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The possibility to control the metabolism of green vegetables and sprouts using light emitting diode illumination

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The influence of different solid-state lighting spectrum on the metabolism, as the index of internal nutritional quality of green vegetables (lettuce, onion leaves, dill, parsley, basil, marjoram, wheat grass, barley grass and leafy radish), was investigated. Plants were illuminated with the basal red 640 nm light emitting diodes, supplemented with 450, 660 and 735 nm components for three to five days in different developmental phases. Reference plants were grown under high-pressure sodium lamps. The content of carbohydrate, nitrate, vitamin C, phenolic compounds and the antioxidant activity of the selected vegetable extracts were evaluated. According to the obtained results, the red light effect on the metabolic system depends on the plant specie. The lettuce, marjoram, wheat grass and leafy radish were found to be the potentially suitable for cultivation under the light emitting diode lighting, due to the positive increase in monosugar content, reduction of nitrates, bigger vitamin C content and promoted antioxidant activity.

Key words: leafy vegetables, light quality, metabolism.

Introduction. Light is one of the most important environmental factors, acting on plants not only as the sole source of energy, but also as the source of external information, affecting their growth, development and metabolism. Plants are empowered with an array of photoreceptors controlling diverse responses to light parameters, such as spectrum, intensity, direction, duration: the red and far-red-absorbing phytochromes, the blue and UV-A light absorbing cryptochromes, phototropins, and other implied photoreceptors, absorbing in UV-A and green regions (Devlin et al., 2007). Spectral light changes evoke different morphogenetic and photosynthetic responses that can vary among different plant species. Such photo-response is of practical importance in recent plant cultivation technologies, since feasibility to tailor illumination spectra purposefully enables one to control plant growth, development, and nutritional quality. The recent progress in solid-state lighting based on light-emitting diodes (LEDs) facilitated and extended the research possibilities in this field and developed the outset for new progressive vegetable-growing technologies.

It is known, that the red light is of major importance for plants (Kopcewicz, Lewak, 1998) – for the development of the photosynthetic apparatus and morphogenetic processes due to light-induced transformations in phytochrome system (Furuya, 1993). Blue light, also essential for plants, affects the formation of chlorophyll, stomata opening, and photomorphogenesis (Dougher, Bugbee, 1998; Shuerger et al., 1997; Heo et al., 2002). Phytochromes, principally thought of as red/far-red reversible pigments, yet they absorb well in the blue portion of the spectrum. Moreover, these receptors are extremely sensitive to all light qualities across the spectrum and will initiate responses to minor illumination form the light qualities across the spectrum (Folta, Maruhnich, 2007). Nevertheless, there are still no clear findings, how light components, such as green, yellow or violet affect plant vitality. Herewith the main growth and development processes, light affects the primary and secondary metabolism reactions, thus acting on plant vital state and the nutritional quality of vegetable food. The knowledge, regarding plant metabolism regulation by light spectral quality is still limited; although it is of economical and ecological importance to explain this effect by scientific findings. Especially for green vegetable cultivation, such as lettuce, onion leaves, dill, parsley et al., which are intensively grown during the seasons of low solar irradiation. Moreover, leafy vegetables and sprouts are the main source of bioactive compounds, positively acting on human organism. Thus, elucidation of the illumination spectrum, which is optimal in the view of vegetable growth rate, healthy development and well-balanced metabolism, is of relevant value. The objective of this study was to evaluate the effect of different light qualities, provided by light emitting diodes (LEDs) on the indices of nutritional quality in green vegetables.

