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Antioxidant Capacity and Shelf Life of Radish Microgreens Affected by Growth Light and Cultivars

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Abstract: Microgreens are young, immature vegetables that contain higher concentrations of active compounds compared to mature vegetables and seeds. Radish microgreens are a good source of antioxidants, phenolic compounds, ascorbic acid, carotenoids, and anthocyanins. The production of microgreens is limited by their short shelf life due to higher dark respiration and accelerated senescence. The study was performed on three radish cultivars (*Raphanus sativus* L.): purple radish (cvP), red radish (cvR), and green radish (cvG). Radish microgreens were grown in chambers with controlled conditions (24 °C and a photoperiod of 16/8 h) under two types of artificial LED light (45 μmol $m^{-2}s^{-1}$): under white light (B:G:R) and a blue/red light combination (B:2R). The effect of the two types of light was examined on the 3rd, 7th, and 14th day after storage at a low temperature (+4 °C). The physiological status of the three cultivars of radish microgreens was examined by measuring the contents of total soluble phenolics, ascorbic acid, proteins, sugars, dry matter, anthocyanins, carotenoids, and chlorophyll as well as the total antioxidant activity. The results revealed that radish microgreens' antioxidant capacity and phytochemical profile depend on the radish cultivar and on the type of LED light used for cultivation. It was shown that B:2R and red cultivar were most beneficial for the synthesis of most of the determined phytochemicals compared to B:G:R, or the purple and green cultivar, respectively. Storage at a low temperature in darkness slowed down most of the metabolic reactions during the first seven days, thus preserving most of the antioxidant activity.

Keywords: *Raphanus sativus* L.; LED light; anthocyanins; total soluble phenolics; ascorbic acid

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1. Introduction

Regular consumption of food of plant origin is necessary for the well-being and health of the human organism. Nowadays, microgreens are gaining popularity in the human diet due to their quick and easy cultivation and rich nutritional value. Especially because of the high concentrations of active compounds such as antioxidants that neutralize free radicals and prevent damage caused by oxidative stress, microgreens are considered as functional food that has positive effects on human health [1–5].

Microgreens are defined as newly sprouted, tender, immature green vegetables, without roots and with fully developed cotyledons or with partially developed first true leaves. The plants are usually harvested 7 to 14 days after sprouting, depending on the species [3,4,6]. The most commonly consumed microgreens are from Brassicaceae, Lamiaceae, and Fabaceae families [2,4,7]. Microgreens are a good source of ascorbic acid, carotenoids, tocopherols, and phenols, as well as macroelements such as K and Ca and microelements Fe and Zn [4,6,7]. It has been shown that they contain higher concentrations of carotenoids, anthocyanins, phenols, vitamins, and minerals in comparison to mature

plants and seeds [3,8,9]. Microgreens are often used for garnishing soups, salads, and sandwiches, and are consumed raw to retain their concentrated flavors, tender textures, vibrant color, and nutrients that are usually lost during heat treatment [4,10,11].

The cultivation of microgreens is carried out in different environments, depending on the scale of production and selection of the plant species. It is influenced by various abiotic factors such as the type of soil for cultivation, fertilization, moisture, light, and temperature [12]. Microgreens are often grown indoors in chambers with artificial lights and under controlled conditions, which enables their cultivation throughout the year and provides protection from potential pollution present in nature. The conditions in the cultivation chambers affect the content of phytochemicals as well as the quality of microgreens after storage [12,13].

The intensity, quality, and duration of light significantly influence the growth and development of plants, including morphogenesis, the normal functioning of the photosynthetic apparatus, and the metabolic pathways [14]. The recent development of light-emitting diode (LED) technology has contributed to ensuring optimal light conditions for plant growth. LEDs provide cheap and cool light sources with a wide range of intensities and wavelengths of light which selectively activate photoreceptors, leading to an increase in the phytochemical content of microgreens [2,15]. Red and blue light are recognized as the most important parts of the spectrum because they are not only the main source of light for photosynthesis, but they also regulate many morphogenic reactions in plants. It has been shown that red and blue lights stimulate plant growth and affect plant quality by stimulating the accumulation of secondary metabolites such as ascorbate, flavonoids, and anthocyanins [2,16,17].

Although the cultivation of microgreens is simple, their production and use are limited by their short shelf life. Therefore, one of the main challenges of the microgreens industry is storage, i.e., maintaining quality after harvesting. Microgreens are difficult to store due to their high surface area to volume ratio and their high rate of respiration and transpiration as well as accelerated senescence [18]. Storage conditions, which include temperature, humidity, and the presence of microorganisms, as well as the type of packaging, can accelerate quality loss and limit their shelf life [19]. Storage temperature is one of the most important factors affecting the physiology and quality of microgreens after harvest [18]. When stored at room temperature, the shelf life of microgreens is three to five days [20], and due to their small size, microgreens freeze quickly at temperatures below 0 °C, which causes significant physical damage to plants. However, storage at a temperature between 0 and 5 °C reduces the rate of respiration and aging, as well as the growth of microorganisms that cause spoilage, significantly reducing the loss of quality and even extending the shelf life for several weeks, depending on the variety and the plant species [18]. An emerging issue in recent years is preventing food waste by extending the shelf life [21]. New biological and biochemical preservation technologies include optimal storage conditions, various preservation technologies, and smart food labeling [22,23] that can extend shelf life and reduce food waste [24]. However, despite the increasing awareness of reshaping the everyday food-consumption practices at the level of individuals and households, it is estimated that 17% of food is wasted at the retail and consumer levels [25].

The nutritional composition of plants is greatly influenced by biochemical changes that occur during their shelf life [3,12,26]. It was shown that post-harvest interventions, e.g., storage temperature, lighting, packaging method, and chlorine wash, can have an impact on the phytochemical profile of microgreens [3,27]. Rocchetti et al. [26] reported significantly a higher content of total phenolics as well as increased total antioxidant capacity in red beet and amaranth microgreens after 10 days storage at 4 °C, depending on the genotype. Yan et al. [28] demonstrated that storage of Tartary buckwheat microgreens at 5 °C combined with selected packaging materials and chlorine + citric acid wash treatment resulted in increased contents of total phenolics, total flavonoids, and antioxidant capacity during the initial 8 days. Furthermore, post-harvest exposure to light has been shown to increase levels of ascorbic acid in radish microgreens when compared to those stored in

the dark [18,19]. On the other hand, radish microgreens stored in the dark had a higher radical scavenging activity and carotenoid retention [18]. Different washing treatments have also been investigated for various microgreens resulting in a significant increase in total phenolics and ascorbic acid during storage [29,30].

Beside the wide range of flavors and colors, as well as high concentrations of bioactive compounds beneficial for human health, quick germination and short growth time are the main reason why the microgreens from the Brassicaceae family became so popular [31]. They have great antioxidant capacity due to numerous antioxidant phytochemicals such as carotenoids, vitamins, mineral elements, and phenolic compounds as well as cancer-fighting glucosinolates [7,31–33]. This makes them excellent dietary sources for human nutrition. However, the composition and content of those phytochemicals vary between the species. Radish microgreens (*Raphanus sativus* L.) are rich in antioxidants, have antimicrobial action, anticarcinogenic properties, and are known as immunostimulants [34]. Various radish cultivars differ by their appearance due to differential contents of chlorophyll and anthocyanins. Green variety daikon radish (*R. sativus* var. *longipinnatus*) contain bioactive compounds beneficial to human health [18] as well as a high phylloquinone content [7]. Red cultivar (*R. sativus* cult. Sango) was reported to have a high content of α-tocopherol while purple cultivar (*R. sativus* cult. China Rose) has the highest amount of total glucosinolates [7] which are known to be involved in plant defense [33].

