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The effect of variety and lighting qualityon wheatgrass antioxidant properties

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Abstract

The aim of this study was to evaluate the effect of light emitting diode providing lighting spectra on the antioxidant properties of the seedlings of different Lithuanian winter wheat varieties (ʽŠirvinta 1', ʽAda', ʽTauras', ʽMilda', and ʽAlma'), used as the source of bioactive compounds in natural medicine. The originally designed light emitting diode unit was used for the wheatgrass illumination. The effect of the sole red 638 nm light, the combination of the four main components (red 638 nm, 669 nm, blue 445 nm and far red 731 nm), and the four main component supplementation with UV (385 nm), green (510 nm) and amber (595 nm) light emitting diodes on the wheatgrass antioxidant properties was investigated. The analysis of free radical scavenging activity, phenolic compounds, vitamin C contents and the composition of selected carotenoids was performed when plants had reached a height of 9–11 cm. ʽŠirvinta 1' and ʽAda' wheats are the most suitable for antioxidant rich green food production, due to significantly higher than the average of the trial the higher free radical scavenging activity, phenolic compound, vitamin C and carotenoid contents. ʽMilda', ʽAlma' and ʽTauras' demonstrated only moderate antioxidant properties. Lighting spectra also had the pronounced effect: the supplemental yellow and green wavelengths promoted the antioxidant properties of ʽŠirvinta 1' and ʽAda' wheats.

Key words: carotenoids, LEDs, lighting spectra, phenols, radical binding activity, vitamin C, *Triticum aestivum* L.

Introduction

Human diet enriched with young parts of plants (so called "green foods") can help to improve the balance of the nutrient intake in a natural way. Green foods, providing nutrients like vitamins, proteins, minerals and antioxidants, are researched for numerous health benefits and already widely used in USA, East Asian countries and

Central Europe /Lee et al., 2003; Marsili et al., 2004; Paulickova et al., 2006/. The cereal grasses (young shoots of grain-bearing plants), including alfalfa, barley, wheat, rye, oat, and kamut, are also beneficial. In the case of wheat, as a major agricultural product, it has been consumed as grains, but due to its fashion in the natural medicine, recently more attention is being paid to the young leaves, rich in antioxidants /Lee et al., 2003, Marsili et al., 2004/, dietary fibers and essential elements /Kulkarni et al., 2007/. Scientific reports on the activity and composition of wheatgrass extracts are still rare: it has been effective in reducing disease activity of ulcerative colitis and some forms of genetic blood diseases involving anemia /Gruenwald, 2009/. However, cultivating of such green foods with improved nutrition quality and functionality for healthy diets and therapeutics is one of the directions in the development of agricultural innovations /McGloughlin, 2008/. At stem elongation stage, cereal plants contain peak concentrations of bioactive compounds and protectants, necessary for their healthy development and for defense from adverse environmental conditions /Jansen et al., 2008; Gruenwald, 2009/. Improvements in amounts of these phytochemicals may well aid both plant productivity and human health /Demmig-Adams, Adams, 2002/, as a common plant acclimation response to a variety of environmental stressors is the accumulation of antioxidants and secondary metabolites /Jansen et al., 2008/. Therefore, nutrient contents depend on variety /Ehrenbergerova et al., 2007/, on where plants are grown, soil quality /Kulkarni et al., 2006/, humidity, and lighting conditions /Wu et al., 2007/ due to differential plant sensitivity to the environmental conditions /Ruzgas, 2001/.

