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Pre-Harvest UVB Irradiation Enhances the Phenolic and Flavonoid Content, and Antioxidant Activity of Green- and Red-Leaf Lettuce Cultivars

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Abstract: As a promising environmental protection technology, the application of ultraviolet B irradiation in vegetable production has been widely considered. However, the effect of UVB irradiation varies with different plant varieties. In this study, we investigated the effects of two UVB intensities (0.7, 1.4 W m^{-2}) on the accumulation of phenolics and flavonoids, and antioxidant activity of green-leaf and red-leaf lettuce (*Lactuca sativa* L.) 7 days prior to harvest. The results indicated that short-term (within 2 days) UVB treatment could promote the increase in total chlorophyll content of red-leaf lettuce and green-leaf lettuce, which increased by 49.8% and 20.6% compared with day zero, respectively, and was beneficial to the synthesis of carotenoids of red-leaf lettuce. Extending UVB exposure time significantly decreased chlorophyll a/b value of green-leaf lettuce from 0.92 to 0.63, and simultaneously increased the accumulation of antioxidant substances such as flavonoids, which were increased by 90.0% and 183.4% compared with day zero for UVB-0.7 and UVB-1.4 treatments of red-leaf lettuce, 84.1% and 110.9% of green-leaf lettuce. In contrast, red-leaf lettuce had a higher accumulation level of secondary metabolites, faster scavenging rate of free radicals, and stronger ability to resist UVB stress. Our results suggest that supplementation of low-dose UVB radiance prior to harvest can improve the secondary metabolite content and antioxidant activity of the two kinds of lettuce. This research provided a theoretical basis for improving lettuce quality by pre-harvest UVB treatment in controlled environmental agriculture.

Keywords: *Lactuca sativa*; ultraviolet B; secondary metabolites; DPPH

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1. Introduction

Ultraviolet B (UVB, 280–320 nm) radiation reaching the earth's surface is increasing due to the depletion of the stratospheric ozone layer [1,2]. UVB light is an important environmental signal that substantially regulates several photomorphogenic and physiological responses of plants. Early studies emphasize the negative effects of excessive UVB irradiation on tomatoes [3] and peas [4], which are potentially resulting in impaired growth and development caused by the deleterious effect on the macromolecules, and photosynthetic apparatus [5].

In response to UVB irradiation, plants employ self-protection mechanisms to accommodate the subsequent adverse impacts; one of the most important reactions is the accumulation of secondary metabolites such as polyphenols and flavonoids [6,7]. Elevated antioxidant bioactive compounds' content improves the antioxidant capacity, delays senescence, and reduces the post-harvest rot of tomatoes [8]. Therefore, the application of targeted UVB irradiation during vegetable production is attracting extensive attention as a kind of promising and environmentally friendly technology to induce an increase in antioxidant activity and improve their health and the nutritional value of lettuce [9].

However, the supplementation of UVB increases carotenoid and chlorophyll concentrations in green-leaf lettuce and decreases these compounds in red-leaf lettuce. Carotenoid content in plants grown under the same conditions varies up to 10-fold by variety, suggesting that selecting specific-leaf lettuce varieties for greenhouse production may improve their nutritional value [10].

Lettuce (*Lactuca sativa* L.), belonging to the Asteraceae family, is an important leafy vegetable that is available all year round in greenhouses and plant factories [11]. According to the Food and Agriculture Organization of the United Nations (FAO), global lettuce production reached 27.6 million tons in 2020 [12]. Lettuce is a low-calorie, low-fat salad vegetable that is rich in fiber and vitamins [13]. Previous studies found that the levels of total phenolics, anthocyanin, luteolin, and quercetin in red-leaf lettuce were all increased from UV block to UV low and UV window, while green-leaf lettuce grown under the same conditions showed no significant change [14]. Aiming to determine the sensitivity and physiological responses of different lettuce genotypes to UVB radiation, two lettuce cultivars, green- and red-leaf Romaine lettuce, were subjected to supplemental UVB radiation prior to their harvest in environmentally controlled growth chambers. The effects of UVB irradiation on photosynthetic pigments (chlorophylls, and carotenoids) and metabolites (flavonoids, phenolics, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2)), as well as antioxidant capacity, were investigated.

2. Materials and Methods

2.1. Plant Material and Cultivation Conditions

The experiments were performed in an environment-controlled growth chamber at China Agriculture University (Beijing, China). Two varieties of *Lactuca sativa* L. (cv. Romaine, green leaf, and cv. purple Romaine, red leaf) were studied in this experiment. Seeds were disinfected and sown in seedling trays (54 × 28 × 4.5 cm) with peat-based substrate (0~6 mm, pH 5.5~6.0). Upon the unfolding of the second true leaf (14 d after sowing), uniform seedlings were selected and transplanted into individual plastic pots (300 mL) for further cultivation. The growth environment was set as 150 µmol m^{-2} s^{-1} white-light-emitting diode (LED), 14 h light photoperiod (8:00–22:00), 18 ± 0.5/25 ± 0.5 °C night/day temperature, and relative humidity of 70–75%. Plants received irrigation with half-dose Hoagland's nutrient solution every day.