The availability of microgreen products is constantly rising, i.e., they are offered for sale in local farmers markets, specialty stores, and in chain grocery stores. However, due to the low demands required for their cultivation and the easily available LED settings, microgreens are increasingly grown on a small scale in homes and after harvesting, they are stored in kitchen refrigerators at 4 °C. Therefore, the aim of this study was to simulate such cultivation and storage conditions to examine the antioxidant capacity of home-grown radish microgreens. The seven-day-old radish microgreens, grown under purple and white LED light, were harvested and stored at 4 °C for two weeks. The measurements of total antioxidant capacity and bioactive substances were conducted on the harvesting day and on the 3rd, 7th, and 14th day of storage.

2. Materials and Methods

2.1. Cultivation and Preparation of Plant Material

Certified ecological seeds of all three radish cultivars (*Raphanus sativus* L.) with different leaf colorations: purple radish (*R. sativus* cult. China Rose, cvP), red radish (*R. sativus* cult. Sango, cvR), and green radish (*Raphanus sativus* var. *longipinnatus*, Japanese white or daikon radish, cvG) were purchased commercially from a local supplier (Lokvina d.o.o., Savska Ves, Croatia). About 2.5 g of seeds of each cultivar were soaked in tap water for 24 h and then sown in a mixture of commercial substrate (Klasman TS2) and quartz sand in a 3:1 ratio in plastic containers (13 × 10 × 6 cm). The plants were grown in two separate chambers with artificial LED light (PPFD = 45 µmol $m^{-2}s^{-1}$, photoperiod 16/8 h, 24 ± 1 °C). The light intensity was measured by using Quantitherm QRT1 light meter (Hansatech Instruments Ltd., King's Lynn, UK). The sensor was placed at about the top of the plants (around 5–6 cm above the soil). Two light spectra were used: a combination of red and blue (B:2R) LED light and white (B:G:R) LED light combined of equal parts of blue, green, and red light with peak wavelengths at ~450 nm, ~540 nm, and ~630 nm, respectively. The experiment was repeated once with three biological replicates (each replicate contained 2.5 g of seeds) and at least three technical replicates of each cultivar were grown under two light conditions (B:2R/cvP, B:2R/cvR, and B:2R/cvG, and B:G:R/cvP, B:G:R/cvR, and B:G:R/cvG). The microgreens were grown for seven days until they reached the stage with fully developed cotyledons. On the seventh day of cultivation, the plants were harvested without washing (composite sample) and divided into four parts. One part was used immediately, as a control group, while the other three parts were stored in plastic containers at +4 °C in the refrigerator for 3, 7, and 14 days in the dark. The plant tissue was ground into powder using liquid nitrogen and used immediately for analyses.

2.2. TBARS Determination

Lipid peroxidation intensity was determined as the amount of thiobarbituric acid reactive substances (TBARS) determined by the TBA reaction [35]. The absorbance was measured at 532 nm spectrophotometrically (Specord 40, Analytik Jena, Jena, Germany) and then, the value for non-specific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol/g fresh weight (FW).

2.3. Total Soluble Phenols Determination

About 0.5 g of powdered plant tissue was extracted by using 2.5 mL of 96% ethanol at −20 °C for 24 h. The reaction mixture for the determination of total soluble phenols contained 10 µL of supernatant, 190 µL of deionized H_2O, 25 µL of Folin−Ciocalteau reagent, and 75 µL of saturated Na_2CO_3 solution [36]. The samples were incubated at 37 °C for an hour and cooled to room temperature. The absorbance was measured at 765 nm on a microplate reader (Tecan, Spark, Männedorf, Switzerland). The total phenol content was expressed as equivalents of gallic acid (GAE) per g of fresh weight (FW).

2.4. Determination of Dry Mass Content

For the dry mass (DM) content, the fresh tissue was measured on analytical balance (AB45, Mettler Toledo, Zagreb Croatia). The tissue was dried at 65 °C for 48 h until reaching a constant weight. The DM content was calculated as the difference between fresh and dried tissue and expressed as a percentage (%) of fresh weight.

2.5. Determination of Total Antioxidant Capacity

The DPPH scavenging activity was determined according to the Brand–Williams method [37], modified according to Bibi Sadeer et al. [38] using the same extract prepared for total soluble phenolic content determination. The reaction mixture was prepared with 20 µL of radish extract and 180 µL of 0.04‰ DPPH (2,2-diphenyl-1-picrylhydrazyl). The absorbance was measured at 517 nm on a microplate reader after 30 min of incubation in the dark at room temperature (RT) with occasional shaking. The total antioxidant activity was determined from the standard curve and expressed as equivalents of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per g of FW.

For the ferric reducing antioxidant power (FRAP) assay [38], the same extract prepared for the total soluble phenolic content determination was used. The reaction mixture contained 5 µL of radish and 180 µL of FRAP reaction mixture. The FRAP reaction mixture was prepared with 0.3 M acetate buffer (pH = 3.6), 10 mM TPTZ (2,4,6−Tris(2−pyridyl) −1,3,5−triazine) solution and 20 mM $FeCl_3 \times 6H_2O$ (10:1:1 *v/v*). The reaction was carried out in the dark for 15 min at 37 °C. The absorbance was measured at 593 nm, using a microplate reader and the antioxidant activity was expressed in equivalents of Trolox µmol per g of FW.

2.6. Ascorbic Acid Content Determination

To determine of the ascorbic acid (AA) content [39], 0.5 g of powdered radish tissue was extracted with 10 mL of distilled H_2O. The reaction mixture was prepared with 300 µL of radish extracts, 100 µL of 13.3% trichloroacetic acid (TCA), 25 µL of deionized water, and 75 µL of 2,4−dinitrophenylhydrozine (DNPH) reagent. The DNPH reagent was prepared by dissolving 2 g of DNPH, 230 mg of thiourea, and 270 mg of $CuSO_4$ dissolved in 100 mL of 5 M H_2SO_4. The blanks were prepared parallel for each sample as described, without the addition of DNPH reagent. Both samples and blanks were incubated for an hour in a water bath at 37 °C followed by the addition of 500 µL 65% H_2SO_4 to all of the samples. The absorbance was measured at 520 nm spectrometrically and the AA concentration was calculated using a standard curve with known AA concentrations and expressed in mg per 100 g of FW.

2.7. Determination of Total Soluble Protein and Sugar Content

To determine the total soluble protein content [40], 0.5 g of powdered radish tissue was extracted with 1 mL of 100 mM potassium phosphate (K-P) buffer (pH 7.0) with 2% polyvinylpyrrolidone (PVP). The reaction mixture was made with 5 µL radish extract and 250 µL of Bradford reagent. The absorbance was measured on a microplate reader at 595 nm, using bovine serum albumin (BSA) as a standard. The protein concentrations were expressed as mg per g of FW.