Object, methods and conditions. Lighting experiments were performed at the Lithuanian Institute of Horticulture in 2006–2008. Different green vegetables were cultivated under solid-state lighting units. Parsley (*Petroselinum crispum* (Miller) Nyman ex A. W. Hill, cv. 'Moss curled'), marjoram (*Majorana hortensis* Moench.), onion leaves (*Allium cepa* L.), lettuces (*Lactuca sativa* cv. 'Grand rapids'), dills (*Anethum graveolens* L. cv. 'Szmaragd') and basil (*Ocimum basilicum* L.) were placed under investigated lighting when plants matured, before gathering and illuminated for three days. Therefore, vegetables were grown in the phytotron chambers in peat substrate according to appropriate agrotechnical conditions. A photoperiod of 18 h was used and the temperature of 21/15 °C (day/night) was maintained throughout the experiment. Wheat grass (*Triticum aestivum* L. cv. 'Pirvinta'), barley grass (*Hordeum vulgare* L. cv. 'Aura') and leafy radish (*Raphanus sativus* L. cv. 'Tamina') were grown under light emitting diode (LED) lighting from the sowing time, as their growth period until consumption is relatively short: plants were kept there for five days after germination. Two treatments under the illumination units, containing different combinations of light emitting diodes were performed as presented in Table. The treatment L1 contained red basal component (640 nm, delivered by AlGaInP LEDs Luxeon™ type LXHL-MD1D, *Lumileds Lighting*, USA)) and three supplemental components: blue (455 nm, LuxeonTM type LXHL-LR5C, *Lumileds Lighting*, USA), red (662 nm, L660-66-60, *Epitex*, Japan,) and far red (735 nm, L735-05-AU, *Epitex*, Japan). The treatment L2 contained single basal 640 nm component. The total photon flux density in both treatments was about 200 µmol m^{-2} s^{-1}. Reference plants were grown under illumination by high-pressure sodium lamps (HPS, Son-T Agro *Philips*, USA).

Table. The light emitting diode combinations and flux densities
Lentelė. Kietakūnių šviestukų kombinacijos ir fotonų srauto tankiai

	Photon flux density Fotonų srauto tankis (μmol $m^{-2}s^{-1}$)			
Treatment Apšvietimo derinys	455 nm	640 nm	662 nm	735 nm
L1	20	167	7	6
L2	-	200	-	-

After the determined illumination period carbohydrate, vitamin C, nitrate, phenolic compound content were determined and the antioxidant activity of selected plants extracts was evaluated.

Samples for determination of carbohydrates were prepared by grinding 1 g of leaf fresh matter and extracting it with 4 mL hot bidistiled water. After 24 h, the extract was filtered through cellulose and membrane (pore diameter 0.2 µm) filters. Chromatographic analysis was carried out using a *Shimadzu* 10A HPLC system with refraction index detector (*Shimadzu*, Japan) and *Adsorbosil* NH_2-column (150 mm × 4.6 mm; *Alltech*, USA) with mobile phase of 75 % aqueous acetonitrile at a flow rate of 1 mL min^{-1}.

Nitrate content in the fresh matter of the leafy vegetables was determined using the potentiometric method described by Geniatakis et al. (2003). Oakton desktop ion meter (*Oakton*, USA) and the nitrate selective electrode (*Cole Parmer*, USA) were used.

Vitamin C content was evaluated using spectrophotometric method, described by Janghel et al. (2007). The extraction was performed by homogenising plant material with 0.2 mol L^{-1} oxalic acid. The ability of extract to reduce methyl viologen in the basic medium was determined and expressed as vitamin C content in the fresh material. The Genesys 6 spectrophotometer was used for analysis (*Thermospectronic*, USA).

Total content of phenolic compounds was determined in the methanolic extracts of fresh leaves by spectrophotometric Folin method (Ragaee et al., 2006). The final concentration of phenolic compound is evaluated according to the gallic acid standard calibration curves.

The antioxidant activity of the methanolic extracts of investigated leafy vegetables was evaluated spectrophotometricaly as the ability to bind ABTS and DPPH radicals (Ragaee et al., Kasparavičienė et al., 2004). The Genesys 6 spectrophotometer was used for analysis (*Thermospectronic*, USA).

The error bars presented in figures and the tables are the standard deviations of the three analytical measurements of the parameter. The data processing was performed using MS Excel software.