After growth for 21 days, mature plants were treated for 7 days with two intensities of supplementary UVB at the plant canopy level, which were 0.7 W m^{-2} and 1.4 W m^{-2} (8:00–14:00), respectively. Day zero (before UVB treatment) was used as the control. The UVB radiation was applied by two Phillips TL 20W/01 lamps (Shanghai, China) with a narrow waveband between 305 and 315 nm and peaks at 311 nm (Figure 1). The UVB radiance was measured by a spectrometer (AvaSpec-ULS2048, Avantes Inc., Apeldoorn, The Netherlands) at 15 cm above the root-to-shoot interface.

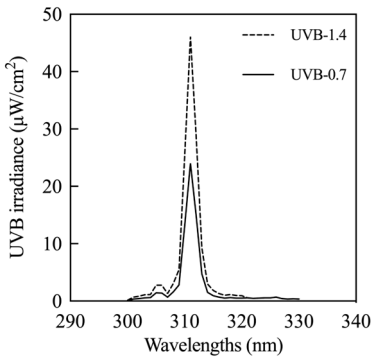

Figure 1. UVB irradiance of two treatments.

The plant samples were collected on the zero, first, second, third, fifth, and seventh days before harvest with three replicates. Each sample was approximately 1 g. All sam-

ples were immediately frozen with liquid and lyophilized at −80 °C for analyzing the biochemical assay.

2.2. Determination of Chlorophyll and Carotenoid

The chlorophylls and carotenoids were extracted with 8 mL of aqueous acetone (80:20, *v*/*v*) at 4 °C for 24 h under dark condition. Absorbance of the extracted supernatants was measured at 663, 645, and 470 nm using a UV-VIS spectrophotometer (UV-2802, Unico Inc., Shanghai, China). The contents of chlorophyll a (Chla), b (Chlb) and the carotenoids (Car) were calculated to μg mL^{-1} using the formulas referring to Wellburn and Lichtenthaler [15].

2.3. Determination of Total Phenolic, Flavonoid Content, and Anthocyanin Relative Content

The phenolics and flavonoids' content was determined following the description of Kaulmann et al. [16]. Homogenized plant material was extracted with 5 mL of methanol: water (80:20, *v*/*v*), and centrifuged at 10, 000 r min^{-1} for 15 min to obtain the extracted supernatant. Phenolic content was assayed with the Folin–Ciocalteu phenol reagent; 200 μL of the supernatant was reacted with 1 mL of Folin–Ciocalteu reagent (10× dilution). After 4 min, 800 μL of 7.5% Na_2CO_3 was added and mixed well, and the mixture was incubated in the dark at room temperature for 2 h. The absorbance of the mixture was measured at 765 nm. The phenolic content was calculated with an external calibration curve of gallic acid and expressed as mg (gallic acid equivalent) g^{-1} (FW).

Flavonoids' content was determined with the aluminum chloride method; a total of 200 μL of the extraction was mixed with 800 μL distilled water and 60 μL $NaNO_2$ (1:20, *w*/*v*), and incubated at room temperature for 5 min. Then, 120 μL of $AlCl_3 \cdot 6H_2O$ (1:10, *w*/*v*) was added and reacted for 6 min. A total of 400 μL of NaOH (1 M) and 400 μL distilled water were then added and mixed uniformly; the mixture was measured at 510 nm. The total flavonoid content was calculated with a rutin external calibration curve and expressed as mg (rutin equivalent) g^{-1} (FW).

The anthocyanin relative content was determined following the description of Mancinelli and Rabino [17]. Homogenized plant material was extracted with 5 mL methanol containing 1% HCL, the mixture was extracted at −4 °C for 24 h in the dark, and then centrifuged at 10,000 r min^{-1} for 15 min to obtain the extracted supernatant. The absorbance of the mixture was measured at 530 nm and 657 nm.

2.4. Determination of DPPH Radical Scavenging Capacity

DPPH radical scavenging activity was determined according to the method of Oueslati et al. [18]. Briefly, methanolic solution of DPPH (1 mM, 0.5 mL) was either reacted with 0.5 mL of extraction (sample) or control solution. Then, the absorbance was measured at 517 nm by a spectrophotometer. The DPPH scavenging activity of the sample was calculated according to Oueslati et al. [18].

