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# Effects of different types of artificial light on the phytochemicals of *Lactuca sativa L. Variety Great Lakes 118* cultivated under aeroponic system

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ABSTRACT

Lettuce is extensively researched in artificial lighting systems due to its efficient growth and phytochemical composition influenced by different spectra photon flux. This research focuses on the *Great Lakes 118* variety and addresses the impact of white-blue (WB), white-red-far-red (WRI), and blue-red-far-red (BRI) light treatments in aeroponic systems. Digital foliar area, transmittance, and fluorescence measurements were completed with lettuce leaf hydromethanolic extract characterization using infrared and gas chromatography-mass spectroscopy (GC-MS). Results showed significant effects on physiological parameters of nitrogen balance index (NBI), chlorophyll (CHL), and anthocyanin (ANTH) contents. WB led to 63.3% and 21.2% higher NBI and CHL than BRI treatment, while WRI showed similar percentages concerning BRI. WB and WRI showed 17.9% and 38.8% upper ANTH contents than BRI. On the other hand, flavonoids (FLAV) did not indicate significant treatment-dependent. BRI exhibited several positive correlations among physiological parameters, suggesting its contribution to improved plant development. Infrared analysis revealed similarities in functional groups between treatments, although WB presented a more significant peak in their infrared spectra. GC-MS evidenced differences in the phytochemical profiles, with 2-imidazolidinethione, guanazine, and 3-ethyl-2,2-dimethyl-oxazolidine being the most abundant compounds in WRI, BRI, and WB-treated plants, respectively. The reported findings suggest that BRI may be the preferred artificial irradiation option.

## 1. Introduction

Agriculture is a fundamental practice for human survival, principally for producing food supplies, and because it forms the backbone of economies, shapes cultures, and influences human societies (Koohafkan & Altieri, 2011). Agriculture employs many techniques tailored to diverse landscapes, climates, and cultural practices. Adopting mechanization and technology practices has played a pivotal role in enhancing agricultural productivity. However, the intensive and constant nature of these practices has caused detrimental effects on natural resources due to contamination, overexploitation, and excessive use (Rohila et al., 2017).

More efficient and sustainable agricultural practices have become imperative to ensure and balance the satisfaction of food demand, the preservation of the environment, and the mitigation of social impacts (Lampridi et al., 2019). Some practices are precision agriculture, Internet of Things (IoT)-based automation, deep learning, artificial intelligence (AI), and Controlled-Environment Agriculture (CEA) (Fussy & Papenbrock, 2022; Papadopoulou et al., 2023; Shafi et al., 2019).

Through technological integration, aeroponics systems emerge as a global solution method within CEA to achieve the mentioned sustainable agricultural objectives and optimize resource utilization for heightened productivity (Ampim et al., 2022). This method allows the growth of plants with their roots suspended in the air to receive controlled nutrient mist applications (Fussy & Papenbrock, 2022). The electronic devices used to control environmental factors such as temperature, humidity, lighting, quality and quantity of available water, pH, and necessary nutrients in this method, have allowed the reduction of crop cycles, creating more extensive and profitable yields, and continuous production throughout the year, regardless of external weather conditions, ensuring a consistent and predictable supply chain (Fussy & Papenbrock, 2022; Garzón et al., 2023). Despite initial

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concerns about costs-including setup, maintenance, and monitoring expenses-, aeroponics proves cost-effective in the long term, primarily through labor savings and resource optimization (Al-Kodmany, 2018; Fussy & Papenbrock, 2022; Garzón et al., 2023). Maintenance costs for aeroponic systems can vary depending on factors such as system complexity, scale, and energy consumption. Tasks such as replacing pumps, cleaning misters, and calibrating sensors contribute to ongoing expenses; however, these are balanced by the system's high water efficiency and yield potential. Additionally, integrating advanced technology may require skilled maintenance and troubleshooting labor, enhancing precision and consistency in crop production (Garzón et al., 2023). Aeroponic systems also support space-efficient designs that maximize available space and resources (Al-Kodmany, 2018; L. Lozano-Castellanos, Navas-Gracia, & Correa-Guimaraes, 2023).

