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IMPACT OF CO_2 ON QUALITY OF BABY LETTUCE GROWN UNDER OPTIMIZED LIGHT SPECTRUM

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Abstract. The cost and yield are two the most important criteria in agriculture by which optimization of environmental factors are needed to carry out. In the present study, we investigated the efficient lighting spectrum and elevated CO_2 concentration for cultivating healthier plants more rapidly. One of the aims of our study is to optimize LEDs light spectrum for healthier vegetable production in greenhouses and maximum economical benefits for growers. The impact of elevated carbon dioxide (CO_2) concentration on antioxidant and nutritional properties of green leaf 'Multigreen 3' and red leaf 'Multired 4' baby leaf lettuce (*Lactuca sativa* L.), grown under optimized light spectrum was investigated. CO_2 concentrations of 0.963 g · $dm^{-3}$and 1.938 g · dm^{-3} were maintained in the growth chambers. Lettuce was grown under four wavelength (640, 455, 660 and 735 nm) light-emitting diode based (LED) illumination. Under 0.963 g · dm^{-3} CO_2 conditions, 'Multired 4' lettuce represented higher antioxidant value due to higher ascorbic acid, anthocyanin, tocopherol contents and higher sucrose concentration, as compared to 'Multigreen 3' lettuce. Higher CO_2 concentration (1.938 g · dm^{-3}) had uneven effect on the quality of both baby leaf lettuce cultivars. Red leaf lettuce reacted to the higher CO_2 level by lowered α tocopherol, ascorbic acid concentrations and significantly higher glucose contents in their leaves, when green leaf lettuce – contrarily – contained higher ascorbic acid and α tocopherol concentrations under 1.938 g · dm^{-3} of CO_2.

Key words: ascorbic acid, carbohydrates, chlorophyll, phenols, tocopherol

INTRODUCTION

CO_2 is one of the most studied environmental stimuli due to the concerns about its rising concentration and possible impact on agro-ecosystems [Ainsworth et al. 2007]. he greenhouse industry has taken advantage of manipulating CO_2 and lighting to the bene-

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fit of crops grown in controlled conditions for extended periods [Lindhout et al. 1990]. Elevated atmospheric CO_2 concentrations have two physiological effects on plants: it stimulate the rate of photosynthesis in leaves because of higher CO_2 concentrations in leaf mesophyll and cause stomata partly to close, thereby reducing the water loss during transpiration. Thereafter, under long-term elevated CO_2 conditions, secondary effects may occur, such as changes in the plant phytochemical composition [Poorter et al. 2003] and antioxidant activity [Wang 2006]. The effect of CO_2 on photosynthesis and dry matter accumulation is mediated by the light availability. In greenhouse crops, after increasing the CO_2 concentration from 0.7 to 1.53 g $\cdot$ dm^{-3}, about 30% lower artificial light flux [Spaargaren 2001] is necessary to maintain the same growth rate and normal development in the same environment due to increased rate of photosynthesis and decreased respiration intensity [Moe 2006]. Light is often limiting factor for plant growth and productivity, and the use of artificial radiation became an important subject for investigations in Northern countries already in early last century. The grow lights available for secondary or supplemental lighting in greenhouses are fluorescent, high-pressure sodium, metal halide lamps [Wheeler 2008]. Light-emitting diodes (LEDs) also represent a promising technology for the greenhouse industry that has technical advantages over traditional lighting sources, but are only recently being tested for horticultural applications [Mitchell et al. 2012]. The LED lighting may be customized for the specific horticultural objectives and optimized for maximum production without wasting energy on nonproductive wavelengths [Tamulaitis et al. 2004, 2005]. The combinations of red and blue light emitting diodes were reported to be efficient enough for successful lettuce growth and photosynthesis [Brazaitytė et al. 2006, Massa et al. 2008, Ilieva et al. 2010]. There are also some data about the improvement of antioxidant activity in baby leaf lettuce [Li et al. 2009, Samuolienė et al. 2012] as well as in other horticultural crops [Saebo et al. 1995, Goins et al. 1997, Wu et al. 2007] invoked by light-emitting diode illumination. However, it is less known about the interaction of elevated CO_2 under solid-state lighting on lettuce antioxidant biosynthesis. In order to apply the results to lettuce quality and production, we considered it important to investigate the light-quality effects when provided as supplemental light rather than as the sole source of light. An optimal strategy of light quality regulation will help in designing the growth chamber light environment to obtain maximum economic benefits for vegetable growers [Kuan-Hung et al. 2013].

