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Effect of End-of-production High-energy Radiation on Nutritional Quality of Indoor-grown Red-leaf Lettuce

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Abstract. **Numerous studies have evaluated the effect of high-energy radiation as means to increase nutritional quality of lettuce (*Lactuca sativa*). However, most research has focused on providing constant radiation quality or quantity throughout the production cycle, which typically results in yield reductions or increases in production costs. End-of-production (EOP) radiation is a cost-effective, preharvest practice that can allow growers to manipulate product quality and thus increase market value of lettuce without negatively affecting plant growth. The objective of this study was to quantify and compare growth and accumulation of secondary metabolites from 'Rouxaï RZ' and 'Codex RZ' red-leaf lettuce grown indoors and exposed to different strategies of EOP high-energy radiation. Plants were grown for 24 days under an average daily light integral (DLI) of 15.8 mol·m^{-2}·d^{-1} (220 µmol·m^{-2}·s^{-1} for 20 h·d^{-1}) using red:blue light-emitting diode (LED) lamps. Four days before harvest (36 days after sowing), plants were exposed to one of three EOP treatments added to red:blue LEDs: 1) ultraviolet-A (EOP-ultraviolet); 2) high blue (EOP-B); or 3) high-intensity (EOP-H) radiation. A fourth treatment was included as a control, with no EOP. Except for EOP-H, all treatments provided a DLI of 15.8 mol·m^{-2}·d^{-1}; EOP-H provided a DLI of 31.7 mol·m^{-2}·d^{-1}. No treatment differences were measured for shoot fresh weight (FW) of 'Rouxaï RZ' but shoot FW of 'Codex RZ' was negatively affected by EOP radiation, indicating potential changes in lettuce yield from applying EOP high-energy radiation during active plant growth. In general, EOP treatments did not affect total phenolic content and total carotenoid concentration of plants, but anthocyanin content and antioxidant capacity were positively influenced by EOP-B and EOP-H, whereas EOP-ultraviolet resulted in similar nutritional quality to control. Findings from this study indicate that EOP high-energy radiation, especially EOP-B, has significant potential to improve the nutritional quality of red-leaf lettuce grown in controlled environments.**

Salad greens such as lettuce (*Lactuca sativa*) are the most common crop type produced in indoor farms (also known as plant factories or vertical farms) because of their relatively short production cycle, small size, and low-radiation intensity requirements. Red-leaf lettuce cultivars are attractive to consumers because these cultivars typically have a high concentration of anthocyanins, which are responsible for their pigmentation, offer health-promoting benefits, and improve aesthetic value (Gazula et al., 2007; Paz et al., 2019; Ryder, 1999). In addition to anthocyanins, red leaves have been correlated with a higher accumulation of other secondary metabolites such as essential oils, carotenoids, and phenolic compounds with different antioxidant functions, which further increase the nutritional value of plants (Kim et al., 2018; Ohashi-Kaneko et al., 2007). However, the limited radiation intensity typically used in indoor farms can prevent leaf pigmentation from being optimally expressed in some red-leaf lettuce cultivars (Paz et al., 2019; Richards et al., 2004; Stagnari et al., 2015; Voipio and Autio, 1995).

Biotic and abiotic stressors are known to trigger the accumulation of secondary metabolites in plant defense pathways (Oh et al., 2009; Thakur et al., 2019). Among various environmental factors that can induce abiotic stress, radiation quantity [i.e., photosynthetic photon flux (*PPF*)] and quality (i.e., color, wavelength) are key regulators of different nutritional compounds that can be easily adjusted in controlled environments (Folta, 2019). In addition to promoting biomass production, high *PPF*s can increase secondary metabolites in lettuce, but effects may differ between green- and red-leaf cultivars (Gent, 2014; Pérez-López et al., 2013, 2014, 2018; Voipio and Autio, 1995; Woltering and Witkowska, 2016; Yan et al., 2019). Furthermore, high-energy radiation with ultraviolet-B (280 to 315 nm), ultraviolet-A (315 to 400 nm), and blue (400 to 500 nm) wavebands has been shown to increase product quality in many plant species (Carvalho and Folta, 2014; Samuolienė et al., 2017).

