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Effects of Supplemental UV-A LEDs on the Nutritional Quality of Lettuce: Accumulation of Protein and Other Essential Nutrients

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Abstract: Light plays an important role in influencing the nutritional quality of food crops, especially with regard to the health-promoting phytochemicals. However, its role in affecting the nutritional quality with regard to the essential nutrients is not well understood. In this study, the effects of preharvest UV-A treatment on the nutritional quality of lettuce (*Lactuca sativa*, cv. red-leaf 'New Red Fire' and green-leaf 'Two Star') in relation to the essential nutrients and health-promoting phytochemicals were explored. Lettuce plants were grown in a growth chamber and were subjected to supplemental UV-A LEDs (peak wavelength 375 nm) for a brief period (3–6 days) prior to harvest. UV-A LEDs were equipped with lenses to control the light dispersion. Many growth indices such as shoot fresh mass, leaf area, and leaf number were unaffected by supplemental UV-A in both varieties while shoot dry mass decreased in response to a 6-day UV-A treatment compared to the control. Leaf chlorophyll and carotenoid concentrations increased significantly in green-leaf lettuce after 3 or 6 days of UV-A treatment, but only after 3 days of UV-A treatment in red-leaf lettuce compared to the control. Leaf protein concentration increased significantly in both lettuce varieties along with a number of essential nutrients such as phosphorus, potassium, calcium, manganese, and sulfur in response to supplemental UV-A. Supplemental UV-A increased the accumulation of protein by approximately 48% in green-leaf lettuce and 31% in red-leaf lettuce compared to the control plants. Moreover, in addition to the above essential nutrients, green-leaf lettuce accumulated higher amounts of magnesium, copper, and zinc compared to the control plants, indicating that green-leaf lettuce was more responsive to preharvest supplemental UV-A treatment than red-leaf lettuce. However, the accumulation of total phenolic compounds and flavonoids in both varieties was lower under supplemental UV-A. Furthermore, the use of LED lenses did not have a consistent impact on most of the plant responses studied. Overall, the results indicate that a brief preharvest exposure of both red- and green-leaf lettuce varieties to UV-A increased their nutritional quality by enhancing the accumulation of protein and other major essential nutrients.

Keywords: essential nutrients; LED lenses; phenolic compounds; phytochemicals; UV-A LEDs

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1. Introduction

Light has a significant impact on the nutritional content of food crops, particularly in relation to the health-promoting phytochemicals. Both visible and ultraviolet (UV) radiation can notably affect the secondary metabolism leading to the accumulation of many health-promoting phytochemicals and nutrients vital for human health [1,2]. Extensive research has been conducted on the effect of light on the health-promoting phytochemicals, especially phenolic compounds in a number of horticultural food crops, including lettuce [3–5]. Numerous phenolic compounds and other phytochemicals in our diet play an important role in promoting human health by preventing many common chronic and

degenerative diseases [6–8]. Indeed, numerous studies have focused on manipulating the light characteristics both in the field and in protective production environments to improve the nutritional quality of food crops including lettuce, especially in relation to the health-promoting phytochemicals [4,5,9–11]. These studies have examined the impact of light in the visible spectrum on the health-promoting phytochemicals in food crops. Lee et al. [2] reported that supplementing the visible spectrum with LEDs affects the accumulation of protein and other essential nutrients in lettuce plants. However, the role of light in the uptake and accumulation of essential nutrients in plants remains unclear. In addition, very little is known about the impact of UV on the accumulation of essential nutrients needed for human health.

Malnutrition resulting from inadequate consumption of many essential nutrients including protein and micronutrients such as calcium, iron, zinc, and many others is a major global health issue [12,13], and hence there is a critical need to find ways to develop nutrient-dense food to prevent the chronic health problems [14]. Thus, enhancing the amount of both essential nutrients as well as health-promoting phytochemicals in food crops can not only help in mitigating the challenges of malnutrition but also alleviate the incidence of some of the commonly occurring chronic and degenerative diseases.

Many horticultural crops are increasingly being cultivated under protective environmental conditions worldwide [9,15,16]. In recent years, the rapid advancements in light-emitting diodes (LEDs) have spurred the adoption of this technology as a preferred lighting source in crop production, especially under controlled environmental conditions [17]. LEDs are solid-state devices, more energy-efficient and long-lasting than conventional lighting. More importantly, they allow for the precise control of spectral quality which is important in modulating the light characteristics for better crop growth with higher nutritional quality. Furthermore, light emission from conventional lighting (e.g., incandescent source) is omnidirectional (360°) while it is more directional (typically 180°) with LEDs, allowing for more precise targeting of light distribution than the conventional lighting sources. Furthermore, light distribution over the canopy can be further precisely targeted using LED lenses which can improve the efficacy of light in inducing light-mediated plant responses. Thus, LEDs are rapidly emerging as the popular source of lighting source for manipulating light characteristics in protective environments such as greenhouses, indoor hydroponic systems, or plant factories where supplement lighting is necessary. Thus, numerous studies have focused on manipulating the visible spectrum to determine its impact on the growth and accumulation of phytochemicals in many crops. However, in contrast, there are only a limited number of studies that examine the effect of UV on the growth and nutritional quality of lettuce. Moreover, the primary focus of these studies has been to explore the impact of a long exposure of lettuce plants to UV-A, often extending over the length of their growing cycle [3,5,9].

