

## Effect of coloured shade nets on some nutritional characteristics of a kapia type pepper grown in plastic tunnel

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**Abstract:** Sweet pepper is the most important vegetable crop of the Hungarian greenhouse industry. Production of red coloured cultivars, having very high nutritive value, is getting popular recently. Shading of plastic tunnels is a common practice in Hungary, but research about its effects on the nutritional characteristics of sweet pepper has just started. The objective of this study was to investigate the effect of different shading methods (shading paint, white, green, yellow and red coloured shade nets) on some nutritional characteristics of a red coloured kapia type pepper, under Hungarian climatic conditions cultivated in unheated walk-in plastic tunnels. Shade net colour slightly affected radiation and hence temperature conditions of the tunnels; especially ratio of supra-optimal temperature values ( $32\text{ }^{\circ}\text{C} <$ ) was changed. Dry matter content was not influenced by shading method, in contrary to sampling time which had a significant effect on this parameter. Vitamin C and total polyphenol contents were affected by both of these factors and also by their interaction; shading effect on these phytonutrients changed with harvest times. However, total carotenoid content was affected just by this latter factor. Heat stress, higher ratio of supra-optimal temperature values, increased total polyphenol concentration and decreased total carotenoid content. In overall, white shade net resulted sweet peppers with the best nutritive quality, but harvest time had a more pronounced effect on content of nutritive constituents than shade net colour.

**Keywords:** *Capsicum annuum*, carotenoids, vitamin C, polyphenols, harvest time

### Introduction

Sweet pepper is by far the most important crop of the Hungarian greenhouse vegetable industry, accounting for about 40% of both total production area and total product (FruitVeB, 2015). Besides the commonly cultivated sweet wax-yellow type, production of red-coloured kapia type peppers is getting popular in Hungary recently. Sweet peppers have one of the highest nutritional values among vegetables (Rubatzky and Yamaguchi 1997). Peppers are not only one of the richest plant sources of vitamin C, but also contain pro-vitamin A carotenes, tocopherols, niacin, riboflavin and thiamine in considerable amounts (Bosland and Votava 2012). Red pepper pods contain twice as much vitamin C and 6 to 90 times more carotenoids than similar green fruits, with capsanthins and capsorubin being the main colour components (Frary and Frary 2012). An even higher, up to seventeen-fold increase in

vitamin C concentration during maturation was observed for chilli pepper hybrids (Nagy et al. 2015). Flavonoid and phenol compounds also contribute to the strong antioxidative property of this vegetable. Antioxidant capacity of pepper pods usually increases with maturity (Frary and Frary 2012). Environmental factors, such as radiation and temperature can greatly affect accumulation of antioxidants, such as carotenoids, vitamin C and polyphenols (Pék et al. 2011).

Low-cost, 2-meter-high walk-in plastic tunnels are still commonly used for vegetable forcing in Hungary, accounting for one third of total greenhouse area (Fodor 2014). Due to high temperatures during late spring, summer and early autumn, overheating could be a serious problem in these tunnels. To reduce the heat load generally ventilation, shading paint, external shade nets and/or mist irrigation are applied (López-Marín et al. 2012; Zhu et al. 2012).

Under high radiation conditions, moderate (20% to 30%) shading results in high bell pepper yield of good quality due to decreased incidence of sunburn and improved water use efficiency (Zhu et al. 2012; Díaz-Pérez 2013; Kitta et al. 2014). Photosensitive shade netting was developed around the beginning of the new century (Fallik et al. 2009). Compared to the traditional method (black shade net), application of red, pearl (white) and yellow nets resulted better yield and fruit quality, reduced infestation by pests and improved post-harvest quality (Stamps 2009; Goren et al. 2011; Shahak 2014). Yellow shade net was found to increase pepper yield and quality compared to traditional black nets in Israel in a field experiment (Fallik et al. 2009). Ambrózy et al. (2016) also reported significantly higher pepper yield under yellow net compared to white net and unshaded control.