Artificial lighting, one of the key parameters controlled in this method, has gained considerable attention in recent years due to its effects on crop growth and phytochemical composition. Examples of spectral combinations with different effects on lettuce development include red and blue light, associated with increased chlorophyll quantity, chloroplasts, heightened nitrate accumulation, and enhanced photosynthetic efficiency of leaves (Frąszczak & Kula-Maximenko, 2021; Mohamed et al., 2021). As monochromatic lighting, each spectrum generally augments leaf area, K, Na, and chlorophyll content, and epidermal cell area but tends to diminish Ca and Mg concentrations, root dry mass, the Soil Plant Analysis Development (SPAD) index, stomatal density, and leaf thickness (Frąszczak & Kula-Maximenko, 2021; Matysiak et al., 2021). Specifically, red light (alongside far-red) induces phytochrome transformations and is pivotal for phytochemical syntheses, such as phenolics and oxalate (Alrajhi et al., 2023; Legendre & van Iersel, 2021; Liu & van Iersel, 2022a; Trivellini et al., 2023). Far-red light triggers shade avoidance responses in plants, promoting stem elongation, leaf expansion, and canopy growth (Lee et al., 2016; Liu & van Iersel, 2022b). This light wavelength enhances light interception, indirectly supporting photosynthesis and biomass accumulation (Van Brenk et al., 2024). Far-red also complements shorter wavelengths to balance photosystems, optimizing photosynthetic efficiency (Carotti et al., 2024). Blue light is crucial for chloroplast development and photomorphogenesis, chlorophyll and anthocyanin regulation (Alrajhi et al., 2023), and the synthesis of bioactive compounds with antimicrobial properties (Ouzounis et al., 2015; Trivellini et al., 2023). Conversely, full-spectrum white light has proven to yield higher outputs, including the plant control morphology and an increased leaf shape index value (Frąszczak & Kula-Maximenko, 2021; Gargaro et al., 2023).

Notable examples of aeroponic systems can be found in different parts of the world, where researchers are actively exploring the effects of different spectral light on various crops (Choi et al., 2022; Chowdhury et al., 2023; Eldridge et al., 2020; He et al., 2023; Islam et al., 2021; M. H. Rahman, Field, et al., 2021).

Research in aeroponic systems spans various fields. In the commercial sector, Japan funds projects to establish companies employing Plant Factories with Artificial Lighting (PFAL), like TS Farm Shirakawa. This company utilizes aeroponic systems to cultivate approximately 4500 lettuces (Hayashi, 2016). In the United States, AeroFarms employs Light-Emitting Diodes (LED) arrays and aeroponic systems to cultivate over 400 leafy green vegetables, microgreens, and herbs (Kozai et al., 2020). At the Signify facility in the Nether-lands, crops such as lettuce, baby leaf greens, herbs, microgreens, edible flowers, Brassicaceae, and fruiting vegetables are exclusively grown using LEDs equipped with blue, red, white, and far-red lights. Cultivation methods include nutrient film technique (NFT), deep flow technique (DFT), aeroponics, and drip irrigation (Kozai et al., 2020). These companies operate research centers focused on technology development through automation and expanding plant phytochemistry to grow high-quality crops.

In the pharmaceutical sector, cultivating plants in controlled environments using artificial light is another field of application that maximizes phytochemical composition, content, and the nutritional and postharvest quality of plants (Samuolienė et al., 2017). LEDs offer both quality and quantity of light, enabling the manipulation of light conditions to enhance the nutritional value of vegetables. This manipulation optimizes the content of essential phytoconstituents or medicinal metabolites, such as ascorbic acid, anthocyanins, carotenoids, phenols, and tocopherols (Samuolienė et al., 2017; Zobayed, 2020). This approach ensures year-round harvesting and maximizes biomass production by optimizing nutrient absorption, temperature, and $CO_2$ concentration (Zobayed, 2020).

In the food industry, LEDs are employed to assess their impact on the nutritional content of cold-stored foods and their ripening rate, as well as to prevent fungal infections and inactivate pathogenic bacteria (D'Souza et al., 2017). Moreover, they are under investigation as regulators of genetic expressions associated with the growth and development of plants. Genetic activation induced by LEDs results in enhanced disease resistance and increased accumulation of carotenoids, phenols, and ascorbic acid, significantly improving crops' commercial and nutritional value (Dutta Gupta & Pradhan, 2017).

On the other hand, the challenge associated with artificial light in CEA lies in managing various factors, such as intensity, exposure time, spectra, and the specific needs of the crop and its variety. Any of these elements can significantly affect the accumulation of phytochemicals and the morphological properties of the plant (Ilić et al., 2017; Ntsoane et al., 2016; Sivakumar et al., 2018; Sofo et al., 2016; Zapata-Vahos et al., 2020). Developing experiments that use different spectral light emerges as a fundamental strategy to obtain a more precise approach to managing lighting conditions and achieving the desired results in cultivation.

This research aims to advance the understanding of the influence of artificial lighting on the phytochemical components of lettuce (*Lactuca sativa* L. Var. Great Lakes 118), one of the most studied and cultivated species in CEA. Given the recognized effectiveness of full-spectrum white light and red and blue light on lettuce yield (M. M. Rahman, Field, et al., 2021), this work focuses on evaluating specific spectral combinations involving two and three colors. It should be noted that the aeroponic system has been chosen as the cultivation method to carry out this study.

## 2. Materials and methods

### 2.1. Plant material

Lettuce plants (Var. "Great Lakes 118″) were supplied by FITOPAL (Palencia, Spain) and obtained after 15 days of growth in a peat-perlite mixture (90:10 *v:v*). The residual substrate on the roots was removed before transferring the plants to rock wool blocks of 4.0 x 2.5 × 2.5 cm (Groho Hidroponía, Madrid, Spain) for the aeroponic system.