Results. The content of primary and secondary plant metabolites, important as the indices of human nutrition quality, is significantly affected by light spectral quality and are dependant on plant species and developmental level.

P a r s l e y. The high flux of red light (Fig. 1) in the L2 treatment, enhanced carbohydrate, especially sucrose, content in the parsley leaves. The reduction in the flux of 640 nm component and the insertion of the blue (455 nm), red (662 nm) and far red (735 nm) in the illumination spectra significantly reduced the accumulation of monosugar, fructose and glucose in the plant material. Another positive effect is the reduction in the nitrate content (Fig. 2). The higher the flux of red light, irrespectively of the presence of other spectral components, the higher the decrease of the nitrate content in parsley. It is consistent with the vitamin C concentration in plant fresh matter. In the treatment L1 it is higher about 10 % as compared with the reference plants; and in treatment L2 it is about 5 % higher, than in the treatment L1.

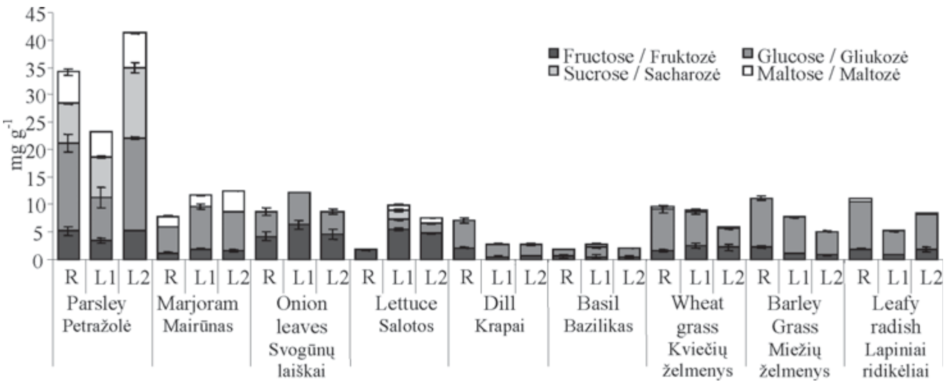

Fig. 1. Carbohydrate content in green vegetables, illuminated with different LED combinations. R – control plants; L1 – illumination contain basal 640 nm and supplemental 455, 662 and 735 nm components; L2 – single basal component

1 pav. Cukrų kiekis žalumyninėse daržovėse, švitintose skirtingomis LED kombinacijomis; R – kontroliniai augalai, L1 – švitinti pagrindine 640 nm komponente ir papildomais 455, 662 ir 735 nm šviestukais; L2 – tik pagrindinė komponentė

M a r j o r a m. The investigated lighting was suitable for marjoram nutrition quality improvement. In both treatments the significant increase in glucose (about 15 %), the monosugar (Fig. 1), and the ~ 50 % reduction in nitrate content (Fig. 2) was observed. Nevertheless, the selected solid-state lighting had no remarkable effect on vitamin C accumulation in leaves (Fig. 3).

O n i o n l e a v e s. The significant effect on photosynthesis product accumulation in leaves was observed in L1 treatment, were the red and blue LED light combinations were investigated. Such lighting promoted monosugar accumulation in leaves (Fig. 1). Nevertheless, vitamin C accumulation in onion leaves was more stimulated by illumination with solely 640 nm red light (Fig. 2). It was increased in this treatment about 16 percents. Nitrate content in onion leaves was not significantly affected by applied illumination.

L e t t u c e. The applied lighting source was found to be the most suitable for lettuce photosynthetic system (Fig. 1). In the treatment L1, where the combination of all four wavelengths was used, the fructose content was about 3 times higher and in treatment L2, where the 640 nm red component was used, the increase was about 2.5 times, in respect to reference, high pressure sodium lamp illumination. In L1 treatment, unlike in other treatments, some sucrose was accumulated in the leaves. Nitrate metabolism was also positively affected by LED lighting (Fig. 2). In this relatively short period 15 % reduction in the lettuce, illuminated with all four components (the 640 nm, 662 nm, 445 nm and 731 nm combination) and 20 % reduction in the lettuce, illuminated with the solely 640 nm light was observed. Vitamin C content was affected negatively – 50 % reduction was measured (Fig. 3).