2.5. Determination of MDA and H_2O_2 Content

The MDA content was determined following the procedure described by Hodges et al. [19] to evaluate the lipid peroxidation of plant leaves. A 1 g sample was extracted with 5 mL of 80% ethanol and centrifuged at 10,000 r min^{-1} for 15 min. A total of 0.5 mL of the extracted supernatant was mixed well with solution A (20% trichloroacetic acid and 0.01% butylated hydroxytoluene), and solution B (20% trichloroacetic acid, 0.01% butylated hydroxytoluene, and 0.65% thiobarbituric acid) in centrifuge tubes, respectively. These mixtures were incubated in a boiling water bath for 25 min, and snap-cooled in ice. Absorbance at 600 nm, 532 nm, and 440 nm was measured spectrophotometrically and used to calculate the MDA content.

The determination of H_2O_2 content was performed according to the method of Murshed et al. [20] with minor modifications. A 1 g sample was extracted with 5 mL of 0.1% trichloroacetic acid solution, and centrifuged at 10,000 r min^{-1} at 4 °C for 15 min. Then, 0.5 mL of supernatant, 0.5 mL of phosphate buffer (0.01 mol L^{-1}, pH = 6.8), and

1 mL of KI solution (1 mol L^{-1}) were reacted in a centrifuge tube at 20 °C for 20 min. The standard curve was obtained from dilutions with 30% H_2O_2. Absorbance at 350 nm was measured and used to calculate H_2O_2 content.

2.6. Statistical Analysis

Data analyses were performed using SPSS 26.0 (IBM Inc., Chicago, IL, USA), and figures were plotted using Prism 9 software (GraphPad Software, La Jolla, CA, USA). Data normality and homogeneity were performed with the Shapiro–Wilk test and Levene's test, respectively. One-way analysis of variance (ANOVA) with the Tukey's test was used to evaluate significant differences. Results are presented as mean ± standard error. Different letters indicate significant differences between treatments at $p \leq 0.05$ level. The Pearson correlation coefficient method was used to conduct pound-wise correlation analysis among the variables, with *r* representing the correlation coefficient.

3. Results

3.1. Effects of UVB Treatment on Chlorophylls' and Carotenoids' Content

As shown in Figure 2B and Figure 2A, total chlorophyll content of red-leaf and green-leaf lettuce increased upon irradiation of UVB at both levels. In comparison with day zero, total chlorophyll content significantly increased at day two, which were 1.47 and 0.86 mg g^{-1} for UVB-0.7 treatment, and 1.45 and 0.78 mg g^{-1} for UVB-1.4 treatment, respectively. With the increase in UVB treatment, chlorophyll content reduced after day two and significantly reduced on day three and day five for the UVB-1.4 treatment.

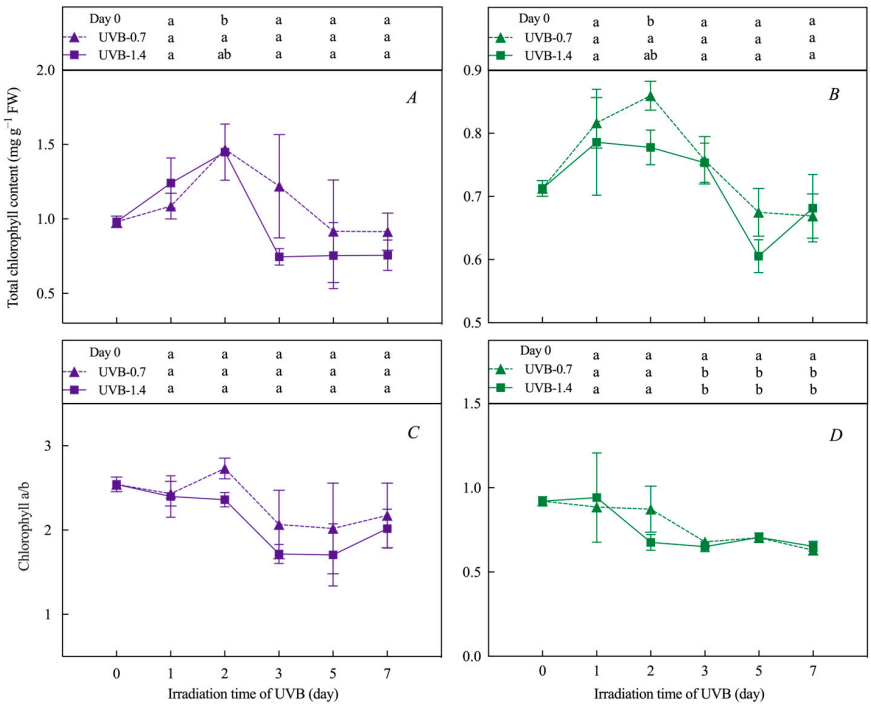

Figure 2. Effects of pre-harvest UVB treatment on total chlorophyll content and chlorophyll a/b of red-leaf lettuce (**A**,**C**) and green-leaf lettuce (**B**,**D**). Data are shown as mean ± standard error. Different letters were statistically dissimilar according to the Tukey test at $p \leq 0.05$ among day zero and the UVB treatment groups on certain days.

