



Light spectrum effects on rocket and lamb's lettuce cultivated in a vertical indoor farming system

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ABSTRACT

Rocket and lamb's lettuce are new leafy vegetables whose consumption in salads is increasing, especially as ready-to-eat products. These plants are often cultivated in greenhouses under artificial light, and are very important due to their pleasant taste, texture, and nutritional value to humans. The present study analyzes the effects of light quality provided by light emitting diodes (LEDs) on the yield and quality of different leafy vegetable species. Lamb's lettuce and rocket plants were grown in the same conditions under 4 light spectrums color fractions (NS-12, AP57, AP673L, and G2) for 27 and 37 days, respectively. Then fresh and dry weight, chlorophyll fluorescence, and chromatic characteristics were determined. The leaf quality of lamb's lettuce and rocket was characterized by measurements of the contents of carbohydrates, organic acids, and amino acids in leaves. Vegetative growth was significantly influenced by light quality and was species-dependent, and treatment with the highest proportion of Red:Blue light (G2) produced the highest fresh weight and maximum efficiency of PSII. The results of the chlorophyll fluorescence parameters were more influenced by the species rather than by the spectrum lights, except for the efficiency of PSII, which was higher in treatments with the lowest Red: Far-red ratio (AP67 and G2). G2 light spectrum reduces chlorophyll and carotenoid content compared to other treatments. Among all the primary metabolites analyzed, non-structural carbohydrates were the most dependent on light quality. Generally, the lights with the greatest effect on the parameters analyzed were those with higher Red:Blue ratios and a higher far-red fraction. No effect of light spectra on glutamate concentration was observed. However, a concentration increase was observed in treatments with a higher proportion of far-red light. Regarding organics acids, the different light spectrums did not affect the concentration of the majority of acids (citrate and malate) in rocket and lamb's lettuce plants. The spectral composition of light has an influence on the quality and quantity of lamb's lettuce and rocket production. Therefore, each species studied responds differently to the light spectrums.

1. Introduction

Rocket (*Eruca sativa*, Mill.) is a commercially important salad crop grown worldwide (Elmardy et al., 2021), and its cultivation is increasing to supply the expanding ready-to-eat market, due to its unique taste (Signore et al., 2020). Similarly, lamb's lettuce (*Valerianella locusta* L.) is a new leafy vegetable that is increasingly consumed in salads, especially as a ready-to-eat product. Moreover, it is particularly important due to its pleasant taste, texture, and nutritional value (Hernández et al.,

2021). Lamb's lettuce is a valued leaf vegetable during the autumn-winter period when it reaches a better and earlier yield. Currently, there is a high market demand for fresh lamb's lettuce all year round (Fabek et al., 2011).

According to the FAO (2022), the future demand for food and cash crops will continue to grow. Meeting this demand will require expanding cropland and improving yields based on new plant varieties and farming technologies. In recent years, the latest technologies have become highly relevant. However, electricity is one of the leading production costs in

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farming systems, with artificial illumination accounting for 21% of production costs (Kozai and Niu, 2020). Therefore, the key to indoor farming systems should be optimized energy conversion into plant yield and quality. One possible way to decrease the electricity consumed in farming systems is to use a minimum photosynthetic photon flux (PPF) and to modulate the light spectrum. There is a strong correlation between PPFD and electrical energy consumed (Massa et al., 2005; Paucek et al., 2020), which is also strongly dependent on the spectral distribution (Kusuma et al., 2020). Moreover, using the minimum energy from artificial light for plant growth contributes to the system's sustainability. On the other hand, advances in photon-to-yield conversion can be achieved by optimizing the spectral effects on plant morphology (Kusuma et al., 2020).

Within lighting systems, the light-emitting diode (LED) possesses singular lighting characteristics for plant growth (Paucek et al., 2020). For example, LED Light allows manipulating and controlling the light's intensity (Mitchell and Stutte, 2017). In addition, the coldness of LED surfaces allows them to be placed close to plant surfaces (Massa et al., 2005). Also, LED lights offer great flexibility for changing the spectral distribution as compared to conventional lamps (Fujiwara, 2020).

Numerous studies have demonstrated the importance of the light spectrum in plant growth (Kozai and Niu, 2020), as it affects both photosynthesis and the morphology of plants (Kubota, 2020). The morphological response is predominantly species and cultivar-specific (Hernández and Kubota, 2016), but could also depend on the plant's growth stage (Chen et al., 2014; Chang and Chang, 2014). In a light spectrum essay on seven plant species, three increased the yield under a red-blue LED light with 9:1 ratio. In contrast, in the other four microgreens species, no significant differences were observed under different ratios of red-blue light (Bantis, 2021). In another vegetable specie as lamb's lettuce, the highest fresh weight of rosettes was obtained under LED lamps with high red-blue ratio (Wojciechowska et al., 2015; 2016). On the other hand, Proietti et al. (2021) observed the highest fresh weight in rocket plants under continuous white LED, whereas the dry weight and leaf dry matter % were enhanced under continuous red-blue LED light.

Light quality also affects the stomatal responses of plants. Research performed by Muneer et al. (2014) and Hernández and Kubota (2016) observed increased stomatal conductance under blue LED light, or as the blue fraction in the light increased. A recent study showed that weak blue light with strong red light induced stomatal opening; conversely, weak blue light by itself promoted stomatal closure (Hosotani et al., 2021). Another parameter that is affected by light wavelengths in their relationship with photosynthesis is chlorophyll fluorescence (Fv/Fm). Chlorophyll fluorescence assesses and quantifies damage to the leaf photosynthetic apparatus, particularly PSII activity, in response to environmental conditions. Lights with a higher fraction of blue light showed higher Fv/Fm values than monochromatic red light in lettuce plants (Son and Oh, 2013). In this sense, Wojciechowska et al. (2013) observed that the Fv/Fm achieved higher values in lamb's lettuce plants with red+blue LED lighting than in plants that grew under ambient light.

The light spectrum also affects the synthesis of many compounds, among them chlorophylls. After germination, etiolated seedlings accumulate the chlorophyll precursor, protochlorophyllide (Pchlde), in the cotyledons. Pchlde is rapidly converted to chlorophyll upon exposure to light (Liu et al., 2017). Fan et al. (2013) observed that the concentration of chlorophyll biosynthesis precursors was higher under red-blue LED light. Moreover, chlorophyll concentration can be modified by the light spectrum, and the response is variable among plant species. The continuous exposure to red-blue LED light decreased the concentration of chlorophylls (a+b) in rocket plants versus white LED light (Proietti et al., 2021). In contrast, Wojciechowska et al. (2013) found that chlorophyll (a + b) in lamb's lettuce plants under red-blue LED light was higher than in white LED and ambient light in the winter season. Other compounds, such as carbohydrates can also be modulated by light.

