

RESEARCH ARTICLE

Bioactive constituents of red and green lettuce grown under colour shade nets

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ABSTRACT

The objectives of this study were to investigate the effects of coloured shade-nets (pearl, blue, or red all with a 50% shade index) compared to non-shaded plants on quality traits on the Discoa and ICE 40102 (green-leaf) and Eglantine (red-leaf) lettuce cultivars. Total chlorophyll content depended on the shading and lettuce genotype. The chlorophyll *a* and *b* contents were higher in shaded than unshaded plants. The cv. Eglantine had the highest total chlorophylls (637.03 $\mu\text{g}\cdot\text{g}^{-1}$ F.M.) content. The highest carotenoid content was in leaves of cv. Discoa under pearl nets (208.89 $\mu\text{g}\cdot\text{g}^{-1}$ F.M.). The highest total phenols content (76.70 \pm 1.9 $\text{mg}\cdot\text{g}^{-1}$ GAE D.M.) was in cv. Discoa under red shade; the highest flavonoids content was for cvs. Discoa (42.97) and Eglantine (42.91 $\text{mg}\cdot\text{g}^{-1}$ RU D.M.) under blue shade. The blue and pearl shade nets resulted in slightly higher flavonoid contents in lettuce leaves compared to unshaded plants. The cv. Eglantine, under blue shade had the highest antioxidant capacity (EC₅₀ - 0.197 $\text{mg}\cdot\text{mL}^{-1}$). Red lettuce cv. Eglantine could be recommended for production due to its higher antioxidant properties. Generally blue shade can be used to retain antioxidant capacity.

Keywords: Antioxidant activity; Flavonoids; Lettuce; Photosynthetic pigments; Phenols

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most produced and consumed leafy vegetables in Serbia, because it is ease acquired and prepared, it is produced throughout the year and has low cost (Ilić et al., 2017a). Lettuce is a high value vegetable, rich in minerals, vitamins and antioxidants (Chatterjee, 2015), usually consumed raw, in fast foods and prepared salads (Mampholo et al., 2016), without any restriction for daily consumption (Kosma et al., 2013). Quality and bioactive compounds in lettuce depend on genotype (Brücková et al., 2016), growing season (Kapoulas et al. 2017), light and temperature (Ilić et al., 2017a), nutrients availability (Slamic and Jug, 2016), agronomic practices (Olasupo et al., 2018; Spehia et al., 2018) and time of harvest (Koukounaras et al., 2016).

High solar radiation, heat stress, drought, desiccating winds, and hail storms are some of the major environmental limitations to optimal productivity and nutritional quality of field-grown lettuce (Ilić et al., 2017a). As a cool-season vegetable lettuce seems to be a model crop studied for

responses to light quality and temperature (Mastilović et al., 2019). Net houses, has the potential to reduce various biotic and abiotic challenges during summer production (Ilić et al., 2017a). Photo-selective, coloured, shade nets provide diverse mixtures of natural, unmodified light and scattered, spectrally modified light (Ilić and Fallik, 2017). Spectral modification promotes physiological responses which affects productivity and quality (Li et al., 2017). Accumulation of phytochemicals depends on many factors, such as light quality and quantity, type of varieties or cultivars, growing season and metabolic factors (Simakumar and Jifon, 2017). High solar intensities and predominance of shorter wavelengths in the light spectrum, in the range of blue and UV light wavelengths, generally increase the amount of flavonoids (Zorrati et al., 2014), phenolic accumulation and antioxidant capacity in green (Zhou et al. 2009) and red lettuce (Hipol and Dionisio-Sese, 2014). Lettuce varieties under pearl and yellow nets produced the highest chlorophyll content, but antioxidant, anthocyanin and ascorbic acid contents recorded under black shade nets. Red light influences the production of flavonoids in lettuce leaves (Nsoante et al., 2016). Significantly higher total

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phenol content was recorded in lettuce plants (cv. Tizian) grown under pearl shade nets and it was accompanied by significantly higher flavonoid content and antioxidant properties in comparison to all other nets (Ilić et al., 2017a).

The aims of the study were to evaluate the effect of shading cultivation of three lettuce cultivars on the content of pigments and bioactive compounds, as well as the antioxidant activity compared to the non-shaded cultivation.