By modifying the spectra and microclimate, coloured shade nets can also affect the concentration of phytonutrients in pepper pods. Coloured shade nets have increased nutritional value of pepper fruits according to several studies. In Israel Kong et al. (2013) reported higher vitamin C contents and increased antioxidant capacity under white net compared to the traditional black one in net house sweet pepper cultivation. In South Africa Selahle et al. (2015) also found that white net was more effective in increasing vitamin C and total polyphenol concentrations, and total antioxidant activity than yellow and red nets. Meanwhile  $\beta$ -carotene and lycopene contents were the highest under the control black net. In an experiment conducted in Hungary, the highest total carotenoid content was also achieved by white shade net (Ambrózy et al. 2016). In contrary of these favourable results Nagy et al. (2016) found that red, white and green nets all decreased vitamin C content of a yellow coloured chilli hybrid compared to the unshaded control.

The above mentioned results were all derived from field experiments. However, Milenkovic et al. (2012) and Ilic et al. (2015) observed that the effect of photosensitive shade net treatments on phytonutrient contents of pepper and tomato

is different if they are combined with plastic sheet cover of tunnels. This phenomenon was also experienced in Hungarian chilli pepper experiments (Nagy unpublished data 2014; Nagy et al. 2016). For example pepper vitamin C content was significantly the highest in the red shade net treatment in plastic tunnels, while in the parallel field experiment this colour resulted significantly lower values than black, white and blue nets (Milenkovic et al. 2012).

The current study aimed to investigate the effect of different coloured shade nets on some nutritional characteristics of a red coloured kapia type pepper cultivated in unheated walk-in plastic tunnels under Hungarian climatic conditions.

## Material and Methods

### Methods of the cultivation

The experiment was carried out in 2012 in a private farm close to Lajosmizse (NL 47°00', EL 19°64'). Kopia type, red coloured 'Kárpia F1' pepper hybrid was cultivated in six unheated walk-in plastic tunnels. Main parameters of the tunnels were the following: 5 m width, 2.3 m height and 40 m length. Tunnels were clad by Extra S-24 type (Solarker Ltd., Kecskemét, Hungary) light stable polyethylene film, having 180  $\mu$ m thickness and 8.5 m width.

Forty-day old seedlings were transplanted on the 24<sup>th</sup> of April in double rows at a distance of 0.3 m and 0.7 m between the rows. Distance between neighbouring plants in the rows was 0.25 m. As a result of this planting design, density reached 8 plants per m<sup>2</sup>. Plants were trained in a one-stem system.

Pepper plants were fertirrigated daily through a drip irrigation system. Tunnel ends and side vents were open over 22 °C day-time and 15 °C night-time tunnel air temperature. Overhead spray irrigation was applied when temperature exceeded 30 °C. Hard, full-grown, red coloured fruits were harvested in 7-10-day intervals.

### Treatments

Treatments consisted of different methods of shading; one tunnel corresponded to one

treatment. Four tunnels were covered with different coloured shade nets (white, green, yellow and red) of 34 g m<sup>-2</sup> weight (Első Magyar Kenderfonó PLC, Szeged, Hungary) at the top of the polyethylene cladding for the whole growing season. One tunnel was shaded by Shadefix paint (Royal Brinkman B.V., 's-Gravenzande, Holland) applied at two occasions during the season with a dose of 100 kg ha<sup>-1</sup>. The sixth tunnel remained unshaded and represented control.

### Measurements

Shading effect of treatments in the spectral range between 325 and 1075nm was measured by a portable spectroradiometer (FieldSpec® HandHeld 2, ASD Inc., Boulder, Colorado, USA) using white reference panel. Four measurements per treatment were recorded outside and inside each tunnel on the 2<sup>nd</sup> of August. Air temperature in the tunnels was determined by thermometers (Conrad Electronic SE, Hirschau, Germany) at every 30 minutes from 27<sup>th</sup> April to 27<sup>th</sup> September. For each tunnel three data loggers were situated in the centre of the tunnels at the height of plant apex. Data loggers were protected from direct sunlight and irrigation, and their situation was adjusted to plant height every second week.

