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LED lighting and seasonality effects antioxidant properties of baby leaf lettuce

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ABSTRACT

We report on the application of supplementary light-emitting diode (LED) lighting within a greenhouse for cultivation of red, green and light green leaf baby lettuces (*Lactuca sativa* L.) grown under natural illumination and high-pressure sodium (HPS) lamps (16-h; *PPFD*-170 μmol m^{-2} s^{-1}) during different growing season. Supplementary lighting from blue 455/470 nm and green 505/530 nm LEDs was applied (16-h; *PPFD*-30 μmol m^{-2} s^{-1}). Our results showed that to achieve solely a positive effect is complicated, because metabolism of antioxidant properties in lettuce depended on multicomponent exposure of variety, light quality or seasonality. The general trend of a greater positive effect of supplemental LED components on the vitamin C and tocopherol contents was in order: 535 > 505 > 455 > 470 nm; on the total phenol content: 505 > 535 = 470 > 455 nm; on the DPPH free-radical scavenging capacity: 535 = 470 > 505 > 455 nm; on the total anthocyanins: 505 > 455 > 470 > 535 nm. Further investigations are needed for understanding the mechanism and interaction between antioxidants and light signal transduction pathways.

1. Introduction

Consumers increasingly demand safe and healthy food of high quality, the favourable ratio between light and temperature in our region offers an opportunity to produce high quality, rich in phytochemicals, products all year-round. Among various environmental factors, light is one of the most important variables affecting the phytochemical concentrations in plant. The usual grow lights available for secondary or supplemental lighting for greenhouses are fluorescent, high-pressure sodium and metal halide lamps (Wheeler, 2008). These lamps vary in spectral quality (Bourget, 2008), hereby resulting in differences in plant growth, development or metabolic response. High-pressure sodium (HPS) lamps mostly emit yellow–orange–red light but they are not rich in blue or green spectral components. In the controlled environment the lighting system is one of the most expensive but also the most effective components. Solid-state lighting technology, which is based on light-emitting diodes (LEDs), offers vast possibilities in horticultural lighting due to its ability to separate and mix different light spectra (Morrow, 2008).

The improvement strategies, through the photosynthetic (chlorophyll and carotenoid) (Hogewoning et al., 2010; Matsuda, Ohasshi-Kaneko, Fujiwara, & Kurata, 2007) and photomorphogenetic (phytochromes and cryptochromes) (Franklin & Whitelam, 2004) light receptors, can be developed for both major (carbohydrates, lipids and proteins) and minor (vitamins and minerals) constituents. The third group of photoreceptors is anthocyanins, red plant pigments, which in addition to other roles prevent photoinhibition and photodamage through the absorption of solar radiation that would otherwise be absorbed by chloroplast pigments (Gitelson, Merzlyak, & Chivkunova, 2001). Moreover, the understanding of the photoprotective function of anthocyanins is essential for physiological studies, besides higher plants vary in ability to synthesise them. According to Grusak (2002), significant quantitative changes are most feasible for minor constituents as they are found in the micrograms range. For example, at the genetic level minimal diversion of precursors and only limited modifications in the plant's ability to store or sequester the target phytochemical is needed. The nutrient content of vegetables is determined by genetic difference, environmental influence or horticultural type and by the interaction of all these components (Mou, 2009). Some antioxidant properties of different crops have been cultured by LED light radiation, such as lettuce (Li & Kubota, 2009; Ohashi-Kaneko, Takase, Kon, Fujiwara, & Kurata, 2007), spinach and komatsuna (Ohashi-Kaneko et al., 2007), pea seedlings (Wu et al., 2007), various seed species (Cevallos-Casals & Cisneros-Zevallos, 2009) or fruit (Giliberto et al., 2005). Thus, the importance of spectrum-depended plant photophysiological responses is quite well established. Although the physiological responses to spectral changes can vary among plant species or varieties, however it is considered that red light is important for photosynthetic apparatus and influences the transport of assimilates (Baroli, Price, Badger, & Caemmerer, 2008). Blue light is important for photosynthesis, chloroplast development, chlorophyll formation and chemical composition of plants, but the response highly depends

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on the dosage of blue light (Hogewoning et al., 2010). The effect of green light is similar to blue light, through the phytochromes and cryptochromes pigment photoreceptor proteins it participates in photosynthesis processes (Swatz et al., 2001). Moreover, the positive response of plant growth, photosynthetic capacity and phytochemicals to both blue and green light is up to 50% of total photon flux density (Baroli et al., 2008; Hogewoning et al., 2010). According to Folta and Maruhnich (2007), green light, through the inductive biological antagonistic systems, tends to reverse the processes established by red or blue light. Besides, as green light is efficiently transmitted through the plant tissues (Sun, Nishio, & Vogelmann, 1998), it may participate in reactions not directly exposed to the light stimulus. Lighting conditions might evoke the photo oxidative changes in plants, which lead to the increased contents and activity of antioxidative enzymes, flavonoid, ascorbate, carotenoid or tocopherol. In recent time many authors state that individual antioxidant compounds act in combination with other antioxidants, as interactions among them can affect total antioxidant capacity, producing synergistic or other effects (Kotíková, Hejtmánková, & Lachman, 2009; Kotíková, Lachman, Hejtmánková, & Hejtmánková, 2011; Niki & Noguchi, 2000). Moreover, the content of phytochemicals may fluctuate with the growing season (Gautier et al., 2005; Hamouz et al., 2010; Koudela & Petříkova, 2008; Mou, 2009). Thus, together with other genetic and agricultural implements, lighting might be the relevant tool for phytochemical-rich vegetable cultivation. It is known how the light irradiance level affect different plants (Carvalho, Santos, Viela, & Amancio, 2008), although the knowledge regarding the effect of light spectral quality for metabolic and photo oxidative processes is still limited. However, it is of economical and nutritional importance to explain this effect by scientific findings.