MATERIAL AND METHODS

Plant material and growing condition

Discoa and ICE 40102 (green-leaf) and Eglantine (red-leaf) lettuce cultivars (cvs) have been tested in greenhouse production during 2018 (in an experimental garden located in the village of Moravac near Aleksinac 21° 42' E, 43° 30' N, altitude 159 m) in the central area of south Serbia. Lettuce were grown in well drained and sandy soil with high organic matter (3.94 %), pH 6.65 (in KCl), and total nitrogen (10 mg · 100g⁻¹) and phosphorus (>40 mg · 100g⁻¹) with good potassium supplies (>40 mg · 100g⁻¹). The plants were grown following the technique usually implemented by the local producers. Seedlings of the cultivars were produced in a greenhouse. Soil preparation is carried out immediately prior to the planting, using power tiller to obtain a finely crumble structures. When plants developed four true leaves they were transferred into the experimental field to soil with a plant density of 8.3 plants m⁻² and cultivated with or without shade. Treatment combinations were replicated 3 times with four treatments including three types of photoselective nets pearl, red and blue, and an unnetted control treatment in a randomized complete block design. The coloured shade-nets were obtained from Polysack Plastics Industries (Nir-Yitzhak, Israel) under the trade mark ChromatiNet. The shading nets were mounted on a structure about 2.2 m in height over the plants (tunnel net house). Plants were watered with drip irrigation (three irrigation cycles per week) at a delivery rate of 2 L·h⁻¹ and an operating pressure of 150 kPa which supplied 450L·m⁻² water throughout the season. Total rainfall during the growing season (June-September) was 243.8 mm. Experiments were repeated 3 times during summer period with seedlings transplanted on 10th June, 10th July and 10th August. Mature lettuce heads were hand-harvested from the central row of each bed 35 days after transplanting.

Light interception by nets

Light intensity was measured around noon of clear days with a Sun Scan SS1-UM-1.05 (Delta-T Devices Ltd., UK). Solar radiation was measured using the Solarimeter-SL 100 (KIMO, France).

The extraction of plant pigments was performed according to Svec, (1978). Concentrations of chlorophylla and carotenoids were determined according to the Holm-Wetsttein equation and expressed in µg g⁻¹ fresh matter (F.M).

The β-carotene concentration (mg · 100 mL⁻¹) was calculated from the equation proposed by Nagata and Yamashita (1992).

Total phenol content was determined according to the Folin-Ciocalteu procedure (Stanojević et al., 2008; Singleton and Rossi, 1999), with modifications. Total phenols were calculated from the calibration developed under the same conditions with standard gallic acid solutions in range from 0.00625 to 0.2 mg·mL⁻¹ and expressed as gallic acid equivalents per dry extract (mg·g⁻¹ GAE D.M.).

Total flavonoid content was determined according to the aluminium chloride colorimetric method (Singleton and Rossi, 1999; Ling and Tang, 2007).

Antioxidant activity. The DPPH test was used to determine the capacity of the compounds from lettuce ethanol extracts to scavenge free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The scavenging capacity was calculated from the equation of Stanojević et al. (2008). From the curve of dependence of calculated DPPH scavenging capacities and concentrations of extract ethanolic solution the concentration of extract needed for neutralization of 50 % of DPPH radical - EC₅₀ (mg·mL⁻¹) was determined.

Statistical analysis

The data was subjected to statistical analysis using statistical package STATISTIC A 13 0 (Dell Inc. 2016). The Duncan's test was used to compare treatment means and treatments declared different at p = 0.05 level of significance.

RESULTS AND DISCUSSION

Light modification by photo-selective nets

Light has been known to be fundamental in plant growth and development. Shade nets have the potential to modify light quality by reducing radiation intensity and the microclimate thermal properties. The relative capacity of these nets to transform direct light into scattered/diffused light, with the pearl net product being the most, while the black missing scattering. Our results showed that during a typical sunny summer day the maximum photosynthetically active radiation (PAR) ranged in the open field from below 200 µmol m⁻²s⁻¹ early in the morning to over 2000 µmol m⁻²s⁻¹ at noon. Compared to open field, the solar radiation was reduced under all shade nets. The reduction ranged from more than 40% early in the morning

to almost 60% in the late afternoon with a gradual increase of the reduction rate during the day. However, shading did not significantly affect the temperature and relative humidity (Table 1).

Results from Fig. 1a show that the maximum solar radiation in the open field, during a sunny day in July reached 874 Wm⁻². Compared to control, the solar radiation at noon (12 h) was significantly reduced under pearl (459 W m⁻²), followed by red (473 W m⁻²) and blue (578 W m⁻²) nets (Fig.1a).

Photosynthetically active radiation (PAR) was significantly lower under different colour shade nets relative to open field (control). The PAR values ranged from 947 in blue, 1100 in pearl, and 1175 μmol s⁻¹m⁻² in red nets (under 50% shading), compared to 2212 μmol s⁻¹m⁻² in control (Fig.1b).

The light intensity values obtained in this study reflect the arid conditions in southern Europe. According to the literature, the light spectrum transmitted by colored shade nets affects the accumulation of secondary metabolites in lettuce (Ilić et al., 2017a) and basil (Stagnari et al., 2018).

Chlorophyll content

Changes in spectral light promote different photosynthetic responses in lettuce plants. Thus, differences in photosynthetic pigments content and composition can be

expected in plants produced under colour shade nets. The total content of chlorophylls in lettuce (Table 2) grown under open field (control) conditions vary significantly across the researched varieties. In our investigation, the cv Eglantine had the highest content of total chlorophyll (637.03 μg g⁻¹ F.M.) compared to the cvs Discoa (573.64 μg g⁻¹ F.M.) and the Ice 40102 (580.76 μg g⁻¹ F.M.) grown under open field conditions (nonshaded-control). The cv Eglantine also recorded the highest chlorophyll *a* (491.18 μg g⁻¹ F.M.) and chlorophyll *b* (145.86 μg g⁻¹ F.M.) content in open field condition (Table 2).