Fruits used for chemical analysis were sampled twice in the season, on 19<sup>th</sup> July and on 25<sup>th</sup> September. One sample was composed of five fully coloured fruits, and four samples were taken

from each treatment. Dry matter was measured after freeze drying of the homogenized plant part materials. Ascorbic acid content was analysed using high performance liquid chromatography (Dong and Pace 1996). Total carotenoids were separated and cleaned on Al<sub>2</sub>O<sub>3</sub> column, and finally quantified spectrophotometrically at 470 nm (MÉTÉ 1977). The analyses of total polyphenols was performed according to the Folin-Denis method, spectrophotometrically at 760 nm, using catechin as standard (AOAC 1990).

### Statistical analysis

Nutritional results were expressed as the mean of the four replications ± standard errors. Air temperature values were evaluated by one-way analysis of variance (ANOVA). Nutritive characteristics were analysed with two-way ANOVA with the factors of treatment and sampling time. Mean separations were performed using Fisher's protected least significant difference test at P ≤ 0.05. Correlation analysis was applied to reveal relations among different parameters using Microsoft Excel 2007 software (Microsoft Inc., Redmond, Washington, USA).

## Results and Discussion

### Radiation and temperature conditions

Considerable differences among the shading effect of the treatments were revealed by the experiment. Compared to the white reference values measured

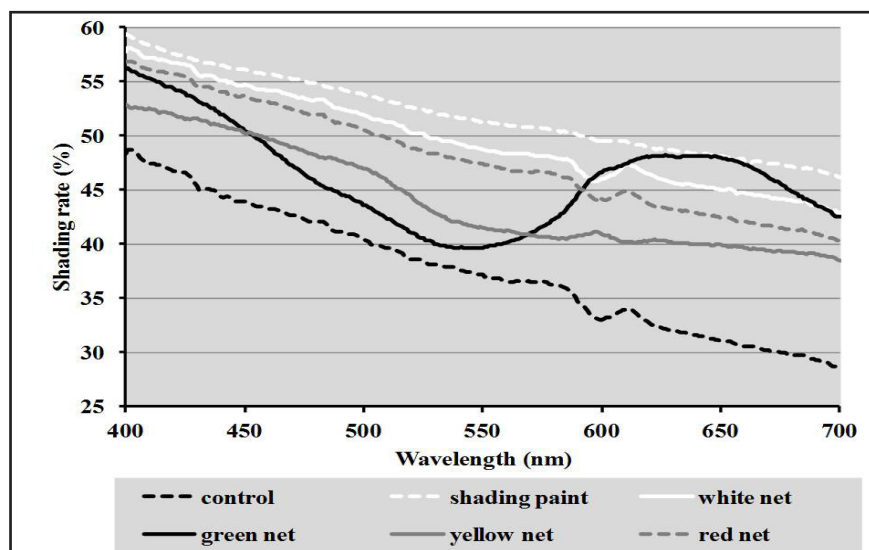


Figure 1. Shading rate of the applied treatments in the PAR range

outside of the tunnel, the cladding material itself showed a 31% cumulative shading ratio for the reflectance spectra between 325 and 1075 nm; while this value was detected 46% for the shading paint, 44% for the white net, 39% for the green net and yellow net, and 42% for the red net treatments. In the photosynthetically active radiation (PAR) range shading effect decreased continuously in relation to increasing wavelength. The order of the treatments was constant in the whole PAR range, except for the green net (Figure 1.). Green shading net resulted in slightly higher shading ratio (47%) in the 600 to 700 nm (red) spectral range than the other three net treatments (40% to 45%).