Each experiment was independently replicated three times, lasting eight weeks per replicate, with eight plants per treatment and replicate. Specimens were pooled based on light treatment to obtain representative samples, which were shade-dried, finely ground with a mill, homogenized, and sieved through a 1 mm mesh.

### 2.2. Growing conditions

The experiments were conducted in the greenhouse at the University of Valladolid, Palencia Campus, Spain, under controlled conditions with an average temperature of 22.2 °C and relative humidity of 58.1% (Climate data logger, Parkside, Germany) using an aeroponic system (Fig. 1) with three isolated production units, each separated by white cellular polycarbonate sheet and a black cover. Each unit housed four rectangular polypropylene growth chambers, with two lettuce plants spaced 20 cm apart per chamber. In the center of each chamber, a CoolNET PRO nebulizer (Regaber, Barcelona, Spain) with dual nozzles provided a 65-μm droplet size under a pressure of 3.0–5.0 bar. Nebulization lasted 5 min with 1-h intervals. The nutrient solution consisted of

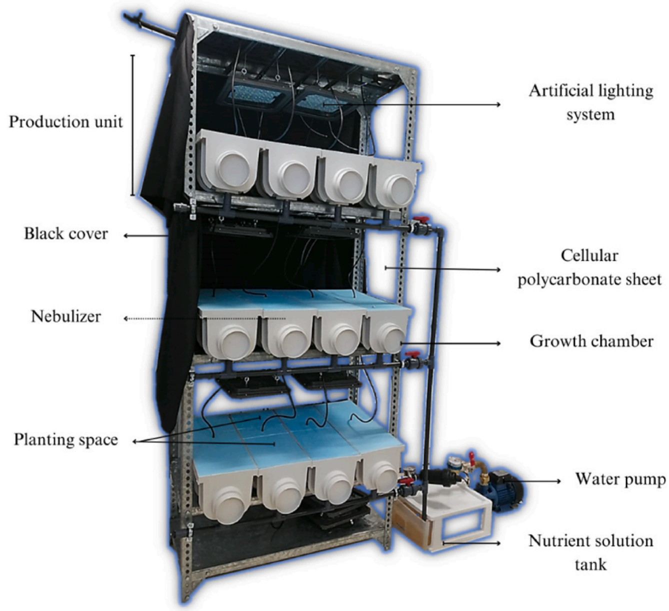


**Fig. 1. The** aeroponic system with artificial illumination.
**Color should be used for all figures in print.

50 mL of biomineral fertilizer Aero Supermix (GroHo Hidroponía, Madrid, Spain) per 25 L of water, recycled through a closed recirculation system.

The artificial lighting system (Boos Technical Lighting Sl, Valladolid, Spain) included two luminaires per production unit, each with four printed circuit board (PCB) plates containing nine red, blue, far-red, and white LEDs. The irradiation treatments were White-Blue (WB), White-Red-Far-red (WRI), and Blue-Red-Far-red (BRI) (Fig. 2), with a Photosynthetic photon flux density (PPFD) of 155 ± 6.2 μmol/s·m$^2$. A 12 h/12 h light/dark cycle was maintained.

The cultivation conditions, including photoperiod and light spectral choices, were selected considering the effects described in the review article by Boros et al. (Boros et al., 2023) on lettuce grown in PFAL. Although photoperiods of 16–18 h are often suggested for optimal growth, a 12-h photoperiod was used in this research based on historical data showing no significant differences as long as the Daily Light Integral (DLI) remains constant (Boros et al., 2023).

Optimal light for lettuce growth typically includes a balanced spectrum with an emphasis on blue (B) (400–500 nm) and red (R) (600–700 nm) wavelengths, which promote photosynthesis and physiological development. Accordingly, one treatment included Far-red (FR, denoted as "I" for this research) in the 700 nm–750 nm range to enhance the synthesis of phytochemical compounds in lettuce leaves. White (W) light, chosen for its high photosynthesis efficiency and cost-effectiveness, was included in two of the treatment combinations: one with only B (WB) and another treatment with R and I (WRI) to separately assess the effects of these specific spectral ranges. Lighting treatments were maintained consistently throughout the experiment.

Irrigation cycles were chosen based on previous studies conducted by the TADRUS research group at the University of Valladolid, which focus on the effects of monochromatic artificial lighting on aeroponically grown lettuce (L. Lozano-Castellanos, Navas-Gracia, &