In the current study, we examine the nutritional quality of lettuce in relation to the accumulation of essential nutrients and health-promoting phytochemicals in response to a preharvest, brief supplemental UV-A exposure (up to 6 days). Lettuce was chosen for this study because it is not only a commonly cultivated leafy vegetable in protected environments, but it is also sensitive to many light-mediated plant responses [2]. Red-leaf and green-leaf lettuce varieties were subjected, prior to harvest, to UV-A LEDs (375 nm) equipped with focus and scatter lenses in a growth chamber study to determine their impact on the nutritional quality of lettuce plants with regard to the essential nutrients and health-promoting phytochemicals.

2. Materials and Methods

2.1. Plant Materials and Growing Conditions

The seeds of two lettuce varieties (*Lactuca sativa* cv. red-leaf 'New Red Fire' and green-leaf 'Two Star') were sown in 72 plug seedling trays containing a soil mix (Metromix 360, Sungro Horticulture, Agawam, MA, USA) in a growth chamber set at 22 °C with a PPFD of 244 μmol/m^2/s (53.19 watts/m^2) with 12 h photoperiod and 60% of relative

humidity. One-week-old seedlings were transplanted into pots (12 cm × 12 cm × 12 cm) containing the above soil mix (1 seedling/pot). Plants were watered every 2 days and fertilized with irrigation water (N:P:K; 20:10:20) at 200 ppm of nitrogen once a week. The plants were rearranged every other day within the growth chamber to reduce the variability in irradiance on the plant canopy.

Six days prior to harvest, plants were subjected to UV-A treatment using custom-built LEDs (peak wavelength 375 nm) with special lenses in a growth chamber with the background fluorescent lighting providing approximately a PPFD of 190 μmol/m^2/s (41.42 watts/m^2). To regulate the distribution of UV-A radiation flux on the plant canopy, the customized optical lenses were built into a LED matrix (4 × 4). The experiment consisted of 4 treatments, namely supplemental LEDs, supplemental LEDs with 5° focus lens (LED + F), supplemental LEDs with scatter lens (LED + S), and white fluorescent background lighting (control). The plants were treated with UV-A for 3 days or 6 days prior to harvest. Optical lenses were used to alter the dispersion of light energy over the canopy. Focus lenses were used to target the UV-A on individual plants while scatter lenses were used to distribute the UV-A uniformly over the plant canopies. Photosynthetically active radiation (PAR) was measured at the canopy level using a Quantum Radiometer Photometer (LI-185B, LI-Cor, Inc., Lincoln, NE, USA). UV-A radiation was measured using a Research Radiometer (ILT 5000, International Light Technologies, Inc., Peabody, MA, USA) at 9 random sites within each treatment. Plants were randomly assigned under each treatment in a completely randomized design with 4 replications.

2.2. Growth Characteristics

Plant growth characteristics including fresh and dry mass of shoots, number of leaves, and leaf area were measured after 3 days and 6 days of treatment. Dry mass was obtained by freeze-drying (Harvest Right, North Salt Lake, UT, USA) the samples up to 16 h or until a constant weight was obtained. The leaf area was determined by a leaf area meter (LI-3100, Li-Cor, Lincoln, NE, USA). Freeze-dried samples were ground into a fine powder using an electric grinder and used for chemical analyses.

2.3. Chlorophyll and Carotenoids

The total chlorophyll and carotenoid concentrations were determined following the procedure by Chen et al. [18] with some modifications. Leaf samples (30 mg) were extracted with 3 mL of 80% acetone in an ultra-sonicator for 20 min. The absorbance of the clear supernatant was read in a microplate reader (Synergy H1, BioTek, Winooski, VT, USA) at 663 nm, 645 nm, and 470 nm. The concentrations of total chlorophyll and carotenoids were derived using the following relationships:

Chl a = 12.72 A663 − 2.59 A645, Chl b = 22.88 A645 − 4.67 A663, Chl (a + b) = 20.3 A645 + 7.22 A663, and carotenoids = (1000 A470 − 3.27Chl a − 104Chl b)/229.

2.4. Essential Nutrients

Freeze-dried ground leaf samples (0.15 g) were used to determine the total carbon and nitrogen concentrations using the TruSpec CN instrument with the LECO TruSpec CN combustion analyzer. Other essential nutrients such as phosphorus, potassium, calcium, magnesium, manganese, iron, copper, and zinc were extracted and analyzed using an ICP spectrometer (Model 720-ES ICP Optical Emission Spectrometer, Varian Australia Pty Ltd., Mulgrave, Australia) following the method by Gieseking et al. [19]. Protein concentrations in the leaves were derived from the leaf nitrogen concentration on a dry weight basis according to Milton and Dintzis [20].