In general, the total chlorophyll content, as well as the contents of both chlorophyll *a* and chlorophyll *b* were significantly higher in shaded leaves of lettuce than in control plants.

Leaves of lettuce cv Discoa, cultivated under red (632.10 μg g⁻¹ F.M.), pearl (793.74 μg g⁻¹ F.M.) and blue (953.32 μg g⁻¹ F.M.) shade nets had the highest total chlorophyll content compared to plants produced in open field (573.64 μg g⁻¹ F.M.), and the differences (at level p=0.05) were statistically significant (Table 2).

The content of total chlorophyll depends on the lettuce genotype and on the shading colour. Thus, cv. Eglantine from open filed (with chlorophyll content of 637.03 μg g⁻¹ F.M.) under different colour nets obtained

Table 1: Influence of shading net on growing environment (06.07.2018.)

Time (h)	PAR				Non-shaded °C	Temperature			Relative humidity			
	Non-shaded μmol m ⁻² s ⁻¹	Reduction, %				Pearl	Red net	Blue	Non-shaded %	Reduction, %		
		Pearl	Red net	Blue						Pearl	Red net	Blue
6:00	182.5	31.24	39.17	46.8	16.7	0.0	0.6	1.8	74.7	-4.1	-5.1	-5.6
9:00	1325.6	46.02	47.97	54.0	24.7	-0.4	0.0	-1.9	71.8	0.0	0.2	-0.5
12:00	2242.2	48.12	50.59	56.8	31.4	-2.2	-3.1	-1.9	47.3	-2.1	-2.9	-3.3
15:00	1684	51.9	51.3	59.8	31.5	-3.4	-1.2	-0.3	48.2	-1.2	-1.8	-2.6
18:00	672	53.9	58.7	67.0	28.3	-1.0	-0.3	0.0	50.4	-0.2	-0.2	0.4

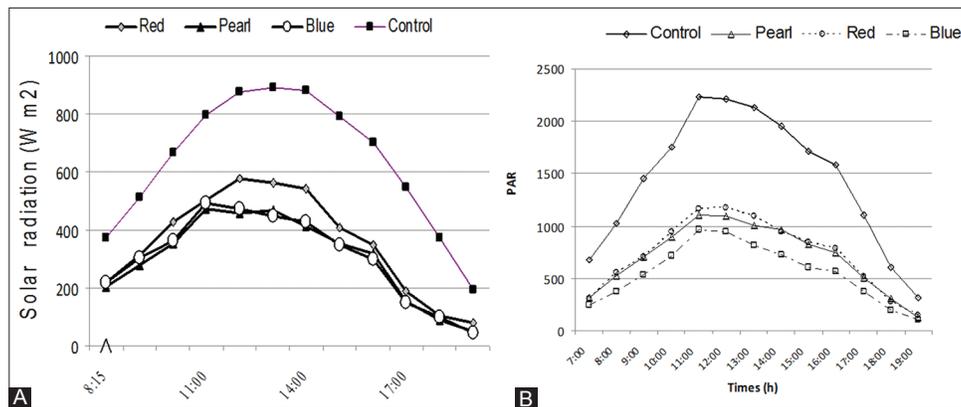


Fig 1. (A-B) Solar radiation (W m⁻²) and PAR-photosynthetically active radiation (μmol m⁻² s⁻¹) under the colour shade nets in comparison to control (average sunny day in July 2018).

Table 2: Content of chlorophylls ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight) in lettuce cultivars as affected by colored shade nets

Treatment	Chlorophyll <i>a</i> $\mu\text{g}\cdot\text{g}^{-1}$ F.M.	Chlorophyll <i>b</i> $\mu\text{g}\cdot\text{g}^{-1}$ F.M.	Chloroph <i>a+b</i> $\mu\text{g}\cdot\text{g}^{-1}$ F.M.	Chl <i>a/b</i>
<i>Open field–Control</i>				
Ice 40102	437 ^h	144 ^g	581 ^h	3.04
Eglantine	491 ^d	146 ^f	637 ^e	3.37
Discoa	439 ^{gh}	135 ^h	574 ⁱ	3.25
<i>Red shade nets</i>				
Ice 40102	508 ^c	179 ^c	687 ^c	2.84
Eglantine	439 ^g	161 ^e	600 ^g	2.73
Discoa	472 ^f	160 ^e	632 ^f	2.96
<i>Pearl shade nets</i>				
Ice 40102	306 ^k	60 ⁱ	365 ^k	5.12
Eglantine	476 ^e	167 ^d	645 ^d	2.82
Discoa	601 ^b	192 ^b	794 ^b	3.13
<i>Blue shade nets</i>				
Ice 40102	429 ^j	143 ^g	572 ^j	2.99
Eglantine	386 ^j	142 ^g	528 ^j	2.71
Discoa	697 ^a	256 ^a	953 ^a	2.72

Values followed by the same letter do not significantly differ between the treatments, at $P=0.05$ according to Duncan's multiple range test
*F.M.-Fresh matter

lower total chlorophyll content (red-599.58; and blue nets - 528.51 $\mu\text{g}\cdot\text{g}^{-1}$ F.M.).