The aim of this study was to examine the effect of different CO_2 concentrations on biochemical constituents and properties relevant for nutritional value of baby leaf lettuce, grown under optimized light spectrum.

MATERIALS AND METHODS

Growth conditions and plant material. Experiments were carried out in 40 m^3 phytotron chambers under controlled environment conditions: the day/night temperatures of 21°C/17°C ±2ºC and a photoperiod of 18 hours were maintained. The relative air humidity was 75–85%. Air temperature and humidity was measured using S3120 temperature and humidity data logger (Pasific Sensor Technologies, Australia). Red leaf

'Multired 4' and green leaf 'Multigreen 3' baby leaf lettuce (*Lactuca sativa* L.) were seeded in peat substrate (pH 5–6), where grew until harvest time (about 22 days). The amount of nutrients (mg dm^{-3}) in substrate was as follows: N 70, P 30, K 160, Ca 250, and Mg 50. Three seeds were sown per 120 ml vessel; 28 cell plastic plug tray for each lettuce cultivar was used. Two CO_2 concentrations of 0.963 g · dm^{-3} and 1.938 g · dm^{-3}were maintained and measured using CO_2 transmitter (Regin CO_2RT, Sweden). The solid-state lighting modules, containing four types of high-power light-emitting diodes (LEDs): Luxeon^{TM} type LXHL-LR3C (peak wavelength λ = 455 nm), Luxeon^{TM} type LXHL-MD1D (λ = 640 nm) (Lumileds Lighting, USA) and L660-66-60 (λ = 660 nm) and L735-05-AU (λ =735 nm) (Epitex, Japan) were used. The generated photosynthetic photon flux density (PPFD) of each type of light-emitting diodes was 16.2, 170, 8.2 and 2.5 µmol · m^{-2} · s^{-1} respectively, and total generated PPFD was 200 µmol · m^{-2} · s^{-1}. The PPFD was measured using a photometer-radiometer RF-100 (Sonopan, Poland). The surface area per light treatment was about 0.5 m^2.

After 22 days after seeding, lettuce plants from the central part of the illuminated area were harvested, excluding marginal plants due to possible side effect. Conjugated biological samples of the green matter of 5 randomly selected plants from each treatment were used for analysis. Three replications were performed for each phytochemical measurement. All data are expressed on a fresh weight basis.

Determination of ascorbic acid content. Ascorbic acid was assessed by a spectrophotometric method [Janghel et al. 2007], which is based on the ability of the ascorbate ion to reduce methyl viologen to stable blue coloured free radical ion. Samples were prepared from 1 g fresh plant material that was homogenized with 10 ml of 5% metaphosphoric acid and centrifuged at 2012 × g for 5 min. 2 ml of methyl viologen and 2 ml of 2 M NaOH were mixed with 1 ml sample extraction. After 2 min, absorption was measured at 600 nm wavelength using a spectrophotometer (Genesys 6, Thermospectronic, USA). The concentration was determined using the calibration data of ascorbic acid standards.

Determination of total content of phenolic compounds. The total content of phenolic compounds was determined in methanol extracts of lettuce leaves (1 g of fresh tissue ground with liquid nitrogen and diluted with 10 ml of 80% methanol) using a colorimetric method [Ragaee et al. 2006]. The extract was shaken for 30 min and centrifuged at 2012 × g for 5 min. 1 ml of extract was diluted with 1 ml of Folin-Ciocalteau 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 spectrophotometer (Genesys 6, Thermospectronic, USA), using water as a blank. Total contents of phenolic compounds were determined according the calibration curve, using gallic as a standard.