As shown in Figure 2C,D, chlorophyll a/b was not affected by UVB treatment for the red-leaf lettuce, while it significantly decreased after 3 days of UVB treatment for the green-leaf lettuce at both UVB intensities.

Carotenoids are important antioxidants that act as a protective substance against ROS accumulation. Before UVB treatment (day zero), the carotenoid contents of red-leaf

lettuce and green-leaf lettuce were 0.26 mg g^{-1} (Figure 3A) and 0.07 mg g^{-1} (Figure 3B), respectively. The carotenoid content of red-leaf lettuce increased with the exposure of UVB, and it reached the greatest concentration at 2 day for UVB-1.4 and at 3 day for UVB-0.7 treatment, and then decreased with the continuation of treatments. However, the carotenoid content of green-leaf lettuce showed a downward trend during the UVB treatment period. Under UVB-0.7 and UVB-1.4 treatments, the carotenoid content of green-leaf lettuce was negatively correlated with treatment time (r = 0.818, $p \leq 0.01$; r = 0.589, $p \leq 0.05$), and increased with treatment time (3~7 days), the carotenoid content of green-leaf lettuce was significantly lower than that before treatment (day zero).

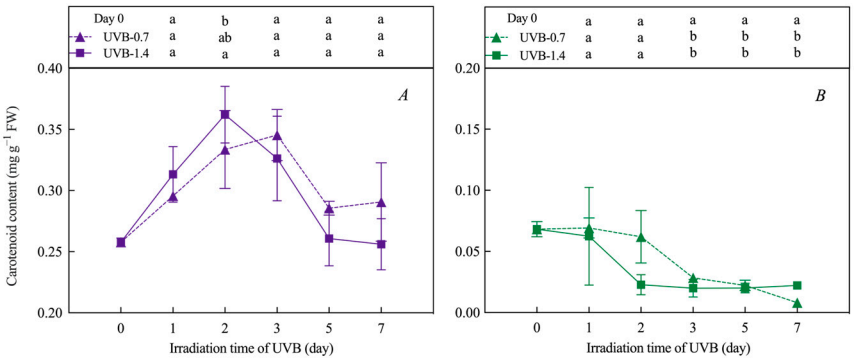

Figure 3. Effects of pre-harvest UVB treatment on carotenoid content of red-leaf lettuce (**A**) and green-leaf lettuce (**B**). Data are shown as mean ± standard error. Different letters were statistically dissimilar according to the Tukey test at $p \leq 0.05$ among day zero and the UVB treatment groups on certain days.

Generally, chlorophyll a is more sensitive to UVB radiation. As shown in Table 1, correlation analysis showed that chlorophyll a of green-leaf lettuce was negatively correlated with radiation time under UVB-0.7 and UVB-1.4 treatment (r =− 0.747, $p \leq 0.01$; r = − 0.534, $p \leq 0.05$). The chlorophyll a content of green-leaf lettuce was always lower than that of chlorophyll b, while that of red-leaf lettuce was higher than that of chlorophyll b (Table 1). Considering that there may be differences in the genotypes of the two experimental materials, their sensitivity to UVB is different, and thus their response to UVB is different.

Table 1. Effects of UVB pre-harvest treatment on chlorophyll a and chlorophyll b content of red-leaf lettuce and green-leaf lettuce.

		UVB-0.7		UVB-1.4	
Variety	**Day**	**Chlorophyll a mg g^{-1}**	**Chlorophyll b mg g^{-1}**	**Chlorophyll a mg g^{-1}**	**Chlorophyll b mg g^{-1}**
Red-leaf lettuce	0	0.70 ± 0.03 ^{a}	0.28 ± 0.01 ^{a}	0.70 ± 0.03 ^{ab}	0.28 ± 0.01 ^{ab}
	1	0.77 ± 0.08 ^{a}	0.32 ± 0.01 ^{a}	0.88 ± 0.14 ^{ab}	0.36 ± 0.04 ^{ab}
	2	1.07 ± 0.01 ^{a}	0.39 ± 0.02 ^{a}	1.02 ± 0.14 ^{a}	0.43 ± 0.05 ^{a}
	3	0.84 ± 0.28 ^{a}	0.38 ± 0.07 ^{a}	0.47 ± 0.05 ^{b}	0.27 ± 0.01 ^{ab}
	5	0.62 ± 0.28 ^{a}	0.29 ± 0.06 ^{a}	0.49 ± 0.17 ^{ab}	0.27 ± 0.05 ^{b}
	7	0.63 ± 0.12 ^{a}	0.29 ± 0.01 ^{a}	0.51 ± 0.09 ^{ab}	0.25 ± 0.02 ^{b}
Green-leaf lettuce	0	0.34 ± 0.01 ^{ab}	0.37 ± 0.001 ^{b}	0.34 ± 0.01 ^{a}	0.37 ± 0.001 ^{a}
	1	0.38 ± 0.02 ^{a}	0.43 ± 0.02 ^{ab}	0.34 ± 0.02 ^{a}	0.45 ± 0.08 ^{a}
	2	0.35 ± 0.03 ^{a}	0.41 ± 0.03 ^{b}	0.31 ± 0.01 ^{a}	0.47 ± 0.03 ^{a}
	3	0.35 ± 0.01 ^{ab}	0.51 ± 0.01 ^{a}	0.30 ± 0.02 ^{a}	0.46 ± 0.01 ^{a}
	5	0.28 ± 0.01 ^{b}	0.40 ± 0.03 ^{b}	0.25 ± 0.01 ^{a}	0.36 ± 0.02 ^{a}
	7	0.26 ± 0.02 ^{b}	0.41 ± 0.02 ^{b}	0.30 ± 0.04 ^{a}	0.46 ± 0.05 ^{a}