Although numerous studies have evaluated the effect of high-energy radiation as a means to increase the nutritional quality of lettuce, most studies have focused on providing constant radiation quantities or qualities throughout the production cycle (Caldwell and Britz, 2006; Li and Kubota 2009; Lin et al., 2013; Ohashi-Kaneko et al., 2007; Viršilė et al., 2020; Woltering and Witkowska, 2016). However, constantly providing high-energy ultraviolet or blue radiation can negatively affect plant morphology and the overall yield of lettuce (Dougher and Bugbee, 2004; Wargent et al., 2009). Similarly, using high *PPF*s can increase production costs associated with sole-source lighting and may induce undesirable photo-oxidative stress (Murchie and Niyogi, 2011; Samuolienė; et al., 2009).

EOP radiation is a cost-effective, preharvest solution to increase the nutritional quality of plant products and thus increase market value without negatively affecting yield. EOP is achieved by exposing plants to supplemental radiation, typically acting as an induced stressor, near the end of the cropping cycle after sufficient growth has already occurred. Previous studies have reported increases in lettuce quality with EOP compared with plants grown under a constant spectrum or *PPF*. However, most research evaluating the effect of EOP on plant quality has focused on greenhouse production (Owen and Lopez, 2015; Samuolienė et al., 2012; Viršilė et al., 2018; Zhang et al., 2019; Žukauskas et al., 2011). Fewer studies have evaluated the effects of EOP using sole-source lighting indoors; and to our knowledge, none have compared the use of high-energy wavebands (Nicole et al., 2016, 2019a, 2019b) vs. higher *PPF*s (Oh et al., 2009; Pérez-López et al., 2018; Zhou et al., 2012). Therefore, our objective was to quantify and compare growth and accumulation of secondary metabolites from two red-leaf lettuce cultivars grown indoors and exposed to different strategies of EOP high-energy radiation. We hypothesized that EOP treatments would not affect plant growth, but that EOP with high-energy wavebands would increase phytochemical accumulation more than EOP, with a high *PPF*.

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Materials and Methods

Plant material and growing conditions. Seeds of 'Codex RZ' and 'Rouxaï RZ' lettuce (Rijk Zwaan, De Lier, The Netherlands) were pregerminated for 48 h in a laboratory and subsequently transplanted into 98-plug sheets (55 mL individual cell volume) of rockwool (A-Ok starter plugs; Grodan, Roermond, The Netherlands). Seedlings were grown for 10 d inside a walk-in growth chamber (C6 Control System with EcoSys Software; Environmental Growth Chambers, Chagrin Falls, OH) using an average DLI of ≈13 $mol·m^{-2}·d^{-1}$ (150 ± 5 $μmol·m^{-2}·s^{-1}$ for 24-h·d^{-1}) provided by red:blue LED lamps (GreenPower; Signify, Somerset, NJ) with peak wavelengths of 660 and 448 nm, which delivered 87% red (600 to 700 nm) and 13% blue radiation as measured with a spectroradiometer (SS-110; Apogee Instruments Inc., Logan, UT). Ambient temperature and relative humidity (RH) were set at 24 °C and 70%, respectively; CO_2 concentration was maintained at ambient levels (405 $μmol·mol^{-1}$). Seedlings were sub-irrigated as needed with tap water that had an electrical conductivity (EC) of 0.4 $mS·cm^{-1}$, a pH of 8.3, and 40 $mg·L^{-1}$ calcium carbonate ($CaCO_3$).

At 12 d after sowing, three uniform seedlings per cultivar were transplanted into a single deep-water culture hydroponic system using 5-cm diameter net cups. Each cylindrical 7.6-L hydroponic system had a white plastic lid with three openings (25 cm apart) that held one net cup each. Plastic barriers were placed inside each reservoir to separate the plant roots. A clear plastic tube attached to an air pump (320 GPH, Dual Diaphragm Air Pump; General Hydroponics, Santa Rosa, CA) provided continuous aeration to the nutrient solution. Plants were grown for 24 d inside two walk-in growth chambers (C6 Control System), each equipped with two opposite shelving units with two compartments (61-cm wide × 183-cm long × 94-cm high) that held four hydroponic systems per cultivar. Each compartment had red:blue LED lamps (GreenPower) that provided an average DLI of 15.8 $mol·m^{-2}·d^{-1}$ (*PPF* 220 ± $μmol·m^{-2}·s^{-1}$; 20-h photoperiod from 0200 to 2200 HR). A white shade screen was used as a curtain (215-cm long × 200-cm high) to prevent radiation pollution between the two opposite shelves in each chamber. Average ambient day (from 0200 to 2200 HR) and night (from 2200 to 0200 HR) air temperature of the chambers were set at 22 °C and 21 °C, respectively. The CO_2 concentration and RH were set at 405 $μmol·mol^{-1}$ and 70%, respectively. Plants were grown using a commercial water-soluble fertilizer (OASIS Hydroponic Fertilizer 16–4–17; Oasis Grower Solutions, Kent, OH) dissolved in tap water at a concentration of 150 $mg·L^{-1}$ N (EC and pH = ≈1.2 $dS·m^{-1}$ and 6.0, respectively). The nutrient solution was refilled with tap water as needed and completely replaced after 2 weeks. Two fans were placed above plant canopy height within each treatment compartment, providing constant air flow to minimize tip burn incidence. Before applying the EOP treatments, all hydroponic systems were randomly rotated three times per week within each chamber to minimize location effects.