Specifically, red-blue light decreased non-structural carbohydrate content versus continuous white LED light in rocket plants (Proietti et al., 2021). On the other hand, red-blue LED light with a ratio of 2:1 can reduce the total soluble solids in some microgreens (Bantis, 2021).

Although previous reports have indicated that light quality affects the agronomic characteristics of leafy vegetables. To our knowledge, little work has been conducted on the interaction of light quality and lamb's lettuce and rocket species, on the growth, physiological response and primary metabolite content of these vegetables. Therefore, the main objective of this work was to study the effect of four different commercial LED light spectrums on yield, physiological parameters and primary metabolite content of two baby leafy vegetables (rocket and lamb's lettuce).

2. Material and methods

2.1. Experimental design, growth conditions, and plant materials

The experiment was set up in a completely randomized design with a factorial structure of 4×2 with 3 replicates. Each replicate consisted of 25 plants. The first factor was the LED light spectrum, which had four levels: NS-12 (control), AP67, AP673L, and G2. The second factor corresponded to the specie: lamb's lettuce and rocket.

The experiment was carried out in a growth chamber under climate control conditions. Rocket (*Eruca vesicaria* (L.) Cav. Var. Prudenzia) and lamb's lettuce (*Valerianella locusta* L. var. Etap) seeds were sown in expanded polystyrene trays containing rock wool with a density of 1000 plant m^{-2} . Seed trays were irrigated with 100% Hoagland's solution (Table 1) and placed in a dark chamber at 22°C and 100% RH. After three days, the germinated trays were transferred to a growth chamber under normal light conditions. Then, seedlings were grown in a recirculated floating hydroponic system under LED light conditions for 27 days for lamb's lettuce and 37 for rocket. The growth conditions of lettuce were set as follows: light with 12 h photoperiod ($150 \mu mol m^{-2} s^{-1}$), 20°C (daytime)/ 18°C (night) 60-80% relative humidity. The nutrient solution was full modified Hoagland's solution, the pH was maintained at 5.5-6, while the electrical conductivity was $2 dS m^{-1}$.

2.2. Light spectrum treatments

Four different commercial LED lights were tested: (i) NS-12 LEDs as a control, (ii) AP67, (iii) AP673L, and (iv) G2. The photosynthetic photon flux densities (PPFD) under each LED lamp were $150 \pm 5 \mu mol m^{-2} s^{-1}$ and daily light integral (DLI) was $6.6 \pm 0.4 mol m^{-2} d^{-1}$ measured with a spectroradiometer (Spectrum Genius Agricultural Lighting, Kempton/Germany) placed 20 cm from the top of the plants. The photoperiod was 12:12 h (12 h light/12 h dark). LED lights were provided by Valoya™ (Helsinki, Finland) and were specially designed for crop cultivation. The relative spectral distributions of LED lighting treatments are detailed in Table 2.

Table 1

Composition of the nutrient solution used in the recycled floating hydroponic system.

| Macronutrients | Concentration (mmol L ⁻¹) | Micronutrients | Concentration (μmol L ⁻¹) |
|-------------------------------|---------------------------------------|----------------|---------------------------------------|
| NO ₃ ⁻ | 14 | B | 25.0 |
| PO ₄ ³⁻ | 1 | Cu | 0.5 |
| SO ₄ ²⁻ | 1 | Fe | 20.0 |
| K | 7 | Mn | 2.0 |
| Ca | 4 | Mo | 0.5 |
| Mg | 1 | Zn | 2.0 |

These nutrients have been added as: KNO₃, Ca (NO₃), KH₂PO₄, MgSO₄, Fe-EDTA, H₃BO₃, MnSO₄·H₂O, ZnSO₄·7H₂O, CuSO₄·5H₂O, and (NH₄)₆Mo₇O₂₄·4H₂O.

Table 2
The relative spectral distributions of LED lighting treatments.

| Light component | Wavelength Nm | NS-12 (control) % | AP67 | AP673L | G2 |
|-----------------|---------------|-------------------|------|--------|-----|
| Ultraviolet | < 400 | 1 | 0 | 0 | 0 |
| Blue (B) | 400-500 | 20 | 12 | 10 | 7 |
| Green | 500-600 | 36 | 16 | 19 | 2 |
| Red (R) | 600-700 | 38 | 56 | 63 | 70 |
| Far Red (FR) | 700-800 | 5 | 16 | 8 | 21 |
| PAR | 400-700 | 94 | 83 | 92 | 75 |
| R:B ratio | | 1.7 | 3.8 | 5.1 | 8.1 |
| R:FR ratio | | 4.6 | 3.3 | 5.5 | 3.1 |

2.3. Fresh and dry weights and dry matter content

At harvest, the leaf's fresh weight (FW) for both species, rocket and lamb's lettuce, was weighed on a digital scale (RADWAG, WLC 2/A2) and recorded. Then, the samples were placed in an oven at 70°C to a constant weight, at which time the dry weight (DW) was recorded. The percentage of dry matter (DM) contents was calculated as a ratio DW/FW X 100.

2.4. Chlorophyll fluorescence, chlorophyll and carotenoid concentrations

The chlorophyll fluorescence parameters were measured using a portable fluorometer FMS-2 (Hansatech Instruments Ltd., United Kingdom) following the protocol of [Alfosea-Simón et al. \(2022\)](#). The chlorophyll fluorescence parameters measured were: i) the quantum efficiency of PSII, $\Phi\text{PSII} = (\text{Fm}' - \text{Fs})/\text{Fm}'$ ii) the antennae efficiency of PSII, $\text{Fv}'/\text{Fm}' = (\text{Fm}' - \text{F}_0')/\text{Fm}'$ and iii) the photochemical quenching coefficient, $\text{qP} = (\text{Fm}' - \text{Fs})/(\text{Fm}' - \text{F}_0')$, where Fm' is the maximal value when all reaction centers are closed after a pulse of saturating light ($12,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.8 s), Fs is the steady-state fluorescence yield, and F_0' is the minimal fluorescence in the light-adapted state that is obtained by turning off the actinic light temporarily and applying a pulse of far-red light (735 nm) to drain the electrons from PSII ([Shin et al., 2020](#)).