For the determination of total soluble sugars, approximately 20 mg of dry tissue was extracted with 4 mL of acetone and the extraction was carried out for 24 h at 4 °C. To the precipitate, 1 mL of 80% ethanol was added, and the extraction was carried out in a water bath for 30 min at 80 °C. The extraction was repeated twice, and 500 µL of combined supernatants was then added to 2 mL tubes and evaporated until dry in a water bath at 85 °C. To the residue, 500 µL of ultra-pure H_2O was added and then used for the measurements. The reaction mixture consisted of 20 µL of sample, 80 µL of purified water, and 200 µL of anthrone dissolved in 95% H_2SO_4. The mixture was incubated for 30 min at 80 °C and cooled to room temperature [41]. The absorbance was measured on a microplate reader at 635 nm in polyethylene microplates, using glucose dilutions as a standard. The concentration of total soluble sugars was expressed in mg per g of DM.

2.8. Determination of Total Monomeric Anthocyanins, Total Chlorophylls, and Carotenoid Content

The concentration of total monomeric anthocyanins was determined by the pH-differential method based on the structural change of anthocyanins in relation to pH = 1.0 and 4.5 [42]. The extraction was carried out with 1 g of fresh powdered radish tissue and 3 mL of methanol in a water bath at 60 °C for 20 min. The extraction was repeated and the supernatants were combined. The combined supernatants were diluted with methanol to a volume of 10 mL, after which 0.5 mL of each sample was separated into two tubes. To one group of tubes, 2 mL of KCl buffer (pH = 1.0) was added, while 2 mL of $CH_3CO_2Na \times 3H_2O$ buffer (pH = 4.5) was added to the second group of tubes. All the samples were incubated at room temperature for 15 min, after which the absorbance at 510 and 700 nm was measured spectrophotometrically. The concentration of monomeric anthocyanins was calculated with the absorbance difference and the molar extinction coefficient, ε = 26,900, and then expressed in mg per g of FW.

Approximately 0.1 g of fresh powdered radish tissue was extracted with 1 mL of 100% acetone for 24 h at −20 °C. After extraction, the samples were diluted and the absorbance was measured spectrophotometrically at three wavelengths, 470 nm, 645 nm, and 662 nm, using pure acetone as a blank. The total chlorophylls (Chl *a*+*b*) and carotenoid (Car) content was calculated using the coefficients according to Lichtenthaler [43].

2.9. Statistical Analysis

Statistical analyses for three radish cultivars before storage (control) and 3, 7, and 14 days after storage (DAS) grown under a combination of blue/red and under white light, respectively, were performed using Statistica software (ver. 14, TIBCO Software Inc., Palo Alto, CA, USA). The results were compared by factorial analysis of variance (ANOVA), followed by Fisher's LSD (the least significant difference) post-hoc test. All three cultivars in were compared mutually regarding light spectra before storage (control) to reveal the influence of light spectra to cultivation. Then, all three cultivars were compared mutually, regarding the storage duration (plants stored at low temperature for 3, 7, and 14 days) in each light spectrum separately. The results are presented as mean ± standard deviation (SD) of the three replicates (n = 3). Differences were considered significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Effect of Low Temperature Storage on TBARS Levels

One of the most critical factor affecting the rate of the postharvest decay of microgreens is temperature [10]. Our investigation included the cultivation of three cultivars of radish microgreen (*Raphanus sativus* L.) under a combination of blue and red light as well as under white LED light (Table 1) and subsequent storage at a low temperature (4 °C).

The level of lipid peroxidation was expressed as relative TBARS content (Figure 1a,b). Generally, the accumulation of TBARS is recognized as a good indicator of oxidative stress [44]. The differential response of the cultivars in this study depended on the duration of storage and on the light type during cultivation. There are two general trends in TBARS change as a response to storage at a low temperature. The first one can be seen in B:2R/cvP, B:2R/cvR, and B:G:R/cvR where storage did not trigger TBARS production, regardless of the duration. The second trend can be seen in B:G:R/cvP and B:G:R/cvG as well as in B:2R/cvG where a shorter period (3 DAS) of storage triggered a decrease in TBARS content while a prolonged time (14 DAS) induced significant an increase compared to the control. It was reported that the exposure of chickpea hypocotyls during 8 days to chilling temperature did not induce lipid peroxidation but triggered a significant increase in MDA levels in the roots [45]. Recently, a review reported [46] that the postharvest of microgreens, regardless of storage temperatures, usually induces higher production of reactive oxygen species (ROS) which trigger lipid peroxidation. However, low temperatures can also reduce the accumulation of ROS and thus, mitigate the intensity of membrane lipid damage as well as preserve the structural integrity of the membranes [47] and this is what could be the case in our investigation in the initial stages of storage.

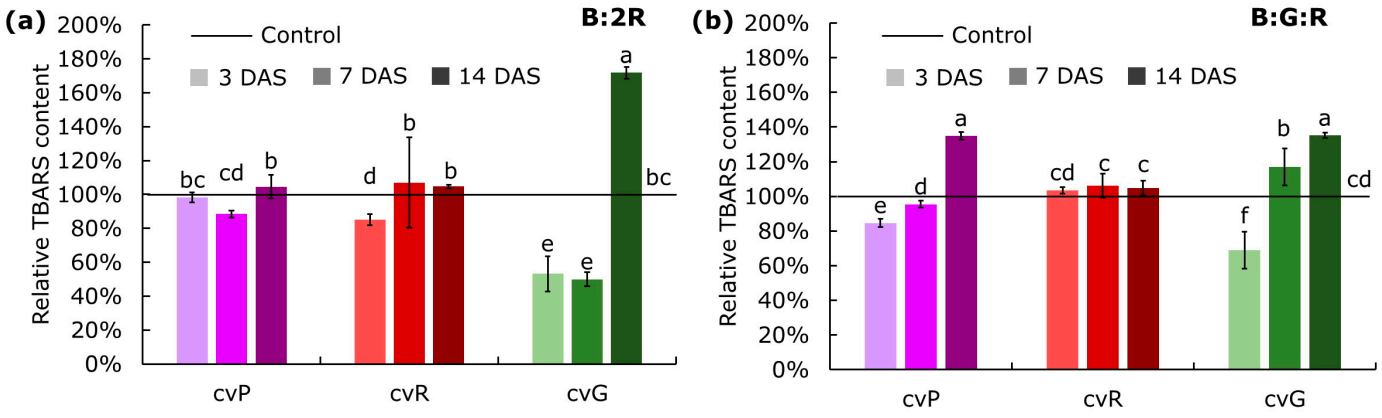

Figure 1. The relative content of TBARS in three cultivars of radish microgreens grown under blue/red light (B:2R; (**a**)) and white (B:G:R; (**b**)) light 3, 7, and 14 days after storage (DAS) at +4 °C. The values were normalized to the corresponding controls and are shown as the relative change to the control (presented as the line at value 100%). The columns show the mean values of the three replicates (n = 3), and the error bars represent the standard deviation (SD). Different letters represent significant differences ($p \leq 0.05$) between the radish microgreens grown under each light type (ANOVA and LSD) compared to the control (letters placed on the line).

Table 1. The contents of total chlorophylls (Chl *a+b*; mg/g_{FW}) and carotenoids (Car; mg/g_{FW}) were measured in radish microgreens grown under red/blue light (B:2R) and white (B:G:R) light 0 (control), 3, 7, and 14 days after storage (DAS) at +4 °C. The data show the mean values of the three replicates (n = 3) ± standard deviation (SD). Different letters represent significant differences ($p \leq 0.05$) between the radish microgreens grown under each light type (ANOVA and LSD).