Treatments. Treatments were applied 36 d after sowing by exposing plants under each compartment to 4 d of the following: 1) 11 ± 1 $μmol·m^{-2}·s^{-1}$ of ultraviolet-A radiation provided by LED lamps (RAY66; Fluence Bioengineering, Austin, TX; peak wavelength of 405 nm) where an additional 55 ± 1 $μmol·m^{-2}·s^{-1}$ were counted as *PPF* of blue wavebands + 165 ± 3 $μmol·m^{-2}·s^{-1}$ from red:blue LED lamps (EOP-ultraviolet); 2) additional blue radiation with 120 ± 2 $μmol·m^{-2}·s^{-1}$ provided by blue LED lamps (RAY66; peak wavelength of 450 nm) + 100 ± 2 $μmol·m^{-2}·s^{-1}$ from red:blue LED lamps (EOP-B); 3) high intensity radiation with 440 ± 3 $μmol·m^{-2}·s^{-1}$ from red:blue LED lamps (EOP-H); or 4) 220 ± 1 $μmol·m^{-2}·s^{-1}$ from red:blue LED lamps as a control with no EOP. Except for EOP-H, all treatments provided a DLI of 15.8 $mol·m^{-2}·d^{-1}$ (220 ± 2 $μmol·m^{-2}·s^{-1}$ of *PPF*; 20-h photoperiod from 0200 to 2200 HR); EOP-H provided a DLI of 31.7 $mol·m^{-2}·d^{-1}$ (*PPF* 440 ± 3 $μmol·m^{-2}·s^{-1}$; 20-h photoperiod from 0200 to 2200 HR). The radiation output to achieve target *PPF*s and a uniform spectral distribution were controlled by adjusting the number of red:blue LED lamps or dimmer settings from each ultraviolet or blue LED lamp. The spectral distribution of the lamps is shown in Fig. 1 and the spectral characteristics of each treatment (combining output from different lamps) are described in Table 1.

Measurements and data collected. For each cultivar, one plant per hydroponic system per treatment was destructively harvested 40 d after sowing. Plants were weighed with an electronic balance to obtain shoot FW. Tissue samples were then collected from the sixth and seventh fully expanded leaves within each plant. Tissue samples were flash-frozen in liquid nitrogen and immediately placed in a freezer at –80 °C. The freeze-dried samples were used to determine dry weight (DW), and an adjusted extraction protocol described by Rodriguez-Saona and Wrolstad (2001) was used to process samples for anthocyanin, antioxidant capacity, and total phenolic content (TPC) following the procedures below. Frozen tissue samples (1 g of FW) were ground using a mortar with liquid nitrogen, and then extracted and re-extracted using 10 mL of acidified methanol (30 mL of 88% to 91% formic acid + 370 mL deionized water + 600 mL of 99.9% methanol) for 20 min.

The extracted sample was centrifuged, and its supernatant was used to determine total monomeric anthocyanin pigment content according to Lee et al. (2005) with some modifications. For each sample, 600-μL aliquots were diluted to 3 mL with a potassium chloride buffer (0.025 M, pH 1.0) and a sodium acetate buffer (0.4 M, pH 4.5). After 15 min, the absorbance was measured at 510 nm with a spectrophotometer (SpectraMax Plus 384; Molecular Devices, Sunnyvale, CA). In addition, absorbance was measured at 700 nm for haze correction. The total monomeric anthocyanin content was expressed as milligrams of cyanidin-3-glucoside equivalents per gram of DW.