The fresh leaf tissue was mixed with acetone (80%) extraction solvent in a 1:5 ratio (w:v; leaf:acetone). The homogenates were centrifuged at $15,000 \times g$ at 4°C for 5 minutes. The supernatant was recovered and diluted ten times with extraction solvent. The concentrations of chlorophyll a (Chl a) and b (Chl b) were determined in the diluted supernatant by reading the absorbance at 663 and 647 nm (A663 and A647), respectively, using a UV/Vis spectrophotometer (PowerWave XS2, BioTek, Winooski, Vermont, USA). Carotenoid (Car) concentration was calculated by reading the absorbance at 470 nm (A470). The concentrations of chlorophyll a and b and carotenoids were obtained according to the equations from [Lichtenthaler and Buschmann \(2005\)](#). The results were expressed in $\mu\text{g g}^{-1}$ FW. Furthermore, the chlorophyll a:b ratio was calculated.

$$\text{Chla} = 12.25A_{663.2} - 2.79A_{646.8}$$

$$\text{Chlb} = 21.50A_{646.8} - 5.10A_{663.2}$$

$$\text{Car} = (1000A_{470} - 1.82\text{Chla} - 85.02\text{Chlb})/198$$

2.5. Chroma index

Leaf color was measured using a Minolta CR400 Chroma Meter (Minolta Camera Co. Ltd., Osaka, Japan) previously calibrated with the internal Minolta standard. Measurements were based on CIELAB parameters: L^* (lightness, ranging from 0 = black to 100 = white), chroma (C^*), which denotes the overall color intensity, was calculated using the following formula: $(a^2 + b^2)^{1/2}$.

2.6. Metabolomic analysis

The leaf samples were ground with liquid nitrogen with a mortar and pestle and lyophilized. Afterward, the samples were prepared for analysis according with the protocol by [Alfosea-Simón et al. \(2022\)](#). For this analysis, a Nuclear Magnetic Resonance (NMR) system coupled to a 500 MHz Bruker spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a broadband 5 mm N2 CryoProbe Prodigy BBO, was used. All the leaf extracts were measured at 300.1 ± 0.1 K without rotation and with 4 test scans.

The acquisition parameters were set in the following manner: the size of the FID = 64K, spectral band = 12.4345 ppm, receiver gain = 28.5, acquisition time = 2.18 s, relaxation delay = 2s, and line broadening = 0.50 Hz. Data acquisition was acquired through the NOESY pulse sequence of pre-saturation (Bruker 1D, noesypr1d) with water suppression through the irradiation of the water frequency during the recycling and mixing times. In the procession of the samples and for each spectrum separately, a reduction of noise is produced, based on the deconvolution of the multi-level signal. Afterward, a correction is performed of the baseline, and to complete the process, an interpolation technique of the areas of the signal is utilized. All of this provides a general view of the metabolites that are most represented produced by the cells at time of harvest, expressing the chemical shifts (δ) in parts per million (ppm). The NMR equipment detects the signals and records them as frequency vs intensity graphic, known as the "acquisition spectrum".

The resulting 1H-NMR spectra were processed with the Chenomx NMR Suite program version 8.3 (Chenomx, Edmonton, Canada), in order to identify and quantify the metabolites of interest. All the samples were calibrated with the signal from the internal standard (IS), the deuterated Trimethylsilylpropionic acid sodium salt (TSP-d4) and the pH was set to a value of around 6. The software utilized includes a broad range of spectrum data which can be utilized to detect the metabolites that are over 5–10 μM : among the metabolites that were found and/or quantified, the following are highlighted: aspartate, asparagine, glutamate, alanine, glutamine, isoleucine, valine, citrate, succinate, acetate, formate, fumarate, malate, fructose, lactate, glucose and sucrose.

2.7. Statistical analysis

The results were reported as the mean values of eight biological replicates ($n = 8$). Data were evaluated by a two-way analysis of variance (ANOVA) (F test $P \leq 0.05$). The normality of the residuals was checked with the Shapiro-Wilk test ($P \leq 0.05$). The homogeneity of variance was checked with Bartlett's test, and the residual independence by descriptive analysis. Differences between means were determined with Tukey's multiple comparison test at $P \leq 0.05$. Statistical analyses were performed with the statistical package SPSS v. 24 (SPSS statistical package, Chicago, IL, USA).

3. Results

The effect of the light spectrum on fresh weight and dry matter content was specie dependent ([Table 3](#)). In both species, a high red fraction in the light spectrum significantly increased FW and DM. Specifically, the rocket plants under G2 had the highest FW and DM content compared to other treatments. Among the light factors, G2 enhanced the fresh weight by 53.4%, 43.4%, and 38.9% with respect to NS-12, AP673L, and AP67, respectively. Also, rocket plants were heavier than lamb's lettuce by 86.5%. The DM content under G2 increased by 60.5, 46.8, and 40.8% as compared to NS-12, AP673L, and AP67, respectively. Between species, rocket plants showed the highest DM content than lamb's lettuce plant by 17%.

Chlorophyll fluorescence parameters did not reveal significant differences for the interaction light spectrum x specie ([Table 4](#)). The maximal photochemical efficiency of PSII (Fv'/Fm') was affected by light and specie independently. In particular, AP673L decreased Fv'/Fm' with

Table 3

Shoot fresh weight and dry matter content in lamb's lettuce and rocket plants under the different LED light spectra.

| Factor | Level | Fresh weight (g) | Dry matter content (%) | |
|---------------------|-----------------|--------------------|------------------------|-------|
| Light spectrum | NS-12 (control) | 99.8c ¹ | 4.3b | |
| | AP67 | 110.2b | 4.9b | |
| | AP673L | 106.8bc | 4.7b | |
| | G2 | 153.1a | 6.9a | |
| Specie | Lamb's lettuce | 79.4b | 4.7b | |
| | Rocket | 148.1a | 5.5a | |
| Interaction (L x S) | Lamb's lettuce | NS-12 | 71.2e | 4.3d |
| | | AP67 | 79.3e | 4.3d |
| | | AP673L | 68.1e | 4.4d |
| | | G2 | 105.1d | 5.9b |
| | Rocket | NS-12 | 120.2cd | 4.3d |
| | | AP67 | 148.4b | 5.4bc |
| | | AP673L | 135.9bc | 4.8cd |
| | | G2 | 201.0a | 7.8a |
| ANOVA | Light spectrum | *** | *** | |
| | Specie | *** | *** | |
| | Light x Specie | *** | *** | |

¹ For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and n.s., non-significant ($p > 0.05$). Mean ($n = 8$).

