Food Chemistry 228 (2017) 50–56

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Blue light dosage affects carotenoids and tocopherols in microgreens

Giedrė Samuolienė [a,c,*], Akvilė Viršilė [a], Aušra Brazaitytė [a], Julė Jankauskienė [a], Sandra Sakalauskienė [a], Viktorija Vaštakaitė [a], Algirdas Novičkovas [b], Alina Viškelienė [a], Audrius Sasnauskas [a], Pavelas Duchovskis [a]

[a] Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kaunas Str. 30, Babtai, LT-54333, Kaunas Dist., Lithuania
[b] Institute of Applied Research, Vilnius University, Saulėtekio dl. 9, bldg. III, LT-10222 Vilnius, Lithuania
[c] Aleksandras Stulginskis University, Studentu 11, Akademija, LT-53361 Kaunas Distr., Lithuania

ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form 30 January 2017
Accepted 31 January 2017
Available online 1 February 2017

Keywords:
Light-emitting diodes
Tocopherols
Xanthophylls
Mustard
Beet
Parsley

ABSTRACT

Mustard, beet and parsley were grown to harvest time under selected LEDs: 638 + 660 + 731 + 0% 445 nm; 638 + 660 + 731 + 8% 445 nm; 638 + 660 + 731 + 16% 445 nm; 638 + 660 + 731 + 25% 445 nm; 638 + 660 + 731 + 33% 445 nm. From 1.2 to 4.3 times higher concentrations of chlorophylls *a* and *b*, carotenoids, α- and β-carotenes, lutein, violaxanthin and zeaxanthin was found under blue 33% treatment in comparison to lower blue light dosages. Meanwhile, the accumulation of metabolites, which were not directly connected with light reactions, such as tocopherols, was more influenced by lower (16%) blue light dosage, increasing about 1.3 times. Thus, microgreen enrichment of carotenoid and xanthophyll pigments may be achieved using higher (16–33%) blue light intensities. Changes in metabolite quantities were not the result of changes of other carotenoid concentration, but were more influenced by light treatment and depended on the species. Significant quantitative changes in response to blue light percentage were obtained for both directly and not directly light-dependent metabolite groups.

1. Introduction

Various types of young vegetables, such as sprouts, microgreens and baby greens, are becoming very popular due to their higher nutritional value than mature plants or seeds. Microgreens are harvested at the first true leaf stage of growth and belong to the group of 'functional foods', and have higher levels of bioactive compounds (Sharma et al., 2012). Grown in a greenhouse with supplemental lighting and heating, microgreens can be produced throughout the entire year.

Assimilation lighting is one of the increasingly important factors for plants grown in a greenhouse. In contrast to photosynthesis, photoresponse is a more qualitative and wavelength-dependent reaction. According to Heuvelink et al. (2006), assimilation lighting, such as red and blue, which are important for the optimisation of photosynthesis, improves product quality, increases production levels and opens possibilities for year-round production.

The most commonly used light sources for supplemental lighting in greenhouses are high-pressure sodium (HPS) lamps; however, they increase energy inputs and CO_2 emission and cause light pollution (Morrow, 2008). Moreover, the main spectral composition of HPS lamps is in the yellow-orange-red region (Morrow, 2008), thus they may not meet plant requirements for the efficient action of photophysiological processes. Potentially more efficient light sources are based on light-emitting diodes (LEDs), due to their advantages, such as: light emission in a narrow band of the spectrum, high efficiency in comparison with traditional lamps, low-voltage operation, the possibility of regulating photosynthetically active photon flux density (PPFD) and low heat emission owing to conduction (Morrow, 2008). LEDs open up the possibility of their use for plant growth in a closed environment.