Antioxidant capacity was determined using the ferric-reducing antioxidant power (FRAP) assay following a modified protocol described by Benzie and Strain (1996). A fresh FRAP solution was prepared by mixing with 25 mL of a 0.3 M sodium acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-Tri (2-pyridyl)-s-triazine solution in 40 mM of hydrochloric acid that was previously prepared, and 2.5 mL of 20 mM ferric chloride. A 4-μL aliquot of the sample extract was mixed with 1 mL of the FRAP reagent (warmed up to 37 °C) and incubated during 30 min in a warm bath. Absorbance was measured at 593 nm using a spectrophotometer; acidified methanol (extract solvent) was used as a blank. The calibration curve was obtained using a series of ferrous sulfate heptahydrate ranging from 2 to 20 mM. Antioxidant capacity was reported as millimolars ferrous iron (Fe^{2+}) equivalents per gram of DW.

TPC was determined using the method described by Ainsworth and Gillespie (2007) using a Folin–Ciocalteau (F–C) reagent. A 100-μL aliquot of the sample supernatant was mixed with 200 μL of 10% (v/v) F–C reagent vortexed thoroughly, after which 800 μL of 700 mM sodium carbonate were added. After incubating for 2 h at room temperature, absorbance was measured using a spectrophotometer at 765 nm. Methanol was used as a blank, and TPC was calculated using a standard curve with multiple gallic acid concentrations and expressed as milligrams of gallic acid equivalents per gram of DW.

The extraction and determination of total carotenoids (xanthophylls and carotenes) was conducted following a modified protocol described by Lichtenthaler and Buschmann (2001). A total of 50 mg of fresh leaf tissue were ground in a mortar with liquid nitrogen, adding 150 mg of magnesium oxide to avoid pheophytin formation. The ground sample was extracted with 5 mL of 100% acetone and after centrifugation, absorbance of the supernatant was measured at 750, 662, 645, 520, and 470 nm using a spectrophotometer. Concentrations of carotenoids were determined by the following equations:

$$c_a(\mu g/mL) = 12.25\,A_{663.2} - 2.79\,A_{646.8}$$

$$c_b(\mu g/mL) = 21.50\,A_{646.8} - 5.10\,A_{663.2}$$

$$c_{(x+c)}(\mu g/mL) = (1000\,A_{470} - 1.82c_a - 85.02c_b)/198$$

Total carotenoids were expressed as milligrams of carotenoids per gram of DW.

Nitrate content was measured by using samples extracted from 1 g of fresh leaf tissue ground in a mortar with liquid nitrogen and 6 mL of deionized water. The mixture was centrifuged for 15 min, and 200 μL of precipitate was mixed with 800 μL of 5% (w/v)

Table 1. Spectral characteristics of end-of-production treatments delivered by red:blue light-emitting diode lamps with or without (control) ultraviolet-A (EOP-ultraviolet), additional blue (EOP-B), or high-intensity (EOP-H) radiation.[z]

Treatment	Photosynthetic photon flux (*PPF*)	Ultraviolet-A (350–400 nm)	Blue (400–500 nm)	Green (500–600 nm)	Red (600–700 nm)	Total photon flux (*TPF*)	Yield photon flux (*YPF*)[y]
EOP-ultraviolet	220 ± 2	11 (5%)	76 (33%)	0.2 (0%)	145 (62%)	232 ± 2	196
EOP-B	220 ± 2	0.2 (0%)	152 (69%)	0.3 (0%)	68 (31%)	220 ± 2	179
EOP-H	440 ± 3	0.2 (0%)	53 (13%)	0.2 (0%)	387 (87%)	440 ± 3	397
Control	220 ± 1	0.2 (0%)	29 (13%)	0.3 (0%)	191 (87%)	219 ± 1	180

[z]Photon flux densities over 1-nm increments were integrated as *PPF* (400–700 nm) and *TPF* (350–700 nm), which includes ultraviolet-A radiation. Numbers outside of parentheses represent photon flux densities in $\mu mol \cdot m^{-2} \cdot s^{-1}$. Numbers in parentheses represent percentages of each waveband's contribution to *TPF*.
[y]*YPF* is the product of *PPF* and relative quantum efficiency calculated based on McCree (1972) and Sager et al. (1988).