Table 4Maximum efficiency of PSII in light-adapted leaves (Fv'/Fm'), photochemical quenching of PSII (qP), and photochemical efficiency of PSII (Φ_{PSII}) in lamb's lettuce and rocket plants under the different LED light spectra.

| Factor | Level | Fv'/Fm' | qP | Φ_{PSII} | |
|---------------------|-----------------|---------------------|-------|---------------|------|
| Light spectrum | NS-12 (control) | 0.77ab ¹ | 0.62 | 0.81 | |
| | AP67 | 0.78a | 0.61 | 0.79 | |
| | AP673L | 0.75b | 0.60 | 0.80 | |
| | G2 | 0.78a | 0.62 | 0.80 | |
| Specie | Lamb's lettuce | 0.73b | 0.53b | 0.73b | |
| | Rocket | 0.81a | 0.70a | 0.87a | |
| Interaction (L x S) | Lamb's lettuce | NS-12 | 0.73 | 0.54 | 0.76 |
| | | AP67 | 0.74 | 0.52 | 0.71 |
| | | AP673L | 0.70 | 0.51 | 0.73 |
| | | G2 | 0.74 | 0.53 | 0.73 |
| | Rocket | NS-12 | 0.81 | 0.71 | 0.87 |
| | | AP67 | 0.81 | 0.70 | 0.86 |
| | | AP673L | 0.80 | 0.69 | 0.87 |
| | | G2 | 0.82 | 0.72 | 0.88 |
| ANOVA | Light spectrum | *** | ns | ns | |
| | Specie | *** | *** | *** | |
| | Light x Specie | ns | ns | ns | |

¹ For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and n.s., non-significant ($p > 0.05$). Mean ($n = 8$).

respect to AP67, G2, and no significant differences were detected with NS-12. Between species, the Fv'/Fm' in rocket plants was significantly higher than in lamb's lettuce plants. On the other hand, the photochemical quenching of PSII (qP) and the photochemical efficiency of PSII (Φ_{PSII}) were affected by the specie factor but not by light quality treatments. All fluorescence parameters were higher in rocket than in lamb's lettuce plants.

The Chl a concentration was affected by light spectrum treatment (Table 5), decreasing as the red fraction increased in the light spectrum. Specifically, G2 significantly decreased Chl a concentration as compared with the control (NS-12) by 12.1%. Regarding Chl b, no significant difference was observed according to light spectrum and specie. Furthermore, the Car concentration under G2 was substantially lower than the other light spectra by 15.5, 16, and 14% as compared to the control (NS-12), AP67, and AP673L, respectively. In addition, rocket plants presented a higher Car concentration than lamb's lettuce plants.

Table 5

Concentration of Chlorophylls and carotenoids in lamb's lettuce and rocket plants under the different LED light spectra.

| Factor | Level | Chl a | Chl b | Car | Chl a:b | |
|---------------------|-----------------|---------------------|-------|-------|---------|--------|
| Light spectrum | NS-12 (control) | 207.2a ¹ | 78.8 | 52.9a | 2.64 | |
| | AP67 | 198.8ab | 76.5 | 53.2a | 2.65 | |
| | AP673L | 194.3ab | 72.7 | 52.0a | 2.74 | |
| | G2 | 182.2b | 70.9 | 44.7b | 2.53 | |
| Specie | Lamb's lettuce | 196.5 | 76.9 | 43.5b | 2.62 | |
| | Rocket | 195.0 | 73.1 | 57.5a | 2.72 | |
| Interaction (L x S) | Lamb's lettuce | NS-12 | 208.3 | 79.2 | 47.5 | 2.63ab |
| | | AP67 | 200.0 | 78.1 | 44.3 | 2.61ab |
| | | AP673L | 199.2 | 70.8 | 45.8 | 2.80a |
| | | G2 | 178.5 | 79.3 | 36.5 | 2.34b |
| | Rocket | NS-12 | 206.5 | 78.4 | 57.6 | 2.61a |
| | | AP67 | 197.4 | 75.4 | 60.9 | 2.70a |
| | | AP673L | 190.6 | 74.1 | 58.2 | 2.62ab |
| | | G2 | 185.3 | 64.5 | 52.9 | 2.73a |
| ANOVA | Light spectrum | * | ns | *** | ns | |
| | Specie | ns | ns | *** | ns | |
| | Light x Specie | ns | ns | ns | ** | |

All parameters are expressed in $\mu\text{g g}^{-1}$ of FW. Chl: Chlorophyll, Car: Carotenoids. ¹ For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and n.s., non-significant ($p > 0.05$). Mean ($n = 8$).

On the other hand, the Chl a:b ratio was affected by the interaction of different factors. In this case, lamb's lettuce under G2 showed the lowest Chl a:b ratio value as compared to the other treatments, but no significant differences were found with the control (NS-12).

Lightness (L^*) and Chroma (C^*) were independently affected by light spectrum and specie (Table 6). G2 and AP67 increased the L^* and C^* as compared to the control (NS-12) and AP673L. Furthermore, rocket plants presented higher L^* and C^* values than lamb's lettuce plants. Also, hue (h) was affected by the interaction between light spectrum and specie factors. Lamb's lettuce under AP673L and control (NS-12) showed the highest h values as compared to the other treatments. Specifically, the control (NS-12) treatment increased the h value significantly as compared with AP67 and AP673L. Moreover, h in lamb's lettuce plant was greater than rocket plants.

The interaction between factors for all non-structural carbohydrates was significant, so the impact of the light spectrum factor was dependent on the species (Table 7). Regarding sucrose, an increasing trend was

Table 6Lightness (L^*), Chroma (C^*), and hue (h) in lamb's lettuce and rocket plants under the different LED light spectra.

| Factor | Level | L^* | C^* | H | |
|---------------------|-----------------|--------------------|-------|---------|---------|
| Light spectrum | NS-12 (control) | 43.4b ¹ | 33.5b | 125.4a | |
| | AP67 | 45.1a | 36.9a | 124.3b | |
| | AP673L | 43.4b | 34.3b | 124.5b | |
| | G2 | 45.8a | 37.2a | 124.7ab | |
| Specie | Lamb's lettuce | 43.2b | 34.3b | 125.81a | |
| | Rocket | 45.6a | 36.4a | 123.63b | |
| Interaction (L x S) | Lamb's lettuce | NS-12 | 42.2 | 33.4 | 126.4ab |
| | | AP67 | 43.9 | 35.6 | 125.3bc |
| | | AP673L | 42.5 | 32.5 | 126.5a |
| | | G2 | 44.3 | 35.9 | 125.0c |
| | Rocket | NS-12 | 44.9 | 33.6 | 124.3cd |
| | | AP67 | 46.0 | 37.9 | 123.3de |
| | | AP673L | 44.1 | 36.0 | 122.5e |
| | | G2 | 47.0 | 38.2 | 124.4cd |
| ANOVA | Light spectrum | * | *** | *** | |
| | Specie | *** | * | *** | |
| | Light x Specie | ns | ns | * | |

¹ For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and n.s., non-significant ($p > 0.05$). Mean ($n = 8$).