Application of different colour shade nets also largely affected the chlorophyll content in cv. Ice 40102, which was higher in plants cultivated under red shade nets (686.98 $\mu\text{g}\cdot\text{g}^{-1}$ F.M.) and lower in lettuce grown under pearl (365.23 $\mu\text{g}\cdot\text{g}^{-1}$ F.M.) and blue nets (572.31 $\mu\text{g}\cdot\text{g}^{-1}$ F.M.) in comparison to plants from the open field (580.76 $\mu\text{g}\cdot\text{g}^{-1}$ F.M.).

The chl *a/b* ratio, in general, were significantly higher in control plants than in shaded plants. The chl *a/b* ratio in lettuce grown under red and blue shade nets (<3) were lower in comparison with non-shaded control plants (>3). Under pearl nets the chl *a/b* ratio in cv. Ice 40102 was the highest (5.1) owing primarily to a very low content of chlorophyll *b* (Table. 2).

The synthesis and degradation of the photosynthetic pigments are associated with the plants 'adaptability' to different environments. The chlorophylls are usually synthesized and photo-oxidized in the presence of light. On the other hand, under deficit light conditions, the plants set a series of compensatory mechanisms into motion such as a substantial increment of the photosynthetic pigments. This response fulfils the function of the photosynthetic antennae absorbing the required light energy considering that the highly pigmented leaves show higher light absorption efficiency per unit of leaf biomass, which may allow the plant to achieve a better carbon balance under light limitation. Besides, it has been reported that the efficiency of photosynthesis during sun flecks is higher in the shade tolerant species (Zervoudakis et al., 2012). Therefore, the decreased chl *a/b* ratio (or increased chl *b/a* ratio) could also be an adaptive response for the light harvesting maximization.

The reason why the Chl *a/b* ratio decreases is that under low light availability plants develop bigger light harvesting complexes (LHCs), Croce and van Amerongen (2014). Both Chl *a* and Chl *b* are found in LHCs yet only Chl *a* is found in the reaction centres. As a result, bigger LHCs as a response to low light lead to increments of both Chl *a* and Chl *b*, still the overall Chl *a/Chl b* ratio drops as there is no increase in the number of Chl *a* molecules forming the reaction centres of the photosystems (Lodish et al., 2000). At high sunlight intensities, chlorophyll degradation rate in plant leaves is higher than the synthesis rate, leading to a decrease in chlorophyll concentration as a result of chloroplast formation inhibition.

In plants under high sunlight intensity occurs high chlorophyll degradation. Red colour lettuce cv. Eglantine with higher chlorophyll content in control, probably due to the presence of the compounds responsible by red coloration protected chlorophyll, avoiding degradation.

Accordingly, shade leaves in comparison with sun leaves tend to show higher chlorophyll concentrations per unit of leaf weight (Fu et al., 2012). Although shade-grown plants are not directly exposed to sunlight, they produce additional chlorophyll *a* and *b* to capture diffuse radiation to produce the carbohydrates needed for the plant to grow, as supported by the results of (Beneragama and Goto, 2010). This study clearly showed that chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids increased in leaves from shaded plants. Our results are supported by a number of publications (Fu et al., 2012; Kosma et al., 2013).

Bioactive compounds and antioxidant activity

Lettuce does not contain exceptionally high concentrations of polyphenols. However, it is usually consumed raw and in

large quantities (Becker, 2016). The phytochemical content and composition can vary among different lettuce varieties and it is important to choose certain type of varieties that are rich in phytochemicals. Phenolic compounds in plants are responsible for oxidative stress scavenging (Lopez et al., 2014).

Table 3 shows the total phenols (TP) and total flavonoids (TF) as well as the antioxidant activity of extracts of the examined lettuce varieties cultivated in different colour shade nets under net-house conditions. Cv Eglantine has the highest content of TP compared to the other two varieties under open-field conditions ($60.20 \pm 2.29 \text{ mg}\cdot\text{g}^{-1}$ GAE D.M). In our study, the levels of TP and TF in lettuce varieties is higher compared to Tizian lettuce variety at same ligh condition (Ilić et al., 2017a). The TP contents in cv Eglantine grown under blue, pearl and red shade nets were lower than in control plants, but differences not significantly lower.

TP content is significantly higher in another two lettuce cultivars from net-house comparing to open field production. Thus, green lettuce cv Ice 40102 grown cover by red and pearl nets obtained significantly higher TP content in comparison to open field production. Similarly, Discoa cultivar grown in red and blue nets contained significantly higher TP content than lettuce from open field. The highest content of TP is in the lettuce cv Discoa cultivated in the red net-house $76.70 \pm 1.9 \text{ mg}\cdot\text{g}^{-1}$ GAE D.M dry extract.