Parameter	Light Spectral Treatments	Cultivar	Storage Duration			
			Control	3 DAS	7 DAS	14 DAS
Chl *a+b* (mg/g_{FW})	B:2R	cvP	0.323 ± 0.026 ^{def}	0.445 ± 0.035 ^{abc}	0.413 ± 0.071 ^{abcd}	0.234 ± 0.004 ^{f}
		cvR	0.298 ± 0.009 ^{ef}	0.505 ± 0.053 ^{a}	0.460 ± 0.074 ^{ab}	0.391 ± 0.025 ^{bcd}
		cvG	0.367 ± 0.024 ^{cde}	0.476 ± 0.061 ^{ab}	0.422 ± 0.083 ^{abc}	0.285 ± 0.006 ^{ed}
	B:G:R	cvP	0.298 ± 0.021 ^{d}	0.423 ± 0.026 ^{c}	0.412 ± 0.035 ^{c}	0.269 ± 0.005 ^{de}
		cvR	0.268 ± 0.049 ^{de}	0.506 ± 0.045 ^{b}	0.507 ± 0.044 ^{b}	0.221 ± 0.011 ^{e}
		cvG	0.320 ± 0.064 ^{d}	0.502 ± 0.048 ^{b}	0.585 ± 0.059 ^{a}	0.324 ± 0.008 ^{d}
Car (mg/g_{FW})	B:2R	cvP	0.060 ± 0.011 ^{d}	0.078 ± 0.006 ^{abc}	0.088 ± 0.016 ^{ab}	0.071 ± 0.001 ^{cd}
		cvR	0.058 ± 0.003 ^{d}	0.090 ± 0.006 ^{ab}	0.090 ± 0.013 ^{a}	0.081 ± 0.018 ^{abc}
		cvG	0.069 ± 0.005 ^{cd}	0.082 ± 0.009 ^{abc}	0.081 ± 0.015 ^{abc}	0.073 ± 0.001 ^{bcd}
	B:G:R	cvP	0.055 ± 0.004 ^{fg}	0.075 ± 0.006 ^{de}	0.089 ± 0.005 ^{bc}	0.066 ± 0.002 ^{ef}
		cvR	0.046 ± 0.006 ^{g}	0.080 ± 0.006 ^{cde}	0.084 ± 0.014 ^{cd}	0.046 ± 0.003 ^{g}
		cvG	0.059 ± 0.017 ^{fg}	0.098 ± 0.009 ^{ab}	0.112 ± 0.012 ^{a}	0.069 ± 0.004 ^{ef}

cvP—purple cultivar (*Raphanus sativus* cult. China Rose); cvR—red cultivar (*R. sativus* cult. Sango); cvG—red cultivar (*R. sativus* var. *longipinnatus*); FW—fresh weight, DM—dry matter, ND—not detected.

3.2. Effect of Low Temperature Storage on Pigment Content

There was no significant difference in total chlorophyll (Chl *a+b*) and carotenoids (Car) content between the cultivars grown under B:2R and B:G:R (Table S1). All three cultivars grown at B:2R showed a significant increase in Chl *a+b* content (Table 1) 3 DAS at low temperature compared to the control. The red cultivar (cvP) revealed significantly higher Chl *a+b* content 7 and 14 DAS, while cvP and cvG showed a decrease in Chl *a+b* content compared to the control values. All three cultivars grown at B:G:R revealed a significant increase in Chl *a+b* content 3 and 7 DAS compared to the control, while the content decreased 14 DAS. Carotenoid content (Car, Table 1) increased 3 and 7 DAS in cvP grown under B:2R compared to the control. CvP grown under B:2R showed higher values while cvG showed no difference in Car 3, 7, and 14 DAS compared to the control. All three cultivars grown at B:G:R showed a significant increase in Car content 3 and 7 DAS at low temperature compared to the control.

Even though Chl and Car have recently become more popular in the human diet, their main role is in photosynthetic processes and as bioactive components, [2] they are often related to the detoxification of ROS [48]. Chlorophyll and carotenoid content is important in the human diet since they play important roles in photooxidative processes by protecting humans against various types of cancer as well as against degenerative diseases [2,49,50]. A combination of red and blue light promotes both chlorophyll and carotenoid synthesis [51] since red and blue photons overlay with the peak absorption spectra of chlorophyll. The white light also contains green photons that have a negative effect on the growth, development, and overall biomass of the seedlings [52]. Recent research on broccoli heads after storage at 4 °C reported an increase in both Chl *a+b* and Car [53]. They suggested that the Chl *a+b* increase might be the result of increased metabolic activity due to immature floral buds' development. The microgreens in our investigation are young, immature plants that initialize the development of first true leaves; therefore, it is reasonable to assume that the synthesis of Chl was encouraged. Most vegetables are still photosynthetically active after storage under light conditions [18,54,55]. Light conditions are also responsible for the various quality parameters of stored vegetables, including the maintaining levels of O_2 and CO_2 inside the package [55]. However, in

our case, after harvesting, microgreens were stored in dark conditions. Despite that, increased accumulation of both Chl *a*+*b* and Car was observed 3 and 7 DAS (Table 1). Dark conditions after storage could induce higher consumption of O_2 due to increased respiratory activity [55]. Even though Chl synthesis is energetically demanding in dark conditions, light-independent Chl biosynthesis has been reported for several species other than conifers [56,57]. Barley plants exposed to light during their development have the ability to synthetize Chl when transferred to dark conditions [56] due to light induction of the enzyme that is responsible for protochlorophyllide reduction in dark conditions [58–60]. This light-independent protochlorophyllide oxidoreductase (DPOR) is sensitive to oxygen levels so it is active in dark conditions [60–62]. It has been reported that in dark conditions, Chl biosynthesis could be activated by DPOR [62]. Storage conditions at low temperature increase ROS, which could promote the synthesis of carotenoids even after harvesting and during storage [63]. A recent study revealed the close relationship between chlorophyll and carotenoid content in black and green tomato fruits after storage [64]. It was suggested that incomplete degradation of chlorophylls in ripe black tomatoes helped maintain high carotenoid and chlorophyll content during their storage. Such results could also explain the parallel increase in Car and Chl *a*+*b* in our investigation.

Our results revealed the degradation of both Chl *a*+*b* and Car 14 DAS in all three cultivars. A recent report on kale exposed to frost suggested that low temperatures support Chl degradation or inhibit its accumulation [65]. On the other hand, low temperatures cause a reduction in metabolic activity which would slow down the degradation of the chlorophylls [18]. Degradation of Chl in microgreens therefore could be an indicator of aging and it is often used to evaluate the quality of stored microgreens [51,66].