Table 7Non-structural carbohydrate concentrations (mg g⁻¹ DW) in lamb's lettuce and rocket plants under the different LED light spectra.

| Factor | Level | Sucrose | Glucose | Fructose | Myo-inositol | Total carbohydrates | |
|---------------------|-----------------|----------------|---------|----------|--------------|---------------------|---------|
| Light spectrum | NS-12 (control) | 7.19b | 3.64b | 1.72b | 0.59b | 13.24b | |
| | AP67 | 8.18b | 4.18b | 1.86ab | 0.43c | 14.8b | |
| | AP673L | 8.26b | 3.83b | 1.28c | 0.41c | 13.85b | |
| | G2 | 10.17a | 5.65a | 2.13a | 0.77a | 18.53a | |
| Specie | Lamb's lettuce | 16.12a | 2.66b | 1.95a | 0.51b | 21.24a | |
| | Rocket | 2.7b | 5.57a | 1.59b | 0.60a | 10.5b | |
| Interaction (L x S) | Lamb's lettuce | NS-12 | 13.18c | 2.3d | 1.51cd | 0.54cd | 17.53c |
| | | AP67 | 15.58bc | 2.67d | 2.31ab | 0.46de | 21.06b |
| | | AP673L | 16.67ab | 2.11d | 1.32d | 0.32e | 20.42bc |
| | | G2 | 19.04a | 3.54cd | 2.66a | 0.71ab | 25.94a |
| | Rocket | NS-12 | 2.71d | 4.64bc | 1.87bc | 0.65bc | 10.02de |
| | | AP67 | 2.63d | 5.3b | 1.52cd | 0.41de | 10.11de |
| | | AP673L | 1.95d | 5.13b | 1.24d | 0.49d | 8.92e |
| | | G2 | 3.52d | 7.23a | 1.74cd | 0.83a | 12.97d |
| | ANOVA | Light spectrum | *** | *** | *** | ** | *** |
| | | Specie | *** | *** | *** | *** | *** |
| Light x Specie | | *** | * | *** | ** | *** | |

For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and n.s., not significant ($p > 0.05$). Mean ($n = 8$).

observed in lamb's lettuce plants as the Red:Blue ratio increased. In particular, sucrose increased significantly by 18.7%, 26.5%, and 44.6% under AP67, AP673L, and G2, respectively, as compared to the control (NS-12). In rocket, the sucrose concentration was significantly lower than lamb's lettuce. Furthermore, no treatment increased the glucose concentration with respect to the control. The glucose concentration significantly increased in rocket plants under AP67 as compared to control (NS-12), by 30.9%, as well as other treatments. On the contrary, no effect of the light spectrum on the glucose concentration of lamb's lettuce was observed. The fructose concentration significantly increased in lamb's lettuce under AP67 and G2 as compared with NS-12 by 52.9% and 76.1%, respectively. Meanwhile, both species showed increases in myo-inositol concentration under the light spectrum treatment with a higher red component (G2) with respect to NS12. Specifically, the concentration of myo-inositol in rocket plants in the G2 treatment increased by 27.7% as compared to the control. Finally, in both species, total carbohydrates increased in the treatment with the higher Red:Blue ratio as compared to the control, although this increase was only significant for lamb's lettuce.

The concentration of amino acids is shown in Table 8. Seven amino acids in common were identified for both species under study. Of these,

the contents of glutamate and glutamine in lamb's lettuce and rocket leaves were relatively high, with both accounting for more than 63% of the total free amino acids identified. On the other hand, amino acid concentration was affected differently by the different light spectrum treatments. Particularly, alanine and glutamine had a significant interaction between species and light spectrum. In contrast, Isoleucine and valine were independently affected by light spectrum and specie, while glutamate, aspartate, and asparagine were differentially significant only between species.

Glutamate concentration increased in lamb's lettuce plants as compared to rocket plants under all light spectrum treatments. With respect to glutamine, the treatment with the highest Red:Blue ratio (G2) significantly increased the concentration of this amino acid as compared with the control in lamb's lettuce. Although not statistically significant, the G2 treatment also increased the concentration of glutamine as compared to NS12 in the rocket plants. The aspartate concentration was markedly higher in lamb's lettuce plants as compared to rocket plants by 32.3%. On the other hand, the asparagine concentration considerably increased in rocket plants as compared to lamb's lettuce plants. No clear effect of the light spectrum on alanine concentration was observed, although the concentration was significantly higher in rocket plants

Table 8Amino acids concentration (mg g⁻¹ DW) in lamb's lettuce and rocket plants under the different LED light spectra.

| Factor | Level | Glutamate | Glutamine | Aspartate | Asparagine | Alanine | Isoleucine | Valine | Total amino acids | |
|---------------------|-----------------|----------------|-----------|-----------|------------|---------|------------|--------|-------------------|-------|
| Light spectrum | NS-12 (control) | 5.23 | 3.93b | 1.59 | 1.18 | 0.69 | 0.07b | 0.12b | 12.74ab | |
| | AP67 | 5.8 | 3.62b | 1.57 | 1.25 | 0.66 | 0.08ab | 0.14ab | 13.13ab | |
| | AP673L | 5.26 | 3.59b | 1.42 | 1.37 | 0.65 | 0.07b | 0.12b | 12.42b | |
| | G2 | 5.88 | 4.57a | 1.61 | 1.39 | 0.67 | 0.09a | 0.15a | 14.27a | |
| Specie | Lamb's lettuce | 8.38a | 4.18a | 1.76a | 0.45b | 0.62b | 0.06b | 0.06b | 15.52a | |
| | Rocket | 3.42b | 3.67b | 1.33b | 1.94a | 0.72a | 0.09a | 0.19a | 11.36b | |
| Interaction (L x S) | Lamb's lettuce | NS-12 | 7.88 | 4.36ab | 1.83 | 0.38 | 0.58b | 0.05 | 0.06 | 15.15 |
| | | AP67 | 8.61 | 3.43bc | 1.75 | 0.49 | 0.67ab | 0.06 | 0.07 | 15.08 |
| | | AP673L | 7.98 | 3.91bc | 1.64 | 0.36 | 0.61b | 0.06 | 0.05 | 14.62 |
| | | G2 | 9.03 | 5.03a | 1.84 | 0.57 | 0.61b | 0.07 | 0.08 | 17.22 |
| | Rocket | NS-12 | 3.24 | 3.50bc | 1.34 | 1.78 | 0.81a | 0.09 | 0.17 | 10.93 |
| | | AP67 | 3.68 | 3.80bc | 1.40 | 1.83 | 0.66ab | 0.09 | 0.20 | 11.67 |
| | | AP673L | 3.23 | 3.27c | 1.19 | 2.13 | 0.70ab | 0.09 | 0.17 | 10.77 |
| | | G2 | 3.51 | 4.12ab | 1.38 | 2.01 | 0.73ab | 0.11 | 0.21 | 12.06 |
| | ANOVA | Light spectrum | ns | * | ns | ns | ns | ** | ** | * |
| | | Specie | *** | * | *** | *** | *** | *** | *** | *** |
| Light x Specie | | ns | * | ns | ns | * | ns | ns | ns | |

For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and n.s., non-significant ($p > 0.05$). Mean ($n = 8$).

than lamb's lettuce plants. The interaction of light spectrum x specie was not significant to the concentration of isoleucine; however, the isoleucine concentration was highest in rocket plants. Finally, valine concentration was higher in the rocket plants versus lamb's lettuce plants under all light spectrum treatments, with the lowest concentration observed in the lamb's lettuce plants under NS-12 and AP673L. When analyzing the sum of amino acids (total amino acids), the effect of the light spectrum and species factors was significant independently. Total amino acids significantly increased under G2 as compared to AP673L, by 15.3%. On the other hand, lamb's lettuce had a higher concentration of total amino acids than rocket by 36.7%.

