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Effect of Light Treatment and Maturity Stage on Biomass Production and Bioactive Compounds of Two Pepper Cultivars under a Deep Water Culture Hydroponic System

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Abstract: Previous pepper studies indicated that biomass production and the amounts of bioactive compounds were dependent on light sources, maturity processes and pepper genotypes. However, the above topic has received little attention in supplemental light versus cultivar combinations under a hydroponic growing system. Therefore, the aim of this study was to evaluate the biomass production (fruit, root, stem and leaf) and fruit bioactive compounds (vitamin C, total flavonoid content and antioxidant capacity-AC-FRAP, total polyphenol-TPC) of two pepper cultivars ('Fehérözön'-Fö and 'Szegedi 80'-S80) in three fruit maturity stages (green, beaker and red) under two LED light treatments (full-F and blue-white-BW spectrums) in a deep water culture hydroponic system. The stem biomass and water use for total and fruit biomass were significantly different for cultivars and light treatments. Light treatments, maturity stages and cultivars had significant effects on fruit biomass production and on all bioactive compounds. However, the results on the bioactive compounds varied according to the green, beaker and red maturity stages of the two pepper cultivars. In correlation analyses, 30 pair-variables correlated significantly and nine showed values r > 0.9 for fruit weight versus (vs.) vitamin C, fruit weight vs. AC-FRAP, fruit weight vs. TPC, vitamin C vs. AC-FRAP, vitamin C vs. TPC, AC-FRAP vs. TPC, and flavonoid vs. TPC. This study suggested that additional lights and maturity features of cultivar genotype strongly determined the biomass and bioactive compounds of pepper under a deep water culture hydroponic system.

Keywords: LED light; *Capsicum annuum*; water use; fresh weight; vitamin C; flavonoid; FRAP; total polyphenol; Pearson correlation

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1. Introduction

Pepper (*Capsicum* sp.) is one of the most important commercially grown vegetable crops in the Mediterranean and temperate zone regions of the world [1–3]. Capsicum species can be classified into various groups based on fruit or pod characteristics such as size, shape, color, pungency and flavor. Despite the great variations, most of the globally cultivated pepper cultivars belong to the *C. annuum* species [2]. A major categorization between the different *C. annuum* types is made by classifying the capsinoid content of fruits differentiating hot and sweet cultivars [2]. Sweet pepper is mainly used for its pleasant flavor. Consumption of pepper enriches the diet considered as of minerals, vitamins, and other food components [3].

Hungary has a long tradition of pepper production. The production area is about 3300 ha, of which approximately 1500 ha is greenhouse surface [4]. The importance of pepper production under protected cultivation systems is constantly growing worldwide [5,6]. Among greenhouse production, the relevance of hydroponic growing systems has been constantly growing in the past decades, due to solving serious plant protection challenges and environmental concerns [7–11]. Conventional (soil-based) cultivation systems are water-consuming mainly due to water loss by excessive irrigation and evaporation. According to Albaho et al. [8], the water use of closed or semi-closed soilless pepper production could be reduced to 43 to 63% compared to the conventional cultivation systems. However, these production methods can consume high amounts of fertilizers, which can be lost in various ways (e.g., leaching, volatilization) in an open irrigation system, causing soil and water contaminations [12]. However, the closed soilless hydroponic system is suitable for pepper cultivation as they have low or zero discharge of fertigation effluents [13].

Light has an impact on plant physiology, flowering, secondary metabolite accumulation, nutrient transport and plant quality [14–19]. Different light spectra can influence the processes of plant part growth. Therefore, the optimum light spectrum for various plant parts (such as for leaf and fruit growth) may be different [15]. Light-emitting diodes (LEDs) are widely used as additional lighting sources in low-light periods [20–22]. The study of Yap et al. [22] demonstrated that pepper fruit yield was higher in the blue light treatment compared to either red or red and blue light treatments. Some studies also investigated the effects of supplemental lighting on sweet pepper production in hydroponic culture [23–25]. However, previous investigation has paid little attention to pepper cultivar biomass of separate plant parts in relation to their water use under supplementary light treatments in a hydroponic system.

Light treatment studies investigated the status of the biochemical components of plants too [26–29]. Azad et al. [26] showed that the total amount of phenolic compounds doubled by red light but far red light decreased the antioxidant activity and ascorbic acid content compared to the control light treatment. The same study confirmed that blue light increased the anthocyanin and chlorophyll contents by 6 and 2 times, respectively [26]. However, the study of Bae et al. [27] used artificial lightening for sweet red pepper and showed that the bioactive compounds (e.g., polyphenols, ascorbic acid, flavonoids, tannins) differed only slightly under various light sources. The amounts of various bioactive compounds were also investigated during the fruit maturity stages of pepper; however, both increasing or decreasing patterns were detected during maturity processes [30–37]. Daood et al. [30] showed that small amounts of tocopherol, ascorbic acid, and P-carotene could be measured in in green mature stage of pepper fruits. During fruit ripening, ascorbic acid content remained constant in the studies of Gnayfeed et al. [31], Martí et al. [34] and Shaha et al. [36], while it increased in the study of Pérez-López et al. [33]. In the case of flavonoids, Shaha et al. [36] found that flavonoid content in the red ripe stage was higher than that in the green mature stage in chili pepper cultivars. An accumulation of polyphenols was shown during ripening in the study of Oney-Montalvo et al. [37], while the study of Oboh and Rocha [32] showed higher polyphenol levels in immature, green fruits of pepper than in the mature ones.

Various results of previous studies indicated that biomass production and the amounts of bioactive compounds were dependent on light sources, maturity processes and pepper genotypes [22,26–37]. However, investigating the biomass production of pepper genotypes together with bioactive compounds and interpreting their intercorrelations have received little attention in previous studies in supplemental light vs. cultivar combinations under a hydroponic growing system.

The aim of this study was to study the biomass production (fruit, root stem and leaf) and fruit bioactive compounds (vitamin C, total flavonoid content and antioxidant capacity) of two remarkably different pepper cultivars (‘Fehérözön’ and ‘Szegedi 80’) in three fruit maturity stages (green, beaker and red) under two LED light treatments (full and blue-white spectrum) in a deep water culture hydroponic system.

2. Materials and Methods

2.1. Experimental Design, Plant Material, Hydroponic System, Nutrient Supply and Fruit Maturity Stage

The experiment was performed on two pepper cultivars (cvs) under two light treatments in a closed hydroponic plant growth system. For both cultivars, three fruit maturity stages were examined. The experiment was conducted with 60 ($15 \times 2 \times 2$) plants replicated four times. The whole experiment was conducted twice.

The two Hungarian cultivars used in the experiment were cv 'Fehérözön' (*Capsicum annuum*), a waxy sweet pepper cultivar with determinate growth habit and yellow-white fruit color and the cv 'Szegedi 80' (*Capsicum annuum* cv. *longum*), an indeterminant sweet spicy pepper with a dark green fruit color during fruit development; 30-day old plantlets of cvs 'Szegedi 80', and 'Fehérözön' were sown in a commercial propagator in rockwool slabs ($5 \times 5 \times 10$ cm, Grodan Prestige, Grodania A/S, Milton, ON, Canada).

For plant growth, a closed deep water culture (DWC) hydroponic plant growth system was used in the experiment. Three plants were placed into each M30 plastic box (Kurucz és Társa Ltd., Debrecen, Hungary), in a 10×10 cm plastic pot each. Three seedlings were placed into one box by inserting the pots into a hole in the top of the box, filling the pots with clay moss, while an aquarium aerator was also placed in the box. The boxes were filled with 30 L of clean water using the concept of reverse osmosis.

A Horti LEDs growing greenhouse lighting set (Hortiled, UAB, Lithuania) was used in the experiment. Two light experiment settings were performed: (i) control (i.e., full light): red: 70%, far red: 2%, white: 10% and blue: 10% and (ii) blue-white: blue: 40% and white: 60%. The PPFD was 320 μmol/m^2/s. The average natural light penetration in the greenhouse was 14,600 lux, 330 μmol/m^2/s. PPFD, 0.131 mW/cm^3 UV on average. Temperature and relative humidity were constantly measured by the Priva greenhouse controlling system (Priva BV, De Lier, The Netherlands).