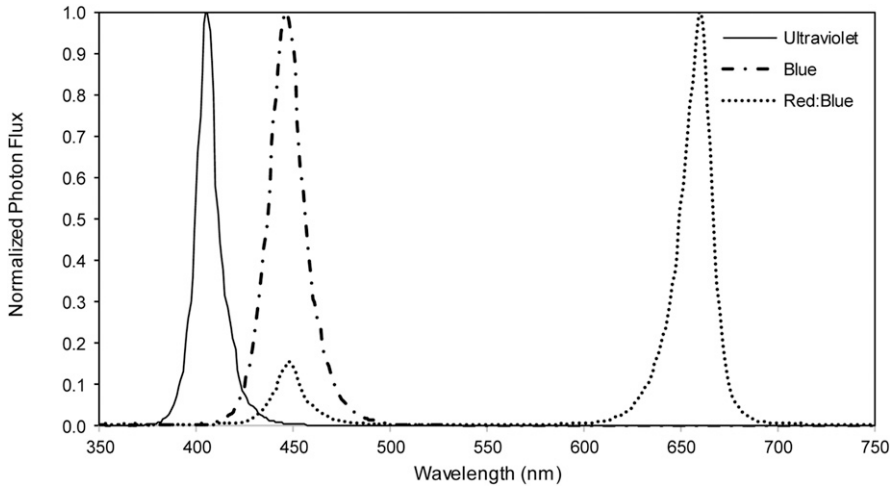

Fig. 1. Normalized spectral power distribution of the lamps used in the experiment. Photon flux ($\mu mol \cdot m^{-2} \cdot s^{-1}$) was measured for every 1 nm.

salicylic acid dissolved in sulfuric acid for 20 min at room temperature, after which 19 mL of 2N sodium hydroxide was added slowly. After allowing the samples to cool at room temperature for ≈30 min, absorbance was measured at 410 nm using a spectrophotometer. Nitrate content determination was performed according to the method of Cataldo et al. (1975) with some modifications. Calculations were made based on the measured absorbance and the nitrate calibration curve, using potassium nitrate as a standard. Nitrates were reported as milligrams of nitrate-nitrogen equivalents per kilogram of FW.

Experimental design and statistical analysis. The experiment was replicated twice over time; and within replication, each chamber was considered one block with four shelves used as treatment replications. Data were analyzed with JMP (version 12; SAS Institute Inc., Cary, NC) as a randomized complete block design with four blocks (two replications in time and two replications in space) and four sub-samples (hydroponic systems) for each cultivar per treatment per block. Results were pooled between replications over time, because the variances between experiments were not different and the statistical interactions between treatment and replication were not significant ($P \geq 0.05$). The influence of the two different categorical independent variables (i.e., cultivar and treatment), and their possible interaction on each of the continuous dependent variables were analyzed by using a two-way analysis of variance (Table 2). Data collected are presented as main effects (n = 8) except for shoot FW, in which the two cultivars are presented separately (n = 4) because of the significant cultivar × treatment interaction ($P \geq 0.05$). Mean separation was performed using Tukey's honestly significant difference test at $P < 0.05$. In addition, cultivar differences are presented in Table 3 for TPC, antioxidant capacity, and total carotenoids (n = 16); mean separation was based on Student's *t* test at $P \leq 0.05$.

Results and Discussion

No treatment differences were measured for shoot FW of 'Rouxaï RZ', which ranged from 67 to 76 g across treatments (Fig. 2A). However, shoot FW of 'Codex RZ' was negatively affected by EOP radiation, because control plants had between 11% and 37% more FW than those treated with EOP—suggesting that plants were still growing when treatments were applied. Correspondingly, 'Codex RZ' plants exposed to EOP-H, which received double the DLI during the EOP treatment, produced 50 g more than those exposed to EOP-ultraviolet. Many studies have shown that higher DLIs linearly increase growth and yield for crops with high-harvest indexes, such as lettuce. In a study evaluating radiation requirements for indoor gardening, Paz et al. (2019) concluded that higher DLIs during the finishing stage increased growth, nutritional quality, and visual appeal of indoor-grown red-leaf lettuce, which corresponds with our findings for EOP-H. Furthermore, others have shown that ultraviolet radiation can reduce biomass accumulation in lettuce, most likely as a response to the high metabolic cost of accumulating photoprotective secondary metabolites (Tsormpatsidis et al., 2008). It is likely that delaying EOP radiation treatments until after 'Codex RZ' plants had surpassed the exponential growth phase would have minimized the differences in the shoot FW measured in our study.

