}	0.04	0.05	0.01	0.00	0.02	0.25	0.38
Red pak choi 'Rubi F_1'							
HPS	1.32±0.02	2.84±0.01	0.38±0.01	0.62±0.00	0.56±0.00	14.50±0.14	13.20±0.06
LED 150	1.29±0.02	3.76±0.04[a]	0.39±0.01	1.18±0.00[a]	0.75±0.01[a]	17.67±0.27[a]	15.30±0.11[a]
LED 250	3.67±0.02[a]	7.90±0.02[a]	1.09±0.01[a]	1.80±0.00[a]	1.81±0.00[a]	59.92±0.24[a]	36.66±0.08[a]
LSD_{05}	0.04	0.06	0.01	0.01	0.01	0.55	0.22
Mustard 'Red Lion'							
HPS	0.36±0.00	0.91±0.01	0.09±0.00	0.25±0.00	0.18±0.00	3.42±0.06	1.49±0.05
LED 150	1.58±0.01[a]	3.55±0.02[a]	0.49±0.00[a]	0.92±0.01[a]	1.05±0.01[a]	23.98±0.22[a]	13.80±0.07[a]
LED 250	1.35±0.01[a]	2.62±0.02[a]	0.39±0.00[a]	0.74±0.00[a]	0.79±0.00[a]	30.63±0.27[a]	10.12±0.06[a]
LSD_{05}	0.01	0.03	0.00	0.01	0.01	0.27	0.17

Values are expressed as mean ± SD (n=3). LSD_{05} - Fisher's protected least significant difference (P≤0.05); a - the value is significantly higher than control (HPS). FW - fresh weight.

Nutritional quality depends not only on antioxidant properties, but also on the mineral elements occurring in plant tissues. The data obtained showed that mineral element content in microgreens can be changed by LEDs lighting (Table 3). The significantly higher contents of Ca, K, Mg, Na, P, Fe and Zn were determined after LED 150 and LED 250 treatments in microgreens. Photophysiological response of mineral elements to LED light may be generated through primary (saccharides) and secondary (flavonoids) metabolite biosynthesis pathways.

Conclusions

The industrially designed LED lamps lighting had a significant impact on *Brassicaceae* microgreen growth and nutritional quality. LED light prevented from undesirable microgreens elongation, led to increased contents of phytochemicals (phenols, total anthocyanin, flavonols and ascorbic acid) and mineral elements (Ca, K, Mg, Na, P, Fe and Zn). A ~150 µmol m^{-2} s^{-1} LED light treatment was rated as the optimal condition for microgreen growth and metabolism processes followed by ~250 µmol m^{-2} s^{-1} irradiation. Microgreens grown under ~250 µmol m^{-2} s^{-1} LED light treatment accumulated the highest contents of ascorbic acid, total phenols, flavonols, also DPPH and ABTS radicals scavenging activity that showed oxidative stress, and nitrates due to intense photosynthesis process.

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