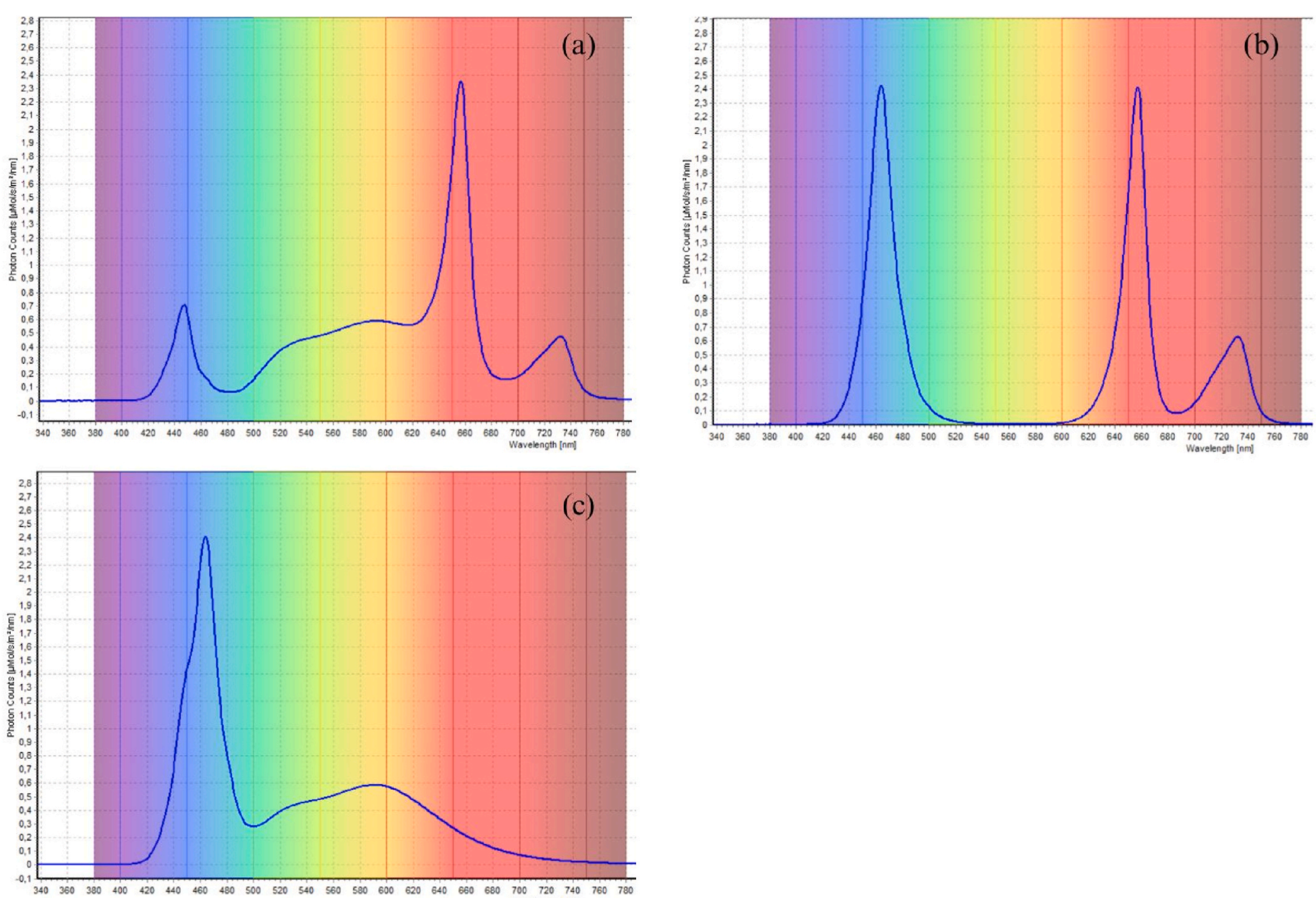


**Fig. 2.** Spectral distribution of light treatments: White-red-far-red (WRI) (a), blue-red-far-red (BRI) (b), and white-blue (WB) (c).

Correa-Guimaraes, 2023).

### 2.3. Extraction procedure

Lettuce powder from plants under WB light (23.9 g) was dispersed in a methanol (HPLC Grade ≥99.8%) procured from Sigma Aldrich Química S.A. (Madrid, Spain), and water solution (1:1 v/v, 50 $H_2O$ + 50 mL of MeOH), heated at 50 °C for 30 min, and sonicated for 5 min in pulse mode with a 1-min breaks every 2.5 min using a UIP1000 hdT ultrasonic probe-type sonicator from Hielscher Ultrasonics (Teltow, Germany). The solution was centrifuged at 9000 rpm (8150×*g*) for 15 min at 4 °C, and the supernatant was filtered through Whatman No. 1 paper. Extract aliquots were lyophilized for FTIR–ATR and GC–MS characterization (5 mg of lyophilized extract dissolved in 1 mL of HPLC-grade MeOH to obtain a 5 mg $mL^{-1}$ solution, which was filtered again). The procedure was applied for lettuce powder from lettuce irradiated with WRI (54.6 g) and BRI (25.6 g) treatments.

### 2.4. Characterization

Light spectra were measured using a StarLine AvaSpec-ULS2048CL spectrometer, EVO Series with CMOS detector (Avantes, Apeldoorn, Netherlands), with an FC-UVIR400-2 optical fiber (400 μm fiber, 2 m length, SMA termination), set to an integration time of 9μs–59s and sampling speed with integrated averaging of 0.38ms for spectral ranges from 360 to 1100 nm.