Variety-specific responses were observed with respect to different coloured shade nets with regards to the TP content. In the similarly experiments, but with butter lettuce cv Tizian, the highest content ($30.78 \text{ mg}\cdot\text{g}^{-1}$ GAE D.M.), of TP (which was generally significantly higher in lettuce plants from open field production compared to colour shade nets), was recorded in the leaves of lettuce cultivated under pearl shade nets (Ilić et al., 2017a).

The TF content in lettuce from open field varies considerably among the researched varieties. Cv Ice 40102 for example, has the lowest content of TF ($3.39 \text{ mg}\cdot\text{g}^{-1}$ RE D.M) compared to the other two varieties in these conditions.

TF content in all cultivars was the lowest in control plants from open field condition while under blue, pearl and red nets higher contents of TF were recorded. In terms of TF content certain varieties specifically react to different color nets. The largest content of TF was displayed in the lettuce cv Discoa ($42.97 \text{ mg}\cdot\text{g}^{-1}$ RE D.M.) and cv Eglantine ($42.91 \text{ mg}\cdot\text{g}^{-1}$ RE D.M.) cultivated in blue net-house. The lowest content of TF was recorded

Table 3: Total phenols content (TPC)^a, total flavonoids content (TFC)^b and EC₅₀ (c) values of lettuce extracts

Treatment	TPC mg·g ⁻¹ GAE D.M. ^a	TFC mg·g ⁻¹ RE D.M. ^b	EC ₅₀ ^c mg·mL ^{-1c}
<i>Open field-Control</i>			
Ice 40102	37.1 ^h	3.4 ^h	1.317 ^a
Eglantine	60.2 ^d	16.2 ^g	0.540 ^{ef}
Discoa	46.3 ^g	21.4 ^f	0.614 ^b
<i>Red nets</i>			
Ice 40102	58.9 ^d	20.6 ^f	0.570 ^c
Eglantine	51.0 ^f	20.8 ^f	0.531 ^g
Discoa	76.7 ^a	23.2 ^f	0.370 ^h
<i>Pearl nets</i>			
Ice 40102	66.1 ^c	23.8 ^{de}	0.620 ^b
Eglantine	47.6 ^g	33.4 ^b	0.383 ^h
Discoa	48.3 ^g	25.2 ^d	0.562 ^{cd}
<i>Blue nets</i>			
Ice 40102	36.4 ^h	30.0 ^c	0.549 ^{de}
Eglantine	54.2 ^e	42.9 ^a	0.197 ⁱ
Discoa	71.4 ^b	43.0 ^a	0.383 ⁱ

Values followed by the same letter do not significantly differ between the treatments, at P=0.05 according to Duncan's multiple range test.

^aTPC-mg·g⁻¹ GAE D.M.=milligram of gallic acid equivalents·g⁻¹ dry matter;

^bTFC-mg·g⁻¹ RE D.M.=milligram of routine equivalents·g⁻¹ dry matter;

^cEC₅₀ⁱ mg·mL⁻¹=concentration of extract necessary to neutralize 50% of initial concentration of DPPH radicals

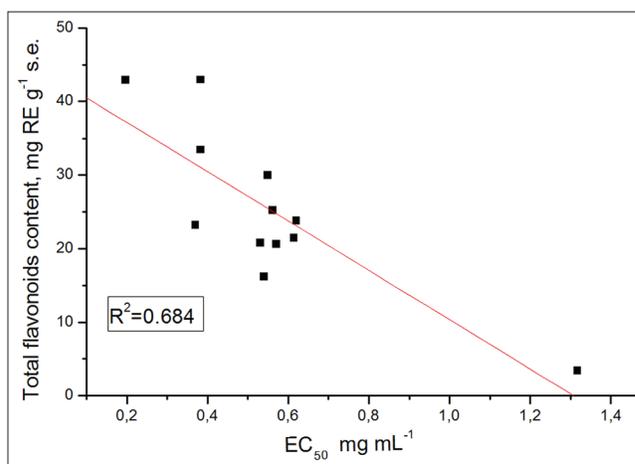


Fig 2. Correlation between content of total flavonoids in extracts and EC₅₀ value.

in the lettuce cv Ice 4010 ($20.62 \text{ mg}\cdot\text{g}^{-1}$ RE D.M and cv Eglantine ($20.80 \text{ mg}\cdot\text{g}^{-1}$ RE D.M cultivated under red nets (Table 3).

Low light intensity generally promoted the accumulation of flavonoids compounds in ginger plant parts (Ghasemzadeh and Ghasemzadeh, 2011). The use of shade netting is acceptable for the production of baby spinach in relation to improving flavonoid concentration and composition (Bergquist, 2006).

EC₅₀ values were determined for each lettuce extract (Table 3).

Eglantine - red cultivar produced from open field showed higher EC₅₀ values than two other green cultivars. The extract of the lettuce cultivated under shade nets has higher antioxidant properties (lower EC₅₀ DPPH values) than the lettuce cultivated under open field conditions. Variety-specific responses were observed with respect to different coloured shade nets with regard to the antioxidant activity. The variety Eglantine grown under blue and pearl nets, respectively, showed higher antioxidant scavenging.