Except for the yellow shade net, average air temperature of tunnels was not affected by the treatments (Table 1), presumably due to proper ventilation and regular misting. Significant correlation was not found between shading ratio and average air temperature. Significantly the highest average air temperature was recorded for the yellow treatment, both for the whole growing period and for the 4-week period prior to the second sampling. These latter results are in accordance with the findings of Selahle et al. (2015). Unfortunately, based on the gathered data we could not find the reason for significantly higher air temperature in the yellow shade net treatment. One possible explanation is that

this shading material captured emitted infrared radiation in the tunnel more than the other ones. Prior to the second sampling average air temperatures were measured 6-7 °C lower compared to the period prior to the first sampling, when air temperatures were considerably higher than the optimal 21-23 °C range of pepper cultivation (Wien 1997). In accordance with the higher average temperature, ratio of supra-optimal temperature values was also the highest under the yellow net, especially prior to the second harvest (Table 1). During the period prior to the first sampling, ratio of high temperature values was the lowest in the white and in the green net treatments. Hence, plants of these tunnels were exposed to less heat stress.

#### Dry matter content

According to the results of the two-way ANOVA, shading did not significantly affect dry matter content of the kapia type sweet pepper fruits ( $P = 0.451$ ) (Figure 2). This result is in agreement with the findings of Goren et al. (2011) and Kong et al. (2013), but is in contrast with the data of Milenkovic et al. (2012) and Selahle et al. (2015). On the other hand, performing correlation analysis on prior to sampling average air temperature and dry matter content data separately for the two sampling times, significant positive correlations were found if results of the yellow shade net treatment were disregarded.

Table 1. Effect of shading method on temperature conditions of walk-in plastic tunnels

Treatment	Average air temperature (°C)			Ratio of supraoptimal (32°C<) temperature values		
	Whole period	Prior to 1 <sup>st</sup> sampling	Prior to 2 <sup>nd</sup> sampling	Whole period	Prior to 1 <sup>st</sup> sampling	Prior to 2 <sup>nd</sup> sampling
	22.04. – 02.10.	24.06. – 19.07.	28.08. – 25.09.	22.04. – 02.10.	24.06. – 19.07.	28.08. – 25.09.
Control	22.8 b*	25.9 ab	18.5 b	15.8% b	29.0% ab	2.3% b
Shading paint	22.7 b	26.6 a	18.9 b	15.3% b	30.9% a	4.1% b
White net	22.6 b	25.6 b	18.9 b	14.3% b	25.2% c	3.8% b
Green net	22.4 b	25.4 b	19.2 b	15.1% b	24.5% c	8.3% b
Yellow net	23.6 a	26.5 a	20.9 a	21.0% a	31.0% a	17.8% a
Red net	22.6 b	25.6 b	18.9 b	15.1% b	26.3% bc	5.2% b
Significance						
P-value	0.001	0.018	0.002	0.049	0.009	0.036

\*mean separation within columns according to Fisher's protected least significant difference test at  $P \leq 0.05$