In this paper we report on the application of supplementary blue and green solid-state lighting within an industrial greenhouse and its effect on antioxidant properties for the cultivation of various baby leaf lettuces (*Lactuca sativa* L.) varieties grown under natural solar illumination and high pressure sodium lamps during different growing seasons.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, Germany), Folin–Ciocalteau reagent (Fluka, Germany), Na_2CO_3 (Sigma–Aldrich, Germany), ascorbic acid (Penta, Check Rep.), oxalic acid (Fluka, Germany), methyl viologen (Sigma–Aldrich, Germany), sodium hydroxide (Delta Chem, Chech Rep.), potassium chloride (Fluka, Germany), sodium acetate (Roth, Germany), HCl (Sigma–Aldrich, Germany), alpha, beta, gamma and delta tocopherol homologues (Supelco, PA, USA), methanol (POCh, Poland), hexane (Sigma–Aldrich, Germany), isopropanol (Merck, Germany).

2.2. Growing conditions and lighting system

Red leaf 'Multired 4', green leaf 'Multigreen 3' and light green leaf 'Multiblond 2' baby leaf lettuce (*L. sativa* L.) were grown to harvest time (about 22 days) within a greenhouse (sowing and harvest time in: November, January and March, Lithuania, lat. 55°N, 2010–2011) in a peat substrate (pH 5–6) under daylight with supplementary lighting provided by standard high-pressure sodium lamps (HPS) (16-h). Short-wavelength single-monochromatic lamps were designed using the four types of high-power (Luxeon III series (3 W), Philips Lumileds Lighting Company, USA) AlInGaN LEDs: 455 nm (LXHL-LR3C), 470 nm (LXHL-LB3C), 505 nm (LXHL-LE3C) and 535 nm (LXHL-LM3C). Supplementary lighting from light-emitting diodes (LEDs) was applied within a 16-h photoperiod. The generated fixed photosynthetic photon flux density (*PPFD*) of each type of solid-state lamps was 30 μmol m^{-2} s^{-1} and the *PPFD* of HPS lamps was 170 μmol m^{-2} s^{-1}. Reference plants were grown under HPS and the *PPFD* was equalised till 200 μmol m^{-2} s^{-1}. The day/night temperature was 17–22/14–17 °C and the relative air humidity was 50–60%. Plants were fertilised with a 0.2% ammonium nitrate solution once a week.

2.3. Determination of total phenolic compounds

The total content of phenolic compounds was determined in methanol extracts of lettuce (1 g of plant tissues grounded with liquid nitrogen and diluted with 10 ml of 80% methanol) using a calorimetric method (Ragaee, Abdel-Aal, & Maher, 2006). The extract was shaked for 30 min, then centrifuged at 2012*g* for 20 min. One millilitre of extract was diluted with 1 ml Folin–Ciocalteau reagent (Folin reagent diluted with bi-distilled water 1:10) and with 2 ml 7.5% Na_2CO_3 solution. The absorbance was measured after 20 min at 765 nm with Genesys 6 spectrophotometer (Thermospectronic, USA) against water as a blank. Gallic acid was used as a standard, and the total phenolics were expressed using a calibration curve.

2.4. DPPH radical-scavenging activity

The antioxidant activity of methanol extracts (1 g of plant tissues grounded with liquid nitrogen and diluted with 10 ml of 80% methanol) of the investigated lettuce was evaluated spectrophotometrically as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity (Ragaee et al., 2006). A Genesys 6 spectrophotometer was used for the analysis (Thermospectronic, USA). The extract was shaked for 30 min, then centrifuged at 2012*g* for 20 min. The absorbance scanned at 16 min at 515 nm was used for the calculation of the ability of seed material to scavenge DPPH free radicals (μmol g^{-1}).

2.5. Determination of vitamin C

Ascorbic acid content was evaluated using a spectrophotometric method (Janghel, Gupta, Rai, & Rai, 2007). Genesys 6 spectrophotometer was used for the analysis (Thermospectronic, USA). One gram of plant tissues was homogenised in 10 ml of 5% oxalic acid in order to avoid the loss of ascorbic acid, and centrifuged (5 min, 1691*g*). One millilitre of extract was mixed with 2 ml of 0.1% methyl viologen and 2 ml 2 M sodium hydroxide. The solution was shaked gently and allowed to stand for 2 min. The coloured radical ion was measured at 600 nm against the radical blank.

2.6. Determination of total anthocyanins

Thirty milligrams of plant material were homogenised in 5 ml of 2% HCl methanol solution for 48 h, and centrifuged (15 min, 1446*g*). The total amount of anthocyanins was determined using spectrophotometric method proposed by Stanciu, Lupşor, and Sava (2009). The pH-differential method is based on coloured oxonium predomination (0.025 M potassium chloride buffer, pH 1) versus colourless hemiketal (0.4 M sodium acetate buffer, pH 4.5) reaction. The absorption values of extracts were measured at 420, 520 and 700 nm wavelengths. Anthocyanins were expressed as mg cyanidin 3-glucoside equivalent 100 g^{-1} fresh weight, using a molar extinction coefficient 25,740 M^{-1} cm^{-1} and a molecular weight of 485 g mol^{-1}.