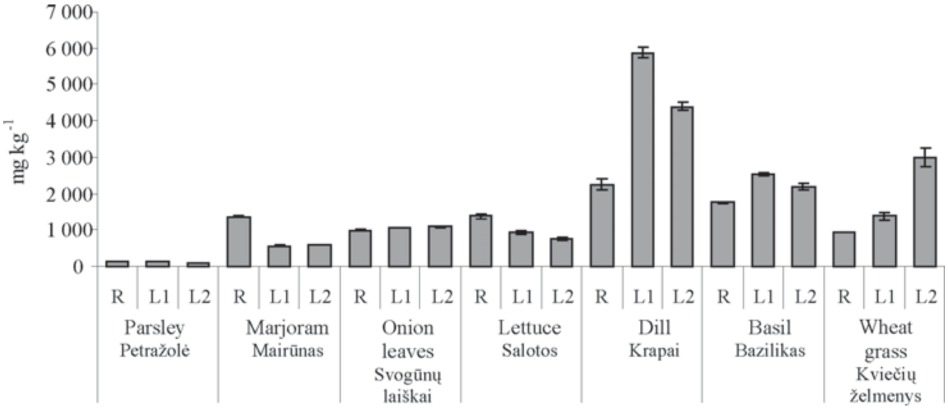

Fig. 2. Nitrate content in green vegetables grown under different illumination. R – control plants; L1 – illumination contain basal 640 nm and supplemental 450, 660 and 735 nm components; L2 – single basal component

2 pav. Nitratų kiekis žalumyninėse daržovėse, švitintose skirtingomis LED kombinacijomis; R – kontroliniai augalai, L1 – švitinti pagrindine 640 nm komponente ir papildomais 455, 662 ir 735 nm šviestukais; L2 – tik pagrindinė komponentė

D i l l. The metabolic system of this plant, from the nutritional viewpoint, reacted negatively to the applied illumination. The fructose content decreased from 2.1 to 0.6–0.7 mg g^{-1} and glucose content decreased form 5.0 to 2.3–2.1 mg g^{-1} (Fig. 1). Another unfavorable effect –50 % increase in nitrate content in treatment L1, and about 30 % increase in treatment L2 (Fig. 2). Vitamin C concentration in leaves also was affected negatively. It decreased about 5 times in the treatment L1 and about 30 % in treatment L2 (Fig. 3).

Basil. The slight increase in monosugar content and traces of sucrose were found in both treatments. In treatment L1, about 0.5 mg g^{-1} of maltose were detected (Fig. 1). However, there were no remarkable effect on vitamin C concentration in leaves (Fig. 3) and tenuous 5–10 % increase in nitrate ion concentration was found in both LED treatments.