Anthocyanin content was more than double in plants treated with EOP-B or EOP-H compared with those treated with EOP-ultraviolet or control (Fig. 2B). Similarly, the highest antioxidant capacity was measured in plants exposed to EOP-B, which was 61% and 47% higher than that produced under EOP-ultraviolet and control, respectively (Fig. 2C). Several studies have reported measurable increases in lettuce quality under different wavebands of EOP (Nicole et al., 2016, 2019a, 2019b; Owen and Lopez, 2015; Samuolienė et al., 2012; Viršilė et al., 2018; Zhang et al., 2019). In addition, Pérez-López et al. (2018) found that 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of EOP radiation provided by warm-white fluorescent lamps increased accumulation of antioxidant capacity in green- and red-leaf lettuce plants compared with those grown under a constant *PPF* of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Žukauskasa et al. (2011) suggested that EOP radiation can affect the accumulation of bioactive compounds in lettuce as an indirect response to preharvest changes in metabolic activity against photo-oxidative stress, essentially acting a protective scavenger against harmful radiation.

Like our findings (Fig. 2B and C), the results of Li and Kubota (2009) showed that blue radiation has a higher capacity to increase anthocyanin content and antioxidant capacity in lettuce compared with ultraviolet radiation. A likely explanation for the significant differences in anthocyanin content and antioxidant capacity measured in our study between EOP-B and EOP-ultraviolet could be related to the different contributions of blue radiation from both treatments (Table 1), with EOP-B providing twice the amount of blue photons compared with EOP-ultraviolet. Although ultraviolet exposure typically triggers the synthesis of ultraviolet-blocking pigments that prevent high-energy radiation from inducing damage via the generation of reactive oxygen species (Murchie and Niyogi,

2011), photo-oxidative stress responses to EOP-ultraviolet could have been limited by the overall lower dosage of high-energy photons compared with that provided by EOP-B. It is also plausible that the ultraviolet dose used in our study was not sufficient to maximize the accumulation of some antioxidant enzymes that contribute to lettuce quality improvements. For example, others have reported increases in proline content with high-energy blue wavebands, which help mitigate stress responses in plants by acting as an osmoregulatory compound that helps stabilize proteins when plants are exposed to stress (Carvalho and Folta, 2014; Zheng and Van Labeke, 2017). In contrast, proline content in lettuce has been reported to be unaffected by ultraviolet radiation (Rajabbeigi et al., 2013). Karvansara and Razavi (2019) concluded that proline production is promoted only at relatively high doses of ultraviolet in some plants, which could help explain the smaller treatment effects measured with EOP-ultraviolet compared with EOP-B.

Khare and Guruprasad (1993) suggested that ultraviolet-B radiation is more effective than ultraviolet-A at enhancing anthocyanin accumulation in plants. However, application of shorter waveband ultraviolet in commercial production facilities may require significant precautions for workers' safety. Additionally, lamps with ultraviolet LEDs are still expensive, and their efficiency can be considerably limited compared with that of LEDs with longer peak wavelengths (Bugbee, 2017). As stated by Mitchell and Sheibani (2020), forthcoming breakthroughs in ultraviolet LED efficiency and efficacy, information from studies to be conducted with ultraviolet, and advancement of technologies to ensure worker safety with ultraviolet radiation may enable the widespread use of EOP-ultraviolet in the future. For now, based on our findings, EOP with additional blue radiation could be considered a more effective strategy to increase anthocyanin content and antioxidant capacity of indoor-grown red-leaf lettuce

Table 2. Significance level of growth and quality parameters measured from 'Codex RZ' and 'Rouxaï RZ' red-leaf lettuce plants grown indoors and exposed to different end-of-production high-energy radiation treatments.[z]

	Variable					
Source	Shoot fresh wt	Anthocyanin	Antioxidant capacity	Total phenolic content	Carotenoids	Nitrate
Replication	NS	NS	NS	NS	NS	NS
Cultivar	***	NS	*	*	***	NS
Treatment	***	*	*	NS	NS	**
Treatment × Cultivar	**	NS	NS	NS	NS	NS

[z]Treatments were delivered by red:blue light-emitting diode lamps with or without (control) ultraviolet-A, additional blue, or high-intensity radiation.
NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 3. Quality parameters measured from two red-leaf lettuce cultivars grown indoors and exposed to different end-of-production (EOP) high-energy radiation treatments.[z]

Cultivar	Antioxidant capacity (mM/g DW)	Total phenolic content (mg/g DW)	Carotenoids (mg/g DW)
Codex RZ	0.47 b[y]	17.5 b	4.8 a
Rouxaï RZ	0.59 a	22.0 a	3.9 b

[z]Data represent a pooled average for plants exposed to EOP treatments delivered by red:blue light-emitting diode lamps with or without ultraviolet-A, additional blue, or high-intensity radiation (n = 16).
[y]Means within column followed by the same letter are not different based on Student's *t* test at $P \leq 0.05$.