2.5. Total Phenolic Compounds and Antioxidant Capacity

Ground leaf samples (40 mg) were used to determine the concentration of total phenolic compounds and the antioxidant capacity. Total phenolic compound concentration was determined using a modified Folin–Ciocalteu method [21]. Samples were extracted in

4 mL of 80% (*v*/*v*) acetone in ultra-sonicator for 20 min. The extract (1.5 mL) was kept in a refrigerator for 12 h and was then centrifuged at 10,000× *g* for 2 min. A total of 50 µL of the supernatant was mixed with 135 µL distilled water, 750 µL 10% Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), and 600 µL 7.5% (*w*/*v*) Na_2CO_3. The mixture was vortexed and incubated in a water bath at 45 °C for 15 min. The absorbance was read at 765 nm (U-1100 Spectrophotometer, Hitachi Ltd., Tokyo, Japan). The results were expressed as gallic acid equivalent.

The antioxidant capacity was determined using the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method [22,23]. To 20 mL distilled water, 27.4 mg of ABTS and 0.4 g of MnO_2 were added to produce ABTS radicals. ABTS was then mixed with sample extract or Trolox standards to start the reaction, and the absorbance was measured at 730 nm using a spectrophotometer (U-1100 spectrophotometer, Hitachi Ltd., Tokyo, Japan). The results were expressed as the Trolox equivalent.

2.6. Individual Phenolic Compounds

Phenolic compounds from lettuce samples (0.1 g) were extracted using 10 mL of 70% MeOH on an orbital shaker overnight in the dark. The extracts were centrifuged (5810R, Brinkman Instruments Inc., Westbury, NY, USA) at 3690 rpm for 30 min. The procedure was repeated twice with 10 mL of 70% MeOH. The pooled supernatant was filtered with qualitative filter paper (11.0 µm, Global Life Science Solution LLC, Marlborough, MA, USA). A total of 2 mL of the filtered extract was dried in a vacuum dryer for up to 4 h. The residue was redissolved in 1 mL of 70% MeOH, and the sample was passed through a 0.22 µm syringe filter (MilliporeSigma, Burlington, MA, USA) before the high-performance liquid chromatography (HPLC) analyses. Phenolic compounds including gallic acid, caffeic acid, luteolin-7-glucoside, quercetin-3-glucoside, and apigenin-3-glucoside were quantified using HPLC (Shimadzu HPLC, Kyoto, Japan). Phenolic compounds were separated using a reverse-phase Waters C18 column (250 mm L × 4.6 mm D, Waters, Milford, MA, USA) at 32 °C at an elution rate of 0.8 mL/min. Mobile phase A consisted of formic acid: deionized water (5:95 *v*/*v*), and mobile phase B consisted of formic acid: methanol (5:95 *v*/*v*). The gradient was set as follows: 0 to 5 min: 10% B; 5 to 25 min: 40% B; 25 to 41 min: 70% B; 41 to 55 min: 100% B; 55 to 65 min: 0% B. The overall procedure followed was according to Woolley et al. [24].

2.7. Statistical Analyses

Treatment differences with regard to growth characteristics, essential nutrients, and phytochemicals were analyzed using ANOVA (SAS 9.4, Cary, NC, USA and XLSTAT, Addinsoft, New York, NY, USA) with light treatments as the independent variable. The pairwise comparisons of means were performed using Duncan's multiple range test at $p < 0.05$, 0.01, and 0.001.

3. Results and Discussion

3.1. Growth Characteristics

Lettuce varieties ('New Red Fire' and 'Two Star') were grown in a growth chamber with a background fluorescent lighting of approximately 190 µmol/m^2/s (41.42 watts/m^2), and the UV-A treatments were started 6 days prior to harvest. Under LEDs, the PAR was approximately 192 µmol/m^2/s (41.86 watts/m^2) while the UV-A irradiance was maximum without any lenses and was the lowest with the focus lens (Table 1).

Growth characteristics of lettuce varieties were measured after 3 and 6 days of supplemental UV-A treatment. There were no significant differences in most of the growth characteristics including shoot fresh mass, leaf area, and the number of leaves in response to the UV-A treatments (Table 2). However, shoot dry mass in 'New Red Fire' and 'Two Star' was suppressed after 6 days of UV treatments. In addition, modifying UV-A LEDs with lenses depressed the dry shoot biomass in 'Two Star' lettuce even after 3 days of UV treatment. These results are in agreement with those of Tsormpatsidis et al. [9] who

observed a negative impact of UV on dry matter accumulation of red-leaf lettuce grown in tunnels under photo-selective films. However, it should be noted that the overall lack of UV-A impact on most of the growth characteristics in this study is perhaps due to the brief preharvest UV-A treatment of plants as they approach the end of the active growing period. Nonetheless, typically, plant growth response to UV has been shown to be highly variable.