3.3. Effect of Low Temperature Storage on Proteins, Sugars, and Dry Matter Content

Total soluble protein (Prot) content measured in control microgreens (Table S1) revealed the highest content in the cvR cultivar, regardless of the light type. CvP reveled the lowest content cultivated under B:G:R compared to cultivation under B:2R and to other cultivars. CvG showed lower Prot content when cultivated under B:2R compared to cultivation under B:G:R. Upon storage, all three cultivars grown under B:2R (Figure 2a) revealed an increase in Prot content 3 DAS followed by a decrease compared to the control values 7 and 14 DAS. Cultivation under B:G:R (Figure 2b) induced a significant increase in Prot in cvP 3 DAS, followed by a significant decrease 7 DAS and a substantial increase 14 DAS compared to the control. In cvR, a significant decrease compared to the control was observed 7 and 14 DAS. In cv G, there was an initial increase 3 DAS, followed by a decrease 7 DAS compared to the control value and again, an increase 14 DAS compared to the control. Dietary proteins of plant origin contain fewer essential amino acids (especially methionine, lysine, and tryptophan) compared to proteins of animal origin; they provide higher amounts of non-essential amino acids (arginine, glycine, alanine, and serine) [67,68]. Sufficient intake of plant proteins has a positive impact on the prevention of various diseases, especially of the vascular system [67]. During the growth of sprouts, there is usually increased synthesis of proteins that are necessary as energy sources [50]. Cultivation of Chinese cabbage under red and blue light promotes the accumulation of soluble proteins [51] which corresponds to our results. Proteins, as well as lipids, are the main targets of oxidative reactions and their oxidation reduces the quality of food [69]. Therefore, a decrease in Prot content might be the result of the protein degradation by ROS.

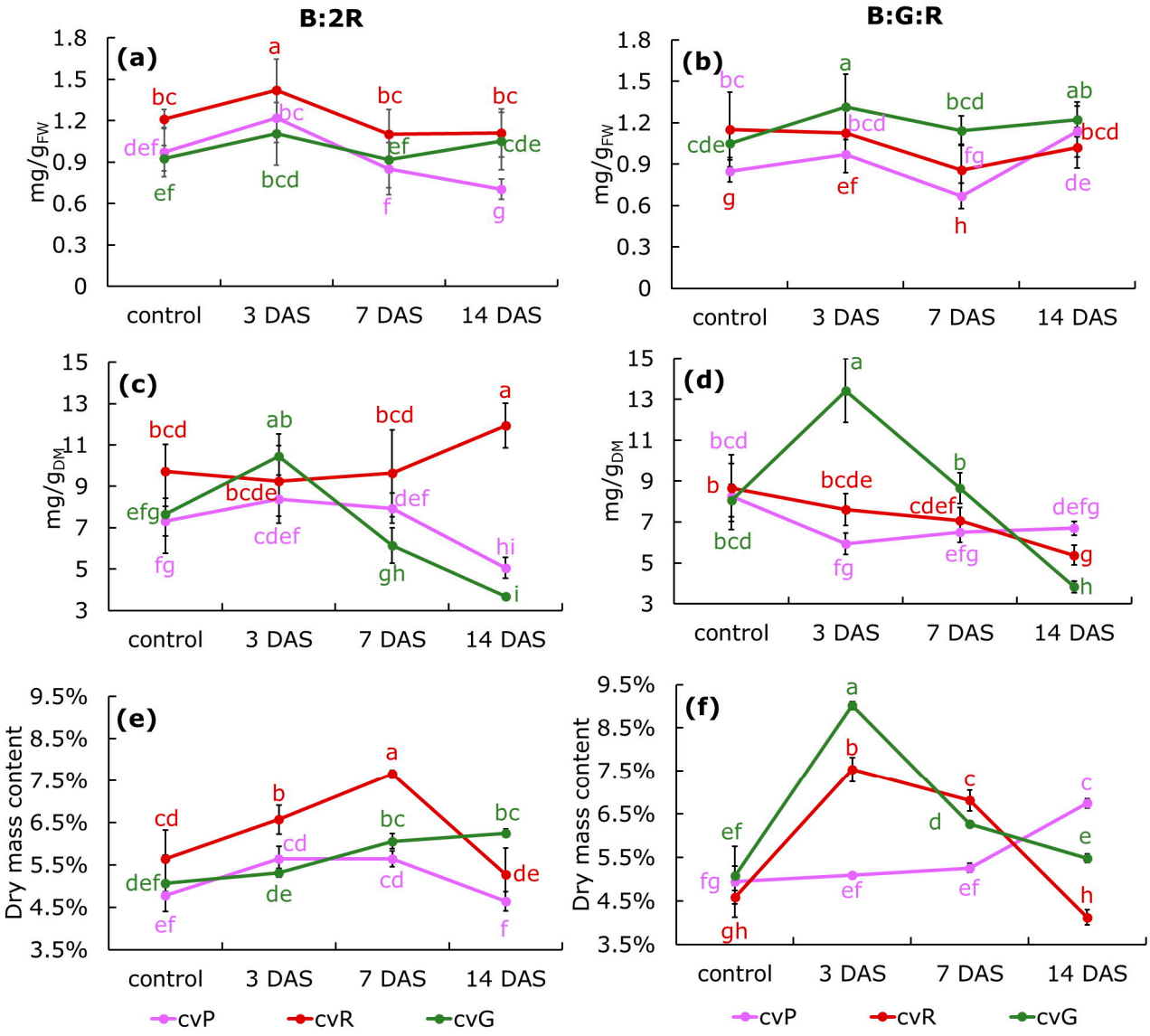

Figure 2. The contents of total soluble proteins (mg/g_{FW}; (**a**,**b**)), total soluble sugars (mg/g_{DW}; (**c**,**d**)) dry mass (%; (**e**,**f**)) measured in radish microgreens grown under blue/red light (B:2R; (**a**,**c**,**e**)) and white (B:G:R; (**b**,**d**,**f**)) light 0 (control), 3, 7, and 14 days after storage (DAS) at +4 °C. The lines show the mean value differences of the three replicates (n = 3), and the error bars represent the standard deviation (SD). Different letters represent the significant differences ($p \leq 0.05$) between the radish microgreens grown under each light type (ANOVA and LSD).

When plants are exposed to certain environmental factors, such as low temperature or changes in the intensity and quality of light, they can acquire increased resistance to such conditions [64,70,71]. Cold-acclimation is often accompanied by changes in gene expression and activation of antioxidant mechanisms, but also by increased accumulation of stress-induced proteins and an increase in the amount of total soluble sugars [72]. One of the important factors that determines the sensory quality of microgreens is total sugar content [11]. Cultivation of microgreens under B:2R and B:G:R did not induce significant changes in total soluble sugars in the control plants. However, there was a significant difference between CvP and cvR grown under B:2R, where cvR revealed the highest and CvP revealed the lowest sugar content (Table S1). Storage at a low temperature revealed various responses related to both light and cultivar. Cultivation under B:2R (Figure 2c) induced in cvP a significant decrease 14 DAS while in cvR, there was a significant increase 14 DAS compared to the control. CvG revealed an initial increase in sugar content 3 DAS

followed by a substantial decrease 7 and 14 DAS compared to the control. Cultivation under B:G:R (Figure 2d) induced a significant initial decrease in sugar content in cvP 3 DAS, followed by stabile low values 7 and 14 DAS compared to the control. In cvR, there was a significant decrease 14 DAS compared to the control, while cvG revealed an initial increase 3 DAS, followed by a decrease compared to the control level 7 DAS and a significantly lower amount 14 DAS compared to the control.