Determination of total content of anthocyanins. The total content of anthocyanins was determined using spectrophotometric method [Stanciu et al. 2009]. 30 mg of fresh tissue grounded with liquid nitrogen and diluted with 5 ml of 2% HCl methanol. After 48 h extraction, the extract was centrifuged at 2012 × g for 5 min. For the spectrophotometric measurements 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) were prepared. The pH-differential method is based on colored oxonium predomination versus colorless hemichelate 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 molar extinction coefficient $\epsilon = 26.900$ and molecular weight of 485 $g \cdot mol^{-1}$.

Determination of alpha, beta, gamma and delta tocopherols. Alpha, beta, gamma and delta tocopherols (α-T, β-T, γ-T and δ-T, respectively) content was evaluated using high-performance liquid chromatography (HPLC) on Pinacle II silica column [Fernandez-Orozco et al. 2003], 5 µm particle size, 150 × 4.6 mm (Restek, USA). Tocopherols were extracted using pure hexane (1:10), centrifuged (5 min, 349 × g) and filtereted through 0.45 µm PTFE membrane syringe filter (VWR International, USA). The HPLC 10A system, equipped with RF-10A fluorescence detector (Shimadzu, Japan) was used for analysis. Peak was detected using an excitation wavelength of 295 nm and emission wavelength of 330 nm. The mobile phase was 0.5% isopropanol in hexane, flow rate – 1 ml min^{-1}.

Determination of soluble sugars. Sucrose, fructose, mannose and glucose were analysed using high performance liquid chromatographic (HPLC) method. About 1 g of fresh plant tissue was grounded and diluted with 4 ml + 70ºC analytical grade water. The extraction was carried out for 24 h. The samples were filtered using cellulose acetate (pore diameter 0.22 µm) syringe filters. Analyses were performed on Shimadzu HPLC 10A (Shimadzu, Japan) chromatograph with a refractive index detector (RID 10A). Separation of carbohydrates was performed on SC-1011 column (300 × 4.6 mm) (Shodex, Japan). Mobile phase – double distilled water, oven temperature was maintained at + 80º, flow rate – 1 ml min^{-1}.

Determination of photosynthetic pigments. Fresh leaf tissue 1g was grounded with 0.5 g $CaCO_3$, diluted 1:250 with pure acetone and filtered through cellulose filter. Chlorophyll *a*, *b* and carotenoids were measured by spectrophotometric method [Gavrilenko 2003]. The absorption was measured at 644 nm, 662 nm and 440.5 nm, for chlorophyll *a, b* and carotenoids respectively.

Determination of the ($DPPH^{\bullet}$) free-radical scavenging capacity. The antioxidant activity of lettuce leaf methanolic was evaluated spectrophotometrically (Genesys 6, Thermospectronic, USA), as the ability to scavenge 2,2–diphenyl–1–picrylhydrazyl ($DPPH^{\bullet}$) free-radicals. 1 g of fresh tissue was ground with liquid nitrogen and diluted with 10 ml of 80% methanol. The extract was shaken for 30 min and centrifuged at 2012 × g for 20 min. The absorbance was scanned at 515 nm for 16 min until the plato phase. The ability of lettuce material to scavenge DPPH free radicals was expressed in $\mu mol \cdot g^{-1}$.

Statistical treatment. Five biological and three analytical replications of all type of analysis were performed for each treatment. Data analysis was processed using one-way analysis of variance Anova test at the confidence level $p \leq 0.05$. Values are presented as the means ± the standard deviation. Data was processed using MS Excel software (version 7.0).

RESULTS

Our results demonstrated that elevated CO_2 concentration with solid-state lighting resulted in slightly decreased total anthocyanin concentration in ‘Multigreen 3’ lettuce, meanwhile remained unaffected in ‘Multired 4’ lettuce (tab. 1).