The concentration of seven organic acids in lamb's lettuce and rocket plants were determined (Table 9). The interaction between light spectrum and species was significant for all the acids studied, except for the major ones (malate and citrate). The G2 light spectrum significantly increased the concentration of malate as compared to NS12, and lamb's lettuce had a higher concentration of this acid than rocket. No significant differences were observed in the concentration of malate or citrate (major acids) in lamb's lettuce plants. The citrate concentration was only modified according to the species cultivated, being significantly higher in lamb's lettuce plants. The maximum lactate concentration was obtained in lamb's lettuce under the NS-12 light spectrum treatment, although this increase was not statistically significant as compared to the rest of treatments. No differences in lactate concentration were observed in rocket plants either. Succinate concentration increased in lamb's lettuce plants under G2 and AP67 as compared to the other two light spectrum treatments. Furthermore, the significant reduction of succinate concentrations in the rocket plants under the treatments with the lowest far-red light fraction (AP673L and G2) is worth noting. Rocket and lamb's lettuce plants under light spectrum treatments with the highest Red:Blue ratio (AP673L and G2) had the highest acetate concentration, being significantly higher than the control treatment in both species. Formate concentration was higher under control (NS12) in lamb's lettuce plants and no significant differences were observed in the concentration of this acid in the rocket plants. Fumarate concentration was significantly affected by light spectrum, significantly increasing in the G2 treatment with respect to the NS12 one. In addition, lamb's lettuce contained a higher concentration of this acid than rocket plants. Finally, lamb's lettuce contained a higher concentration of organic acids than rocket plants. Lastly, a clear effect of the G2 light spectrum on the increase of total organic acids was observed.

4. Discussion

Light is an essential environmental factor that modulates the growth, physiology, anatomy, and morphology of plants (Lin et al., 2013). Light is sensed by distinct photoreceptors (Casal et al., 2014), for example red/far-red light is perceived by phytochromes (Sánchez-Lamas et al., 2016), whereas blue light is sensed by cryptochromes (Liu et al., 2011). However, there may be cross-talk and synergy between the red and blue photoreceptor signaling pathways, which are independent in some cases but interactive in others (Chen et al., 2019). Our research showed a positive interaction between specie and spectrum on plant growth. Rocket plants showed high growth under G2 spectrum, increasing a 67% the fresh weight compared to NS-12 light spectrum. On the other hand, lamb's lettuce showed a lower growth (47%) when both spectra were compared. Therefore, the rocket has a better growth response to G2 spectrum than lamb's lettuce. Fresh weight and dry matter content increased as the red-light fraction increased in both species, being highest in rocket plants under G2 spectrum, in which the Red:Blue ratio was highest (table 3). In terms of yield, after G2 light, the best shoot growth for both species was under AP67 light spectrum. That is, the two light spectra with the highest percentage of far-red (AP67 and G2) produced the highest amount of fresh weight in both species. Probably far-red could increase the photosynthetic efficiency by the increasing the quantum yield of photosystem II (Table 4), attenuating the non-photochemical quenching of fluorescence and reducing the dissipation as heat (Zhen and Van-Lersel, 2017). In this sense, Li et al. (2021) observed the increase of fresh weight in two lettuce cultivars in plant factory supplemented with far-red light. The treatment NS12 (the only one with ultraviolet light 1%) showed the lowest fresh weight values compared to the other treatments for both species. Therefore, no effect of ultraviolet light on fresh weight was observed for both species. Although in other plant species, it has been observed that supplemental UV-light can enhance biomass (Lee et al., 2019). In our study, UV-light had no effect on growth, probably because the NS-12 light spectrum has the lowest R: B ratio of all light spectrums and the percentage of UV-light added (1%) is not enough to have effects on plant growth.

Regarding the chlorophyll fluorescence parameters, under G2, it was observed that Fv'/Fm' had a value of 0.78, which indicated a high PSII maximum efficiency. Similar results were obtained by Wang et al. (2016), whose lettuce plants under monochromatic red light had an Fv'/Fm' value of 0.77. Also, the effect of the specie on the fluorescence parameter is significant, with rocket plants obtaining higher qP and $\Phi PSII$ values than lamb's lettuce plants (Table 4). Photochemical

Table 9
Organic acids concentration ($mg\ g^{-1}\ DW$) in lamb's lettuce and rocket plants under the different LED light spectra.

| Factor | Level | Malate | Citrate | Lactate | Succinate | Acetate | Formate | Fumarate | Total Organic acids | |
|---------------------|--------------------------|----------------|---------|---------|-----------|---------|---------|----------|---------------------|-------|
| Light spectrum | NS-12 (control) | 8.85b | 5.24 | 0.25 | 0.08c | 0.06 | 0.03a | 0.06 ab | 14.58b | |
| | AP67 | 9.22b | 4.98 | 0.24 | 0.17a | 0.06 | 0.03b | 0.06 ab | 14.74b | |
| | AP673L | 8.8b | 5.22 | 0.25 | 0.08c | 0.07 | 0.03ab | 0.05 b | 14.51b | |
| | G2 | 10.74a | 5.55 | 0.25 | 0.14b | 0.07 | 0.03b | 0.07 a | 16.84a | |
| Specie | Lamb's lettuce | 16.19 | 8.32a | 0.26a | 0.17 a | 0.1a | 0.05a | 0.10a | 25.19a | |
| | Rocket | 4.31 | 2.95b | 0.24b | 0.08 b | 0.04b | 0.01b | 0.03b | 7.65b | |
| Interaction (L x S) | Lamb's lettuce | NS-12 | 15 | 8.45a | 0.29a | 0.14b | 0.09 c | 0.06a | 0.10a | 24.14 |
| | | AP67 | 16.25 | 7.91a | 0.23ab | 0.19a | 0.10 b | 0.05ab | 0.11a | 24.83 |
| | | AP673L | 15.33 | 8.23a | 0.28ab | 0.15b | 0.10 b | 0.05ab | 0.09a | 24.24 |
| | | G2 | 18.16 | 8.67a | 0.23ab | 0.19a | 0.11 a | 0.04b | 0.12a | 27.53 |
| | Rocket | NS-12 | 4.24 | 2.84b | 0.22b | 0.03d | 0.03 e | 0.01c | 0.03b | 7.41 |
| | | AP67 | 3.94 | 2.78b | 0.24ab | 0.15b | 0.03 e | 0.01c | 0.03b | 7.18 |
| | | AP673L | 3.9 | 2.97b | 0.22b | 0.03d | 0.05 d | 0.01c | 0.03b | 7.21 |
| | | G2 | 5.17 | 3.21b | 0.26ab | 0.11c | 0.05 d | 0.01c | 0.03b | 8.82 |
| | | Light spectrum | *** | ns | ns | *** | ns | *** | * | ** |
| | | Species | *** | *** | ** | *** | *** | *** | *** | *** |
| ANOVA | Light spectrum x Species | ns | ns | *** | *** | *** | *** | ns | ns | |