Data are expressed in the form of "mean ± standard error". The content of chlorophyll a and chlorophyll b of lettuce in different treatment periods was analyzed by the Tukey method, and the difference between different letters was significant ($p \leq 0.05$, n = 3).

3.2. Effects of UVB Treatment on Total Phenolic, Flavonoid Content, and Anthocyanin Relative Content

Red-leaf and green-leaf lettuce showed differences in their phenolic and flavonoid content. On day zero, the total phenolic content of red-leaf lettuce and green-leaf lettuce were 1.46 mg g^{-1} (Figure 4A) and 0.29 mg g^{-1} (Figure 4B), respectively, while the flavonoid content was 2.43 (Figure 4C) and 0.35 (Figure 4D) mg g^{-1}, respectively.

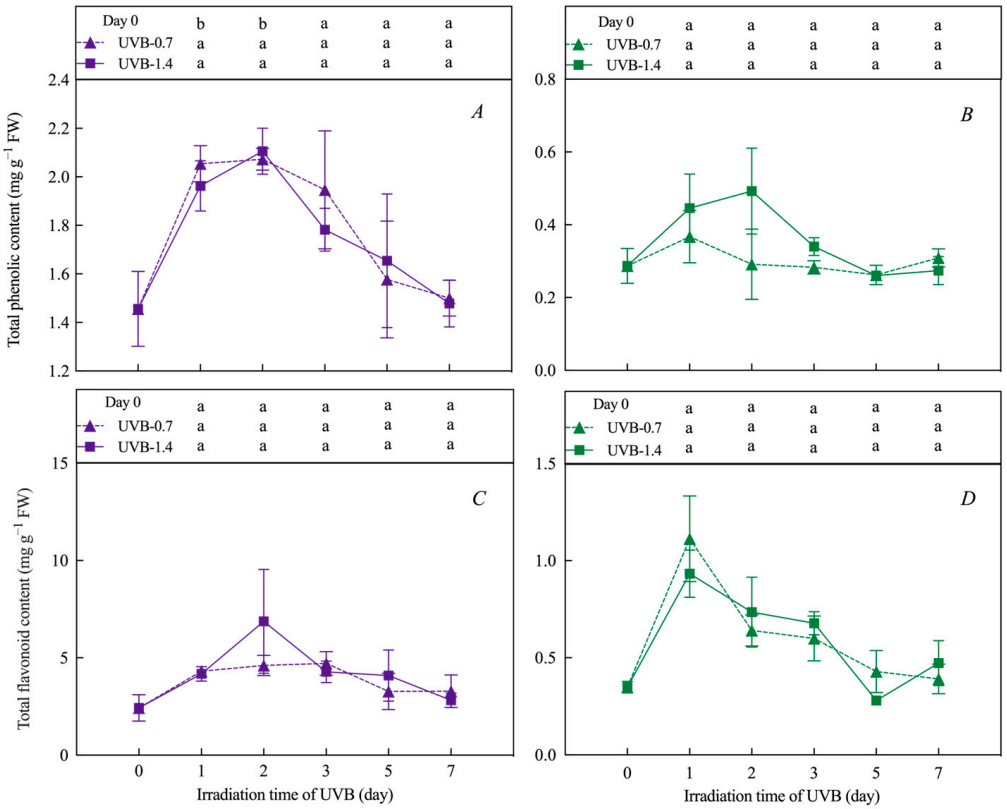

Figure 4. Effect of pre-harvest UVB treatment on total phenolic content and flavonoid content of red-leaf lettuce (**A**,**C**) and green-leaf lettuce (**B**,**D**). Data are shown as mean ± standard error. Different letters were statistically dissimilar according to the Tukey test at $p \leq 0.05$ among day zero and the UVB treatment groups on certain days.