Table 1. Average PAR and UV-A levels (with S.E, n = 9) under LEDs, LEDs + focus lens (LED + F), LEDs + scatter lens (LED + S), and control.

	Treatments			
	Control	**LED**	**LED + F**	**LED + S**
PPFD (μmol/m^2/sec)	190 ± 1.9	192 ± 3.1	197 ± 4.8	192 ± 4.4
UV-A (watts/m^2)	-	1.37 ± 0.03	0.814 ± 0.1	1.15 ± 0.04

Table 2. Shoot fresh mass, dry mass, leaf area, and leaf number of lettuce varieties, 'New Red Fire' and 'Two Star', subjected to supplemental UV-A radiation for 3 or 6 days before harvest. Treatments included LEDs, LEDs + focus lens (LED + F), LEDs + scatter lens (LED + S), and control (Con). Measurements were made at 0, 3, and 6 days of treatment.

		New Red Fire				**Two Star**			
		Shoot				**Shoot**			
Day		**Fresh Mass (g)**	**Dry Mass (g)**	**Leaf Area (cm^2)**	**Number of Leaves**	**Fresh Mass (g)**	**Dry Mass (g)**	**Leaf Area (cm^2)**	**Number of Leaves**
0		43.3	2.8	1056.8	12.7	40.2	3.8	779.32	16.0
3	Control	53.7	3.8	1198.3	14.0	48.5	5.7a	941.94	18.0
	LED	61.5	3.5	1492.3	16.0	58.6	4.9ab	1093.97	16.7
	LED + F	54.7	3.2	1327.7	15.0	50.8	4.1b	959.20	16.3
	LED + S	56.3	2.8	1246.3	14.0	55.4	4.4b	1034.40	15.3
	Significance	ns	ns	ns	ns	ns	*	ns	ns
6	Control	62.5	5.1a	1554.7	15.7	54.5	7.7a	1027.27	19.7
	LED	61.7	3.5b	1478.7	14.8	60.3	5.1b	1201.00	18.5
	LED + F	59.0	3.4b	1493.3	15.0	49.6	4.3c	974.61	17.3
	Led + S	67.6	3.9b	1636.0	16.0	56.0	4.4c	1077.79	16.5
	Significance	ns	**	ns	ns	ns	***	ns	ns

Data followed by different letters in a column are significantly different. Significant differences are presented at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). NS stands for no significant difference.

Many studies have shown positive impacts of UV-A [25,26] while others have reported negative or no impacts [3,27–29]. For example, a study by Li and Kubota [5] on lettuce showed that supplemental UV-A did not have any effect on many of its growth characteristics including leaf fresh and dry weights, leaf number, and leaf size. Similar results were noted in dropwort plants, where their biomass accumulation was not affected by UV-A treatment [30]. Thus, these results demonstrate that the impact of UV-A on plant growth and plant functions is complex as it is affected by many factors including genotype, environmental factors, and perhaps more importantly, the spectral balance of radiation [3,27,31].

3.2. Chlorophyll and Carotenoids

After exposing plants to UV-A LED for 3 days, leaf chlorophyll concentration increased in 'New Red Fire' while longer exposure (6 days) did not result in higher chlorophyll concentration in these plants (Figure 1). Moreover, optical lenses did not have any impact on the chlorophyll concentration compared to the control; however, the chlorophyll concentration was lower compared to that in plants treated with LEDs alone (with no optical lenses). In contrast, all the UV-A treatments in 'Two Star' increased the concentration of chlorophyll

after 3 or 6 days of treatment. However, the chlorophyll concentration decreased in the control plants over the 6-day period, indicating that older plants have lower chlorophyll content. Accumulation of carotenoids in 'New Red Fire' in response to the UV-A treatments was similar to that of chlorophyll accumulation in that it produced significant increases in the carotenoid concentration only after 3 days of treatment (Figure 1). In contrast, longer exposure to UV-A decreased the chlorophyll and carotenoid concentrations in 'New Red Fire' indicating that these compounds are sensitive to longer UV-A exposure. The response of 'Two Star' to the UV-A treatments (with or without lenses) parallels that of chlorophyll accumulation in that all the UV-A treatments for 3 or 6 days enhanced the carotenoid accumulation in the leaves. Like chlorophyll, the carotenoid concentration also decreased in the control plants over the 6-day period. The optical lenses had no impact on the concentrations of either chlorophyll or catenoids in 'Two Star' lettuce. This may be due to the reduction in the levels of UV-A plants received when lenses were used (Table 1). These results support the finding of Tezuka et al. [25] who found that UV-A radiation can enhance chlorophyll content in radish. In contrast, the study by Li and Kubota [5] showed that supplemental UV-A had no impact on either chlorophyll or carotenoid contents in the leaves of the 'Red Cross' lettuce variety. Thus, the results show that UV-A response is variable and depends on the plant species and varieties.