Light is one of the most important environmental factors, which acts on plants not only as the sole source of energy. Complex, multiple photoreception system (chlorophylls, carotenoids, phytochrome, cryptochrome, phototropins, and other) /Chen et al., 2004; Devlin et al., 2007/ respond to light quantity and quality, duration, intermittence, and other parameters, thus determining plant morphogenetic changes, functioning of the photosynthetic apparatus, and the trend of metabolic reactions. Moreover, lighting conditions might evoke the photoxidative changes in plants, which lead to the altered action of antioxidant defense system: increased contents and activity of antioxidative enzymes, carotenoid, tocopherol, flavonoid, ascorbate. Thus, in combination with other agrotechnical means, light, optimizing the irradiation conditions, or creating the mild photostress, might be an effective tool for phytochemical-rich vegetable cultivation. It is known, how the light irradiance level affects different plants /Carvalho et al., 2008; Stanelonia et al., 2008/, although the knowledge regarding the effect of light spectral quality for metabolism is still limited.

Solid-state lighting based on light-emitting diodes (LEDs) is one of the largest potential advancements in horticultural lighting in the last decades /Morrow, 2008/. Efficiency, longevity, wavelength specificity and versatile application possibilities are the features of LEDs which, when properly employed, are capable of providing performance well beyond any conventional lighting source /Bourget, 2008; Massa et al., 2008/. This technology expanded the possibilities to analyze the effect of lighting parameters on physiological processes in plants and to explore the effect of the light spectral quality on the metabolic and photooxidative processes.

Therefore, the objective of our work was to evaluate the effect of light emitting diode providing lighting spectra on the antioxidant properties of seedlings of different Lithuanian winter wheat varieties.

Materials and methods

Lighting experiments were performed at the Lithuanian Institute of Horticulture in 2008, in the phytotron chambers, under controlled environmental conditions. The originally designed /Tamulaitis et al., 2005/ light emitting diode based lighting units, consisting of commercially available wavelengths: red basal component (640 nm, delivered by AlGaInP LEDs Luxeon^{TM} type LXHL-MD1D, "Lumileds Lighting", USA) and additional blue (455 nm, LuxeonTM type LXHL-LR5C, "Lumileds Lighting", USA), red (669 nm, L670-66-60, "Epitex", Japan), far red (735 nm, L735-05-AU, "Epitex", Japan), amber (595 nm, LuxeonTM type LXHL-MLAC, "Lumileds Lighting", USA), green (510 nm, LuxeonTM type LXHL-MM1D, "Lumileds Lighting", USA) and UV (380 nm, NCCU001E, "Nichia", Japan) LEDs were used. The LED combinations are presented in Table 1. L1 treatment contained the sole red light; treatment L2 contained red, blue and far red, "the main" components. In treatments L3–L5 the main components were supplemented with UV, amber and green light. Reference plants were grown under high-pressure sodium lamps (HPS) ("Son-T Agro", "Philips", USA) rich in orange and yellow wavelengths. Photosynthetic photon flux density of about 200 µmol m^{-2} s^{-1} and 18 h photoperiod was maintained in LED and reference lighting treatments.

Table 1. The light emitting diode combinations and flux densities
1 lentelė. *Kietakūnių šviestukų kombinacijos ir fotonų srauto tankiai*

Treatments *Deriniai*	Photosynthetically active flux density µmol m^{-2} s^{-1} *Fotosintetiškai aktyvios spinduliuotės srautas µmol m^{-2} s^{-1}*						
	Blue *Mėlyna* 445 nm	Red *Raudona* 638 nm	Red *Raudona* 669 nm	Far red *Tolimoji raudona* 731 nm	UV 385 nm	Green *Žalia* 510 nm	Amber *Gintarinė* 595 nm
L1	–	200	–	–	–	–	–
L2	33	154	7	6	–	–	–
L3	32	147	7	6	8	–	–
L4	32	144	7	6	–	11	–
L5	32	144	7	6	–	–	11

The day/night temperature was maintained at +21/17°C. Lithuanian winter wheat (*Triticum aestivum* L.) varieties (developed at the Lithuanian Institute of Agriculture), 'Širvinta 1', 'Ada', 'Tauras', 'Milda' and 'Alma' were sown in peat substrate. Lighting was applied starting from sowing and lasted for 7 days until harvesting when the seedlings had reached a height of 9–11 cm. After harvesting, vitamin C, phenolic compounds content was assessed and the antioxidant activity of winter wheat leaf extracts was evaluated.