Table 1. The variation of antioxidant compounds in baby-leaf lettuce, cultivated under different CO_2 concentrations

Cultivar	CO_2 concentration	DPPH• µmol · g^{-1} FM	Total phenols mg · g^{-1} FM	Ascorbic acid mg · g^{-1} FM	Total anthocyanins mg · g^{-1} FM
Multired 4	0.963 g · dm^{-3}	10.05 ± 0.09	1.38 ± 0.02	3.22 ± 0.27	80.55 ± 1.63
	1.938 g · dm^{-3}	9.85^{a} ± 0.07	1.29 ± 0.03	2.65 ± 0.02	79.78 ± 3.11
	$LSD_{0.05}$	0.11	0.08	0.71	8.01
Multigreen 3	0.963 g · dm^{-3}	9.99 ± 0.04	1.29 ± 0.02	0.99 ± 0.06	71.22 ± 2.54
	1.938 g · dm^{-3}	9.36^{a} ± 0.01	1.09 ± 0.04	1.69^{a} ± 0.21	58.11^{a} ± 1.34
	$LSD_{0.05}$	0.15	0.17	0.44	9.12

Letters a indicate significant differences at $p \leq 0.05$. FM – fresh mass

Table 2. The content of tocopherol (µg · g^{-1}, FW) in baby-leaf lettuce cultivated under different CO_2 concentrations

Cultivar	CO_2 concentration	Alfa µg · g^{-1} FM	Beta µg · g^{-1} FM	Gama µg · g^{-1} FM	Delta µg · g^{-1} FM
Multired 4	0.963 g · dm^{-3}	2.25 ± 0.05	0.02 ± 0.01	14.36 ± 0.14	0.19 ± 0.01
	1.938 g · dm^{-3}	0.93^{a} ± 0.01	0.01^{a} ± 0.01	7.02^{a} ± 0.08	0.13^{a} ± 0.01
	$LSD_{0.05}$	0.44	0.01	1.13	0.04
Multigreen 3	0.963 g · dm^{-3}	0.69 ± 0.04	0.01 ± 0.01	9.98 ± 0.12	0.14 ± 0.01
	1.938 g · dm^{-3}	1.82^{a} ± 0.03	0.02^{a} ± 0.01	9.37 ± 0.17	0.12^{a} ± 0.01
	$LSD_{0.05}$	0.17	0.01	1.22	0.01

Mean differences with the same letters are significantly ($p \leq 0.05$). FM – fresh mass

Table 3. The soluble carbohydrates in baby-leaf lettuce cultivated under different CO_2 concentrations

Cultivar	CO_2 concentration	Sucrose mg · g^{-1} FM	Glucose mg · g^{-1} FM	Fructose mg · g^{-1} FM
Multired 4	0.963 g · dm^{-3}	2.08 ± 0.05	0.23 ± 0.01	2.64 ± 0.07
	1.938 g · dm^{-3}	2.26^{a} ± 0.04	1.06^{a} ± 0.09	3.40^{a} ± 0.08
	$LSD_{0.05}$	0.05	0.28	0.15
Multigreen 3	0.963 g · dm^{-3}	4.44 ± 0.13	0.44 ± 0.01	2.58 ± 0.02
	1.938 g · dm^{-3}	3.73 ± 0.12	0.58^{a} ± 0.02	2.43 ± 0.14
	$LSD_{0.05}$	0.63	0.08	0.37

Letters a indicate significant differences at $p \leq 0.05$. FM – fresh mass

The DPPH• free-radical scavenging activity significantly decreased in both lettuce cultivars (tab. 1). However, the elevated CO_2 concentration had no significant effect on total phenols content in baby leaf lettuce. The content of ascorbic acid significantly increased in green leaf lettuce, but remained unaffected in red leaf lettuce (tab. 1). In

contrary to ascorbic acid, the content of the total anthocyanins significantly decreased in green leaf lettuce and remained unaffected in red leaf under elevated CO_2 conditions. The trend of changes in ascorbic acid content (tab. 1) was similar to changes in total content of tocopherols. Under 1.938 g · dm-3 CO_2 conditions, red leaf lettuce contained less of all tocopherol isomers, when green leaf lettuce contained significantly higher amount of α tocopherols, as compared to lettuce, cultivated under 0.963 g · dm^{-3} CO_2 conditions (tab. 2).