According to Mizuno, Amaki, and Watanabe (2011), three general photoreactions sense wavelengths from UV to the far-red light region, and the photophysiological response leads to specifically activated signalling pathways responsible for changes in photosynthesis, metabolism, biomass or development: (I) red and blue light are used in photosynthesis; (II) blue light is used in cryptochrome and phototropin reaction systems and by three members of the Zeitlupe family; and (III) red and far-red light enables reversible switching of the phytochrome system. Blue and red light are absorbed by both photosynthetic (chlorophylls and carotenoids) and photomorphogenetic (cryptochromes, phytochromes) receptors. On the other hand, far-red light is transmitted through plant

* Corresponding author at: Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kaunas Str. 30, Babtai, LT-54333, Kaunas Dist., Lithuania.
E-mail address: g.samuoliene@lsdi.lt (G. Samuolienė).

http://dx.doi.org/10.1016/j.foodchem.2017.01.144
0308-8146/

tissues causing indirect response from bioactive compounds, while other lights are absorbed (Hogewoning et al., 2010).

The importance of spectrum-dependent plant photophysiological responses is quite well established. In most studies, blue light is associated with biomass production and photosynthesis (Johkan, Shoji, Goto, Hahida, & Yoshihara, 2010). It is well known that for normal plant growth the optimum red to far-red ratio and a particular proportion of blue light are needed (Hogewoning et al., 2010; Matsuda, Ohashi-Kaneko, Fujiwara, Goto, & Kurata, 2004; Yorio, Goins, Kagie, Wheeler, & Sager, 2001). Blue LEDs, used alone or in combination with red light, increase the chlorophyll ratio (Li, Tang, Xu, Liu, & Han, 2012; Mizuno et al., 2011), the chlorophyll content (Li & Kubota, 2009; Samuolienė et al., 2011), and the level of phenolic compounds (Długosz-Grochowska, Kołton, & Wojciechowska, 2016; Liu et al., 2016). Analysis of dose-response curves allows a better understanding of plant environment interaction; whether the enhancement effect of blue light is a qualitative (photosynthetic capacity) or a quantitative (such as chloroplast movement, stomatal conductance) response or a combination of both (Hogewoning et al., 2010). Moreover, the irradiance level affects the leaf area, chlorophyll index and primary photosynthesis metabolites in microgreens (Samuolienė et al., 2013). A large number of photobiological studies have shown the importance of red and blue light on synergistic action of phytochromes and cryptochromes (Liu, Liu, Zhao, Pepper, & Lin, 2011; Mizuno et al., 2011; Yu, Liu, Klejnot, & Lin, 2010). Moreover, the action of antenna pigments (carotenoids, chlorophylls *a* and b) differs in light-harvesting reactions. Chlorophyll *a* acts uniquely as a primary electron donor and also transfers resonance energy in the antenna complex, ending in the reaction centre where specific receptors P680 and P700 are located. β-Carotene and lutein play a central role in PS II, harvesting blue light and transferring energy to photosystem reaction centres and protecting the photosynthetic apparatus against photo-oxidative damage (Jahns & Holzwarth, 2012). The effect of changing the amounts of carotenoids was also demonstrated by Bhandari and Sharma (2006), and was achieved by a combination of blue and red light.

The optimisation of light quality application, based on advancements in LED technology, suggests a light formula concept, which is defined as an optimised light quality component aimed at optimal photophysiological response, leading to high productivity and nutritional quality. Moreover, a light formula is cultivar-specific, dynamic and adjustable, and accompanied by the corresponding biological processes of plants (Liu, 2012). Knowledge about the response of photosynthesis metabolites to specific narrow-band light spectra is scarce. However, an opportunity to change light quantity, quality, duration or combination enables the use of LEDs for plant growth, to modulate development or metabolism and to influence a desired final product (Carvalho & Folta, 2014).

Carotenoids protect plants from photo-oxidative damage through thermal dissipation by means of the xanthophyll cycle (converting violaxanthin to zeaxanthin) (Stange & Flores, 2012). Chlorophylls are the precursors of tocopherols (Edelenbos, Christensen, & Grevsen, 2001), which are distinguished by their antioxidant properties. Thus, the goal of this study was to improve the antioxidant quality of microgreens by changing blue light dosage, with the main attention on carotenoids and tocopherols.