Ascorbic acid content was evaluated using a spectrophotometric method /Janghel et al., 2007/. The total content of phenolic compounds was determined in methanolic extracts of fresh leaves using a spectrophotometric Folin method /Ragaee et al., 2006/. The antioxidative activity of methanolic extracts of the investigated leafy vegetables was evaluated spectrophotometricaly 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 spectrophotometrical measurements were performed in five replications and data was processed using one-way analysis of variance *Anova* /Tarakanovas, Raudonius, 2003/, the Fisher's LSD test to trial mean at the confidence level $P = 0.05$.

The carotenoids (violoxanthin, zeaxanthin, α-carotene (not detected) and β-carotene) content was determined using the HPLC method with gradient elution and diode array detection /Edelenbos et al., 2001/. Extraction was performed in 80% iced acetone. Analysis was carried out using the "Shimadzu 10A HPLC" system ("Shimadzu", Japan) and a "Zorbax Extended-C18" column (150 mm x 4.6 mm; "Agilent Technologies", Germany). The error bars presented in Fig. are the standard error mean values of five analytical measurements of a parameter. Data was processed using *MS Excel* software and Student's t-test for a confidence range of $P = 0.05$.

Results and discussion

Although antioxidant activity of wheatgrass is well believed, the exact source of the activity is not well established /Kulkarni et al., 2006/. According to our results, the nutritional value is similar to that of other green vegetables /Urbonavičiūtė et al., 2008/. However, different winter wheat varieties possess different antioxidant properties and they also depend on the LED lighting quality (Table 2). Generally, ʻŠirvinta 1' and ʻAda' wheats are distinguished by 22% and 39% higher than the average of the trial free DPPH radical scavenging activity, while ʻMilda' and ʻAlma' have lower antioxidant activity. This could be related to the overall differences in wheat genotype-environment interaction, resulting in the sensitivity to draught, diseases, and yield stability – ʻŠirvinta 1' and ʻAda' surpass the other investigated varieties in adaptability /Ruzgas, 2001/. However, including the lighting effect, the results are varied. Wheatgrass, grown under HPS lighting (R), rich in orange and yellow irradiance possessed relatively high antioxidant properties. ʻAlma' had the highest DPPH radical scavenging activity, as compared to LED lighting treatments. Single red light (L1) suppressed the antioxidant properties of all wheat varieties, ʻŠirvinta 1' and ʻAda' were more resistant. The supplementation of the red LED with blue, another red and far red components (L2) compensated the negative effect (Table 2). The opposite, positive red LED light effect on the antioxidant activity of pea seedlings was described by Wu et al. (2007). Red light possibly acts as photostressor for plants, creating the misbalance in phytochorme system /Dougher, Bugbee, 2001; Wu et al., 2007/, thus the supplementation of the lighting spectra with the light components of different colours (though maintaining the same lighting flux) normalized the action of phytochrome and, possibly, activated the more diverse array of photoreceptors and resulted in the more pronounced effect on the free radical scavenging activity.

Low flux of supplemental UV 385 nm component inhibited the free radical scavenging activity, as compared to treatment L2 (without UV light). Amber 595 nm and green 510 nm lights, being the close components in the spectra, had the differential effect. The green light (L4) had the pronounced positive effect on the antioxidant properties of 'Tauras', 'Milda', wheat leaves; 'Ada' had the highest antioxidant effect under such lighting. Amber light (L5) had the best antioxidant effect on 'Širvinta 1' wheats. 'Alma', having the lowest free radical scavenging activity, was the least insensitive for the different LED lighting spectra (Table 2).