The quality of light can regulate carbohydrate metabolism in plants. Red, blue, and red/blue light contributed the most to the accumulation of soluble sugars in the Chinese cabbage seedlings compared to white light [51]. Stressful conditions can provoke an increased accumulation of soluble sugars that act as osmoprotectants and participate in cellular respiration [73]. Besides their role in the regulation of osmotic pressure, sugars also have a cryoprotective role since low temperatures usually trigger an increase in the level of total soluble sugars [65]. It has also been suggested that changes in the content of soluble sugars are related to cold tolerance. On the other hand, a decrease in sugar content can be the result of respiration processes that consume accumulated sugars [46].

Dry mass (DM) content depends on the crop cultivar and it is subjected to numerous factors [65]. There was no significant difference in DM content between the CvP and D cultivars grown under B:2R and B:G:R; however, cvR revealed the highest value under B:2R and the lowest values under B:G:R (Table S1). Storage at a low temperature (Figure 2e,f) provoked an increase in DM 3 and 7 DAS in B:2R/cvP, B:2R/cvR, B:G:R/cvR, and B:G:R/cvG compared to the control. The increase in DM content was observed in B:G:R/cvP 14 DAS, and B:2R/cvG 7 and 14 DAS compared to the control. In Chinese cabbage seedlings, white light provoked the highest content of dry mass in comparison to cultivation under red and blue light [51]. Different ratios of red and blue photons can have a significant impact on the increase in plant dry mass [74]. Such results suggest that B:2R in our investigation was the beneficial light type for the cultivation of radish microgreens. In microgreens, the DM could reach values over 18% [11,65]; however, in our investigation, all of the cultivars revealed values lower than 6% and this is considered as relatively low. Nevertheless, a similar percentage of dry weight was reported for daikon, red radish, and China rose cultivars in a study comparing six genera belonging to the Brassicaceae family [32]. Accelerated vegetative growth, which is a distinctive microgreens' feature, usually results in a low DM due to specific adaptations preventing them from water loss [4,46]. However, storage at low temperatures usually intensifies water loss due to the continuous respiration or physical injuries of microgreens [46] suggesting an increase in DM content which was also revealed in our investigation. Moreover, the loss of water during storage with the subsequent DM increase (Figure 2) could explain the increased concentration of both Chl *a*+*b* and Car in radishes (Table 1).

3.4. Effect of Low Temperature Storage on Total Antioxidant Capacity

To estimate the total antioxidant capacity, several methods can be used [38] and they are based on the ability of various antioxidants to reduce free radicals. In our research, DPPH and FRAP assays were used. TAC determined by the DPPH assay (Table S1) revealed the highest values in cvR regardless of the cultivation light type, while the lowest value was observed in B:G:R/cvG. For TAC determined by the FRAP assay (Table S1), the highest values were measured in B:2R/cvP and B:2R/cvR, while the lowest were measured in B:G:R/cvp and B:G:R/cvG. Upon storage, there was a significant decrease in TAC measured by the DPPH assay (Figure 3a) 3 DAS compared to the control in all B:2R grown cultivars. In cvR, there was significant increase in TAC 7 DAS followed by a decrease; however, it was not lower compared to the control. In cvP, after an initial decrease, a slight increase was observed 7 and 14 DAS; however, the values were lower than the control. In cvG, an initial decrease 3 DAS was followed by an increase 7 and 14 DAS compared to the control level. Cultivation under B:G:R (Figure 3b) revealed a significant increase in TAC 3 DAS in cvP and cvG. In cvP, the TAC decreased 7 and 14 DAS compared to the control level, while in cvG, the TAC decreased slightly compared to the control level 7 DAS and this was followed by a

significant increase 14 DAS compared to the control. CvR showed a significant decrease in TAC 3 DAS, followed by a substantial increase 7 DAS, and finally a decrease 14 DAS compared to the control level. The TAC measured by the FRAP assay measured in B:2R cultivated radishes (Figure 3c) revealed a significant decrease 3 and 7 DAS in cvP, while at 14 DAS, the TAC showed a slight increase; however, the activity was still lower than the control. A significant decrease 3 DAS in cvR was followed by an increase compared to the control level 7 DAS and a significant increase 14 DAS compared to the control. In cvG, the TAC revealed slight changes; however, they were not significant compared to the control. Cultivation under B:G:R (Figure 3d) revealed a significant increase in TAC measured by the FRAP assay in cvP and cvG 3 DAS. CvP revealed a decrease 7 DAS compared to the control level followed by an increase 14 DAS compared to the control. For CvG however, after initial an increase, a substantial decrease compared to the control level 14 DAS was revealed. In cvR, there was a significant decrease in TAC 3 DAS after which the TAC increased compared to the control level 7 and 14 DAS. An initial increase in antioxidative capacity 3 DAS in cvP and cvG grown under B:G:R light suggested the intensified initial redox potential of antioxidants that can inactivate ROS and neutralize the harmful consequences of their actions even before visible symptoms. A low temperature could protect the antioxidants from degradation and thus diminish the loss of antioxidant activity [75]. Recently it was shown that the synthesis and metabolism of antioxidants are possible even during storage, but there are often changes in the profile of bioactive compounds that can lead to changes in the total antioxidant capacity of fruits and vegetables [18,76]. A decrease in DPPH and FRAP was reported recently in *Helianthus tuberosus* inulin extracts [77], lettuce [75], and sunflower microgreens [29] stored at low temperatures which was a result of a low metabolic rate. It was suggested recently that the DPPH and FRAP decrease might imply lower stability during storage [78]. Moreover, continuing respiration and senescence were reported to reduce the antioxidant capacity of several microgreens [79].

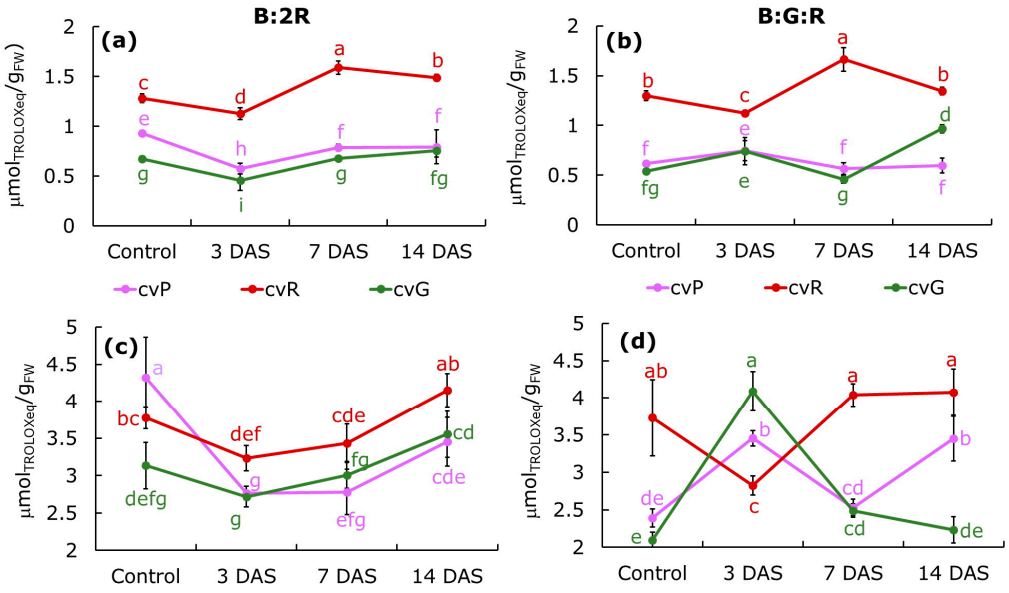

Figure 3. The total antioxidant capacity of radish microgreens grown under blue/red light (B:2R; (**a**,**c**)) and white (B:G:R; (**b**,**d**)) light 0 (control), 3, 7, and 14 days after storage (DAS) at +4 °C was evaluated by DPPH scavenging activity (µmol$_{TROLOXeq}$/g$_{FW}$; (**a**,**b**)) and FRAP assay (µmol$_{TROLOXeq}$/g$_{FW}$; (**c**,**d**)). The lines show the mean value differences of the three replicates (n = 3), and the error bars represent the standard deviation (SD). Different letters represent the significant differences ($p \leq 0.05$) between the radish microgreens grown under each light type (ANOVA and LSD).