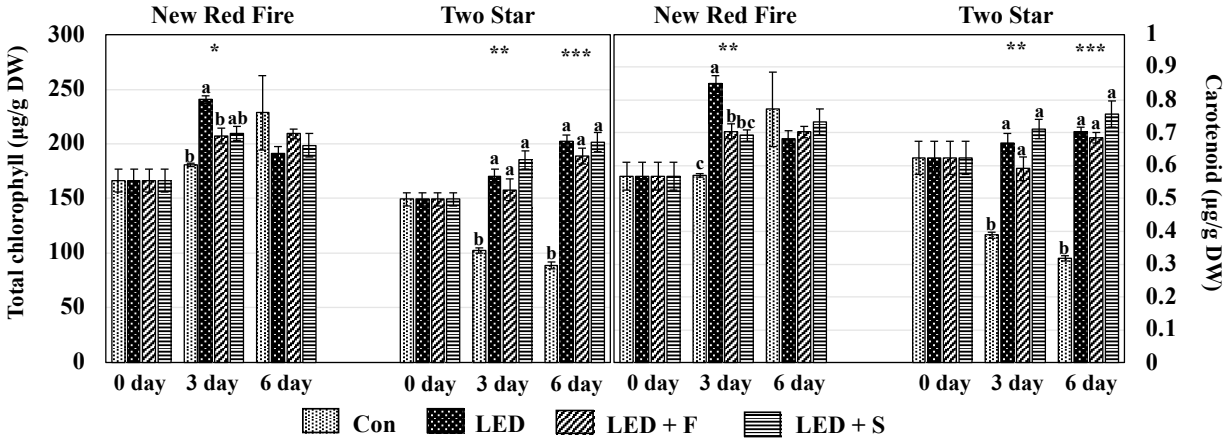

Figure 1. Total chlorophyll and carotenoid concentrations of lettuce varieties, 'New Red Fire' and 'Two Star', subjected to supplemental UV-A radiation. Treatments included LEDs, LEDs + focus lens (LED + F), LEDs + scatter lens (LED + S), and control (Con). Measurements were made at 0, 3, and 6 days of treatments. Vertical bars indicate standard errors (n = 4). Bars with different letters are significantly different. Significant differences are presented at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

3.3. Protein and Other Essential Nutrients

Supplemental UV-A LED produced a significant impact on the accumulation of many essential nutrients in both lettuce varieties. Protein is an important nutrient as it is acutely deficient in the human diet in many parts of the world, and it is especially a major challenge in most developing countries. Longer exposure of both varieties of lettuce (6 days) increased the protein concentration in the leaves (Figure 2 and Table 3). Supplemental UV-A treatment (LED without lenses) for 6 days enhanced the amount of protein by approximately 31% in the leaves of 'New Red Fire' and by around 48% in the leaves of 'Two Star' lettuce. These findings are consistent with our previous greenhouse study where supplementing solar radiation with UV-A and UV-B significantly increased the accumulation of protein in green-leaf lettuce, 'Two Star' [32].

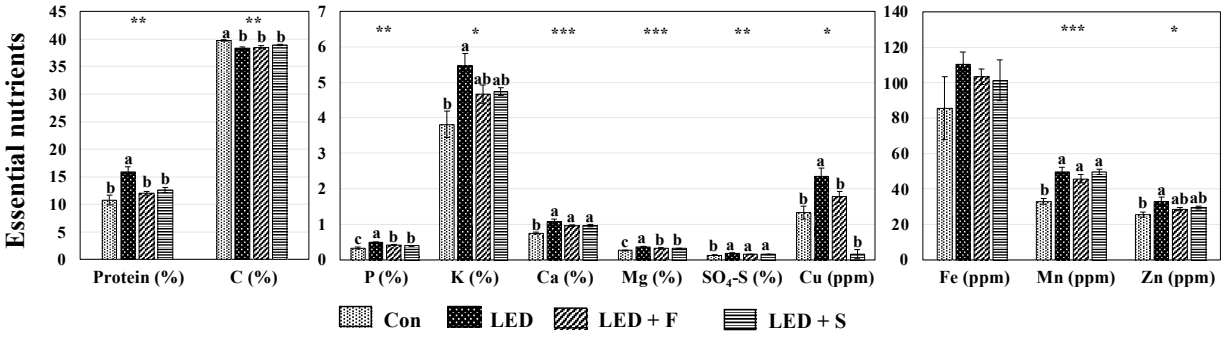

Figure 2. Concentrations of essential nutrients in 'Two Star' lettuce subjected to supplemental UV-A radiation. Treatments included LEDs, LEDs + focus lens (LED + F), LEDs + scatter lens (LED + S), and control (Con). Measurements were made after 6 days of treatments. Vertical bars indicate standard errors (n = 4). Bars with different letters are significantly different. Significant differences are presented at $p < 0.\ 05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Table 3. Concentrations of essential nutrients in lettuce variety 'New Red Fire' subjected to supplemental UV-A radiation. Treatments included LEDs, LEDs + focus lens (LED + F), LEDs + scatter lens (LED + S), and control (Con). Measurements were made after 3 and 6 days of treatments.