Table 2. The effect of variety and lighting treatment on the antioxidant properties of winter wheat extract expressed as the ability to scavenge free DPPH radicals (μmol g^{-1})
2 lentelė. *Veislės ir apšvietimo derinių įtaka žieminių kviečių antioksidacinėms savybėms, išreikštoms geba imobilizuoti laisvuosius DPPH radikalus (μmol g^{-1})*

Variety (Factor A) *Veislė (A veiksnys)*	Lighting treatments (Factor B) *Apšvietimo deriniai (B veiksnys)*						Average (B) *Vidurkis (B)*	LSD_{05} (B) *R_{05} (B)*
	R	L1	L2	L3	L4	L5		
'Širvinta 1'	3.554	2.454	3.340	2.345	2.262	3.987	2.99	0.441
'Ada'	3.161	2.466	3.926	3.500	3.973	3.395	3.403	0.074
'Tauras'	2.595	0.903	2.249	0.984	2.590	2.188	1.918	0.126
'Milda'	2.635	0.572	1.568	1.131	2.538	3.246	1.948	0.167
'Alma'	3.053	0.998	2.242	1.328	2.086	2.079	1.964	0.191
Average (A) *Vidurkis (A)*	3.000	1.478	2.665	1.857	2.690	2.979	2.445	
LSD_{05} (A) / *R_{05} (A)*	0.102	0.100	0.127	0.111	0.603	0.146		

Phenols are attributed to the signaling and defensive functions in plants; their concentration was found to be increased in highly irradiated plant tissues /Treutter, 2006/, thus certifying their main role in the protection from photooxidative damage. Similarly to the free radical scavenging activity, the higher contents of phenolic compounds (Table 3) were determined 9% and 18% higher than the average of the trial in 'Širvinta 1' and 'Ada' wheatgrasses (the more environment resistant varieties) respectively, as compared to other varieties. The same trend was observed in the lighting effect, when sole red 638 nm light (L1) and supplemental UV light reduced the contents of phenolic compounds in wheat leaves, in comparison to L2 treatment, where red light was supplemented with blue and far red light. Green (L4) and amber (L5) light had the analogous effect, both enhancing the contents of phenolic compounds in 'Širvinta 1', 'Ada' and 'Milda'.

The vitamin C contents in young leaves of 'Širvinta 1' and 'Ada' wheat, having the higher antioxidant potential, was 7% and 40% higher than the average of the trial concentration (Table 4). 'Tauras', 'Milda', 'Alma', were less enriched with vitamin C. The wheat varieties, grown under reference lighting (R) and under L2 treatment, accumulated less of vitamin C. Sole red 638 nm light (L1), despite having the negative effect on the free radical scavenging ability, stimulated the increase in vitamin C contents. This could be related to the photooxidative damage, generated by the high flux of red light, and the protective action of ascorbate /Conklin, 2001/. Varieties, naturally containing higher concentration of vitamin C ('Širvinta 1', 'Ada') accumulated more of vitamin C when illuminated with UV, green and amber supplemental components, as compared to illumination with only four basal components (L2). 'Milda' and 'Alma', naturally having the lower contents of vitamin C, accumulated more of vitamin C under the lighting with only four basal components (L2). Altered vitamin C concentration acts as a primary "crosstalking" signal that coordinates the activity of defense networks complementary to antioxidant system /Pastori et al., 2003/, thus the varieties, having the lower levels of vitamin C responded to the stressful lighting conditions by increased contents of other antioxidants – phenolic compounds.

Table 3. The effect of variety and lighting treatment on the contents of phenolic compounds in the young winter wheat leaves (mg g^{-1})
3 lentelė. *Veislės ir apšvietimo derinių įtaka fenolinių junginių kiekiui jaunuose žieminių kviečių lapuose (mg g^{-1})*