Leaf area (LA) was measured weekly using the digital image processing program ImageJ (Java) to track growth over the experimental period. Individual photos were taken above the canopy, and this was a non-destructive measurement. Chlorophyll (CHL), flavonols (FLAV), anthocyanins (ANTH), and nitrogen balance index (NBI) measurements were also taken weekly with the Dualex METOS® leaf sensor (Pessl Instruments, Werksweg, Austria), employing a non-destructive method under a transmittance measurement system and fluorescence screening effect of chlorophyll.

Infrared vibrational spectra were collected using a Fourier transform Nicolet iS50 from Thermo Scientific (Waltham, MA, USA) with a diamond attenuated total reflection (ATR) covering 400-4000 $cm^{-1}$, at a spectral resolution of 1 $cm^{-1}$, based on 64 scans.

Hydro-methanolic extracts were analyzed by GC–MS at the Support for Research Services (STI) of the University of Alicante using an Agilent Technologies gas chromatograph model 7890A coupled with a quadrupole mass spectrometer model 5975C. Chromatographic conditions: injection volume = 1 μL; injector temperature = 280 °C, in splitless mode; initial oven temperature = 60 °C for 2 min, followed by a ramp of 10 °C/min to a final temperature of 300 °C for 15 min. The chromatographic column used for compound separation was an Agilent Technologies HP-5MS UI column with a length of 30 m, a diameter of 0.250 mm, and a film thickness of 0.25 μm. Mass spectrometer conditions were as follows: electron impact source temperature = 230 °C; quadrupole temperature = 150 °C; ionization energy = 70 eV. Component identification were identified by comparing mass spectra and retention times to reference compounds and matching with the National Institute of Standards and Technology (NIST11) database.

### 2.5. Statistical analysis

The experimental design was a fully randomized factorial design with three light treatments (WB, WRI, and BRI) and three replicates per treatment. Statistical analyses were conducted using Python, including repeated measures ANOVA and temporal correlations, to evaluate variations in NBI, CHL, ANTH, FLAV, and LA over time. Repeated measurements within subjects provided a robust temporal analysis, capturing the dynamic responses of plants to different light treatments.

Results are expressed as the mean ± standard deviation (SD) of the three replicates. A significance level of $p \leq 0.05$ was used to determine statistically significant differences. $p \leq 0.05$ is considered statistically significant, while $p > 0.05$ indicates non-significant differences.

## 3. Results

### 3.1. Characterization of chlorophyll, polyphenols and leaf area

Upon characterization using the transmittance measurement system and the chlorophyll fluorescence detection for NBI, CHL ANTH and FLAV, and utilizing the ImageJ program for LA analysis, temporal variability across treatments can be visualized in Fig. 3. Following this, the analysis of variance (ANOVA) (Table 1) reveals a statistically significant effect for NBI with a p-value of 0.0061 in the Test for Between-Subject Effects, indicating sensitivity to distinct light conditions. Similarly, CHL exhibits a highly significant influence (p-value: 0.0004), emphasizing its responsiveness to diverse light spectra. In contrast, FLAV and LA reveal p-values of 0.4937 and 0.3119, respectively, suggesting no significant effect in the between-subject context. ANTH presents significant impacts with p-values of 0.0005.

The Test for Within-Subject Effects explores the impact of time on the dependent variables across measures. NBI significantly impacts (p-value: 0.0010), emphasizing its responsiveness to temporal variations. ANTH and LA display p-values of <0.0001. These results suggest nuanced temporal dynamics, where both variables exhibit a more pronounced response over time, while FLAV and CHL exhibit changes that are not statistically significant within subjects.

The discrepancy between the two tests in LA is particularly interesting. While the Test for Between-Subject Effects yielded a p-value of 0.3119, indicating no significant impact of light treatments, the Test for Within-Subject Effects revealed a highly significant p-value of <0.0001, indicating a substantial influence of temporal variations on LA within subjects. This finding suggests that the dynamics occurring within individual subjects contribute significantly to the overall effect, and such nuances might not be apparent when examining data collectively.

For CHL, the smaller p-value within subjects compared to the between-subject analysis suggests a more noticeable response over time. This indicates that changes in CHL are more discernible when considering individual subjects across different time points, as opposed to looking at the aggregated data across all subjects.

Conversely, FLAV presents p-values more prominent than 0.05 in both tests, suggesting no significant changes within or between subjects.

Additionally, the percentage change in each variable for WB and WRI light treatments concerning the baseline treatment BRI over the eight weeks indicates that for NBI, the percentage change for WB ranges from −10.4% to 63.3%, while for WRI, it varies from −14.2% to 34.4%. In CHL, WB changes from −21.2% to 21.2%, and WRI exhibits variations from −52.7% to −1.1%. Analyzing FLAV, WB demonstrates changes from −24.0% to 28.5%, and WRI shows variations from −45.0% to 29.6%. Regarding ANTH, WB changes from −10.8% to 20.1%, and WRI displays variations from 10.8% to 38.8%. Finally, WB demonstrates changes from −36.8% to 21.1% for LA, and WRI exhibits variations from −55.4% to −14.0%. The variables with the most minor percentage change over the eight weeks are ANTH and CHL for WB and WRI treatments.