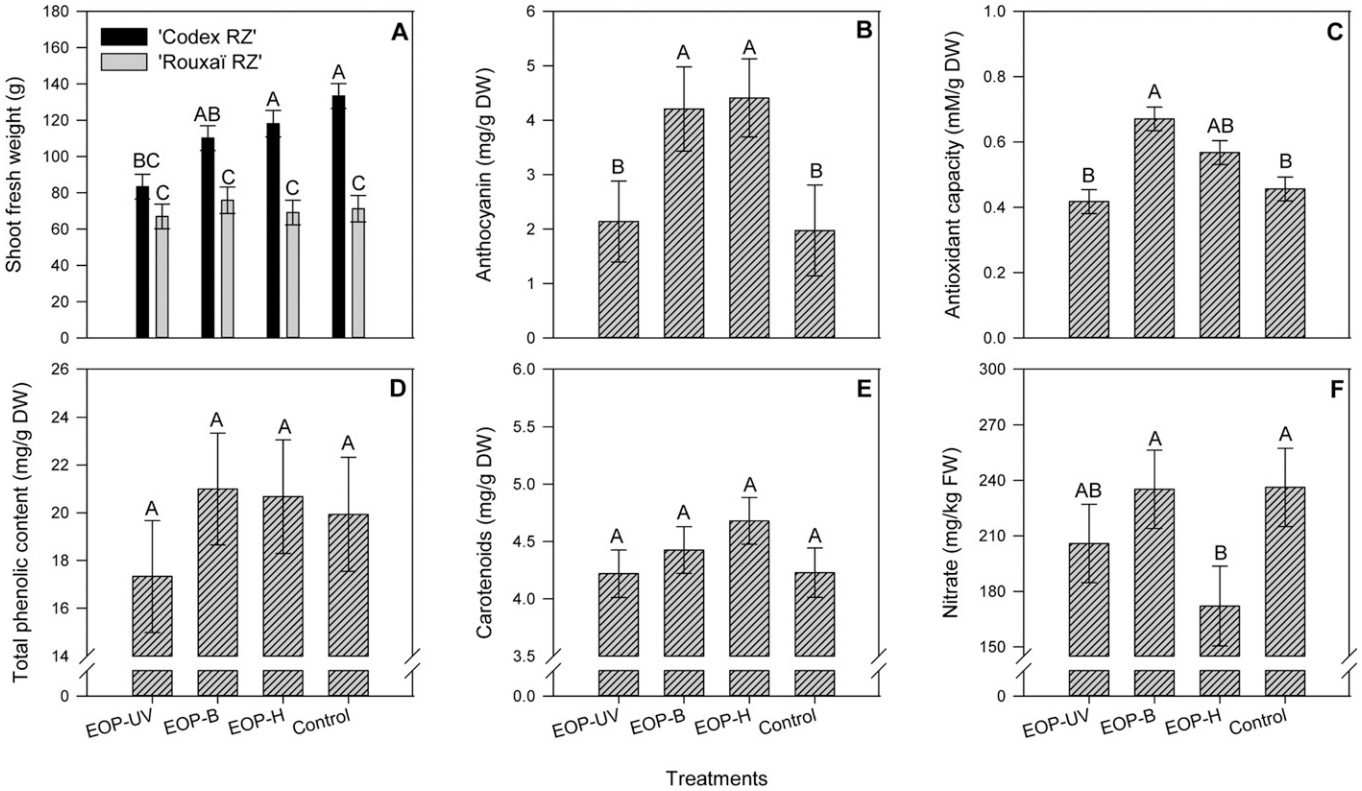

Fig. 2. Growth (**A**) and quality (**B**–**F**) parameters measured from 'Codex RZ' and 'Rouxaï RZ' red-leaf lettuce plants exposed to end-of-production treatments delivered by red:blue light-emitting diode lamps with or without (control) ultraviolet-A (EOP-UV), additional blue (EOP-B), or high-intensity (EOP-H) radiation. Except for shoot fresh weight (n = 4), bars represent the mean ±SE of four replications and two cultivars (n = 8). Means within graphs with the same letter are not different based on Tukey's honestly significant difference test at $P \leq 0.05$.

plants compared with EOP-ultraviolet (Fig. 2B and C). Furthermore, while EOP-H can positively affect lettuce quality, providing high *PPF*s can be more energetically expensive than exposing plants to high-energy blue wavebands.