Table 4. The content of photosynthetic pigments in baby-leaf lettuce cultivated under different CO_2 concentrations

Cultivar	CO_2 concentration	Chlorophyll *a* mg · g^{-1} FM	Chlorophyll *b* mg · g^{-1} FM	Carotenoids mg · g^{-1} FM
Multired 4	0.963 g · dm^{-3}	0.48 ± 0.05	0.32 ± 0.10	0.22 ± 0.02
	1.938 g · dm^{-3}	0.35 ± 0.07	0.20 ± 0.02	0.17^{a} ± 0.01
	LSD_{0-05}	0.15	0.14	0.05
Multigreen 3	0.963 g · dm^{-3}	0.44 ± 0.02	0.26 ± 0.10	0.19 ± 0.01
	1.938 g · dm^{-3}	0.34 ± 0.06	0.20 ± 0.02	0.15 ± 0.02
	LSD_{0-05}	0.10	0.20	0.05

Letters a indicate significant differences at $p \leq 0.05$. FM – fresh mass

Under elevated CO_2, the significant increase of all soluble carbohydrates was determined in 'Multired 4' lettuce: the amount of sucrose was determined 1.08 times, glucose – 4.5, fructose – 1.28 times higher. In 'Multigreen 3' lettuce only increase in glucose contents by 1.3 times was observed (tab. 3). Different carbon dioxide concentrations had no significant effect on the contents of photosynthetic pigments; only the content of carotenoids under elevated CO_2 concentration was significantly lower in 'Multired 4' lettuce (tab. 4).

DISCUSSION

It is known, that CO_2, light intensity, and other environmental parameters have a significant influence on greenhouse vegetable growth, productivity and quality attributes such as color and taste [Gruda 2005] and can affect the antioxidant content and antioxidant activity Wang 2006]. Our results demonstrated that elevated CO_2 concentration with solid-state lighting resulted in slightly lower total anthocyanin concentration in 'Multigreen 3' lettuce, unaffected anthocyanin contents in 'Multired 4' lettuce. The $DPPH^{\bullet}$ free-radical scavenging activity significantly decreased in both lettuce varieties (tab. 1). If plants exposed to elevated CO_2 experience less oxidative stress thus reduce the need for antioxidant protection [Vurro et al. 2009]. However, the elevated CO_2 concentration had no significant effect on total phenols of baby leaf lettuce. The content of

ascorbic acid significantly increased in green leaf lettuce, but remained unaffected in red leaf lettuce (tab. 1). Meanwhile, the trend of ascorbic acid content changes is similar to changes in total content of tocopherols (tabs 1, 2). Thus, the increased amount of ascorbic acid in green leaf 'Multigreen 3' lettuce (tab. 1) might contribute to the elevated CO_2 concentration, cultivar, or ascorbate synthesis from glucose and significantly effected the accumulation of total tocopherol and its homologues. However, opposite results with 'Multired 4' lettuce were observed (tab. 2). On the other hand, elevated CO_2 allows for greater antioxidant synthesis via enhanced rates of photosynthesis and soluble carbohydrates production [Rao et al. 1995].

The impact of elevated CO_2 concentrations on the oxidative status of leaves has been examined in various plant species [Cheeseman 2006, Qiu et al. 2008] where it seems to cause a decrease in the activity of some antioxidant enzymes and in the concentration of some antioxidants like tocopherols [Wustman et al. 2001]. According to our results, the enhanced CO_2 concentration had significant effect on accumulation of α and β tocopherol and negative effected on the content of δ tocopherol in 'Multigreen 3' lettuce, whereas the significant increase of all tocopherol isomers was observed in 'Multired 4' lettuce (tab. 2).