The correlation for the WB treatment reveals strong patterns in the relationships between physiological variables (Fig. 4). The NBI shows a high positive correlation with LA (0.88) and a substantial negative correlation with FLAV (−0.84) and ANTH (−0.67). The CHL also shows a positive correlation with NBI (0.81) and LA (0.49) but a negative correlation with FLAV (−0.61) and ANTH (−0.93). FLAVs have a positive correlation with ANTH (0.49) but a negative correlation with NBI (−0.84). The ANTH exhibits a negative correlation with CHL (−0.93).

In the WRI treatment group, the correlation shows different patterns (Fig. 5). NBI demonstrates a positive correlation with ANTH (0.49) and a negative correlation with FLAV (−0.98) and CHL (−0.83). CHL has a strong positive correlation with FLAV (0.90) and a negative correlation

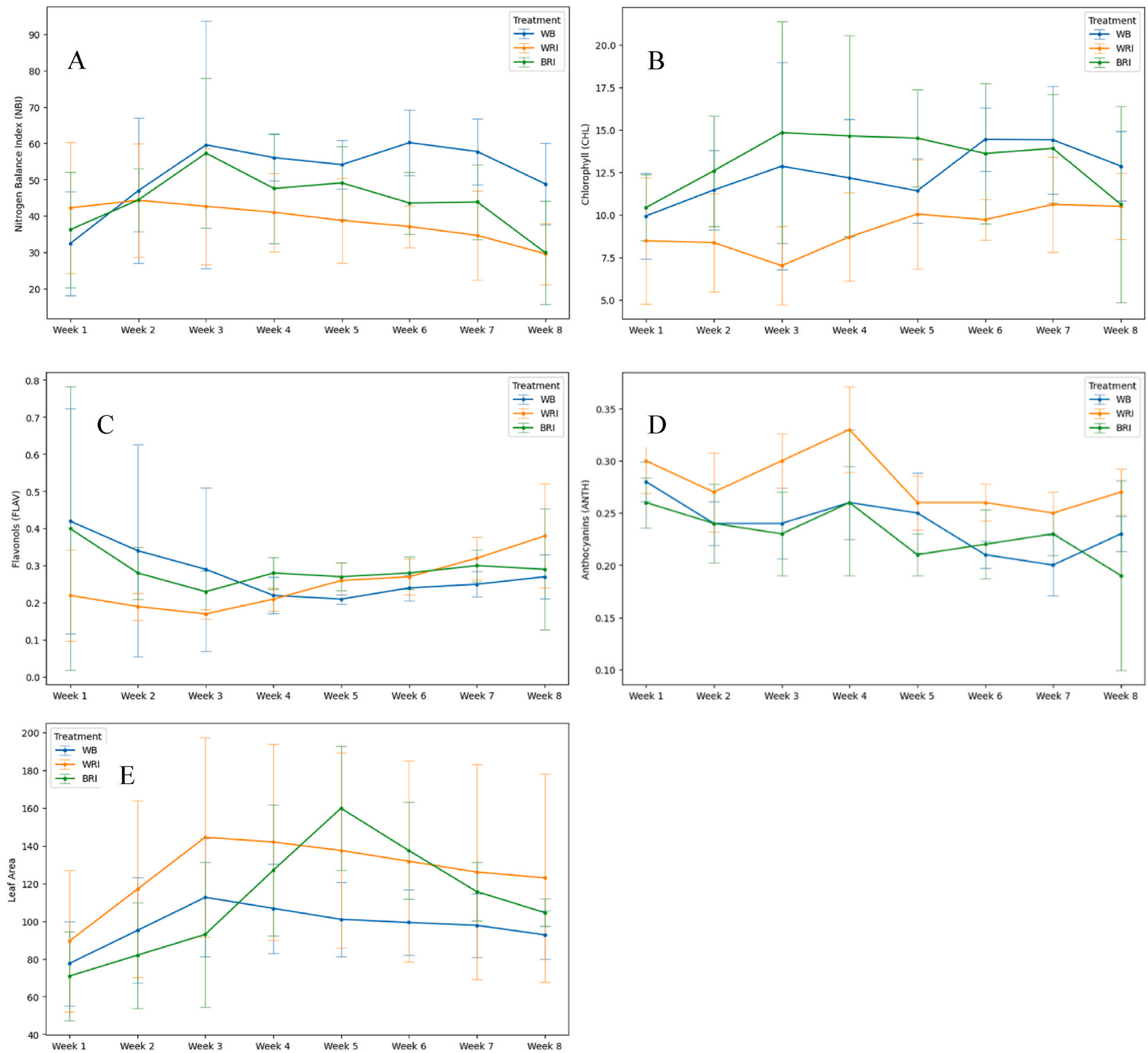


**Fig. 3.** Temporal variability of Nitrogen Balance Index (NBI) (A), Chlorophyll (CHL) (B), Flavonols (FLAV) (C), Anthocyanins (ANTH) (D), and Leaf Area (E) in aeroponic lettuce irradiated for eight weeks under White-Blue (WB), White-Red-Far-red (WRI), and Blue-Red-Far-red (BRI) light treatments.
Note: Vertical lines represent standard deviation.

with NBI (−0.83) and ANTH (−0.66). ANTH shows a negative correlation with CHL (−0.66) and a positive correlation with NBI (0.49). LA has a weak correlation with all the variables.