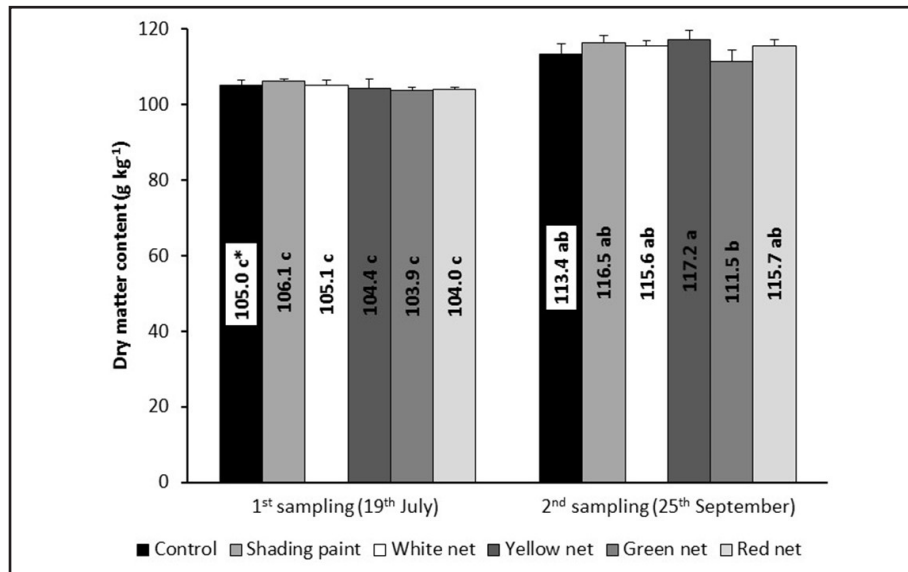


Figure 2. Effect of shading method and sampling time on dry matter content of kapia type red sweet pepper fruits \* Mean separation was performed using Fisher's protected least significant difference test at  $P \leq 0.05$ .

Hence despite of the ANOVA results, it can be stated that in this experiment colour of shading net affected dry matter content of pepper pods in some extent. Meanwhile, fruits sampled at the end of September had significantly higher dry matter content than the ones harvested in July ( $P = 2.28 \times 10^{-11}$ ). It is presumed that less irrigation and misting in September, may have resulted in higher dry matter accumulation in the pepper pods. The treatment x sampling time interaction did not have a significant effect on the dry matter content ( $P = 0.728$ ).

#### Vitamin C content

As sampling time had a significant effect on dry matter content, beside the fresh weight (FW) base, the investigated nutritional characteristics were also expressed on dry weight (DW) base, for ensuring better comparability (Table 2). Measured vitamin C values varied quite considerably, ranging between 1.29 and 2.40 g kg<sup>-1</sup> FW, which is considered around the average value for red coloured sweet pepper cultivars (Table 2). Vitamin C contents were affected by both shading and sampling time and also by their interaction. Kong et al. (2013) and Nagy et al. (2016) also found that shading effect on ascorbic acid content of sweet pepper changed with harvest times. On average of the two sampling times white and yellow

shade nets resulted significantly the highest DW based vitamin C content, while the red net treatment and the control had the lowest values (Table 2.). This order of the treatments is in good agreement with the findings of field experiments of Kong et al. (2013), Tinyane et al. (2013) and Selahle et al. (2015), but partly contradicts to results of Milenkovic et al (2012). Latter authors found significantly the highest ascorbic acid concentration under red shade net in plastic tunnels, while the same red net produced significantly the lowest vitamin C value under field conditions. Mashabela et al. (2015) assumed that more favourable red/far red (R/FR) photon ratio under the white net could improve the ascorbic acid content and the antioxidant scavenging activity in sweet pepper fruits. Among the coloured shade nets white has the best light-diffusing capability in the PAR range, resulting in deeper light penetration into the canopy (Kong et al. 2013). This effect can increase the lower vitamin C content of pepper pods situating in more shaded parts of the plants (Milenkovic et al. 2012). High vitamin C content under the yellow shade net could be the consequence of higher air temperature in this treatment (Lee and Kader 2000).

Difference among the treatments was proved considerably lower at the first sampling than for the second one. On average of the six shading

Table 1. Effect of shading method and sampling time on some phytonutrient contents (mean  $\pm$  SE) of kapia type red sweet pepper fruits