¹For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and n.s., non-significant ($p > 0.05$). Mean ($n = 8$).

quenching of PSII (qP) indicates the proportion of open reaction centers in PSII (Jiménez-Suancha et al., 2015; Murchie and Lawson, 2013) meanwhile, Φ PSII is the photochemical efficiency of PSII, and can be used to estimate the rate of electron transport through PSII (Wang et al., 2016; Lichtenthaler et al., 2005). Therefore, G2 in rocket plants promoted a higher operating efficiency of PSII, which could induce the optimal activity of photosystems and activate electron transport between photosystem II and photosystem I.

The content of photosynthetic pigments is one of the important indexes that show the capability of photosynthesis. Red light promoted the growth of leaves and restrained the synthesis of chlorophyll, and on the contrary, blue light enhanced the synthesis of chlorophyll and carotenoids (Senger, 1982), which accumulated when the blue fraction was present in the light spectrum (Długosz-Grochowska et al., 2017; Ying et al., 2020). Our results with both species were consistent with the above. The treatment with the lowest Red:Blue ratio (NS12) increased the contents of chlorophylls and carotenoids, which increased the amount of light energy absorbed by plant leaves, resulting in higher rates of photosynthesis. Moreover, it is well known that 5-aminolevulinic acid (5-ALA) is the precursor of chlorophyll biosynthesis in plants. Therefore, this action of blue light could favor chlorophyll accumulation under the NS-12 treatment. On the other hand, the low chlorophyll concentration under the light spectrum with the highest Red:Blue ratio (G2) could be due to the reduced synthesis of glutamate-1-semialdehyde, and the consequent reduction in 5-ALA observed by Sood et al. (2005) under red light. However, other studies observed the highest chlorophyll concentration under light with the highest red fraction in rocket plants (Elmardy et al., 2021). However, the lettuce seedling's chlorophyll concentration was affected by the interaction between the light spectrum and the cultivar (Hernández-Adasme et al., 2022). The diverse results found in the literature seem to indicate that the chlorophyll concentration is dependent on species. According to Yue et al. (2021), different light quality conditions changed the chlorophyll a:b ratio (Chl a:b). Our results (Table 5) showed that the interaction between light and species in the Chl a:b ratio was significant. Lamb's lettuce plants under light with the highest Red:Blue ratio (G2) showed the lowest Chl a:b, which was due to less Chl a and more Chl b. A similar result was observed by Długosz-Grochowska et al. (2017) in two varieties of lamb's lettuce under the higher Red:Blue ratio light. The above indicated that individual PSII complexes developed bigger light harvest complexes II per reaction center, suggesting that lamb's lettuce plants under G2 had a lower light-harvesting efficiency.

Carotenoids are a group of pigments with colors ranging from yellow to red. They are related to light collection and can reduce the damage to plants caused by excessive light (Yue et al., 2021). The light with the highest Red:Blue ratio (G2) decreased carotenoid concentration, which could be due to the red light causing a decreased induction of various genes related to carotenoid synthesis; in contrast, blue light promotes the induction of these compounds (Frede et al., 2019). Also, the reduction in carotenoids may be due to a decrease in specific molecules, as noted by Długosz-Grochowska et al. (2017); these authors observed a significant decrease of xanthophylls, lutein, and β -carotene concentrations under lights with a high Red:Blue ratio versus lights with a low Red:Blue ratio in two lamb's lettuce cultivars. On the other hand, in rocket plants, Elmardy et al. (2021) observed a decline in the concentration of carotenoids under light with a combination of red, green, and blue ratios (R:G:B) = 5:2:3, as compared to R:G:B = 7:0:3 and 3:0:7 ratios independently of intensity or photoperiod. In other species of leafy vegetables such as lettuce, Hernández-Adasme et al. (2022) observed a decrease in carotenoid concentration under red light, although the effect of the light spectrum was dependent on the cultivar. Thus, the influence of specific light spectra on carotenoids seems to be species-dependent.

Color is an essential factor in consumer vegetable selection (Bantis, 2021), as it is often used as an indicator of food quality/defects (Cömert et al., 2019). This study indicated that the control treatment (NS-12)

with the lowest Red:Blue ratio promoted leaves that were greener than other light treatments due to the highest hue value (Table 6). At the same time, color has a relationship with plant pigments; according to Yue et al. (2021), chlorophyll and carotenoids are components of plant coloring. Therefore, the highest hue could be due to the greater chlorophyll concentration observed under control conditions (NS-12). Similarly, Bantis (2021) observed a higher hue in four of seven microgreens under light with a lower Red:Blue ratio than for a higher Red:Blue ratios.

Sucrose, glucose, and fructose constitute the majority of the soluble sugars in rocket and lamb's lettuce (Table 7). Moreover, the type and concentration of carbohydrates have an impact on the sensorial characteristics of vegetables, thus determining the quality of the vegetables (Bell et al., 2017). As shown in Table 7, the light spectrum treatments could influence the composition of sugars in rocket and lamb's lettuce. An interesting interaction was observed on sugar content. Lamb's lettuce sucrose under G2 light spectrum, increase 44% compared to NS-12. However, in rocket plants only was of 29%. Regarding to glucose, both species obtained similar increases under G2 compared to NS-12 light spectrum (around 54%). The response of fructose concentration was different between species, a significantly increased was observed in lamb's lettuce plants (76%) under G2 light spectrum compared to NS-12 spectrum. On the contrary, the fructose concentration in rocket plants under G2 light spectrum decreased 7% compared to NS-12 light spectrum. These sugars are important carbohydrate with influences of the sensory taste of vegetables, so an increase on fructose could further improve the nutritional benefits which is most likely to be of value to consumers. Therefore, depending the specie and the objective of production (yield or quality), it's a key point to choose the correct spectrum of light. Although there was a significant interaction in light x species, the results of the effects of light quality showed that the treatments with a higher percentage of far-red light (AP67 and G2) obtained higher concentrations of fructose, glucose, and sucrose. This is perhaps because far-red light increases leaf expansion, canopy size, and fresh weight (Table 3), which in turn causes increases in the cumulative incident light and biomass (Legendre and Lersel, 2021), resulting in a higher synthesis of carbohydrates in plants.