According to our data under elevated CO_2 concentration the significant increase of all soluble carbohydrates was determined in 'Multired 4' lettuce, while only increase of glucose was observed in 'Multigreen 3' lettuce (tab. 3). It suggests that the changes in atmospheric CO_2 concentration affect the balance of carbohydrate metabolism [Urbonavičiūtė et al. 2006]. Nevertheless, the elevated CO_2 concentration stimulated soluble carbohydrate accumulation; it had no significant effect on the content of photosynthetic pigments [Juknys et al. 2011]. Only the content of carotenoids under elevated CO_2 concentration was significantly lower in 'Multired 4' lettuce (tab. 4).

CONCLUSIONS

In summary, red leaf 'Multired 4' lettuce represented higher antioxidant value due to higher ascorbic acid, anthocyanin, tocopherol contents, and lower sucrose concentration, as compared to green leaf 'Multigreen 3' lettuce, cultivated under 0.963 g · dm^{-3} CO_2 concentration. Higher CO_2 concentrations also had uneven effect in both baby leaf lettuce cultivars. Red leaf lettuce reacted to the higher CO_2 level by lowered α tocopherol, ascorbic acid concentrations and significantly lower glucose contents in their leaves, when green leaf lettuce – contrarily – contained higher ascorbic acid and α tocopherol concentrations under 1.938 g · dm^{-3} of CO_2. Based on our results, elevated atmospheric CO_2 concentration under LED_S lighting affected the balance of antioxidant and carbohydrate content in lettuce plants.

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WPŁYW JAKOŚCI CO_2 NA MŁODĄ SAŁATĘ UPRAWIANĄ POD ZOPTYMALIZOWANYM SPEKTRUM ŚWIATŁA

Streszczenie. Koszty i plon to dwa najważniejsze kryteria w rolnictwie, według których należy wybierać optymalne czynniki środowiska. W niniejszym badaniu oceniano spektrum światła i podwyższone stężenie CO_2 w celu szybszej hodowli zdrowych roślin. Jednym z celów badania było zoptymalizowanie spektrum światła LED w celu produkcji zdrowszych warzyw w cieplarniach oraz dla maksymalnych korzyści ekonomicznych hodowców. Badano wpływ podwyższonego stężenia dwutlenku węgla (CO_2) na cechy anytoksydacyjne i żywieniowe młodej sałaty zielonej ‘Multigreen 3’ i czerwonej ‘Multired 4’ (*Lactuca sativa* L.) hodowanych w warunkach zoptymalizowanego spektrum światła. W komorach wzrostu utrzymywano stężenia CO_2 wynoszące 0,963 g · dm^{-3} oraz

1,938 g · dm^{-3}. Sałata wzrastała oświetlana diodą emitującą światło (LED) o czterech długościach fal (640, 455, 660 i 735 nm). W warunkach 0,963 g · dm^{-3} CO_2 sałata 'Multired 4' miała większą wartość antyoksydacyjną ze wzdględu na wyższy poziom kwasu askorbinowego, antocyjanin, tokoferolu orac wyższe stężenie sacharozy w porównaniu z sałatą 'Multigreen 3'. Wyższe stężnie CO_2 (1,938 g · dm^{-3}) miało nierówny wpływ na jakość obu odmian sałaty. Sałata czerwona reagowała na wyższy poziom CO_2 obniżonym α-tokoferolem i stężeniem kwasu askorbinowego oraz istotnie wyższą zawartością glukozy w liściach, natomiast zielona sałata miała wyższe stężęnie kwasu askorbinowego i α-tokoferolu w warunkach 1,938 g · dm^{-3} of CO_2.

Słowa kluczowe: kwas askorbinowy, węglowodany, chlorofil, fenole, tokoferol

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