For nutrient supply, the applied products were Bionova A, Bionova B, Bionova Ca, Bionova MgO10 and Bionova MicroMix (Bio Nova International B.V., Waalwijk, The Netherlands). Nutrients were supplied on a daily basis according to the manufacturer's recommendation. The nutrition solution was optimized based on the EC and pH according to the plant development and nutrient status.

At harvest, the fruit biomass production and the four bioactive components were measured for three fruit maturity stages (green, beaker and red).

2.2. Biomass Determination with Water Use Efficiency

The biomass of fruits (placenta with seeds and pericarp), stems, leaves and roots were weighed at the harvest time in each treatment. Water use (g/L) for total biomass or fruit biomass was calculated as the total biomass or fruit yield was divided by the consumed water amount.

2.3. Analytical Assays of Bioactive Compounds

2.3.1. Vitamin C Determined by the α-α′ Dipyridyl Method

Vitamin C was determined by the α-α′ dipyridyl method according to Stevens et al. [38]. Samples of 5 g fresh hydroponic pepper were acidified with 1 mL galactialacetic acid in a mortar with quartz sand. The homogenized solution was filtered through to Whatman filter paper (Merck, Darmstadt, Germany, WHA1001325) to the 100 mL Erlenmeyer flask and was filled to 100 mL with distilled water; 0.2 g ascorbic acid (Merck, Germany) was dissolved in 0.5 mL glacial acid and was diluted to 100 mL for the calibration curve; 1 mL dissolved ascorbic acid was supplemented with 0.3 mL 40% H_3PO_4, 0.1 mL 1% $FeCl_3$ and 0.25 mL α-α dipyridyl dissolved in 96% ethanol.

All samples were prepared in the same way and were placed in the dark for 30 min. Samples were diluted to 10 mL. Samples were measured by spectrophotometry at 496 nm wavelength. The blind sample was considered as the buffer of additional reagents without samples.

2.3.2. Total Flavonoid Content Determined by Spectrophotometric Method

Total flavonoid content determined by spectrophotometric method according to da Silva et al. [39]. Samples of 5 g of fresh pepper fruits were homogenized in mortar in dd H_2O: methanol (20:80); 1 mL aliquot of the diluted sample or catechin standard solutions (0.2, 0.4, 0.6, 0.8 and 1.0 mg/10 mL) was put into a 10 mL flask containing 4 mL dd H_2O. 0.3 mL 5% $NaNO_2$ was added to the flask at the initial time. Then after 5 and 6 min 0.3 mL 10% $AlCl_3$ and 2 mL 1 M NaOH was added to the mixture, respectively. Immediately, the flask was diluted to a volume with the addition of 2.4 mL of dd H_2O and mixed. The absorbance of the mixture was determined at 510 nm (pink in color) compared to the water blank. Total flavonoids of pepper fruits (fresh weight basis) were expressed as mg/100 g catechin equivalents (CE).

2.3.3. Antioxidant Capacity Determined by FRAP Method

Reagents included (i) 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine, Fluka Chemicals, Buchs, Switzerland) in 40 mmol/L HCl (BDH); (ii) 300 mmol/L acetate buffer, pH 3.6 (3.1 g $C_2H_3NaO_2 \times 3H_2O$ (Merck, Germany) and 16 mL $C_2H_4O_2$ (WVR, Debrecen, Hungary) per L of buffer solution) and (iii) 20 mmol/L $FeCl_3 \times 6H_2O$ (BDH). The FRAP reagent was prepared by mixing 2.5 mL TPTZ solution, 25 mL acetate buffer and 2.5 mL $FeCl_3 \times 6H_2O$ solution.

For the samples: aqueous solutions of Fe(II) concentration were used for calibration in the range of 100–1000 mmol/L ($FeSO_4 \times 7H_2O$; Riedel de Haen, Berlin, Germany). Pepper fruit samples were mixed with 300 mL FRAP reagent and were heated to 37 °C when a reagent blank reading was conducted at 593 nm. Then 10 mL of sample with 30 mL H_2O were added. Absorbance measures were conducted after 0.5 s and every 15 s thereafter during the measuring period. Changes in absorbance (DA 593 nm) between the final and the reagent blank readings were calculated for all samples. All solutions and measurements were prepared on the same day. Antioxidant capacity was then quantified and expressed as ascorbic acid (ASA) mg/100 g dry matter.

2.3.4. Total Polyphenol Content—TPC

The total polyphenol content (TPC) was assessed by the Folin–Ciocalteu method [40,41]. The absorbance of the pepper sample mixtures was measured at 765 nm using a SPECTROstar® Nano device (BMG Labtech, Ortenberg, Germany). TPC was quantified and expressed as gallic acid equivalent (GAE) mg/100 g dry matter.

2.4. Statistical Analysis

Significant differences among mean values were determined by ANOVA for light treatments, maturity stages and cultivars in order to compare the means of the fruit biomass and bioactive parameters using $LSD_{0.05}$ values with the program of Statistica for SPSS version 13. ANOVA was also performed for light treatments and cultivars in order to compare the means of stem, root, leaf biomass and WU using $LSD_{0.05}$ values.

Pearson correlation coefficients (r) and their associated significance levels (p = 0.05 and 0.01) were determined for the relationships among fruit biomass parameters and the four bioactive compounds separately for full and blue-white light treatments. Pearson correlation coefficients for the five parameters were also determined separately for the two cultivars. Then the strongest correlated pairs were selected.

3. Results

3.1. Effect of Light and Cultivar on Biomass of Fruit, Root, Stem and Leaf

The datasets of biomass of fruit and stem were significantly different for the two light treatments and for the two cultivars at $p < 0.05$ according to ANOVA. ANOVA showed a significant effect for light treatments and cultivars at $p < 0.05$ on the. The two-way interaction was non-significant. However, ANOVA showed non-significant effects for light treatments and cultivars at $p < 0.05$ on the dataset of biomass of root and leaf (Figure 1b,d).