The correlation for the BRI treatment group (Fig. 6) reveals that NBI has a strong positive correlation with CHL (0.89), a moderate positive correlation with LA (0.26) and ANTH (0.25), and a negative one with FLAV (−0.64). CHL exhibits a weak positive correlation with LA (0.59) and ANTH (0.09) and a negative correlation with FLAV (−0.72). FLAVs show a moderate positive correlation with ANTH (0.42) but a negative correlation with LA (−0.44). ANTH has a negative correlation with LA (−0.40).

### 3.2. Infrared vibrational analysis

The leading bands in the infrared spectra of lettuce extracts irradiated by different light treatments are summarized in Fig. 7 and Table 2. The WB treatment revealed a more significant number of prominent peaks, particularly within the Fingerprint region, compared to the other treatments, WRI being the one with the smallest amount.

Notably, the WRI treatment did not display significant peaks associated with high flavonoid presence or development. However, a marked increase in alcohol and phenol concentrations was observed, particularly in the spectral range from 3300 to 3630 $cm^{-1}$.

The three treatments demonstrated the presence and development of chlorophyll and carotenoids, with WRI and BRI exhibiting the peaks at 1523 $cm^{-1}$ approx. and WB at 2998 $cm^{-1}$. Only BRI revealed the presence of nitro compounds in its leading bands. Furthermore, the WB treatment shared the same number of peaks with WRI at 1100.691 $cm^{-1}$ associated with C-O stretching, alcohols, carboxylic acids, esters, and ethers, and at 3625.095 $cm^{-1}$ with BRI associated with O-H stretch, H

**Table 1**
Repeated measures ANOVA for Nitrogen Balance Index (NBI), Chlorophyll (CHL), Flavonols (FLAV), anthocyanins (ANTH) and Leaf area (LA) in aeroponic lettuce irradiated for eight weeks under White-Blue (WB), White-Red-Far-red (WRI), and Blue-Red-Far-red (BRI) light treatments.