For both cultivars, EOP treatments did not significantly affect TPC, but the general trend shows that plants exposed to EOP-ultraviolet had lower TPC compared with those treated with EOP-B or EOP-H (Table 2, Fig. 2D). The lack of significant treatment differences was unexpected, as radiation-quality responses to TPC tend to follow similar trends as those from antioxidant activities (Carvalho and Folta, 2014; Li and Kubota, 2009; Mulabagal et al., 2010; Oh et al., 2009; Son and Oh, 2013). Furthermore, Pérez-López et al. (2018) and Oh et al. (2009) reported significant increases in the accumulation of phenolic compounds when lettuce plants were exposed to EOP-H. It is likely that the short-term treatments used in this study were not sufficient to induce an increased synthesis of phenolic compounds. In contrast, cultivar effects indicated that 'Rouxaï RZ' plants had significantly higher antioxidant capacity and TPC than 'Codex RZ' plants (Table 3). It is not uncommon for net changes in lettuce quality to be cultivar-specific, which may be more pronounced in plants with lower basal antioxidant capacities (Llorach et al., 2008; Pérez-López et al., 2014; Žukauskas et al., 2011). Therefore, genetic variability is most likely responsible for the differences measured for antioxidant capacity and TPC between the two cultivars used in our study.

No treatment effect was measured for total carotenoid concentration; but in general, values were similar under EOP-ultraviolet and control, which were slightly lower than those measured under EOP-B or EOP-H (Table 2, Fig. 2E). Although others have shown higher carotenoid concentration in lettuce with blue and/or red LEDs compared with white radiation (Carvalho and Folta, 2014; Johkan et al., 2010; Li and Kubota, 2009), red-leaf lettuce plants are known to produce lower carotenoid concentration than green-leaf or romaine lettuce (Mou, 2005). Accordingly, we found that 'Codex RZ' plants had more total carotenoids than 'Rouxaï RZ' (Table 3), which could be related to the greener shoot pigmentation observed in that cultivar (data not shown). There seems to be significant genetic variation in carotenoid concentration among different lettuce genotypes (Mou, 2005). Thus, lack of treatment differences and significant cultivar effects for total carotenoid concentration may be related to the specific genetics of the cultivars used in our study.

Plants treated EOP-B or control had 37% higher nitrate content than those exposed to EOP-H (Fig. 2F). In accordance with our findings, others have shown that higher *PPF*s tend to reduce nitrate accumulation in lettuce leaves (Bian et al., 2018; Blom-Zandstra and Lampe, 1985; Escobar-Gutierrez et al., 2002; Paz et al., 2019; Samuolienė et al., 2009). In contrast, blue radiation has been reported to increase nitrate content of plants (Carvalho and Folta, 2014). High nitrate content is often considered a negative quality attribute of lettuce (Bruning-Fann and Kaneene, 1993); and although data on the potential long-term health risk of nitrate are contradictory (Gangolli et al., 1994; Walker, 1990), regulatory agencies are setting maximum-allowed nitrate levels in vegetables (Santamaria, 2006). Accordingly, recommended levels for nitrate content in lettuce range between 3000 to 5000 $mg \cdot kg^{-1}$ of FW (Colonna et al., 2016), which are an order of magnitude above the values measured in any of our treatments. Therefore, although nitrate content was differently affected by EOP radiation, all plants had acceptable levels for human consumption.

In conclusion, no treatment differences were measured for shoot FW of 'Rouxaï RZ', but shoot FW of 'Codex RZ' was negatively affected by EOP radiation, indicating potential changes in yield when applying EOP high-energy radiation during active plant growth. In general, EOP treatments did not affect TPC and carotenoid concentration of plants; but anthocyanin content and antioxidant capacity were positively influenced by EOP-B and EOP-H, whereas EOP-ultraviolet resulted in similar nutritional quality to control. Cultivar differences indicated that 'Rouxaï RZ' plants have a higher antioxidant capacity and accumulate more TPC than 'Codex RZ', but the opposite cultivar trend was measured for total carotenoid concentration. Based on our findings and considering potential implications on production costs, EOP with additional blue radiation is a more effective strategy to increase anthocyanin content and antioxidant capacity of indoor-grown red-leaf lettuce plants than EOP-H and EOP-ultraviolet.

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