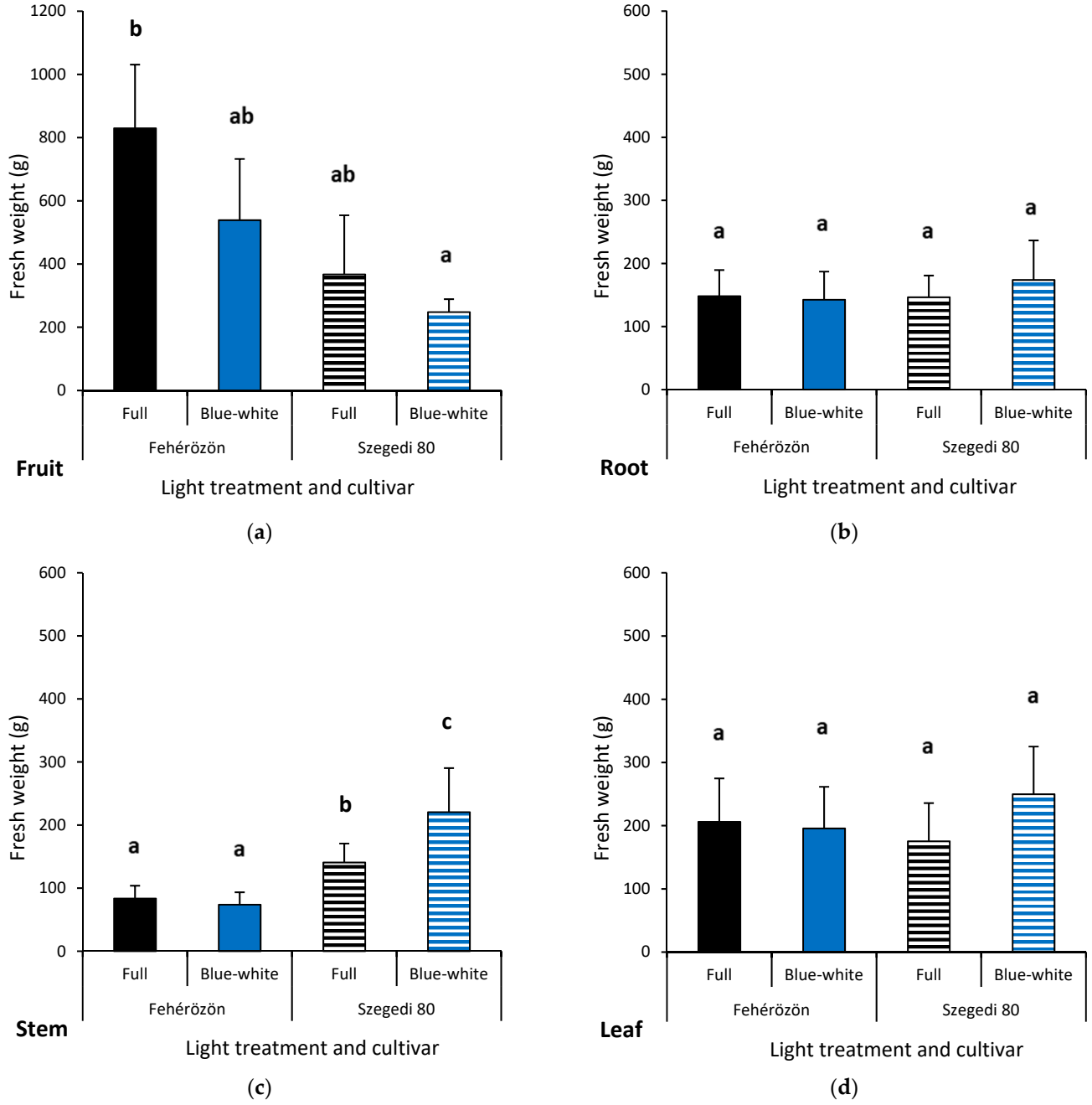


Figure 1. The effect of two light treatments (full and blue-white) and two pepper cultivars ('Fehérözön' and 'Szegedi 80') on fresh weight (g) for fruit (**a**), root (**b**), stem (**c**) and leaf (**d**). Data are means of three replicates. Differences among light treatments and cultivar were based on $LSD_{0.05}$ values at $p = 0.05$. In figure (**a**–**d**), different letters above each column are significantly different among the light treatments and cultivars. On the columns, bars indicate standard deviation (SD) values.

The full light treatment showed the highest fruit fresh weight (829.4 g) for cv. 'Fehérözön', whereas the lowest fresh weight value was 73.8 g for the stem of cv. 'Fehérözön' (Figure 1a,c).

In the case of fruit biomass, fresh weight was significantly different between full light treatment for cv. 'Fehérözön' (829.4 g) and blue-white treatment for cv. 'Szegedi 80' (247.8 g) (Figure 1a). However, these two treatments were not significantly different from either full light treatment for cv. 'Szegedi 80' and blue-white treatment for cv. 'Fehérözön'.

In the case of stem biomass, fresh weight was significantly different between cvs. 'Fehérözön' (83.5 and 73.8 g)and 'Szegedi 80' (140.6 and 220.4 g) in both light treatments

(Figure 1c). Stem fresh weight values were significantly different between full and blue-white treatments for cv. 'Szegedi 80'; however, stem fresh values were not significantly different between full and blue-white treatments for cv. 'Fehérözön'.

3.2. Effect of Light, Cultivar and Maturity on Fruit Biomass

The values of fruit biomass were significantly different for the two light treatments and for the two cultivars at $p < 0.05$ according to ANOVA. The two- and three-way interactions were non-significant (Table 1).

Table 1. Analysis of variance for the effects of light treatment, cultivar and maturity stage on fruit weight, vitamin C, flavonoid, AC-FRAP and TPC content in two light treatments (full and blue and white) and two pepper cultivars ('Fehérözön' and 'Szegedi 80').

Variance Sources	df	Fruit Weight		Vitamin C		Flavonoid		AC-FRAP		TPC	
		MS	*p*	MS	*p*	MS	*p*	MS	*p*	MS	*p*
Light treatment (L)	1	80,701	0.000	32,525,125	0.000	13,486	0.000	11,028,999	0.000	34,052,216	0.000
Cultivar (C)	1	1600	0.048	235,435	0.021	3032	0.009	324,391	0.042	577,676	0.002
Maturity (M)	2	3029	0.039	363,293	0.004	3151	0.012	436,568	0.032	267,561	0.011
L × C	1	2633	0.052	8241	0.067	620	0.080	61,376	0.081	47,087	0.057
L × M	2	1023	0.064	3243	0.137	156	0.256	34,542	0.098	31,541	0.069
C × M	2	480	0.422	2601	0.231	131	0.237	45,372	0.077	34,527	0.065
L × C × M	2	294	0.691	3581	0.112	151	0.251	35,887	0.099	53,022	0.052
Error	24	311		4237		82		24,371		6451	
Total	36										

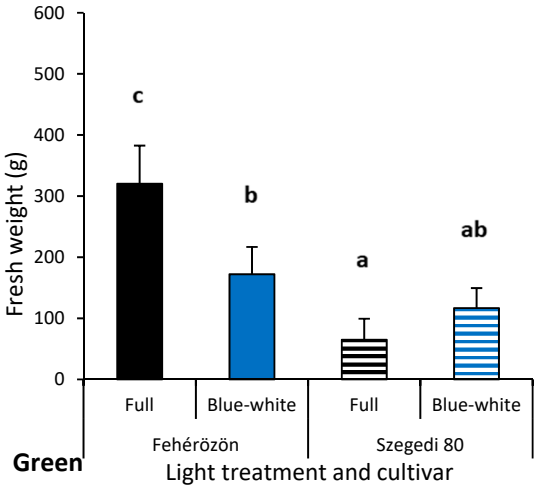
df: degrees of freedom. *p*: the probability values associated with the F-tests. MS: mean squares. AC-FRAP: antioxidant capacity measured with FRAP method, TPC: total polyphenol content.

The full light treatment showed the highest fruit fresh weight (320 g) for the green maturity stage of cv. 'Fehérözön' in the full light treatment, whereas the lowest fruit fresh weight value was 15.2 g for the red maturity stage of cv. 'Szegedi 80' in the blue-white light treatment (Figure 2a,c).

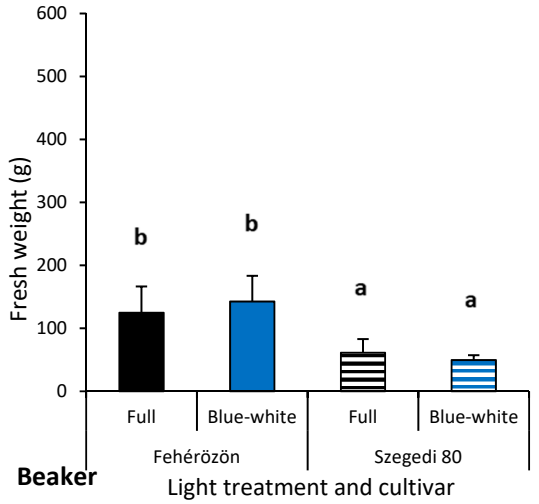
In the case of the green maturity stage, fresh fruit weight was significantly different among full light treatment for cv. 'Fehérözön' (320.1 g), blue-white light treatment for cv. 'Fehérözön' (172.2 g) and full light treatment for cv. 'Szegedi 80' (65.0 g) (Figure 2a). The green fruit fresh value for the blue-white treatment for cv. 'Szegedi 80' was not significantly different from either blue-white light treatment for cv. 'Fehérözön' or full light treatment for cv. 'Szegedi 80'.

In the case of the beaker maturity stage, fresh fruit weight was significantly different between cvs. 'Fehérözön' (124.8 and 142.6 g) and 'Szegedi 80' (61.2 and 49.6 g) for both light treatments (Figure 2b). However, the two light treatments did not differ from each other for either cv. 'Szegedi 80' or cv. 'Fehérözön'.