For the total phenolics, the red-leaf lettuce resulted in a significant increase after 2 days of UVB treatment; on day two, it increased by 42.3% and 44.6% compared with day zero for UVB-0.7 and UVB-1.4 treatments of red-leaf lettuce. Then, it showed a downward trend for the last four days, while there was no significant difference for the green-leaf lettuce.

For the total flavonoids, there was no significant differences for both the red-leaf and green-leaf lettuce. There were numeric increases in the total flavonoid content. On day two, it increased by 90.0% and 183.4% compared with day zero for UVB-0.7 and UVB-1.4 treatments of red-leaf lettuce ($p = 0.103$), 84.1% and 110.9% of green-leaf lettuce ($p = 0.057$).

The anthocyanin content showed a positive correlation with the UVB treatment days ($r = 0.701$, $p \leq 0.01$; $r = 0.767$, $p \leq 0.01$) for the UVB-0.7 and UVB-1.4 treatment of the red-leaf lettuce, respectively (Figure 5). The anthocyanin content on day seven of UVB-0.7 was significantly higher than that on day zero. For UVB-1.4 treatment, the content of the last day was significantly higher than that of day zero, and the maximum value appeared on day five. The value of UVB-1.4 on day five and UVB-0.7 on day seven were four- and three-folds higher than those on day zero, respectively.

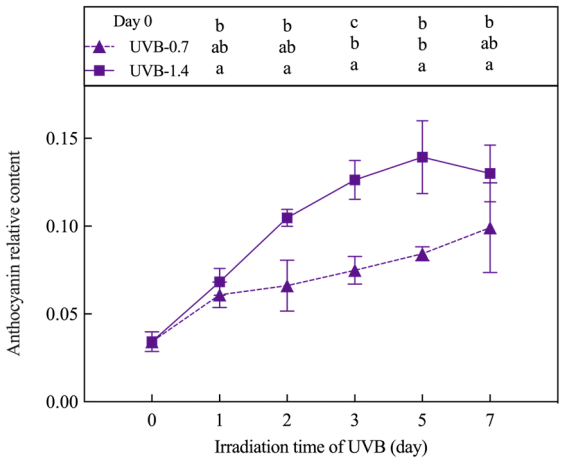

Figure 5. Effect of pre-harvest UVB treatment on anthocyanin relative content of red-leaf lettuce. Data are shown as mean ± standard error. Different letters were statistically dissimilar according to the Tukey test at $p \leq 0.05$ among day zero and the UVB treatment groups on certain days.

3.3. Effects of UVB Treatment on Antioxidant Capacity

As shown in Figure 6, for red-leaf lettuce, there was no significant difference between the two UVB treatments. Meanwhile, for green-leaf lettuce, the higher intensity UVB treatment was more effective. The DPPH free radical scavenging rate of red and green lettuce peaked within day one and day two, respectively, under high UVB radiation treatment, and the flavonoids' content reached the maximum within 2 days. It may be explained that UVB radiation will produce a variety of free radicals, leading to lipid peroxidation and destruction of cell structure. At this time, the level flavonoids' content gradually increased to eliminate free radicals, to reduce the damage caused by UVB and enhance antioxidant capacity. However, the maximum value of flavonoid content and the DPPH free radical scavenging rate did not appear at the same time, indicating that flavonoid content could not represent the tolerance ability of plants to UVB stress.

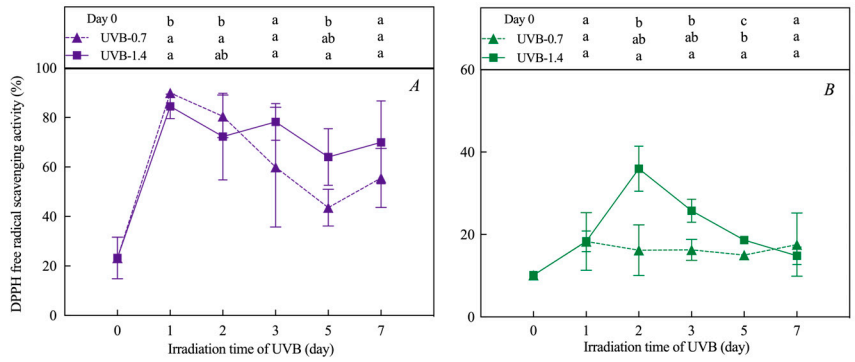

Figure 6. Effect of pre-harvest UVB treatment on DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging capacity of green-leaf (**A**) and red-leaf (**B**) lettuce. Data are shown as mean ± standard error. Different letters were statistically dissimilar according to the Tukey test at $p \leq 0.05$ among day zero and the UVB treatment groups on certain days.