The observed higher flavonoid concentrations in lettuce varieties under specific coloured nets could be associated with the higher antioxidant scavenging activity. Our study shows an increase of antioxidant scavenging activity in all lettuce cultivars under the blue nets. It is clearly evident in this study that light quality influences the biosynthesis and accumulation of phytochemical parameters of lettuce. Our data suggest the possible use of light quality during production to improve the phytochemical quality of lettuce varieties.

Negative linear correlations were observed between TP content and the EC₅₀ values of the tested antioxidant activity assays for all the studied cultivars. However, the low values of determination coefficients ($R^2 = 0.243$) for TP do not suggest significant correlations between the studied parameters, which may be attributed to secondary metabolites that could not be detected in the present study, such as sesquiterpene lactones, antioxidant vitamins and glutathione. A higher correlation was observed for TF content ($R^2 = 0.684$) indicating that higher content of flavonoid compounds result in higher antioxidant activity (Fig. 2).

The antioxidant capacity of lettuce from summer production under different shade-nets in our experiments is higher than in the earlier examined butter varieties of lettuce cultivated during summer (Ilić et al., 2017a), and similarly to the cos lettuce from spring production (Mladenović et al., 2013). Llorach et al. (2008) described that the red and darker green types of lettuce possess higher antioxidant activity than the green type lettuce. They assumed that the high antioxidant activity of red lettuce was due to the existence of anthocyanin, which was also a strong antioxidant.

Changes in light intensity through the utilisation of shade nets were able to change the synthesis of phenolic compounds in plants. However, different plants had diverse reactions to shade levels, which alter the production of TP and TF. Previous studies showed that change in light intensity was able to modify the production and accumulation of TF and TP in herbs (Buthelezi et al., 2016).

Table 4: Total carotenoids, β -carotene ($\mu\text{g g}^{-1}\text{F.M.}$) and chlorophyll/carotenoide content ratio of three lettuce varieties growing with or without colored shade nets

Treatment	Carotenoids $\mu\text{g}\cdot\text{g}^{-1}$ F.M.	β -carotene	Total chl/car
<i>Open field - Control</i>			
Ice 40102	145 ^g	76 ^l	4.00
Eglantine	123 ^j	57 ^k	5.18
Discoa	130 ⁱ	88 ^b	4.42
<i>Red shade nets</i>			
Ice 40102	200 ^b	77 ^{ef}	3.43
Eglantine	176 ^e	82 ^c	3.41
Discoa	136 ^h	80 ^{cd}	4.65
<i>Pearl shade nets</i>			
Ice 40102	122 ^k	60 ^l	3.00
Eglantine	183 ^d	93 ^a	3.53
Discoa	209 ^a	69 ^h	3.80
<i>Blue shade nets</i>			
Ice 40102	159 ^f	72 ^g	3.60
Eglantine	120 ^j	79 ^{de}	4.40
Discoa	194 ^c	67 ⁱ	4.91

Values followed by the same letter do not significantly differ between the treatments, at $P=0.05$ according to Duncan's multiple range test

Carotenoids and β -carotene content

In general, the carotenoid content of lettuces cultivated under shade nets was higher than control. Control plants exhibited the lowest carotenoid contents and depending on the variety ranged from 122.98 $\mu\text{g g}^{-1}$ F.M. (cv Eglantine) to 145.18 $\mu\text{g g}^{-1}$ F.M. (cv Ice 40102). The highest carotenoid content was found in leaves of cv. Discoa cultivated under pearl nets (208.89 $\mu\text{g/g}$ F.M.), see Table 4.

Under red nets (cvs Ice 40102 and Eglantine), pearl nets (cvs Eglantine and Discoa) and blue nets (cv Discoa) the total carotenoids content was significantly higher ($p=0.05$) compared to the un-shaded (control) plants. The total carotenoid content is generally reported to be lower in leaves that are exposed to sun compared to shade leaves. Ilić et al. (2017b) reported that the highest content was found in pepper plants under pearl nets (291.8 $\mu\text{g g}^{-1}$ FM). Carotenoids play a role in photosynthesis as an accessory pigment that absorbs at different wavelengths from the chlorophylls and transfers the absorbed light energy to the chlorophylls. As such, they are involved in light harvesting and protection against excessive light energy (Strzalka et al., 2003). The content of β -carotene was dependent on the lettuce genotype. The greatest amount of it is in the cv Discoa lettuce (88.20 $\mu\text{g g}^{-1}$ F.M.), and the smallest in the cv Eglantine lettuce (56.55 $\mu\text{g g}^{-1}$ F.M.). Along with genetic variability, there also appears to be an environmental influence on β -carotene accumulation in the lettuce. Only cv. Eglantine under colour shade nets recorded significantly higher amount of β -carotene than unshade plants from open field. Plants grown under black nets contain high levels of β -carotene (Ntsoane et al., 2016).

The values determined in the stated lettuce (β -carotene) are in accordance with the data obtained by other authors (Zdravković et al., 2014, Llorach et al., 2008; Mladenović et al., 2013). The increased levels of β -carotene provide photoprotection by dissipation of the excess energy of the excited chlorophyll, thereby removing the reactive oxygen species.