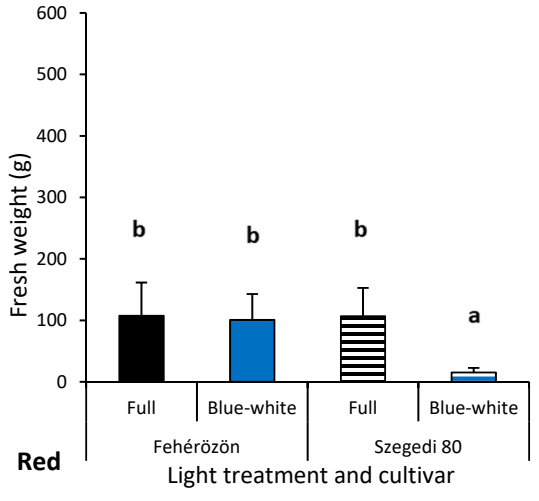
In the case of the red maturity stage, the fresh fruit weight value of the blue-white treatment for cv. 'Szegedi 80' (15.2 g) was significantly different from all other treatments (full light treatment for cv. 'Szegedi 80', full light treatment for cv. 'Fehérözön', blue-white light treatment for cv. 'Fehérözön', Figure 2c). However, these three treatments were not significantly different from each other.



(**a**)



(**b**)



(**c**)

Figure 2. The effect of two light treatments (full and blue-white) and two pepper cultivars ('Fehérözön' and 'Szegedi 80') on fresh weight (g) for three different fruit maturity stages: green (**a**), beaker (**b**) and red (**c**). Data are means of three replicates. Differences among light treatments and cultivar were based on $LSD_{0.05}$ values at $p = 0.05$. Different letters above each column are significantly different among the light treatments and cultivars. On the columns, bars indicate standard deviation (SD) values.

3.3. Water Use for Total and Fruit Biomass

The datasets of water use for total and fruit biomass were significantly different for the two light treatments and for the two cultivars at $p < 0.05$ according to ANOVA. The two-way interaction was non-significant .

The total biomass for cv. 'Fehérözön' showed the highest water use (33 g/L) in the full light treatment, whereas the lowest water use value was 6.5 g/L for the fruit biomass of cv. 'Szegedi 80' in the blue-white light treatment (Figure 3a,b).

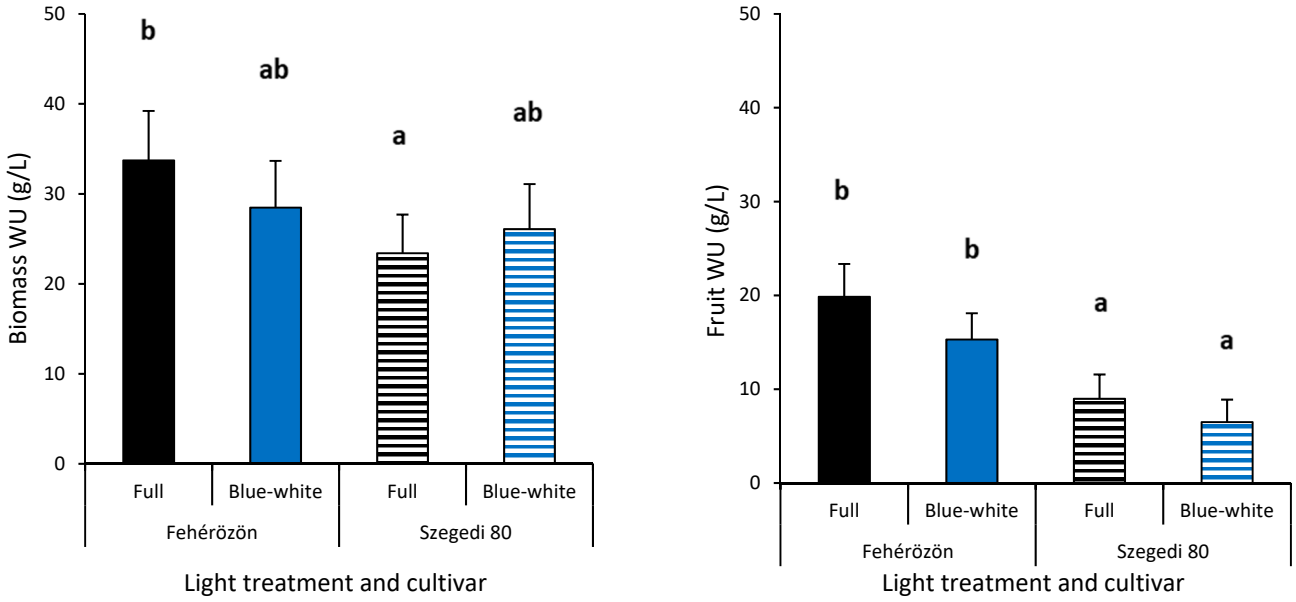


Figure 3. The effect of two light treatments (full and blue-white) and two pepper cultivars ('Fehérözön' and 'Szegedi 80') on water use (WU, g/L) for total biomass (**a**) and fruit yield (**b**). Data are means of three replicates. Differences among light treatments and cultivar were based on $LSD_{0.05}$ values at $p = 0.05$. Different letters above each column are significantly different among the light treatments and cultivars. On the columns, bars indicate standard deviation (SD) values.

In the case of total biomass, water use was significantly different between cvs. 'Fehérözön' (33.7 g/L) and 'Szegedi 80' (23.4 g/L) in the full light treatment (Figure 3a). However, the full light treatment for cv. 'Szegedi 80' did not differ significantly from the blue-white light treatments for cv. 'Fehérözön' or cv. 'Szegedi 80'.

In the case of fruit yield, water use was significantly different between cvs. 'Fehérözön' (19.8 and 15.3 g) and 'Szegedi 80' (9.0 and 6.5 g) for both light treatments (Figure 3b). However, the two light treatments did not differ from each other for either cv. 'Szegedi 80' or cv. 'Fehérözön'.

3.4. Vitamin C

The values of vitamin C were significantly different for the two light treatments and for the two cultivars at $p < 0.05$ according to ANOVA. The two- and three-way interactions were non-significant (Table 1).

The full light treatment showed the highest vitamin C values (1080.9 mg/100 g) for the beaker maturity stage of cv. 'Fehérözön' in the full light treatment, whereas the lowest vitamin C value was 332.9 mg/100 g for the green maturity stage of cv. 'Szegedi 80' in the full light treatment (Figure 4a).