3.4. Effects of UVB Treatment on MDA and H_2O_2 Content

H_2O_2 contents were also determined to investigate ROS levels in red-leaf lettuce, and they are presented in Figure 7C. The content of H_2O_2 experienced a sharp increase during the first two days; the amount of H_2O_2 was 3.1-folds and 3.5-folds greater in day two compared with day zero. Then, it remained stable in UVB-1.4 treatment and showed a downward trend in UVB-0.7 treatment during the last five days. For the green-leaf lettuce, the maximum value of H_2O_2 appeared on day three, when H_2O_2 levels were 4.0-folds

and 3.7-folds greater compared with day zero, and immediately dropped on day five. The decrease was 64.1% and 30.2% compared with day zero, respectively. However, H_2O_2 content in treated lettuce was lower than that of day zero on day five and seven.

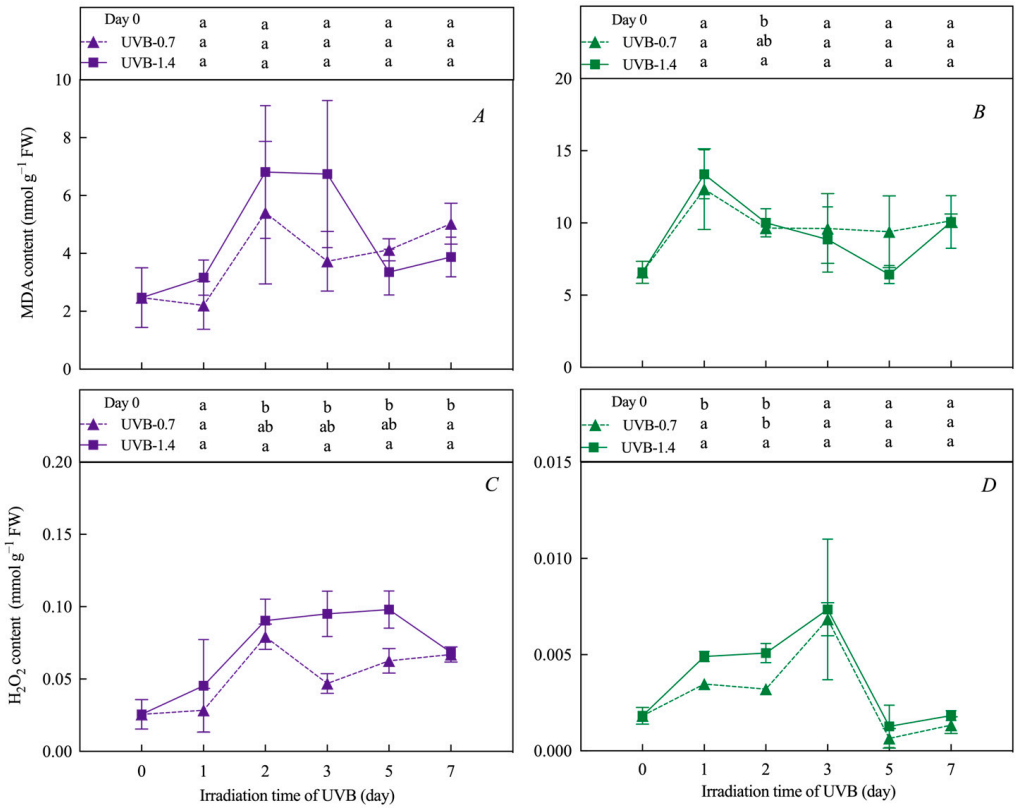

Figure 7. Effects of pre-harvest UVB treatment on MDA (**A**), H_2O_2 (**B**) content of red-leaf lettuce and MDA (**C**), and H_2O_2 (**D**) content of green-leaf lettuce. Data are shown as mean ± standard error. Different letters were statistically dissimilar according to the Tukey test at $p \leq 0.05$ among day zero and the UVB treatment groups on certain days.

4. Discussion

In leafy vegetables, chlorophylls are important primary photosynthetic pigments functioning in light energy capture, while they exhibit a green color appearance and thus are a parameter of vegetable quality and freshness [21,22]. In this study, total chlorophyll content increased significantly after 2 days of treatment with 0.7 W m^{-2} UVB radiation. This result is consistent with previous studies on stored broccoli. A certain intensity of UVB irradiation was suggested to delay chlorophyll degradation by suppressed chlorophyll-degrading enzyme activities [23]. With the elongation of treatment days, the chlorophyll content in the leaves of the jack bean decreased, and this is explained by the damage effects of excessive UVB that inhibited the chlorophyll synthesis substances such as aminolevulinic acid and protochlorophyllides [24].