The ratio chlorophyll/carotenoids was for cultivars Ice 40102 and Eglantine high in plants grown in open field indicating that for these cultivars shading nets favoured carotenoid synthesis while for the cultivar Discoa the influence of shading nets was opposite (Table 4).

CONCLUSIONS

This research demonstrated that photo-selective nets create modified light conditions and increase the synthesis of health-promoting metabolites in green and red lettuce cultivars. The combination of cultivar variation and responsiveness to specific light modifications can create opportunities for the lettuce production with improved antioxidant properties. Among the different varieties of lettuce, the red cv Eglantine lettuce showed the highest total antioxidant levels. Blue nets can be recommended for all three varieties to retain the antioxidant capacity. The EC_{50} values of DPPH radical scavenging activity highly correlated with total flavonoid content. However, further studies are needed to analyze the effect of the light spectra modifications to other nutritional properties, and to elucidate the molecular mechanisms behind the detected differences between the lettuce cultivars.

Author's contribution

Authors have contributed equally. All authors read and approved the final manuscript. Thank Mr. Dušan Marinković from Victoria seed Company, 119b Bower rd Ethelton 5015 South Australia Hm.CLAUSE 165 Templestowe rd Lower Templestowe 3107 providing seeds of lettuce for experiments.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Becker, C. 2016. Flavonoids and phenolic acids in lettuce: How can we maximize their concentration? And why should we? *Acta Hort.* 1142: 1-10.
- Beneragama, C. K. and K. Goto. 2010. Chlorophyll a: b ratio increases under low-light in "shade-tolerant" *Euglena gracilis*. *Trop. Agric. Res.* 22: 12-25.
- Bergquist, S. 2006. Bioactive Compounds in Baby Spinach (*Spinacia oleracea* L.). Effects of Pre and Postharvest Factors. Doctoral Dissertation. Swedish University of Agricultural Sciences, Alnarp, Sweden.
- Buthelezi, M. N. D., P. Soundy., J. Jifon. and D. Sivakumar. 2016. Spectral quality of photosensitive nets improves phytochemicals and aroma volatiles in coriander leaves (*Coriandrum sativum* L.) after postharvest storage. *J. Photoch. Photob. B.* 161: 328-334.
- Chatterjee, R. 2015. Influence of nutrient sources on growth, yield and economics of organic lettuce production under foothills of eastern Himalayan region. *Emir. J. Food Agric.* 27: 460-462.
- Croce, R. and H. van Amerongen. 2014. Natural strategies for photosynthetic light harvesting. *Nat. Chem. Biol.* 10: 492-501.
- Ghasemzadeh, A. and N. Ghasemzadeh. 2011. Effects of shading on synthesis and accumulation of polyphenolic compounds in ginger (*Zingiber officinale* Roscoe) varieties. *J. Medic Plants Res.* 5: 2435-2442.
- Hipol, R. L. B. and M. L. Dionisio-Sese. 2014. Impact of light variation on the antioxidant properties of red lettuce. *Elect. J. Biol.* 1: Ilić, S. Z and E. Fallik. 2017. Light quality manipulation improve vegetables quality at harvest and postharvest: A review. *Environ. Exper. Bot.* 139: 79-90.
- Ilić, Z. S., L. Milenković, A. Dimitrijević, L. Stanojević, D. Cvetković, Ž. Kevrešan, E. Fallik and J. Mastilović. 2017a. Light quality manipulation by color nets improve quality of lettuce from summer production. *Sci. Hortic.* 226: 389-397.
- Ilić, S. Z., L. Milenković, L. Šunić, S. Barać, J. Mastilović, Ž. Kevrešan and E. Fallik. 2017b. Effect of shading by color nets on yield and fruit quality of sweet pepper. *Zemdirbyste-Agric.* 104: 53-62.
- Fu, W., P. Li and Y. Wu. 2012. Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. *Sci. Hortic.* 135: 45-51.
- Kapoulas, N., A. Koukounaras and S. Z. Ilić. 2017. Nutritional quality of lettuce and onion as companion plants from organic and conventional production in north Greece. *Sci. Hortic.* 219: 310-318.
- Kosma, C., V. Triantafyllidis, A. Papanavvas, G. Salahas and A. Patakas. 2013. Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season. *Emirates J. Food Agric.* 25: 974-979.
- Koukounaras, A., A. S. Siomos, D. Gerasopoulos and K. Karamanoli. 2016. Genotype, ultraviolet irradiation, and harvesting time interaction effects on secondary metabolites of whole lettuce and browning of fresh-cut product. *J. Hort. Sci. Biotech.* 91: 491-496.
- Li, T., G. Bi, J. Lecompte, C. Barickman and B. B. Evans. 2017. Effect of colored shade cloth on the quality and yield of lettuce and snapdragon. *Hortic. Tech.* 27: 860-867.
- Lin, J. Y. and C. Y. Tang. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables: As well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.* 101: 140-147.
- Llorach R., A. Martinez-Sanchez and F. A. Tomas-Barberan. 2008. Characterisation of polyphenols and antioxidant properties of