Variety (Factor A) *Veislė (A veiksnys)*	Lighting treatments (Factor B) *Apšvietimo deriniai (B veiksnys)*						Average (B) *Vidurkis (B)*	LSD_{05} (B) *R_{05} (B)*
	R	L1	L2	L3	L4	L5		
'Širvinta 1'	0.742	0.623	0.805	0.610	0.731	0.817	0.721	0.005
'Ada'	0.673	0.707	0.885	0.750	0.847	0.855	0.786	0.009
'Tauras'	0.785	0.406	0.580	0.587	0.643	0.512	0.585	0.024
'Milda'	0.703	0.335	0.695	0.560	0.689	0.751	0.622	0.032
'Alma'	0.775	0.418	0.890	0.505	0.501	0.555	0.607	0.016
Average (A) *Vidurkis (A)*	0.736	0.498	0.771	0.602	0.682	0.698	0.664	
LSD_{05} (A) / *R_{05} (A)*	0.012	0.023	0.027	0.011	0.021			

Table 4. The effect of variety and lighting treatment on the contents of vitamin C in the young winter wheat leaves (mg g^{-1})
4 lentelė. *Veislės ir apšvietimo derinių įtaka vitamino C kiekiui jaunuose žieminių kviečių lapuose (mg g^{-1})*

Variety (Factor A) *Veislė (A veiksnys)*	Lighting treatments (Factor B) *Apšvietimo deriniai (B veiksnys)*						Average (B) *Vidurkis (B)*	LSD_{05} (B) *R_{05} (B)*
	R	L1	L2	L3	L4	L5		
'Širvinta 1'	0.406	0.735	0.470	1.481	0.920	1.101	0.852	0.067
'Ada'	0.432	1.803	0.689	1.222	1.367	1.182	1.116	0.165
'Tauras'	0.524	0.781	0.649	0.951	0.651	0.951	0.751	0.075
'Milda'	0.530	0.538	0.908	0.633	0.449	0.633	0.615	0.067
'Alma'	0.546	0.484	0.776	0.858	0.584	0.598	0.641	0.051
Average (A) *Vidurkis (A)*	0.488	0.868	0.698	1.029	0.794	0.893	0.795	
LSD_{05} (A) / *R_{05} (A)*	0.048	0.079	0.046	0.086	0.162	0.104		

Vitamin C also acts in plants as a key component in excess photonic energy dissipation mechanisms such as the xanthophylls cycle /Yabuta et al., 2007/. The increase in the vitamin C contents coincide with the increased content of carotenoid violaxanthin pool (Fig.): varieties, having the higher free radical binding activity and higher concentration of vitamin C ('Širvinta 1' and 'Ada'), also contained the higher concentration of carotenoid violaxanthin (Fig.), as compared to other varieties with the lower antioxidant potential. However, the stressful red light treatments (L1) inhibited the violaxanthin accumulation in the leaves of all varieties. The ʽAlma' wheatgrass antioxidant ability was relatively insensitive for different lighting treatments, it might be associated with the relatively high concentration of beta carotene (Fig.), the precursor of vitamin A, acting as the protecting agent in the photosystems I and II /Havaux, Niyogi, 1999/. However, the interpretation of these results is complicated due to the possible synergistic effects between these individual antioxidants /Stahl, Sies, 2005/.