Carotenoids have been related to the photoprotective effect of photosynthetic organisms [25]; the biosynthesis of carotenoids stimulated by UV radiation directly provides protection of the photosystems to such stress [26]. In this study, the accumulation of carotenoids was found in the red-leaf lettuce under UVB exposure until day three. This result is in accordance with previous reports that investigated the reaction of plants exposed to UVB in several lettuce varieties [10] and tomato fruits [27]. In the view of functional foods, adequate consumption of vegetable source carotenoids may reduce the risk of cardiovascular diseases and specific kinds of cancer [28]; enhancement of carotenoids thus increases the food nutritional value. Nevertheless, with the increase in UVB dose, carotenoid content of green-leaf and red-leaf lettuce decreases, and the rate of decrease in

UVB-1.4 treatment was higher than that in UVB-0.7 treatment. Under higher UVB radiation, carotenoid biosynthesis could be indirectly affected via non-specific signaling cascades associated with DNA damage and reactive oxygen species (ROS) production [29].

UVB irradiance is correlated with the synthesis of ROS in plant tissues; over accumulation of intracellular ROS is likely to reduce membrane stability, increase membrane permeability, and cause protein degradation [30]. ROS production in response to UVB is indicated by the H_2O_2 content in this study, which was greater under higher doses of UVB. High ROS content was also observed in Chlorophytum borivillianum and grapevines under exposure to a higher amount of UVB [31,32]. Increases in H_2O_2 production induces membrane lipid peroxidation, which is indicated by the level of MDA content [33]. In this study, MDA content under UVB radiation was numerically greater than that on day zero, which may be attributed to the free radical accumulation.

Generally, plants have the potential to scavenge the ROS though antioxidant enzymes (superoxide dismutase, catalase) and antioxidant substances (flavonoids, carotenoids) [34]. One of the most important responses to UVB radiation is the accumulation of secondary metabolites such as phenolics and flavonoids, which behave as UV-absorbing "sunscreen" to prevent cell damage [35,36]. Soybean [37] and Arabidopsis mutants [38] lacking phenolics showed oxidative damage earlier under UVB stress. A previous study determined the concentration of phenolics and flavonoids in different tissues of red lettuce (cv. Lollo Rosso) and found that they were three-fold and six-fold greater in the red tissue compared with the green tissue [39]. This agrees with our result that red-leaf lettuce had a higher accumulation of phenolics and flavonoids compared with green-leaf cultivar.

The phenylpropanoid pathway is influenced by UVB photoreceptor and related genes (*PAL*, *C4H*, *4CL*, *CHS*) [40,41]. Upregulation of genes associated with UVB promotes the photoprotective mechanism by inducing polyphenol synthesis through the phenylalanine pathway, particularly the flavonoid pathway [42,43]. This explains the accumulation of flavonoids in both red-leaf and green-leaf lettuce cultivars. Manipulation of UVB radiation level of protected horticultural crops by lighting or alteration of greenhouse covering materials are beneficial in improving flavonoid content for lettuce [44] and individual flavonoids, including luteolin and quercetin in rocket salad [45].

The higher the UVB dose, the higher the stimulation of anthocyanin detected in this study for the red-leaf lettuce. This was in line with previous studies, which found that sun-exposed pears receiving higher UVB usually show higher anthocyanin levels than shaded fruit [46]. Anthocyanin shares the same upstream pathway of flavonoid biosynthesis; The up-regulated expression of chalcone synthase (*CHS*), flavanone 3-hydroxylase (*F3H*), and dihydroflavonol 4-reductase (*DFR*) genes were reported upon irradiance of UVB, and thereby promoted anthocyanin accumulation in the red coloration of lettuce leaves [47]. UVB effects on secondary plant metabolites are dose dependent. Previous studies have shown that the content of secondary metabolites in plants increases under UVB radiation, but a higher intensity of UVB radiation could also reduce it [48].

The vegetable source antioxidants influence DPPH free radicals' scavenging ability. Under UVB treatment, the scavenging rate of DPPH free radicals of the two lettuce genotypes resulted in a certain increase. High-intensity UVB treatment is beneficial to the accumulation of antioxidant substances in red-leaf lettuce and improves its antioxidant capacity. Correlation analysis showed that under UVB-1.4 treatment, the DPPH radical scavenging activity of red-leaf lettuce was positively correlated with the relative content of total flavonoids and anthocyanins ($r = 0.509$, $p \leq 0.05$; $r = 0.552$, $p \leq 0.05$). Agreed in previous studies in leafy vegetable including lettuce [14], white cabbage [49], and broccoli sprouts [50], this result indicates that the irradiation of UVB prior harvest is beneficial in improving the antioxidant capacity of vegetables by the enhancement of secondary metabolites.

5. Conclusions

We conclude that supplementation of low-dose UVB radiance, prior to the harvest of two lettuce cultivars, changes the secondary metabolism including phenolics and flavonoids

biosynthesis, and thereby increases the antioxidant capacity of these leafy vegetables. In contrast, red-leaf lettuce has a stronger accumulation capacity of secondary metabolites. The optimal application was treated with 1.4 W m^{-2} UVB for 2 days (from 8:00 a.m. to 14:00 p.m.). As a pollution-free and residual-free environment regulation method, UVB has excellent potential to improve the quality and flavor characteristic of lettuce before harvest.

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