	Vitamin C		Total carotenoids		Total polyphenols	
	g kg <sup>-1</sup> FW	g kg <sup>-1</sup> DW	mg kg <sup>-1</sup> FW	mg kg <sup>-1</sup> DW	g kg <sup>-1</sup> FW	g kg <sup>-1</sup> DW
1 <sup>st</sup> sampling (19.07.)						
Control	1.76 $\pm$ 0.04 d*	16.8 $\pm$ 0.2 cd	75 $\pm$ 11 c	712 $\pm$ 104 c	2.54 $\pm$ 0.04 a*	24.21 $\pm$ 0.18 a
Shading paint	2.04 $\pm$ 0.09 bc	19.3 $\pm$ 0.9 ab	80 $\pm$ 3 c	754 $\pm$ 34 c	2.49 $\pm$ 0.03 ab	23.37 $\pm$ 0.10 ab
White net	1.70 $\pm$ 0.03 d	16.1 $\pm$ 0.2 d	103 $\pm$ 14 c	974 $\pm$ 120 c	2.48 $\pm$ 0.03 abc	23.59 $\pm$ 0.17 ab
Green net	1.77 $\pm$ 0.05 d	17.0 $\pm$ 0.3 bcd	81 $\pm$ 2 c	778 $\pm$ 39 c	2.36 $\pm$ 0.07 bcd	22.58 $\pm$ 0.54 bc
Yellow net	1.74 $\pm$ 0.06 d	16.8 $\pm$ 0.6 cd	86 $\pm$ 5 c	833 $\pm$ 52 c	2.31 $\pm$ 0.06 cd	22.26 $\pm$ 0.52 bcd
Red net	1.80 $\pm$ 0.05 cd	17.3 $\pm$ 0.5 bcd	91 $\pm$ 3 c	874 $\pm$ 26 c	2.37 $\pm$ 0.10 abcd	22.83 $\pm$ 0.94 abc
2 <sup>nd</sup> sampling (25.09.)						
Control	1.34 $\pm$ 0.10 e	11.9 $\pm$ 1.0 e	181 $\pm$ 19 ab	1586 $\pm$ 136 ab	2.33 $\pm$ 0.07 bcd	20.53 $\pm$ 0.51 e
Shading paint	1.83 $\pm$ 0.08 cd	15.7 $\pm$ 0.6 d	204 $\pm$ 8 ab	1756 $\pm$ 77 ab	2.43 $\pm$ 0.09 abc	20.88 $\pm$ 0.82 de
White net	2.40 $\pm$ 0.16 a	20.8 $\pm$ 1.4 a	221 $\pm$ 24 a	1915 $\pm$ 206 a	2.53 $\pm$ 0.06 ab	21.86 $\pm$ 0.43 cde
Green net	2.18 $\pm$ 0.15 ab	18.6 $\pm$ 1.4 abc	174 $\pm$ 27 b	1479 $\pm$ 204 b	2.48 $\pm$ 0.06 abc	21.11 $\pm$ 0.18 de
Yellow net	1.74 $\pm$ 0.03 d	15.6 $\pm$ 0.3 d	169 $\pm$ 17 b	1521 $\pm$ 156 b	1.92 $\pm$ 0.08 e	17.17 $\pm$ 0.28 f
Red net	1.29 $\pm$ 0.08 e	11.2 $\pm$ 0.7 e	197 $\pm$ 12 ab	1710 $\pm$ 125 ab	2.20 $\pm$ 0.04 d	19.05 $\pm$ 0.41 f
Significance (P-value)						
Treatment	2.85*10 <sup>-7</sup>	2.99*10 <sup>-6</sup>	0.14	0.12	3.32*10 <sup>-6</sup>	3.37*10 <sup>-6</sup>
Sampling time	0.88	1.3*10 <sup>-3</sup>	9.05*10 <sup>-15</sup>	4.47*10 <sup>-14</sup>	4.13*10 <sup>-3</sup>	8.25*10 <sup>-13</sup>
Treatment x time	3.94*10 <sup>-8</sup>	1.77*10 <sup>-7</sup>	0.72	0.74	3.31*10 <sup>-3</sup>	5.97*10 <sup>-3</sup>

\*mean separation within columns according to Fisher's protected least significant difference test at  $P \leq 0.05$

treatments DW based vitamin C content was significantly higher for the July sampling than for the samples gathered in September, under cooler conditions. This result is in accordance with the general assumption that heat stress usually results in higher vitamin C accumulation (Lee and Kader 2000). Another factor could be the higher irradiation during summer compared to that in September, as higher solar radiation favours the biosynthesis of ascorbic acid (Tinyane et al. 2013). In previous pepper studies positive correlation between total soluble solids and vitamin C content was found (Milenkovic et al. 2012). However, in this present experiment dry matter and vitamin C content correlation could not be proved. Neither significant correlation between shading ratio and vitamin C content was revealed, in contrary to the findings of Tinyane et al. (2013).