Amino acids (AAs) are involved in primary and secondary metabolism, and participate in a wide range of cellular enzymatic reactions that have an influence on diverse phenological and physiological processes such as vegetative growth, fruit ripening, signaling, and activation of defense systems against different stresses and osmotic adjustment, and also act as a reserve source of nitrogen (Teixeira et al., 2017). The amino acids concentration showed that quality light changed the aminogram of the rocket and lamb's lettuce plants. The amino acids that were found in the highest concentrations in both species were glutamate, glutamine, and aspartate. In addition, these amino acids play key roles in physiological and metabolic processes in plants, as they intervene in the assimilation of nitrogen, and are also involved in the synthesis of the rest of the amino acids (Alfosea-Simón et al., 2022). It is known that amino acids are a key feature of the umami taste (one of the core five tastes) (Chaudahari et al., 2009). Moreover, glutamate is commonly produced through two pathways, both of which result in the overall conversion of 2-oxoglutarate to glutamate. One route uses ammonium as the nitrogen donor via glutamate dehydrogenase (GDH), and another uses glutamine as the nitrogen donor catalyzed by glutamate synthase (GltS) (Walker and van der Donk, 2016). According to Eprintsev et al. (2022), irradiation with far-red light increased GDH activity, while GltS activity was modulated by a phytochrome via a reversible red/far-red reaction (Yoneyama and Siuzuki, 2020; Siuzuki et al., 2001). Therefore, the higher glutamate concentration in both species could be due to the stimulus provided by the red:far red ratio on enzymatic activity. Our results showed that the light spectrum has no effect on the two amino acids (glutamate and aspartate) that play a key role in the flavor of different plants and foods. In addition, our results showed that the light spectrum affects the amino acid profile, depending

on the species. Overall, the highest Red:Blue ratio increase the concentration of single and total amino acids. Thus, the utilization of a higher red fraction in the light during plant growth could increase their quality.

The results in the current work demonstrated the positive effect of far-red light on amino acid accumulation (Table 8). The ammonium produced from the reduction of nitrate in plants is assimilated rapidly into organic nitrogen through the GS/GOGAT pathway, which is the most active ammonium assimilation pathway in photosynthetic plants. Glutamine is synthesized through the action of GOGAT to complete the assimilation of ammonium. In this process, light quality plays an important regulatory role in enzyme activity (Ning et al., 2019). Interestingly, it is possible that far-red light may inhibit protein biosynthesis as the precursor of amino acids, thereby resulting in their accumulation.

The metabolomic analysis (H-NMR) also identified the organic acids malate, acetate, citrate, lactate, formate, fumarate, and succinate (Table 9). Among them, citrate, malate, and fumarate acids are important because they are involved in the Krebs cycle (Igamberdiev and Eprintsev, 2016). For the organic acids from the Krebs cycle, the most abundant was malate, whose concentration comprised 64% and 58% of the total acids quantified in lamb's lettuce and rocket, respectively. Our results showed that malate accumulation was dependent on light x species interaction. Generally, malate is the most-accumulated acid and plays many functions in plant cells, one of which is its role as an osmolyte and an anion, which compensates for the positive charge of potassium, being particularly important in the regulation of stomata. Relative to the species studied, much more malate accumulated in lamb's lettuce than in rocket. On the other hand, AP67 followed by G2 light, both with the lowest R:FR ratio, obtained the highest malate concentration. According to Simanavičius and Viršilė (2018), malic acid content was one of the most sensitive to the differences in the light spectrum. In particular, increased blue or red fractions decreased malate concentration in tatsoi. In contrast, Samuolienė et al. (2021) found no significant differences in its content under blue and/or red LED in lettuce. Kasperbauer et al. (2001) found that far-red light increased organic acids in strawberries. The relationship between red light and far-red light regulates numerous processes (Demotes-Mainard et al., 2016). In lamb's lettuce and rocket, the accumulation of malate could result from the enhancement of the activity of NAD-malate dehydrogenase, which is under the control of red and far-red light (Thum et al., 2004) and could account for the increase in its concentration under low red:far-red ratio in AP67 and G2 lamps. Moreover, a significant interaction between light spectrum and species was observed in succinate content. Both species increased the succinate content under G2 compared to NS-12 light spectrum. Rocket plants increased by more than 200% whereas lamb's lettuce only increased a 35%. In addition, treatments with the highest far-red proportion (AP67 and G2) showed the highest concentration of succinate. It has been demonstrated that succinate content is regulated via phytochrome A, which is related to far-red light (Popov et al., 2010). On the other hand, the literature results confirm that the effect of the light spectrum on organic acids depends on the species, and is a key factor in enhancing the quality of leafy vegetables.

5. Conclusion

In this work, two leafy vegetables (rocket and lamb's lettuce) grown under four light spectrum treatments were analyzed to characterize their agronomic and metabolic profiles to discover how the light spectrum affected the cultivation of these varieties when grown under artificial light in a vertical and hydroponic cultivation system. The treatments with the highest Red:Blue ratio (G2) produced the highest fresh weight in lamb's lettuce and rocket. Color is an important quality index, and should be preserved until the vegetable is sold. The green color of leafy vegetables depends on many factors (among them the spectrum of light), and when it is lost, browning and chlorophyll and carotenoids

degradation take place. The treatments with the highest far-red fraction (AP67 and G2) significantly improved the brightness and chroma of the species studied. The concentration of metabolites analyzed largely depended on the species studied, although it is true that the light spectrum significantly influenced the concentration of non-structural carbohydrates and organic acids, where the light spectrum with highest Red:Blue ratio (G2) generally increased their concentration.

Certainly, additional studies may be needed to evaluate the effect of these light spectra (NS12, AP67, AP573L and G2) on the quality and metabolomic profiles (carbohydrates, organic acids, amino acids) of rocket and lamb's lettuce, also in relation with the determination of the optimal light spectrum of LED management, to identify the best cultivation conditions in accordance with the production objectives, whether yield or quality. Moreover, this study provided interesting preliminary data on the use of different commercial light spectra for the production of rocket and lamb's lettuce, a species that has been little studied to date for this type of production.

CRediT authorship contribution statement

A. Frutos-Totosa: Conceptualization, Investigation, Formal analysis. **C. Hernández-Adasme:** Conceptualization, Investigation, Formal analysis, Writing – original draft. **V. Martínez:** Conceptualization, Writing – review & editing, Supervision. **T. Mestre:** Conceptualization, Writing – original draft. **H.M. Díaz-Mula:** Formal analysis, Writing – original draft. **M.A. Botella:** Writing – review & editing, Supervision. **P. Flores:** Writing – review & editing, Supervision. **A. Martínez-Moreno:** Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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