2. Materials and methods

2.1. General

α-Carotene, β-carotene, lutein, neoxanthin, violaxanthin and zeaxanthin were purchased from ChromaDex, USA; α-, β-, γ- and δ-tocopherol from Supelco, USA; acetone, methanol and *n*-hexane from Merck, Germany; ethyl acetate and $CaCO_3$ from Sigma-Aldrich, Germany.

2.2. Growing conditions

Microgreens of mustard (*Brassica juncea* L., 'Red Lion'), beet (*Beta vulgaris* L., 'Bulls Blood') and parsley (*Petroselinum crispum* Mill., 'Plain Leaved or French') were grown to harvest time (10, 14 and 13 days respectively) within a growth chamber in a peat substrate (Profi 1, JSC Durpeta, Lithuania) (pH 6, accuracy ± 0.01 pH units). The average amounts of nutrients (mg l^{-1}) in the substrate were as follows: N, 110; P_2O_5, 50; K_2O, 160. The microelements Fe, Mn, Cu, B, Mo and Zn were also present. Electrical conductivity (EC) varied between 1.0 and 2.5 m S/cm (±0.03 m S/cm). Depending on their size and weight, 1 g of mustard, 3 g of beet and 1 g of parsley seeds (CN Seeds, Ltd., UK) were sown per vessel (13 × 20 × 10 cm), which represented one replicate. Four vessels for each species were used. Vessels were arranged randomly and systematically rotated every day to improve the uniformity of the light environment. Plants were watered as needed. Experiments were performed in walk-in controlled-environment growth chambers (4 × 6 m). Day/night temperatures of 21 ± 2/17 ± 2 °C were established with a 16 h photoperiod and relative humidity of 50–60%. After the lighting experiment, conjugated biological samples of fresh matter from randomly selected plants were used for phytochemical and biometrical analyses.

2.3. Lighting system

Microgreens were cultivated under custom-made lighting equipment containing five separate modules for parallel growth runs under individually controlled illumination conditions. Light-emitting diode (LED) based lighting units, consisting of commercially available LEDs with emission wavelengths λ of blue (λ = 447 nm, LZ1-00B200, LED Engin Inc., USA), red (λ = 638 nm, LXHL-LD3C and λ = 665 nm, LXM3-PD01-0300, Philips Lumileds, USA) and far-red (λ = 731 nm, L735-05-AU, Epitex, Japan) were used for microgreen lighting. A photosynthetic photon flux density (PPFD) of 300 ± 3 μmol m^{-2} s^{-1} was maintained. The spectral composition was changed by adding a blue component from 0% up to 33% changing the *PPFD* level of red (638 nm) light (Table 1). PPFD was measured and regulated at the vessel level using a photometer–radiometer (RF-100, Sonopan, Poland).

2.4. Determination of carotenoids

Concentrations of α-carotene, β-carotene, lutein, neoxanthin, violaxanthin and zeaxanthin were evaluated according to the methods of Edelenbos et al. (2001) using HPLC on a YMC carotenoid column (3 μm particle size, 150 × 4.0 mm) (YMC, Japan). Carotenoids were extracted using 80% acetone (1 g of sample ground with liquid N 10 ml^{-1} of solvent), centrifuged (5 min, 349*g*) and filtrated through a 0.45 μm nylon membrane syringe filter (VWR International, USA). An HPLC 10A system (Shimadzu, Japan) equipped with a diode array (SPD-M 10A VP) detector was used for analysis. Peaks were detected at 440 nm. The identification of each compound was carried out by comparing retention time and spectra of the peaks with those previously obtained by the injection of standards. The mobile phase consisted of A (80% methanol, 20% water) and B (100% ethyl acetate). Gradient: 0 min; 20% B, 2.5 min; 22.5% B, 20–22.5 min; 50% B, 24–26 min; 80% B, 31–34 min; 100% B, 42–47 min; and 20% B, flow rate 1 ml min^{-1}.