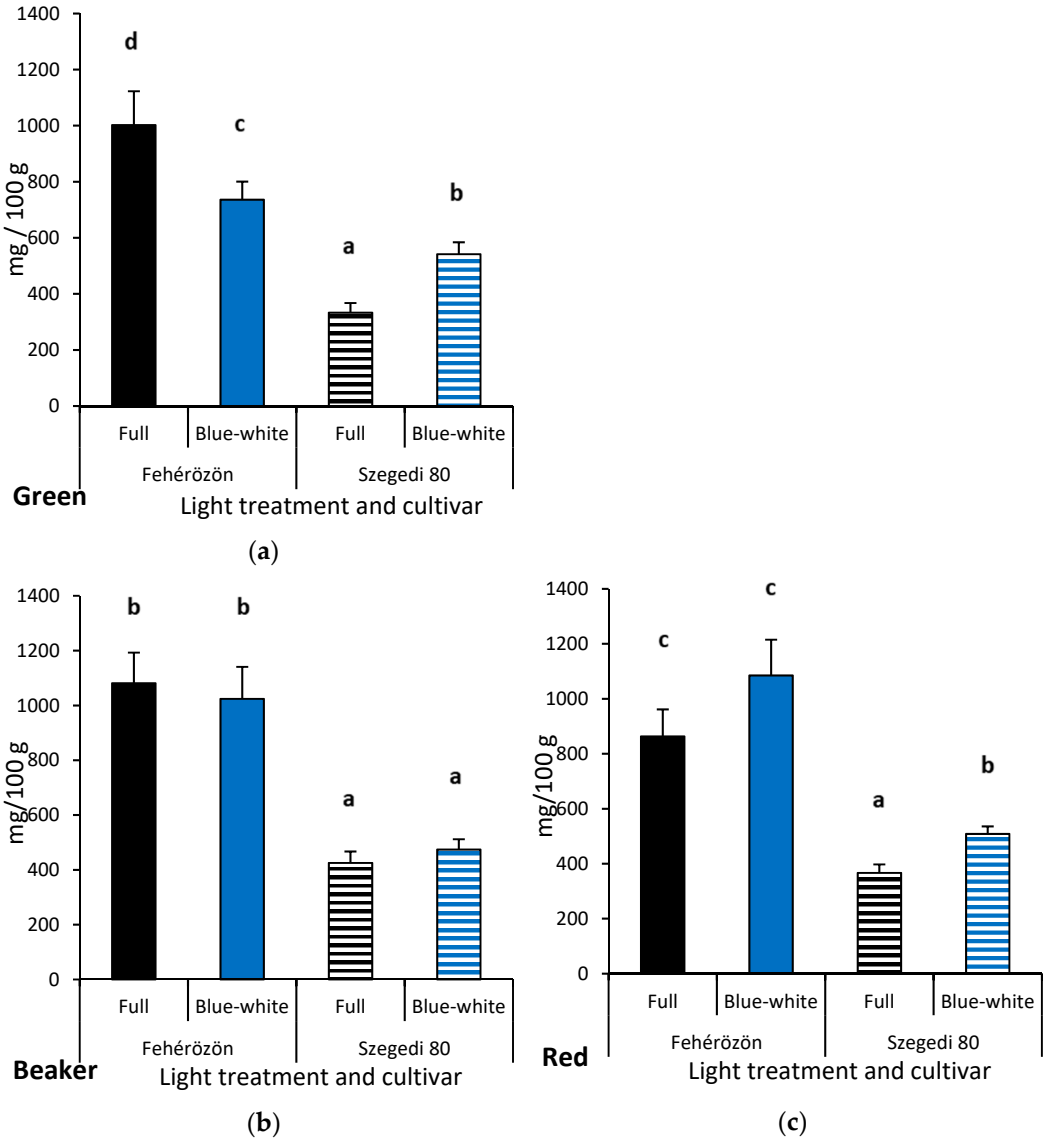


Figure 4. The effect of two light treatments (full and blue-white) and two pepper cultivars ('Fehérözön' and 'Szegedi 80') on vitamin C content (mg/100 g) of fruit for three different fruit maturity stages: green (**a**), beaker (**b**) and red (**c**). Data are means of three replicates. Differences among light treatments and cultivar were based on $LSD_{0.05}$ values at $p = 0.05$. Different letters above each column are significantly different among the light treatments and cultivars. On the columns, bars indicate standard deviation (SD) values.

In the case of the green maturity stage, the vitamin C values were significantly different for both light treatments and two cultivars (Figure 4a).

In the case of the beaker maturity stage, vitamin C content was significantly different between cvs. 'Fehérözön' (1081.0 and 1024.0 mg/100 g) and 'Szegedi 80' (425.0 and 473.9 mg/100 g) for both light treatments (Figure 4b). However, the two light treatments did not differ from each other for either cv. 'Szegedi 80' or cv. 'Fehérözön'.

In the case of the red maturity stage, vitamin C content was significantly different between cvs. 'Fehérözön' and 'Szegedi 80' for both light treatments (Figure 4c). However, the two light treatments did not differ from each other for cv. 'Fehérözön' (863.1 and 1084.9 mg/100 g) while the two light treatments differed from each other for cv. 'Szegedi 80' (36.4 and 508.4 mg/100 g).

3.5. Flavonoid

ANOVA showed a significant effect for light treatments, cultivars and maturity at $p < 0.05$ on the values of flavonoid content. The two- and three-way interactions were non-significant (Table 1).

The full light treatment showed the highest flavonoid content values (116.4 mg/100 g) for the beaker maturity stage of cv. 'Fehérözön' in the full light treatment (Figure 5b), whereas the lowest flavonoid content value was 37.2 mg/100 g for the red maturity stage of cv. 'Szegedi 80' in the full light treatment (Figure 5c).

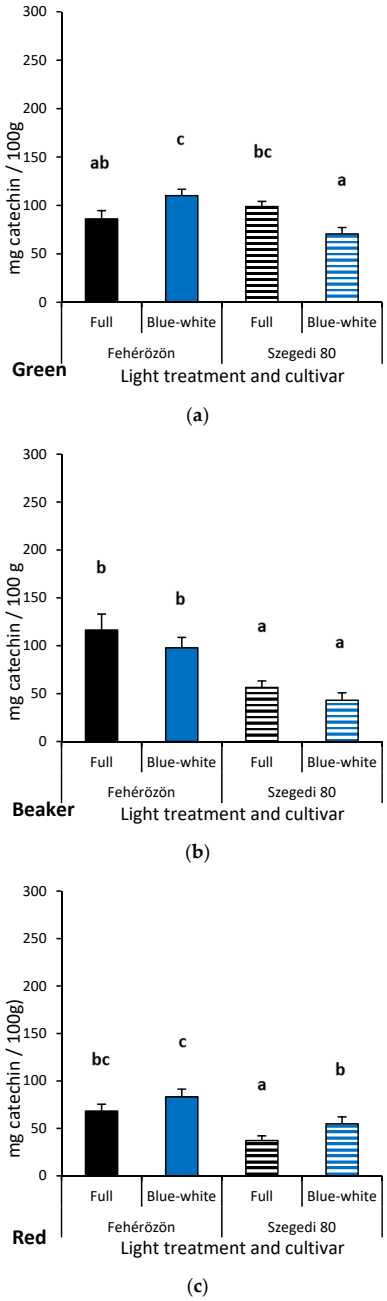


Figure 5. The effect of two light treatments (full and blue-white) and two pepper cultivars ('Fehérözön' and 'Szegedi 80') on flavonoid content (mg catechin/100 g) of fruit for three different fruit maturity stages: green (**a**), beaker (**b**) and red (**c**). Data are means of three replicates. Differences among light treatments and cultivar were based on $LSD_{0.05}$ values at $p = 0.05$. Different letters above each column are significantly different among the light treatments and cultivars. On the columns, bars indicate standard deviation (SD) values.

In the case of the green maturity stage, the flavonoid value of cv. 'Szegedi 80' in the blue-white treatments (70.5 mg catechin/100 g) was significantly lower than the values of cv. 'Fehérözön' in the blue-white light treatment (110.1 mg catechin/100 g) and in the full light treatment for cv. 'Szegedi 80' (98.8 mg catechin/100 g) (Figure 5a). The flavonoid content values did differ significantly between full light treatment for cv. 'Szegedi 80' and blue-white light treatment for cv. 'Fehérözön' as well as between full light treatment for cv. 'Fehérözön 80' and blue-white light treatment for cv. 'Szegedi 80' (Figure 5a).

In the case of the beaker maturity stage, flavonoid content was significantly different between cvs. 'Fehérözön' (116.4 and 97.8 mg catechin/100 g) and 'Szegedi 80' (56.5 and 43.1 mg catechin/100 g) for both light treatments (Figure 5b). However, the two light treatments did not differ from each other either for cv. 'Szegedi 80' or cv. 'Fehérözön'.

In the case of the red maturity stage, flavonoid content was significantly different among blue-white light treatments for cvs. 'Fehérözön' (83.3 mg catechin/100 g) and 'Szegedi 80' (54.8 mg catechin/100 g) as well as for full light treatment for cv. 'Szegedi 80' (37.2 mg catechin/100 g) (Figure 5c). The full light treatment for cv. 'Fehérözön' did not differ from the two light treatments for cv. 'Szegedi 80'.

3.6. AC-FRAP

ANOVA showed a significant effect for light treatments, cultivars and maturity at $p < 0.05$ on the values of AC-FRAP content. The two- and three-way interactions were non-significant (Table 1).