#### Total carotenoid content

According to the results of two-way ANOVA, significant effect of shading treatment on total carotenoid content was not demonstrated. However, according to Fisher's post-hoc test, white shade net resulted in significantly higher carotenoid content compared to the green and the yellow ones at the second sampling time (Table 2). This result is in accordance with the results of Selahle et al. (2015) who found significantly higher  $\beta$ -carotene and lycopene content in red peppers under white shade net compared to red and yellow nets. Also, in the experiment of Ambrózy et al. (2016) the highest total carotenoid content was measured under the white shade net treatment. Light level and quality can affect carotenoid accumulation in red pepper pods (Kim et al. 1978; Russo and Howard 2002). For tomatoes it was found that

fruit-localized phytochromes, sensitive to R/FR ratio, mediate lycopene accumulation in the berries (Alba et al. 2000). Sampling time had a highly significant effect on the carotenoid content. On the average of the six treatments carotenoid level in the fruits of the July sampling was only half of that of the September sampling. This result is in good agreement with the findings of Ambrózy et al. (2016). This phenomenon can be explained by the temperature conditions, as it is well known that supraoptimal temperatures can inhibit carotenoid synthesis in fruits of tomato, an another Solanaceous vegetable (Helyes et al. 2007). In this study, a significant negative correlation was found between the ratio of supraoptimal temperature prior to harvest and total carotenoid content ( $r = -0.90$ ,  $N = 12$ ,  $P < 0.001$ ). Meanwhile correlation was not revealed between shading ratio and carotenoid results.

#### Total polyphenol content

Similarly to the vitamin C results, total polyphenol content was significantly affected by both investigated factors and also by their interaction (Table 2). In the average of the two sampling times the red and the green shading nets resulted significantly lower total polyphenol content than the other four treatments. Tinyane et al. (2013) and Selahle et al. (2015) also found inferior polyphenol content under red shade net in tomatoes and red peppers, respectively. Mashabela et al. (2015) supposed that reduced R/FR ratio could be responsible for lower total polyphenol content. As for the sampling time, total polyphenol content was significantly higher in July than in September, especially when it was expressed on DW basis (Table 2). Polyphenol content increasing effect of heat stress is a well-known phenomenon (Bita and Gerats, 2013). Accordingly, in the present experiment a significant positive correlation between ratio of supra-optimal temperature values prior the samplings and DW based total polyphenol content was found ( $r = +0.59$ ,  $N = 12$ ,  $P < 0.05$ ).

#### **Conclusion**

Effects of shade net colour and sampling time on nutritional characteristics of kapia type sweet pepper cultivated in plastic tunnels were investigated. Net colour influenced light level, light quality and tunnel temperature, while sampling time had a much bigger effect on temperature conditions. Shade net colour significantly affected vitamin C and total-polyphenol contents of pepper fruits, presumably due to modification of light quality. Considering vitamin C, total carotenoid and total polyphenol contents, white shade net improved nutritional value of pepper the most in average of the two sampling times compared to the control treatment. This favourable effect is probably due to higher ratio of diffused light and more favourable R/FR ratio under this net. Hence, if the main goal is producing pepper with better nutritional quality, this colour of shade net is advised to use. It is intriguing that this simple growing practice can significantly improve nutritional quality of sweet pepper produced in plastic tunnels, especially in Hungary where per capita pepper consumption is very high. Our results were in good accordance with previous field and net house experiments but not with a previous research conducted in shade net covered plastic tunnels. Hence, more detailed research is necessary for investigating the combined effect of shade net and plastic sheet covers of tunnels. During these future researches special attention should be paid to the R/FR photon ratios to understand physiological mechanisms. Harvest time influenced nutritive characteristics at a much greater extent than shade net colour, due to significant differences in ratio of supra-optimal temperature values. Heat stress increased total polyphenol concentration and decreased total carotenoid content of pepper pods.

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