	Light Source	Protein (%)	C (%)	P (%)	K (%)	Ca (%)	SO_4-S (%)	Mn (ppm)
3 days	Control	13.25	37.93	0.48 b	5.78 b	0.81	0.166 b	68.08
	LED	15.75	37.31	0.56 ab	6.76 ab	0.86	0.203 ab	70.78
	LED + F	16.33	36.88	0.65 a	7.35 a	0.95	0.237 a	70.23
	LED + S	15.52	36.73	0.61 a	7.11 a	0.96	0.216 a	71.1
	Significance	ns	ns	*	*	ns	*	ns
6 days	Control	14.42 c	38.42 a	0.52 c	6.10 b	0.87 b	0.19 b	59.3 b
	LED	18.92 a	36.86 b	0.62 a	7.26 a	1.04 a	0.22 a	77.1 a
	LED + F	16.81 b	37.07 b	0.58 b	7.16 a	0.94 ab	0.21 ab	70.6 ab
	LED + S	16.81 b	37.34 b	0.55 bc	7.08 a	0.96 ab	0.21 ab	84.1 a
	Significance	***	***	***	**	*	*	*

Data followed by different letters in a column are significantly different. Significant differences are presented at $p < 0.\ 05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). NS stands for no significant difference.

Similar results were also observed in radish where soluble protein levels increased in response to UV-A radiation [25]. These findings also support the observation that lettuce plants grown in high tunnels typically receive less solar UV-A and UV-B radiation than in the open field, leading to a lower accumulation of protein in these plants [33]. The use of optical lenses to modify UV-A irradiance had no impact in 'Two Star' while it produced lower protein accumulation in 'New Red Fire' compared to that in LED treatment (without the lenses). Nevertheless, LED lenses produced a higher accumulation of protein in 'New Red Fire' than in the control plants. Improving protein content in our diet is notably important as it is an essential nutrient in the human diet. Its deficiency is widespread in many parts of the world, especially in developing countries where children are often at a greater risk of acute protein deficiency [34].

Longer exposure of 'New Red Fire' to supplemental UV-A LED (6 days) resulted in a higher accumulation of many essential nutrients including phosphorus (by 19%), potassium (by 19%), sulfur (by 15%), calcium (19%), and manganese (by 30%) compared to the control plants (Table 3).

Other essential nutrients examined including magnesium, iron, copper, and zinc were not affected by the UV treatments (data not presented). Similarly, in 'Two Star', longer exposure produced a significant increase in phosphorus (by 50%), potassium (by 42%), calcium (44%), magnesium (38%), copper (by 78%), manganese (by 53%), and zinc (by 29%) compared to the control plants (Figure 2). The UV-A treatment also resulted in a smaller but significant increase in sulfur in both varieties. However, accumulation of these nutrients

due to supplemental UV-A LED treatments was not significant for 3-day exposure in 'Two Star' (data not presented). Dispersion of UV-A irradiance with lenses had either variable or no impact on the accumulation of essential mineral nutrients in lettuce varieties. The results showing that supplemental UV-A can produce a positive response in the accumulation of essential nutrients in both lettuce varieties are consistent with a previous study where supplementing solar radiation with UV-A increased the accumulation of many mineral essential nutrients in both green-leaf lettuce and red-leaf lettuce [32]. Numerous studies have explored the impact of visible light with regard to light intensity, spectral quality, and photoperiod on the accumulation of many nutrients and the possible mechanisms involved in the uptake of these nutrients in several plant species [1]. However, there is little information on the role of UV in influencing the accumulation of essential nutrients in plants.

3.4. Total Phenolic Concentration and Antioxidant Capacity

The total phenolic concentrations in the leaves of 'New Red Fire' were reduced after 6 days of supplemental UV-A treatments (Figure 3). The reduction in concentration ranged from 24% to 51% under LEDs compared to the control plants. Similarly, in 'Two Star', all the supplemental UV-A treatments decreased the amount of total phenolic compounds with 3 or 6 days of treatment. The antioxidant capacity did not change with 3 days of supplemental UV-A treatment in both varieties.