The blue-white light treatment showed the highest AC-FRAP values (2125.7 mg ASA/100 g) for the red maturity stage of cv. 'Fehérözön', whereas the lowest AC-FRAP value was 430.5 mgASA/100 g for the red maturity stage of cv. 'Szegedi 80' in the full light treatment (Figure 6c).

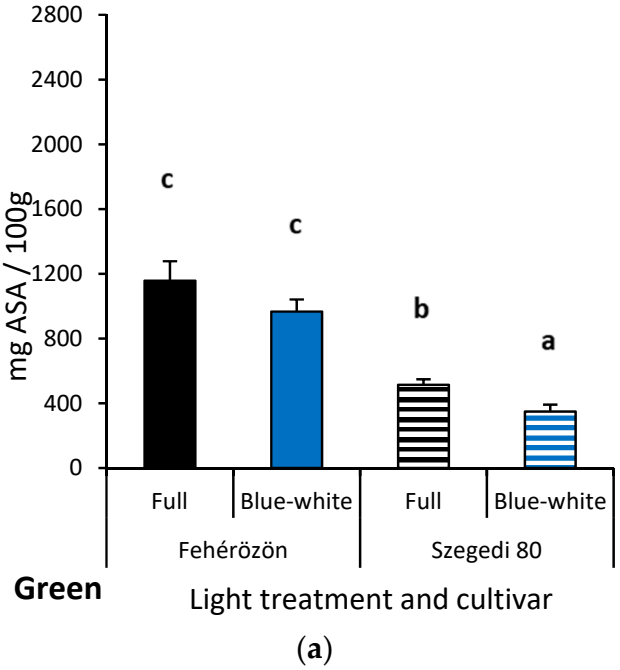
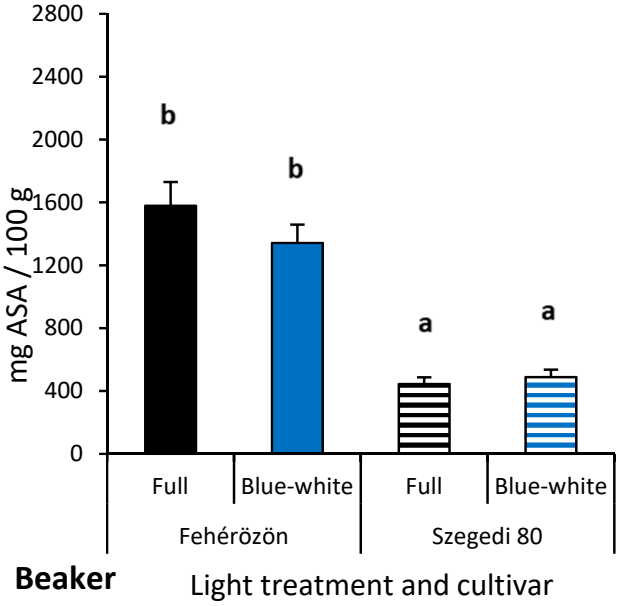
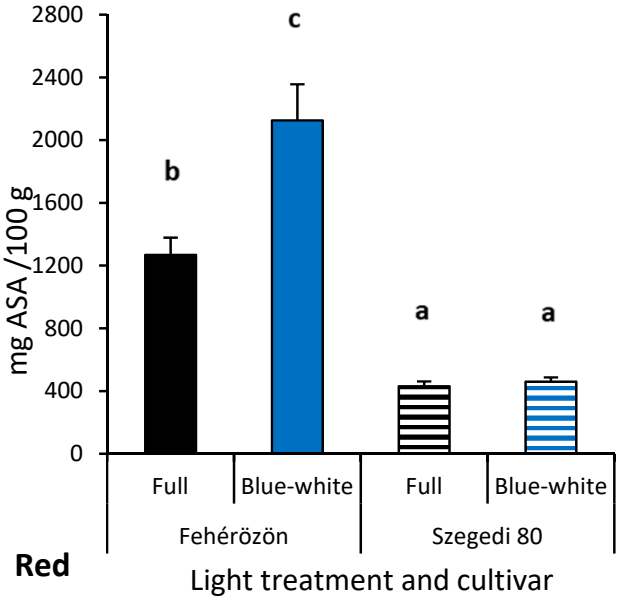


Figure 6. *Cont.*



(**b**)



(**c**)

Figure 6. The effect of two light treatments (full and blue-white) and two pepper cultivars ('Fehérözön' and 'Szegedi 80') on AC-FRAP content (mg ASA/100 g) of fruit for three different fruit maturity stages: green (**a**), beaker (**b**) and red (**c**). AC-FRAP: antioxidant capacity measured with FRAP method. Data are means of three replicates. Differences among light treatments and cultivar were based on $LSD_{0.05}$ values at $p = 0.05$. Different letters above each column are significantly different among the light treatments and cultivars. On the columns, bars indicate standard deviation (SD) values.

In the case of the green maturity stage, AC-FRAP content was significantly different between cvs. 'Fehérözön' (1157.0 and 966.8 mg ASA/100 g) and 'Szegedi 80' (513.9 and 348.7 mg ASA/100 g) for both light treatments (Figure 6a). However, the two light treatments did not differ from each other for cv. 'Fehérözön' while the two light treatments differed from each other for cv. 'Szegedi 80'.

In the case of the beaker maturity stage, AC-FRAP content was significantly different between cvs. 'Fehérözön' (1578.4 and 1342.0 mg ASA/100 g) and 'Szegedi 80' (455.1

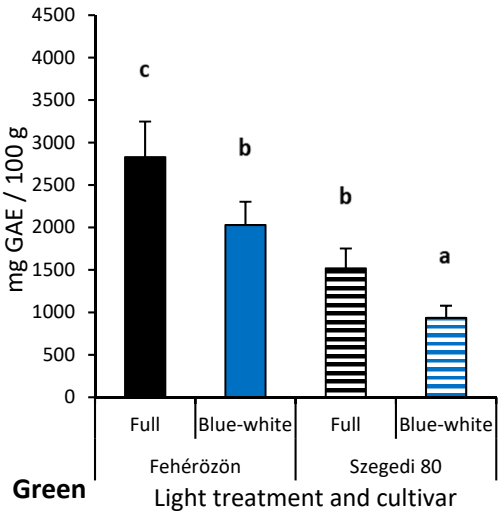
and 488.1 mg ASA/100 g) for both light treatments (Figure 6b). However, the two light treatments did not differ from each other for either cv. 'Szegedi 80' or cv. 'Fehérözön'.

In the case of the red maturity stage, AC-FRAP content was significantly different between cvs. 'Fehérözön' (1267.8 and 2125.7 mg ASA/100 g) and 'Szegedi 80' (430.5 and 459.4 mg ASA/100 g) for both light treatments (Figure 6c). However, the two light treatments did not differ from each other for cv. 'Szegedi 80' while the two light treatments differed from each other for cv. 'Fehérözön'.

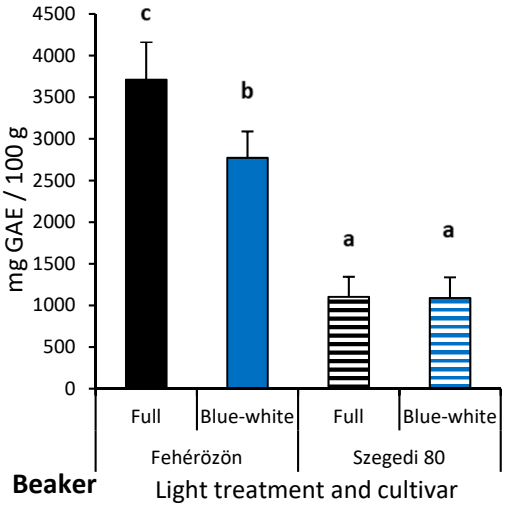
3.7. TPC

The values of TPC content were significantly different for the two light treatments and for the two cultivars at $p < 0.05$ according to ANOVA. The two- and three-way interactions were non-significant (Table 1).

The full light treatment showed the highest TPC values (3710.6 mgGAE/100 g) for the beaker maturity stage of cv. 'Fehérözön' in the full light treatment (Figure 7b), whereas the lowest vitamin C value was 791.3 mgGAE/100 g for the red maturity stage of cv. 'Szegedi 80' in the full light treatment (Figure 7c).