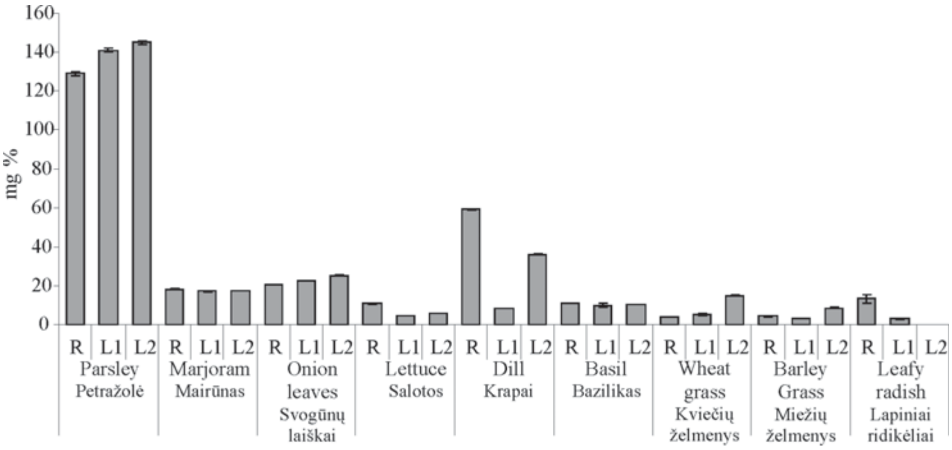

Fig. 3. Vitamin C content in green vegetables grown under different illumination. R – control plants; L1 – illumination contain basal 640 nm and supplemental 450, 660 and 735 nm components; L2 – single basal component

3 pav. Vitamino C koncentracija žalumyninėse daržovėse, švitintose skirtingomis LED kombinacijomis; R – kontroliniai augalai, L1 – švitinti pagrindine 640 nm komponente ir papildomais 455, 662 ir 735 nm šviestukais; L2 – tik pagrindinė komponentė

W h e a t g r a s s. L1 treatment with all four investigated LED components was of somewhat similar effect on sugar metabolism as compared to HPS lamps. Nevertheless, the higher flux of red 640 nm component light reduced glucose concentration in wheat grass in two times (Fig. 1). Photosynthetically active radiation promoted the vital activity if the sprouts and the nitrate uptake from soil. In the treatment L1 the ~ 20 % increase in nitrate concentration was determined; and the high flux of red light enhanced nitrate content by 65 % (Fig. 2). The inverse trend was observed in vitamin C concentration (Fig. 3). In L1 treatment the slight increase in vitamin C concentration was determined, and in the treatment L2 vitamin C concentration rose almost three times. The light effect was significant for phenolic compound content in sprouts (Fig. 4). Since the combination of different red and blue lights showed the same effect for phenol accumulation in leaves, the wheat grass, grown under solely red light accumulates about 10 % less of them. The antioxidant activity, according to the ABTS radical scavenging activity (Fig. 5 A) was about 10 % higher in LED treatments, as compared to HPS. Nevertheless, the DPPH radical scavenging activity (Fig. 5 B) was opposite, about 20 % lower.

Barley grass. The lighting effect, similar to wheat grass was observed in barley grass. Plants, grown under LED illumination for 5 days, accumulated only a half of fructose, as compared to HPS and less of glucose: in the treatment L1 6.2 mg g^{-1} of glucose were quantitated, in L2 – 3.5, when in reference plants – 7.5 mg g^{-1} of fructose were detected (Fig. 1). Vitamin C content in the treatment L1 was about 20 % higher than in reference plants, and in the L2 treatment – about two times higher (Fig. 3). Despite the positive effect on vitamin C content in leaves, in the treatment L1 was observed the 20 %, and in treatment L2 – ~ 10 % decrease in phenolic compound concentration (Fig. 4), as compared to reference plants. Changes in the ABTS radical scavenging activity (Fig. 5 A) were within the limits of the error; the DPPH radical scavenging activity (Fig. 5 B) was found to be about 1.8 times higher in the treatment L1.

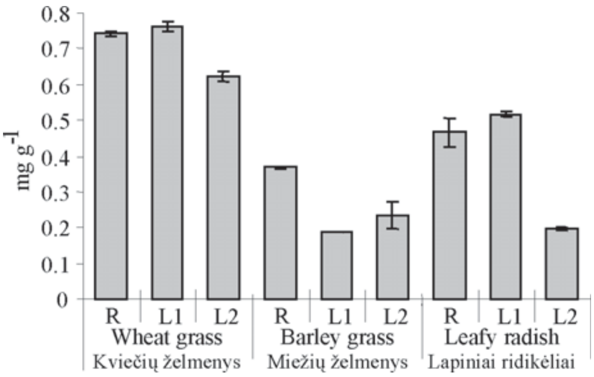

Fig. 4. The content of phenolic compounds in green vegetables and sprouts grown under different illumination; R – control plants, L1 – illumination contain basal 640 nm and supplemental 450, 660 and 735 nm components, L2 – single basal component