3.5. Effect of Low Temperature Storage on Bioactive Compounds

In our investigation, all three cultivars revealed higher AA content when grown under B:2R, compared to cultivation under B:G:R, where B:2R/cvP revealed the highest content and B:G:R/cvG revealed the lowest content (Table S1). In microgreens cultivated under B:2R (Figure 4a), 3 DAS there was a significant decrease in AA content in cvP and a significant increase in cvG, while cvR revealed no change compared to the control. However, all three cultivars revealed a significant decrease 7 and 14 DAS compared to the control. Cultivation under B:G:R (Figure 4b) induced significant a increase 3 DAS in cvP followed by a substantial decrease compared to the control. In cvR, there was a significant decrease 3, 7, and 14 DAS compared to the control. CvG revealed a decrease in AA content 3 and 7 DAS, while 14 DAS, an increase on control group was observed. Cultivation under light can significantly increase the AA content in sprouts and microgreens compared to cultivation in darkness [14,80]. Certain combinations of red and blue LED lights might increase the AA concentration in young spinach leaves compared to cultivation under standard HPS lamps [15]. Similar results were reported on the influence of red LED light on the AA content in Brassicaceae family microgreens. Antioxidant metabolism is variously sensitive to different wavelengths of light, which is also dependent on the natural level of antioxidant compounds in plant leaves [81]. In addition, the metabolic pathway of ascorbic acid interacts with the photosynthetic and respiratory chains of electron transport, so the amount and quality of light affect the accumulation of ascorbic acid [82] thus suggesting that B:2R had a beneficial influence on AA synthesis during cultivation. Despite the positive impact of light during cultivation, storage at low temperatures in the dark induced significant changes in AA content. Cultivation under red LED light could delay senescence in broccoli after storage, suggesting that in such conditions the antioxidant efficiency in detoxifying ROS was enhanced compared to cultivation under a white LED light [83]. A decrease in AA after storage was also reported for sunflower microgreens [29]. Ascorbic acid is a thermo-unstable vitamin, sensitive to changes in temperature during storage, so it can be used as a chemical indicator of quality and shelf life [84]. The synthesis of bioactive compounds requires an adequate supply of minerals, water, and light which regulate numerous physiological and biochemical reactions to maintain efficient enzyme activity. Microgreens lack basic factors that could slow down metabolic reactions after harvesting so excessive amount of ROS can be produced. Some bioactive compounds, including vitamins and pigments, usually detoxify ROS by degrading themselves [79]. A low temperature during storage slows down metabolic processes leading to a deficiency in certain essential factors in the metabolic pathways involved in AA synthesis [29]. Since our microgreens were stored in darkness, respiration took place instead of photosynthesis which probably led to increased ROS production which might cause the reduction and/or degradation of bioactive substances, including ascorbic acid.

Low temperature is known to affect the quality of microgreens after storage by regulating metabolic activities associated with the senescence process such as respiration rate or water loss [19]. Damaged tissue increases the rate of respiration which consequently increases the production of reactive oxygen species (ROS) involved in the regulation of various processes. Cells activate antioxidant defense mechanisms to diminish the harmful effects of ROS [44,85]. The total soluble phenols (Phe) determined in this investigation (Table S1) showed higher values measured in cvP and cvG cultivated under B:2R compared to B:G:R cultivation, while cvR revealed higher values measured under B:G:R compared to the ones grown under B:2R. Storage at a low temperature in the dark induced a differential response in Phe content (Figure 1a,b). Cultivation under B:2R (Figure 4c) induced a significant decrease in Phe content in cvP 3 DAS and continue to decrease 7 and 14 DAS compared to the control. In cvR and cvG, the significant decrease compared to the control was observed 7 and 14 DAS. In the microgreens cultivated under B:G:R (Figure 4d), there was a significant increase in cvP 3 DAS followed by a decrease 7 DAS compared to the control level and beyond 14 DAS. In cvR, a significant decrease in Phe content was observed 7 and 14 DAS, while in cvG, there was stabile Phe content during 14 days of storage at low

temperatures. The content of phenolic compounds is an important index of the quality of sprouts and microgreens, and the accumulation of phenolic phytochemicals can be stimulated by cultivation under different wavelengths of LED lighting [2] as well as with exposure to illumination by dark grown microgreens [14]. Light, especially the blue part of the electromagnetic spectrum is well known to induce phenol synthesis since it stimulates some key enzymes that mediate the synthesis of phenolic compounds [86]. Phe content depends on the light quality as well as on the cultivar. Recent research on red and green *Ocimum* cultivars confirmed that LED light with a 2R:1B ratio, the same as B:2R in our investigation, induced the highest Phe content in a green *Ocimum* cultivar [74]. Moreover, the same investigation reported that a ratio of equal parts of blue and red light induced higher Phe content in a red *Ocimum* cultivar by suggesting that green tissues were more stimulated by higher amounts of red light than blue light. In addition, gene expression for certain phenolic compounds is light-regulated and it could differ between green and red tissues in the same species such as in *Perilla* plants [87]. Higher levels or increases in certain phenols are sometimes correlated with stabile levels of lipid peroxidation products [45]. Upon harvest, the antioxidant content decreases, especially if the temperature, light, and packaging during storage were not adequate. Therefore, storage at low temperatures is often used to manipulate plant metabolic processes by slowing them down and prolonging their shelf life [75,76,79]. The Phe decline after storage in sunflower microgreens suggested that damage to cell structures and senescence could be responsible for tissue electrolyte leakage that could induce Phe loss [29]. Low temperatures could also slow down the decrease in Phe content; however, polyphenol oxidase can usually oxidize phenolic component which can be seen as a decrease in overall Phe content [75]. Phe content usually increases at low temperatures, though, such an increase could be seen as the result of a higher accumulation of lignin, suberin, or anthocyanins [88]. As mentioned before, light is a key factor in Phe synthesis since it activates key enzymes in the Phe metabolic pathway, such as phenylalanine ammonia lyase (PAL). In fact, different response in red and green *Ocimum* cultivars might be explained by the different regulatory mechanisms of PAL in red and green tissues [11,74]. In addition, storage under light conditions does not lead to a decrease in Phe content [18]. Such reports suggest that upon storage at low temperatures in darkness, the synthesis of certain Phe components might be disrupted at the expense of some other ones, such as anthocyanins, chlorophylls, or carotenoids.