(**a**)



(**b**)

Figure 7. *Cont.*

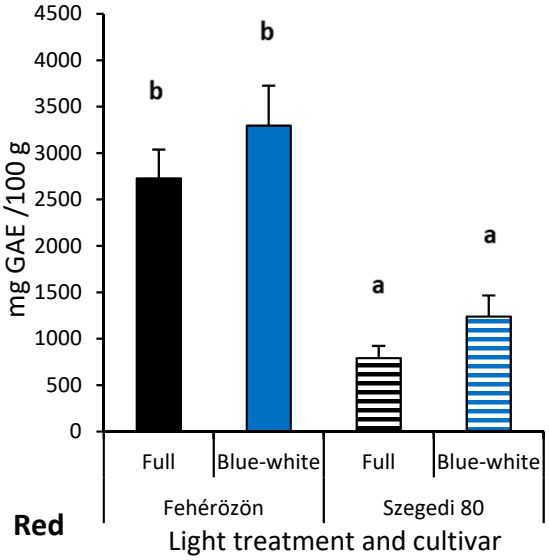


Figure 7. The effect of two light treatments (full and blue-white) and two pepper cultivars ('Fehérözön' and 'Szegedi 80') on TPC content (mg GAE/100 g) of fruit for three different fruit maturity stages: green (**a**), beaker (**b**) and red (**c**). TPC: total polyphenol content. Data are means of three replicates. Differences among light treatments and cultivar were based on $LSD_{0.05}$ values at $p = 0.05$. Different letters above each column are significantly different among the light treatments and cultivars. On the columns, bars indicate standard deviation (SD) values.

In the case of the green maturity stage, TPC content was significantly different among full light treatment for cvs. 'Fehérözön' (2826.7 mgGAE/100 g) and 'Szegedi 80' (1518.2 mgGAE/100 g) as well as blue-white light treatment for cv. 'Szegedi 80' (935.1 mgGAE/100 g) (Figure 7b). However, blue-white light treatment for cvs. 'Fehérözön' did not differ from the full light treatment for cv. 'Szegedi 80'.

In the case of the beaker maturity stage, TPC content was significantly different between cvs. 'Fehérözön' (3710.6 and 2771.8 mgGAE/100 g) and 'Szegedi 80' (1102.3 and 1089.2 mgGAE/100 g) for both light treatments (Figure 7b). However, the two light treatments did not differ from each other for cv. 'Szegedi 80' while the two light treatments differed from each other for cv. 'Fehérözön'.

In the case of the red maturity stage, TPC content was significantly different between cvs. 'Fehérözön' (2727.0 and 3295.4 mgGAE/100 g) and 'Szegedi 80' (791.3 and 1238.9 mgGAE/100 g) for both light treatments (Figure 7c). However, the two light treatments did not differ from each other for either cv. 'Szegedi 80' or cv. 'Fehérözön'.

3.8. Correlation among Parameters

Among the 60 pair-variables, 30 (9, 6, 3, 9, 6 and 1) pair-variables correlated significantly at $p = 0.05$ level in the treatments of full light, cv. 'Fehérözön' in full light, cv. 'Szegedi 80' in full light, blue-white, cv. 'Fehérözön' in full light, and cv. 'Szegedi 80' in full light, respectively (Table 2).

Table 2. Pearson's correlation coefficients (r) among 5 measures for two light treatments (full and blue-white) on two pepper cultivars ('Fehérözön' and 'Szegedi 80').

Full Light	**Vitamin C**	**Flavonoid**	**AC-FRAP**	**TPC**
Fruit weight	0.953 **	0.598 **	0.953 **	0.935 **
Vitamin C		0.511	0.929 **	0.939 **
Flavonoid			0.607 *	0.714 **
AC-FRAP				0.970 **
cv. 'Fehérözön' in full light	**Vitamin C**	**Flavonoid**	**AC-FRAP**	**TPC**
Fruit weight	0.563	0.594	0.603 *	0.579
Vitamin C		0.682 *	0.253	0.652 *
Flavonoid			0.676 *	0.823 **
AC-FRAP				0.827 **
cv. 'Szegedi 80' in full light	**Vitamin C**	**Flavonoid**	**AC-FRAP**	**TPC**
Fruit weight	0.507	0.058	0.380	0.153
Vitamin C		−0.496	−0.154	−0.360
Flavonoid			0.714 **	0.972 **
AC-FRAP				0.746 **
Blue-white light	**Vitamin C**	**Flavonoid**	**AC-FRAP**	**TPC**
Fruit weight	0.691**	0.757**	0.624 **	0.772 **
Vitamin C		0.643**	0.892 **	0.924 **
Flavonoid			0.479	0.599 *
AC-FRAP				0.922 **
cv. 'Fehérözön' in blue-white light	**Vitamin C**	**Flavonoid**	**AC-FRAP**	**TPC**
Fruit weight	−0.511	0.053	−0.452	−0.263
Vitamin C		−0.626 *	0.767 **	0.802 **
Flavonoid			−0.752 **	−0.842 **
AC-FRAP				0.791 **
cv. 'Szegedi 80' in blue-white light	**Vitamin C**	**Flavonoid**	**AC-FRAP**	**TPC**
Fruit weight	0.315	−0.179	0.582	0.726 *
Vitamin C		0.464	−0.126	−0.024
Flavonoid			−0.583	−0.455
AC-FRAP				0.431

Significant ($p < 0.05$ and 0.01) correlation coefficient values are presented as * and **, respectively. Vitamin C: vitamin C content of fruit, Flavonoid: flavonoid content of fruit, AC-FRAP: antioxidant capacity measured with FRAP method, TPC: total polyphenol content. Data were combined for four replicates and maturity stages. Values of 'r' above 0.9 were highlighted in bold.

Among these 30 significant pair-variables, 18 were presented in the Full light and the Blue-white treatment (Table 2). Among these 30 significant pair-variables, 3 were correlated negatively and 27 were correlated positively (Table 2).

Among the 30 significant pair-variables, 9 pair-variables showed values r > 0.9 for Fruit weight vs. vitamin C, Fruit weight vs. AC-FRAP, Fruit weight vs. TPC, vitamin C vs. AC-FRAP, vitamin C vs. TPC, AC-FRAP vs. TPC and Flavonoid vs. TPC (Table 2).

4. Discussion

4.1. Light and Cultivar Effect on Plant Parts, Fruit Yield and Maturity Stages

In this study, full light vs. blue-white light gave various results on the two different types of pepper cultivars depending on plant parts and fruit maturity stage (Figures 1 and 2). Previous studies showed that pepper yield increased by adding various compositions of supplemental lights [21,27,42]. These previous results were also confirmed in this study by the results of fruit fresh weight of cv. 'Fehérözön' (Figure 1) under full light conditions which coupled with the highest water use for total biomass for cv. 'Fehérözön' under the full light treatment (Figure 3).

This study showed that the fruit and stem were affected by the light treatments while the leaf and root were affected by the light treatments (Figure 1). The effect blue light spectrum was the highest for stem and the lowest for fruit, while the effect of full light was the opposite (Figure 1a,c). Our results on stem fresh weight (Figure 1c) were partially confirmed by a previous study by Liu et al. [43], who showed that supplementary UV-A and blue light significantly improved the quality of pepper seedlings, resulting in healthier pepper plants with stronger stems and this positively affected yield after transplanting probably due to better photosynthesis of plants. In relation to this, Bagdonavičienė et al. [44] confirmed that supplemental cyan (505 nm) and blue (470 nm) LED lights had a significant effect on the photosynthetic activity of the sweet pepper under greenhouse conditions.

In this study, fruit weight was the lowest in the blue-white light treatments (Figure 1a). In previous studies, 6–12% of blue supplemental light rate [45,46] can be advantageous for fruit yield but a higher percentage of blue light might be suboptimal for yield. Our results confirm this statement as the blue light rate was 40% in our blue-white light treatments which caused pepper fruit yield reduction in this study.