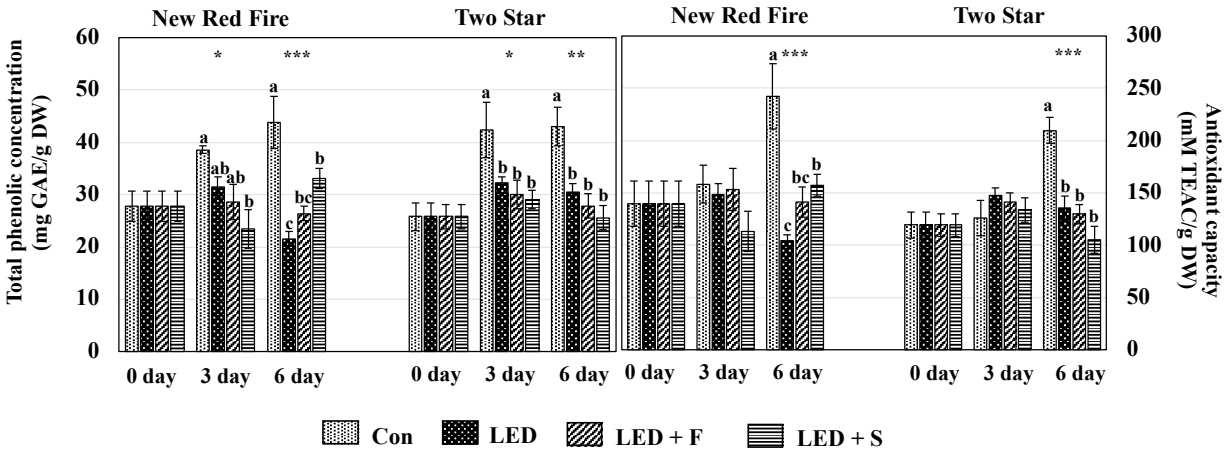

Figure 3. Total phenolic concentration and antioxidant capacity (TAEC, Trolox equivalent antioxidant capacity) of lettuce varieties, 'New Red Fire' and 'Two Star', subjected to supplemental UV-A radiation. Treatments included LEDs, LEDs + focus lens (LED + F), LEDs + scatter lens (LED + S), and control (Con). Measurements were made at 0, 3, and 6 days of treatments. Vertical bars indicate standard errors (n = 4). Bars with different letters are significantly different. Significant differences are presented at $p < 0.\ 05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Over the 6-day period, the antioxidant capacity in the control plants was higher in both varieties than that in the UV-A-treated plants. However, with 6 days of supplemental UV-A treatment, the antioxidant capacity decreased in both varieties. These results support the observations made on basil grown under supplemental UV-A where the total phenolic compounds and antioxidant capacity were reduced in purple-leaf basil while in green-leaf basil they were higher in response to UV-A [35]. In microgreens and dropwort, supplemental UV-A improved the concentration of total phenolic compounds along with other antioxidant compounds [26,30]. In our previous greenhouse study with higher PAR (791 mmol/m^2/s), we observed an increase in the amount of total phenolic compounds and the antioxidant capacity in red-leaf lettuce [32]. In contrast, Li and Kubota [5] found that the total phenolic compound accumulation was not affected by the supplemental UV-A in red-leaf lettuce grown in a growth chamber with fluorescent background lighting (300 mmol/m^2/s). Krizek [31] suggested interactions of UV-A, PAR, and UV-B can impact

plant response to UV, and it is likely that the level of PAR may play an important role in plant response to UV-A. In addition, considering the inconsistency of UV-A response to the accumulation of total phenolic compounds, Verdaguer et al. [36] suggested that individual phenolic compounds should be the focus, instead of the total phenolic compounds in determining the UV response in plants.

3.5. Individual Phenolic Compounds

Accumulation of gallic acid and caffeic acid was monitored after 3 and 6 days of UV treatments in both lettuce varieties. In 'Two Star' control plants, the accumulation of phenolic acids decreased with age (Table 4). After 6 days of supplemental UV-A treatment (with scatter lens) of 'Two Star' lettuce, the accumulation of gallic acid increased significantly (approximately 74%). There was no significant impact of supplemental UV-A treatments on these phenolic acids in 'New Red Fire' lettuce (data not presented). Overall, UV-A treatment had a negative impact or no impact on the accumulation of flavonoids (Figure 4). This is perhaps due to the low PAR levels found in the growth chamber. It is worth noting that supplemental UV-A-induced flavonoid accumulation in plants is affected by the background PAR levels [15,31]. In our previous greenhouse study, supplementing solar radiation with UV-A resulted in a higher accumulation of several flavonoids in both red- and green-leaf lettuce [32]. This is perhaps due to higher PAR levels (719 $\mu mol/m^2/s$) found in the greenhouse than in a typical growth chamber.

Table 4. Concentrations of individual phenolic compounds in lettuce variety 'Two Star' subjected to supplemental UV-A radiation. Treatments included LEDs, LEDs + focus lens (LED + F), LEDs + scatter lens LED + S), and control (Con). Measurements were made after 3 and 6 days of treatments.

	Phenolic Acids (μg/g DW)			
Light Source	**3 Days after UV Treatments**		**6 Days after UV Treatments**	
	Gallic Acid	**Caffeic Acid**	**Gallic Acid**	**Caffeic Acid**
Control	10.24 ab	51.32 a	4.49 b	33.07
LED	14.99 a	40.45 ab	5.07 b	21.24
LED + F	12.57 ab	43.05 ab	3.93 b	20.61
LED + S	6.74 b	33.17 b	7.85 a	17.75
Significance	*	**	*	ns

Data followed by different letters are significantly different. Significant differences are presented at $p < 0.05$ (*) and $p < 0.01$ (**). NS stands for no significant difference.