4 pav. Fenoliniu junginių koncentracija žalumyninėse daržovėse ir želmenyse, švitintose skirtingomis LED kombinacijomis; R – kontroliniai augalai, L1 – švitinti pagrindine 640 nm komponente ir papildomais 455, 662 ir 735 nm šviestukais, L2 – tik pagrindinė 640 nm komponentė

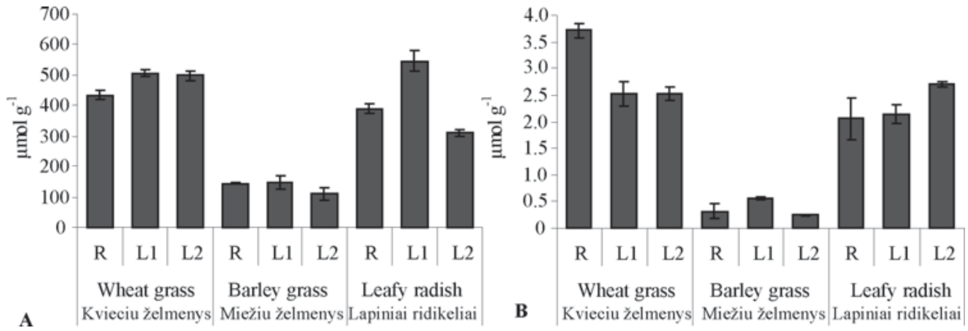

Fig. 5. The antioxidant activity of green vegetables and sprouts grown under different illumination; A – the ABTS radical scavenging activity; B – the DPPH radical binding activity, R – control plants, L1 – illumination contain basal 640 nm and supplemental 450, 660 and 735 nm components, L2 – single basal component

5 pav. Žalumyninių daržovių ir želmenų žaliavos antioksidacinis aktyvumas, juo švitinant skirtingomis LED kombinacijomis; A – ABTS radikalų surišimo aktyvumas, B – DPPH radikalų surišimo aktyvumas, R – kontroliniai augalai, L1 – švitinti pagrindine 640 nm komponente ir papildomais 455, 662 ir 735 nm šviestukais, L2 – tik pagrindinė 640 nm komponentė

L e a f y   r a d i s h. LED illumination significantly inhibited sugar accumulation in leafy radish (Fig. 1); Glucose and sucrose concentration was more than two times lower, although in L2 treatment (sole red light) sugar concentration was closer to reference. Negative effect was for vitamin C content (Fig. 3): in plants, grown under reference illumination, it was about 13 mg g^{-1} and in plants, grown under LED illumination it was ~ 3 mg g^{-1}. The total content of phenolic compounds (Fig. 4) and DPPH radical scavenging activity (Fig. 5 B) varied within the limits of the error in the L1 and reference treatments. Although high flux of red light inhibited accumulation of phenolic compounds in leaves and enhanced DPPH radical scavenging activity. In the ABTS scavenging activity (Fig. 5 A) the opposite trend was observed.