In our study, full light treatments had the largest effect on the fresh weight of the green maturity fruit stage of cv. 'Fehérözön' and was significantly higher compared to the corresponding blue-white light treatments in the green maturity stage of fruit (Figure 2). In addition, this cultivar under full light treatment showed significantly higher fresh weights than that of cv. 'Szegedi 80' under the blue-white light treatments in all three maturity stages (Figure 2). These results are in line with previous results of Kaiser et al. [45] who showed that adding 24% blue light could be suboptimal for growth and yield. However, the possible reason for the positive effect of full light treatment on fruit weight is that the red light part within the full spectrum of supplemental light may play an essential role in the development and maturity of pepper fruit. This was confirmed by Naznin et al. [46] who showed that significantly higher numbers of pepper flowers and fruit were measured under the 95% red + 5% blue light treatment including a high chlorophyll content in the plant. Kim et al. [21] and Schuerger et al. [42] also confirmed that adding far-red light to red light increased the yield of sweet pepper fruits in greenhouses.

Our study, however, clearly indicated that the effect of blue light on maturity stages can be largely affected by various pepper genotypes. Pepper cultivars have different light requirements and their reaction to various light compositions is also affected by the canopy architecture of the given genotypes [47].

4.2. Bioactive Compounds: Vitamin-C, Flavonoid, AC-FRAP and TPC

Pepper fruit is an important source of vitamin C (50–1400 mg/100 g)) for human consumption [48], and in this study it ranged between 332.9 and 1080.9 mg/100 g in the light vs. cultivar treatments combinations (Figure 4). These values in various maturity stages were consistent with the results of the studies of [30,49]; however, some studies reported a higher vitamin C content of pepper fruit such as Gnayfeed et al. [31], where cv. 'Szegedi 178' had 1142.6 and 1256.7 mg/100 g vitamin C content at the beaker and red maturity stages, respectively.

In this study, the two pepper genotypes showed remarkable differences in vitamin C content as cv. 'Fehérözön' had significantly higher vitamin C content than cv 'Szegedi 80' in all light treatments and maturity stages (Figure 4). This probably originates in the different growing and fruit maturity features of the two cultivars.

The cv. 'Szegedi 80' has indeterminant growing features 'with dark green fruit features during most maturity stages and it showed relatively low but consistent vitamin C content in the three fruit maturity stages (Figure 4). Our results are consistent with some previous results [31,34–36] where authors concluded that vitamin C remained constant during fruit maturation depending on the pepper species/cultivars.

The determinant cv. 'Fehérözön' with yellow-white fruit color of most maturity stages showed considerable variation among maturity stages and light treatments too compared to cv. 'Szegedi 80'. Some previous studies showed a maturity stage-dependent increase in

vitamin C content of some pepper genotypes [33,35]. The reported increasing tendencies in ascorbic acid content during pepper ripening were associated with the role of ascorbate as a photoprotector [33].

In previous studies, the amount and intensity of light during the growing season influenced the amount of ascorbic acid in the plant parts [50]. Ascorbic acid is synthesized from sugars supplied through photosynthesis in plants and fruits exposed to direct sunlight contain a higher amount of vitamin C compared to shaded fruits on the same plant [50]. However, the study of Azad et al. [26] showed that ascorbic acid content was reduced with supplemental light, while Ohashi-Kaneko et al. [51] showed that supplemental blue light significantly increased the ascorbic acid concentration compared to control treatment. Overall, various results indicate that the mechanisms of the light dependence on the formation of vitamin C in pepper fruit needs further investigation.

Total flavonoid content showed light-dependent changes in the three maturity stages of this study (Figure 5). These results were in line with the previous study of Bhandari et al. [35] who stated that nutrients in the plants have their own unique pattern of accumulation and/or degradation processes during the fruit-maturing stages. In the case of cv. 'Szegedi 80', the flavonoid content was higher in the green mature stage than in the beaker or red maturity stages, while cv. 'Fehérözön' showed much less maturity dependence on the flavonoid content of fruit (Figure 5). However, Shaha et al. [36] found that flavonoid content in the red ripe stage was higher than that in the green mature stage in chili cultivars. In addition, light effects on the flavonoid content of pepper fruit varied in the three maturity stages of this study (Figure 5). These results are in line with the study of Gangadhar et al. [28], where there were significant differences in the production of various metabolites among the different LED illumination treatments.

The AC-FRAP values were similar for the cv. 'Szegedi 80' in both light treatments in all maturity stages (Figure 6) and were significantly lower compared to cv. 'Fehérözön' in all cases (Figure 6). The difference in antioxidant capacity among sweet pepper cultivars was confirmed by the study of Martí et al. [34] and Palma et al. [52]. For example, the study of Palma et al. [52] showed that the pungent cultivars' antioxidants had different behavior during the maturation process compared to the sweet ones, and these cultivars could have a different reaction to the light quality. In this study, cultivar differences of AC-FRAP were similar to that of the vitamin C content (Figures 4 and 6). In addition, strong associations were found between the two parameters the correlation coefficient values for 'Full light' (r = 0.511–0.970) and 'blue-white light' (r = 0.599–0.924) analyses (Table 2). This was in line with the work of Kim et al. [23] who showed that the bioactive compounds of pepper correlated with the antioxidant activity.

The TPC content varied during fruit ripening under the two light treatments in both cultivars (Figure 7). In the case of cultivars, the cv. 'Fehérözön' had more TPC in all maturity stages compared to cv. 'Szegedi 80' in the full light treatments (Figure 7). However, significant accumulations of polyphenols could not be detected either in the order of green, beaker and red maturity stage or the other way around. Previous studies by Oney-Montalvo et al. [37] showed that polyphenols accumulated during ripening; however, Oboh and Rocha [32] showed higher polyphenol levels in immature, green fruits of pepper than in the mature ones. The contradiction in TPC accumulation results suggests that several other factors may play a role in the TPC content of pepper fruit maturity stages such as light compositions, growing conditions, and cultivar genotypes. In addition, in both the full and blue-white light treatments, TPC had strong correlations with fruit weight (r = 0.935) and all the other three bioactive compounds (vitamin C, flavonoid content and AC-FRAP; r= 0.939, 0.714, 0.970, respectively) (Table 2). A previous study by Bhandari et al. [35] confirmed also positive correlations among antioxidant activity, vitamin C (r = 0.610 **) and total phenol content (r = 0.595 **) throughout the entire ripening process. This indicates that accumulation patterns of bioactive compounds are likely to fairly complicated as all these parameters may have multi-connections in the light-regulated alteration of the biosynthetic pathways.

Our results suggest that future research on genetic and molecular analysis of light-regulated alteration in biosynthetic pathways will provide better insight into the growth and maturity characteristics of pepper genotypes.

5. Conclusions

This study showed that light treatment and maturity stage had a significant effect on biomass production and bioactive compounds of pepper under a deep water culture hydroponic system. However, the effects were various for the two cultivars that differed in their growing and maturity features.

The majority of best combinations of cultivar, treatment and physiological stage were provided by the full light treatment for cv. 'Fehérözön' in most maturity stages. However, the differences in pepper genotypes were detected for almost all parameters including fruit fresh weight, vitamin C, flavonoid and AC-FRAP and TPC values but at various levels. These results indicated that cultivar genotype is a strong determining factor in parameters of biomass production and in bioactive compounds regardless of maturity stage or light treatment.

The maturity stage showed large differences in the amounts of bioactive compounds between the two cultivars. However, differences between the two light treatments were less consistent and/or varied within a given maturity stage. These results clearly indicated that light treatment has to be adjusted to a given maturity stage and to the specific light requirements of a pepper cultivar.

The highest correlation ($r = 0.972$) was achieved between TPC and flavonoid content for cv. 'Szegedi 80' in the full light treatment. However, correlations among parameters showed strong relationships in several cases among fruit weight and bioactive compounds but the significance of these correlations was largely dependent on light treatment and cultivar.

Overall, this study emphasizes that the effect of additional lights on biomass and bioactive compounds of pepper features are strongly determined by the cultivar genotype under a deep water culture hydroponic system.

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