A balance between PAR and UV radiation can determine the response of plants to UV-A and UV-B, and lower PAR levels are likely to suppress the positive response to UV not only in growth but also in the accumulation of phenolic compounds [31,36]. Thus, it is important to use some caution in comparing UV studies conducted in growth chambers with those conducted in greenhouses or under field conditions.

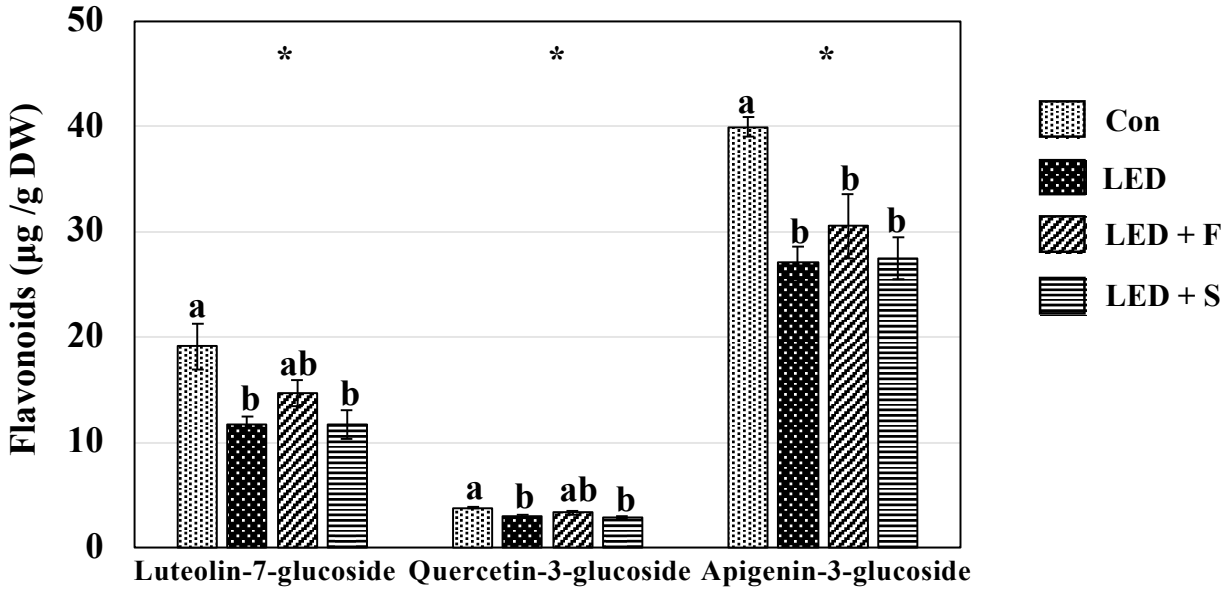

Figure 4. Concentrations of individual phenolic compounds in lettuce variety 'New Red Fire' subjected to supplemental UV-A radiation. Treatments included LEDs, LEDs + focus lens (LED + F), LEDs + scatter lens (LED + S), and control (Con). Measurements were made after 6 days of treatments. Vertical bars indicate standard errors (n = 4). Bars with different letters are significantly different. Significant differences are presented at $p < 0.05$ (*).

4. Conclusions

In summary, the results show that UV-A has a positive impact on the nutritional quality of both red-leaf and green-leaf lettuce, and it significantly enhanced the accumulation of protein in the leaves. Moreover, it enhanced the accumulation of several essential nutrients such as phosphorus, potassium, calcium, manganese, and sulfur in both varieties. However, the green-leaf lettuce was more responsive to UV-A with regard to the accumulation of essential nutrients than the red-leaf variety. In addition to the above essential nutrients, the green-leaf lettuce also accumulated higher amounts of magnesium, copper, and zinc in response to UV-A. Overall, UV-A did not impact growth characteristics; however, total leaf chlorophyll concentration increased in green-leaf lettuce, and in red-leaf lettuce after 3 days of treatment. Similarly, the leaf carotenoid concentration increased in response to UV-A in both varieties.

Six days of UV-A exposure reduced the accumulation of the total phenolic compounds in both varieties. While flavonoid accumulation was not affected in the green-leaf lettuce, it declined in the red-leaf lettuce in response to UV-A treatment. Overall, the impact of LED lenses was variable and inconsistent. The results show that a brief preharvest UV-A treatment can enhance the nutritional quality notably by increasing the accumulation of protein and many other essential nutrients in both red- and green-leaf lettuce.

Author Contributions: C.B.R. was the principal investigator of this study and was responsible for managing the research activities and revising the manuscript. M.L. and J.-H.L. conducted all the experiments, and M.L. was involved in data collection and analyses and in the writing of the manuscript. J.K. designed and built the LEDs with optical lenses and was responsible for setting up the treatments. Preliminary work on the use of LEDs with lenses was conducted by M.-M.O. All authors have read and agreed to the published version of the manuscript.

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