The highest amount of monomeric anthocyanins (Anth) was detected in cvR, while the content was higher in cvR grown under B:G:R than in those grown under B:2R (Table 1). Furthermore, cvP revealed lower values compared to cvR, regardless of growth light, while in cvG, Anth was not detected. After storage, cvP grown under B:2R (Figure 4e) revealed a significant increase 14 DAS. In cvR, a significant decrease 3 DAS compared to the control was observed, followed by an increase 7 and 14 DAS. Cultivation under B:G:R in cvP (Figure 4f) provoked an increase 14 DAS compared to the control, while cvR showed was a significant increase 7 and 14 DAS compared to the control. Red lettuce cultivar showed stimulated plant growth and an accumulation of Anth under supplemental blue LED radiation, applied in shorter intervals and in combination with moderate light intensity [89]. In our case, equal ratios of B:G:R light induced higher Anth content in the red cultivar, while in the purple cultivar, this was characterized with green leaves and purple stems; the higher Anth content, although not significant, was detected under B:2R light. This is supported by investigation on red and green *Ocimum* cultivars, where a 2R:B combination induced highest Anth content in the red cultivar and it was correlated with higher antioxidant activity [74]. Red and green forms of *Perilla* express differential genes involved in anthocyanins' accumulation with higher expression in the anthocyanin-producing red form [87]. Moreover, Anth synthesis is light-regulated; however, each transcriptional factor that controls Anth biosynthesis responds to differential stimuli and is facilitated by both blue and red light receptors [90]. An Increase in Anth content in strawberries was also detected 10 days after storage at a temperature of 0 °C [91]. Anthocyanins are non-enzymatic antioxidants and low temperatures promotes the synthesis and increased accumulation of Anth in numerous

plant species [70,85]. An increase in Anth content caused by low temperatures is involved in Chl protection from cold conditions by preventing its overexcitation [70]. Recent research has shown that low temperatures promote anthocyanin biosynthesis due to the increased regulation and expression of the genes involved in anthocyanin biosynthesis [92]. In addition to antioxidant properties, anthocyanins have shown a role in protecting the model lipid membrane from oxidation [93]. Therefore, the increased content in anthocyanins seems to have a beneficial influence in cvR and cvP cultivars to alleviate the adverse consequences of harmful radicals.

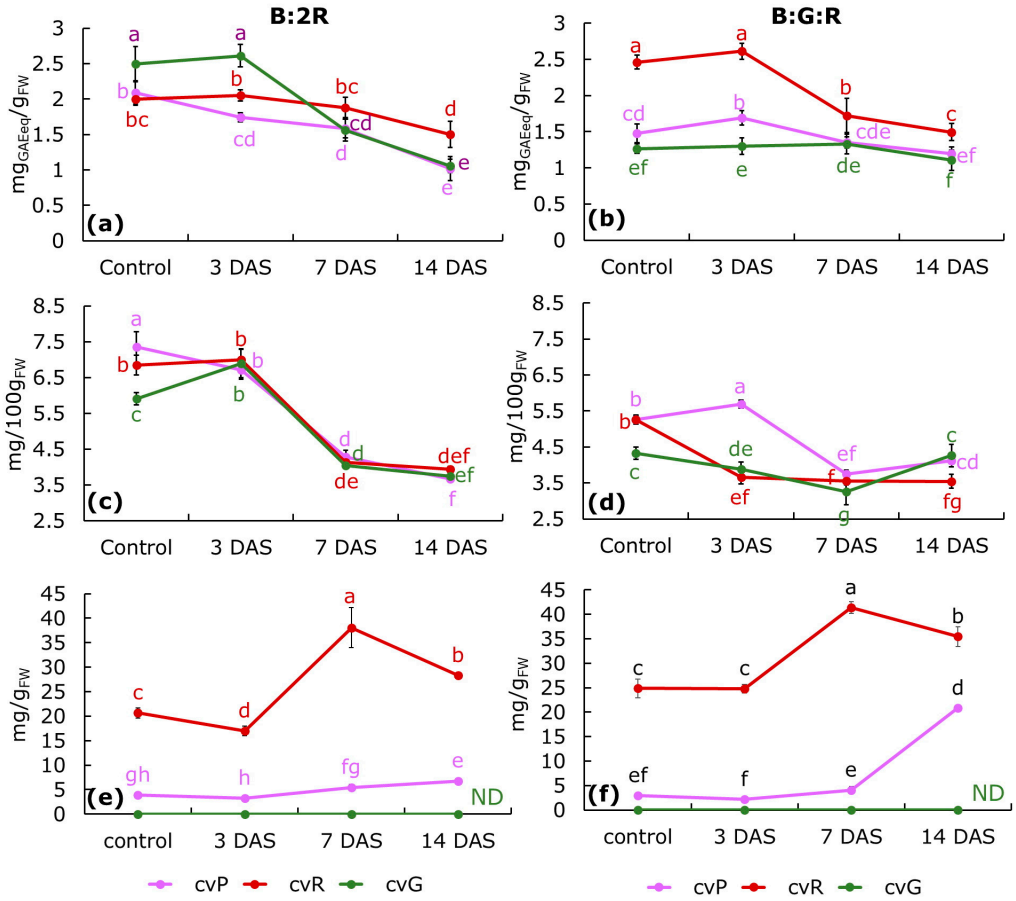

Figure 4. The content of ascorbic acid (mg/100g_{FW}; (**a**,**b**)) total soluble phenolic content (mg_{GAEeq}/m_{FW}; (**c**,**d**)) and the content of monomeric anthocyanins (Anth; mg/g_{FW}; (**e**,**f**)) measured in radish microgreens grown under blue/red light (B:2R; (**a**,**c**,**e**)) and white (B:G:R; (**b**,**d**,**f**)) light 0 (control), 3, 7, and 14 days after storage (DAS) at +4 °C. The lines show the mean value differences in the three replicates (n = 3), and the error bars represent the standard deviation (SD). Different letters represent the significant differences ($p \leq 0.05$) between the radish microgreens grown under each light type (ANOVA and LSD). ND—not detected.

4. Conclusions

Radish microgreens' antioxidant capacity and phytochemical profile depends on the radish cultivar and on the type of light spectral composition used for cultivation. A combination of blue and red LED light (B:2R) was shown to be more beneficial on the synthesis of total soluble phenolics, ascorbic acid, proteins, and sugars as well as on the dry matter content and total chlorophyll and carotenoids. Consequently, an increase in those phytochemicals induced a better overall antioxidant capacity in the plants grown under B:2R LED light. Such results suggest that microgreens cultivated in this way would be the best for consumption immediately after harvesting. The highest contents of total soluble phenolics, proteins, and sugars, dry matter, and monomeric anthocyanin content, as well as higher

overall antioxidant capacity determined in the red radish cultivar (cvR), distinguished this cultivar as the most desirable for human consumption regardless of the cultivation light spectrum. Storage at low temperature in darkness slowed down most of metabolic reactions during first seven days, thus preserving most of the antioxidant activity. Trends of changes in the antioxidant capacity in all three radish cultivars during storage indicate the complexity of bioactive compounds in such conditions. Changes in cultivation methods and in combination of certain storage methods can increase the production of antioxidants; therefore, further investigations are needed to reveal the best cultivation and storage practices for radish microgreens to increase the synthesis of desirable phytochemicals and to extend their shelf life.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9010076/s1, Table S1: The influence of blue/red light (B:2R) and white (B:G:R) LED light on measured parameters in three seven-day-old radish microgreen cultivars determined before storage.

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