Discussion. The selected solid-state lighting differentially affected the metabolic system of the investigated green vegetables. The most sensitive response was in the sugar, the main photosynthesis product, and accumulation in green leaves. Carbohydrate metabolism lies at the very heart of the sensitive self-regulatory system of plant development (Koch, 2004), therefore the light changes not only affect sugar, as the index of nutritional quality, content, but also participate as the signaling molecule in the regulation of important vital processes. Notwithstanding, a high total concentration of carbohydrates is one of desirable parameters in view of food quality. The nutritional quality also depends on the percentage of monosugars. However, the positive effect both of the solely 640 nm red light illumination and in the combination with other red light wavelengths and blue light was observed in lettuce, onion leaves and marjoram. In parsley, positive effect was seen only growing them under high flux of red light (L2 treatment). Negative reduction in sugar content was detected in dill, wheat grass and barley grass. Although it is stated that red light positively affects the formation and performance of photosynthesis system (Spalding, Folta, 2005), it is evident, that this effect is specie-dependant. This presumption is confirmed analyzing the results of the nitrate metabolism. Reduction of nitrates content is definitely of importance for improvements of nutritional quality of vegetable food. Nitrate reductase activity and the synthesis of this enzyme are also red-light sensitive and dependant on genetically determined features (Appenroth et al., 2000; Lillo, 2004). The nitrate content significantly decreases in lettuce and marjoram; due to nitrate reductase activity nitrates are reduced to nitrite ions and incorporated to the content of ammonium and amino acids. Sole 640 nm red light had the more pronounced effect on nitrate metabolism, as compared to the investigated combination of other light wavelengths. However, no significant light effect was detected in parsley and onion. Therefore in dill, basil and wheat grass the high flux of red solid-state light stimulated vital activity of plants, nitrate uptake and the increase in its concentration in leaves.

Biologically active compounds are quite sensitive to lighting conditions. One of them, vitamin C, significantly increases in parsley, onion leaves, wheat grass and barley grass. This could be associated with the stating, that vitamin C actively accumulates in metabolically active tissues and acts as signaling molecule, coordinating the performance of protective mechanism of antioxidant system (Pastori et al., 2003). Significant light effects on the content of phenolic compounds and antioxidant activity, as the rate of the radical scavenging, was observed only in leafy radish. The enhanced antioxidant activity could be associated to the expression of antioxidant defence genes, defending the plant cells against light-induces photooxidative damage (Wu et al., 2007).

Conclusions. Light effect is a subsequence of the action of complex signal transduction network, including different enzymes, primary and secondary metabolites and messengers. Therefore, it could be employed as the tool for the purposeful plant metabolism, herewith the nutritional quality of vegetable food regulation. However, it is difficult to attain comprehensively positive effect, because different metabolic systems differentially react to the lighting conditions. Moreover, there are contradiction between the natural plant needs, metabolic homeostasis and the agronomic, nutritional objectives. The lettuce, marjoram, wheat grass and leafy radish were found to be the potentially suitable for growing under the light emitting diode lighting, due to the positive increase in monosugar content, reduction of nitrates, higher vitamin C content and promoted antioxidant activity. The light quality requirements of other plants are different, although the combination of different red and far red lights with the blue light had the superior effect, due to activation of more diverse array of light-sensitive receptors.

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Galimybė kontroliuoti žalumyninių daržovių ir želmenų metabolizmą kietakūnės šviesos pagalba

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Santrauka

Tirtas skirtingo kietakūnės šviesos spektro poveikis lapinių daržovių (salotų, svogūnų laiškų, krapų, petražolių, bazilikų, mairūno, kviečių, miežių želmenų ir lapinių ridikėlių) metabolizmo, apsprendžiančio daržovių maistinę kokybę, rodikliams. Augalai skirtinguose augimo tarpsniuose švitinti 3–5 dienas pagrindine 640 nm bangos ilgio šviesa, papildyta 450, 660 ir 735 nm šviesą emituojančiais diodais. Palyginamieji augalai auginti po aukšto slėgio natrio lempomis. Nustatytas angliavandenių, vitamino C, nitrato jonų ir fenolinių junginių kiekis bei antioksidacinis aktyvumas. Pagal gautus rezultatus, tirto apšvietimo poveikis metabolizmui labai priklauso nuo augalo rūšies bei išsivystymo laipsnio. Salotos, mairūnai, kviečių želmenys ir lapiniai ridikėliai potencialiai tinkami jų maistinės kokybės gerinimui kietakūnės šviesos pagalba, dėl pvitinant padidėjusio monocukrų, vitamino C kiekio, antioksidacinio potencialo ir reikšmingos nitratų kiekio redukcijos žaliavoje.

Reikšminiai žodžiai: lapinės daržovės, šviesos kokybė, metabolizmas.