- five lettuce varieties and escarole. *Food Chem.* 108: 1028-1038.
- Lodish, H., A. Berk, S. L. Zipursky, P. Matsudaira, D. Baltimore and J. Darnell. 2000. In: *Molecular Cell Biology*. 4th ed. W. H. Freeman, New York.
- Lopez, A., G. Javier, J. Fenoll, P. Hellin and P. Flores. 2014. Chemical composition and antioxidant capacity of lettuce: Comparative study of regular-sized (Romaine) and baby-sized (Little Gem and Mini Romaine) types. *J. Food Comp. Anal.* 33: 39-48.
- Mampholo, B. M., M. M. Maboko, P. Soundy and D. Sivakumar. 2016. Phytochemicals and overall quality of leafy lettuce (*Lactuca sativa* L.) varieties grown in closed hydroponic system. *J. Food Qual.* 39: 805-815.
- Mastilović, J., Ž. Kevrešan, A. Jakšić, I. Milovanović, M. Stanković, Trajković, R. Milenković, L and S. Z. Ilić. 2019. Influence of shading on postharvest lettuce quality: Differences between exposed and internal leaves. *Zemdirbyste-Agric.* 106: 65-72.
- Mladenović, J., G. Acamovic-Djokovic, R. Pavlovic, J. Zdravkovic, P. Maskovic and M. Zdravkovic. 2013. Antioxidant and Antimicrobial Activities of Lettuce. 4th International Symposium Agrosym 2013 Jahorina. Book Procced, pp. 619-624.
- Nagata, M. and I. Yamashita. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *J. Food Sci. Technol.* 39: 925-928.
- Ntsoane, L. M., P. Soundy, J. Jifon and D. Sivakumar. 2016. Variety-specific responses of lettuce grown under the different-coloured shade nets on phytochemical quality after postharvest storage. *J. Hortic. Sci. Biotech.* 91: 520-528.
- Olasupo, I. O., I. O. O. Aiyelaagbe, E. A. Makinde and W. A. O. Afolabi. 2018. Growth, yield, and nutritional composition of plastic tunnel-grown lettuce in response to poultry manure. *Int. J. Veg. Sci.* 24: 526-538.
- Spehia, R. S., M. Devi, J. Singh, S. Sharma, A. Negi, S. Singh, N. Chauhan, D. Sharma and J. C. Sharma. 2018. Lettuce growth and yield in hoagland solution with an organic concoction. *Int. J. Veg. Sci.* 24: 557-566.
- Singleton, V., R. Orthofer and R. M. Lamuela-Raventos. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 299: 152-175.
- Sivakumar, D. and J. Jifon. 2017. Influence of photoselective shade nettings on postharvest quality of vegetables. In: M. W. Siddiqui M.W. (Ed.), *Preharvest Modulation of Postharvest Fruits and Vegetables Quality*, AAP-CRC Press, Elsevier Inc., USA, pp. 121-138.
- Slamic, B. and T. Jug. 2016. Lettuce growth in extreme conditions. *Emir. J. Food Agric.* 28: 398-401.
- Stagnari, F., C. Di Mattia, A. Galienia., V. Santarella., S. D'Egidioa, G. Pagnania and M. Pisante. 2018. Light quantity and quality supplies sharply affect growth, morphological, physiological and quality traits of basil. *Ind Crops Prod.* 122: 277-289.
- Stanojević, L. P., M. Z. Stanković, V. D. Nikolić and L. B. Nikolić. 2008. Antioxidative and antimicrobial activities of *Hieracium pilosella* L. extracts. *J. Serb. Chem. Soc.* 73: 531-540.
- Strzałka, K., A. Kostecka-Gugała and D. Latowski. 2003. Carotenoids and environmental stress in plants: Significance of carotenoid-mediated modulation of membrane physical properties. *Russ. J. Plant Physiol.* 50: 168-173.
- Svec, W. A. 1978. The isolation, preparation, characterization and estimation of the chlorophylls and the bacteriochlorophylls. In: D. Dolphin (Ed.), *The Porphyrins*. Academic Press, Cambridge, pp. 341-399.
- Zdravković, J., G. Aćamović-Djoković, J. Mladenović, R. Pavlović and M. Zdravković. 2014. Antioxidant capacity and contents of phenols, ascorbic acid, β -carotene and lycopene in lettuce. *Hem. Ind.* 68: 193-198.
- Zervoudakis, G., G. Salahas, G. Kaspiris and E. Konstantopoulou. 2012. Influence of light intensity on growth and physiological characteristics of common sage (*Salvia officinalis* L.). *Braz Arch. Biol. Technol.* 55: 89-95.
- Zhou, Y. H., Y. Y. Zhang, X. Zhao, H. J. Yu, H. K. Shi and J. Q. Yu. 2009. Impact of light variation on development of photoprotection, antioxidants, and nutritional value in *Lactuca sativa* L. *J. Agric. Food Chem.* 57: 5494-5500.
- Zoratti, L., K. Karppinen, A. L. Escobar, H. Häggman and L. Jaakola. 2014. Light-controlled flavonoid biosynthesis in fruits. *Front Plant Sci.* 5: 16.