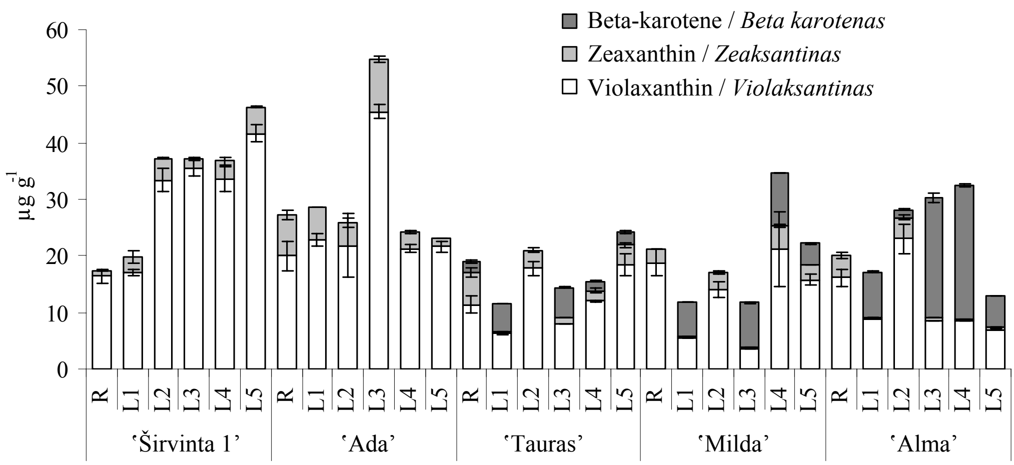

Figure. The effect of variety and lighting treatment on carotenoid contents in winter wheat leaves
Paveikslas. *Veislės ir apšvietimo spektro įtaka karotenoidų sudėčiai jaunuose žieminių kviečių lapuose*

Conclusions

Different winter wheat varieties differ in antioxidant properties. ʽŠirvinta 1' and ʽAda' wheats are the most suitable for antioxidant rich green food production, due to the higher free radical scavenging activity (by 22% and 39% higher than the average of the trial, respectively), phenolic compound (by 9 and 18% higher), vitamin C (by 7% and 40%) and carotenoids violaxanthin (by 85 and 60%), zeaxanthin (by 20% and 110%) contents. ʽMilda', ʽAlma' and ʽTauras' exhibited only moderate antioxidant properties. The antioxidant properties also depend on the lighting spectra. The supplementation of the red light with the wavelengths of far red, blue, yellow and green spectral regions resulted in significantly higher radical scavenging ability, vitamin C and violaxanthin contents in ʽŠirvinta 1' and ʽAda' wheats, as compared to sole red light. However, the results of our pilot study underlie trends for the further optimization of the lighting conditions, because more comprehensive metabolism and gene expression investigations are needed for the production of wheatgrass as the safe material with improved nutritional quality.

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Veislės ir apšvietimo spektro kokybės įtaka kviečių želmenų antioksidacinėms savybėms

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Santrauka

Tyrimų tikslas – įvertinti kietakūnio apšvietimo spektro įtaką žieminių kviečių lietuviškų veislių ('Širvinta 1', 'Ada', 'Tauras', 'Milda' ir 'Alma') želmenų, naudojamų natūralioje medicinoje, antioksidacinėms savybėms. Apšvietimui naudotas originalios konstrukcijos kietakūnių šviesą emituojančių diodų šviestuvas. Tirta raudonos (638 nm), keturių pagrindinių apšvietimo komponenčių (raudonos 638 nm bei 669 nm, mėlynos 445 nm ir tolimosios raudonos 731 nm) ir keturių pagrindinių komponenčių derinių su UV (385 nm), žalios (510 nm) bei geltonos (595 nm) srities bangų ilgių įtaka antioksidacinėms savybėms. Tirta ekstraktų geba imobilizuoti laisvuosius radikalus, fenolinių junginių, vitamino C kiekis ir karotenoidų sudėtis želmenims pasiekus 9–11 cm aukštį. Kviečių veislės 'Širvinta 1' ir 'Ada' tinkamiausios daug antioksidantų turintiems želmenims auginti, o 'Milda', 'Alma' ir 'Tauras' pasižymėjo tik vidutinėmis antioksidacinėmis savybėmis. Reikšminga ir apšvietimo spektro įtaka: pagindinių apšvietimo komponenčių spektrą papildžius geltonos ir žalios srities bangų ilgiais, veislių 'Širvinta 1' ir 'Ada' kviečiuose nustatyta daugiau antioksidacinėmis savybėmis pasižyminčių fenolių, vitamino C ir karotenoido violaksantino.

Reikšminiai žodžiai: karotenoidai, kietakūniai šviestukai, fenoliai, radikalų imobilizavimo geba, vitaminas C, *Triticum aestivum* L.