| Variable | Treatment | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 |
|---|---|---|---|---|---|---|---|---|---|
| NBI | WB | 32.4 ± 14.2$^{a}$ | 46.9 ± 19.9$^{a}$ | 59.5 ± 34.0$^{a}$ | 56.0 ± 6.4$^{a}$ | 54.1 ± 6.6$^{a}$ | 60.2 ± 8.9$^{a}$ | 57.7 ± 9.0$^{a}$ | 48.8 ± 11.1$^{a}$ |
| | WRI | 42.2 ± 17.9$^{a}$ | 44.3 ± 15.6$^{a}$ | 42.6 ± 15.9$^{a}$ | 41.0 ± 10.7$^{a}$ | 38.7 ± 11.7$^{ab}$ | 37.0 ± 5.8$^{b}$ | 34.6 ± 12.2$^{ab}$ | 29.6 ± 8.4$^{b}$ |
| | BRI | 36.2 ± 15.9$^{a}$ | 44.4 ± 8.5$^{a}$ | 57.3 ± 20.6$^{a}$ | 47.5 ± 15.0$^{a}$ | 49.1 ± 9.9$^{b}$ | 43.5 ± 8.6$^{b}$ | 43.8 ± 10.2$^{b}$ | 29.9 ± 14.2$^{b}$ |
| | WB/BRI | −10.4% | 5.8% | 4.0% | 17.9% | 10.2% | 38.2% | 31.7% | 63.3% |
| | WRI/BRI | −14.2% | 0.2% | 34.4% | 16.0% | 26.7% | 17.5% | 26.6% | 1.0% |
| | *p*−value between treatments: 0.0061 | | | | | | | | |
| | *p*−value within treatments: 0.0010 | | | | | | | | |
| CHL | WB | 9.9 ± 2.5$^{a}$ | 11.4 ± 2.3$^{ab}$ | 12.8 ± 6.0$^{ab}$ | 12.1 ± 3.4$^{ab}$ | 11.4 ± 1.9$^{ab}$ | 14.4 ± 1.8$^{a}$ | 14.4 ± 3.1$^{a}$ | 12.8 ± 2.0$^{a}$ |
| | WRI | 8.4 ± 3.7$^{a}$ | 8.3 ± 2.9$^{b}$ | 7.0 ± 2.3$^{b}$ | 8.7 ± 2.5$^{b}$ | 10.0 ± 3.2$^{b}$ | 9.7 ± 1.1$^{b}$ | 10.6 ± 2.8$^{a}$ | 10.5 ± 1.9$^{a}$ |
| | BRI | 10.4 ± 1.9$^{a}$ | 12.5 ± 3.2$^{a}$ | 14.8 ± 6.5$^{a}$ | 14.6 ± 5.9$^{a}$ | 14.5 ± 2.8$^{a}$ | 13.6 ± 4.1$^{a}$ | 13.9 ± 3.2$^{a}$ | 10.6 ± 5.7$^{a}$ |
| | WB/BRI | −4.8% | −9.0% | −13.3% | −16.8% | −21.3% | 6.1% | 3.6% | 21.2% |
| | WRI/BRI | −18.7% | −33.6% | −52.7% | −40.6% | −30.8% | −28.6% | −23.7% | −1.1% |
| | *p*−value between treatments: 0.0004 | | | | | | | | |
| | *p*−value within treatments: 0.0554 | | | | | | | | |
| FLAV | WB | 0.4 ± 0.3$^{a}$ | 0.3 ± 0.2$^{a}$ | 0.2 ± 0.2$^{a}$ | 0.2 ± 0.05$^{a}$ | 0.2 ± 0.01$^{a}$ | 0.2 ± 0.03$^{a}$ | 0.2 ± 0.03$^{a}$ | 0.2 ± 0.06$^{a}$ |
| | WRI | 0.2 ± 0.1$^{a}$ | 0.1 ± 0.04$^{a}$ | 0.1 ± 0.01$^{a}$ | 0.2 ± 0.03$^{b}$ | 0.2 ± 0.05$^{b}$ | 0.2 ± 0.05$^{a}$ | 0.3 ± 0.06$^{b}$ | 0.3 ± 0.1$^{a}$ |
| | BRI | 0.4 ± 0.3$^{a}$ | 0.2 ± 0.07$^{a}$ | 0.2 ± 0.06$^{a}$ | 0.2 ± 0.04$^{b}$ | 0.2 ± 0.04$^{a}$ | 0.2 ± 0.04$^{a}$ | 0.3 ± 0.04$^{ab}$ | 0.2 ± 0.1$^{a}$ |
| | WB/BRI | 3.5% | 23.6% | 28.5% | −21.8% | −24.0% | −12.4% | −17.5% | −7.3% |
| | WRI/BRI | −45.0% | −30.8% | −26.6% | −22.3% | −4.7% | −4.1% | 4.7% | 29.6% |
| | *p*−value between treatments: 0.4943 | | | | | | | | |
| | *p*−value within treatments: 0.0569 | | | | | | | | |
| ANTH | WB | 0.2 ± 0.02$^{ab}$ | 0.2 ± 0.02$^{a}$ | 0.2 ± 0.03$^{b}$ | 0.2 ± 0.04$^{a}$ | 0.2 ± 0.04$^{a}$ | 0.2 ± 0.01$^{b}$ | 0.2 ± 0.03$^{ab}$ | 0.2 ± 0.02$^{a}$ |
| | WRI | 0.3 ± 0.03$^{a}$ | 0.2 ± 0.04$^{a}$ | 0.3 ± 0.03$^{a}$ | 0.3 ± 0.04$^{a}$ | 0.2 ± 0.03$^{a}$ | 0.2 ± 0.02$^{a}$ | 0.2 ± 0.02$^{a}$ | 0.2 ± 0.02$^{a}$ |
| | BRI | 0.2 ± 0.02$^{b}$ | 0.2 ± 0.04$^{a}$ | 0.2 ± 0.04$^{b}$ | 0.2 ± 0.07$^{a}$ | 0.2 ± 0.02$^{b}$ | 0.2 ± 0.03$^{b}$ | 0.2 ± 0.02$^{b}$ | 0.1 ± 0.09$^{a}$ |
| | WB/BRI | 7.2% | 1.2% | 5.1% | 0.5% | 20.1% | −5.1% | −10.8% | 17.9% |
| | WRI/BRI | 16.0% | 12.7% | 31.0% | 24.5% | 24.3% | 17.9% | 10.8% | 38.8% |
| | *p*−value between treatments: 0.0005 | | | | | | | | |
| | *p*−value within treatments: <0.0001 | | | | | | | | |
| LA | WB | 77.5 ± 22.3$^{a}$ | 95.0 ± 27.9$^{a}$ | 112.5 ± 31.2$^{a}$ | 106.7 ± 23.6$^{a}$ | 100.9 ± 19.7$^{ab}$ | 99.3 ± 17.3$^{a}$ | 97.7 ± 16.7$^{a}$ | 92.7 ± 12.9$^{a}$ |
| | WRI | 89.5 ± 37.6$^{a}$ | 116.9 ± 46.7$^{a}$ | 144.4 ± 52.8$^{a}$ | 141.9 ± 52.0$^{a}$ | 137.4 ± 51.6$^{a}$ | 131.7 ± 53.3$^{a}$ | 125.9 ± 57.0$^{a}$ | 122.9 ± 55.2$^{a}$ |
| | BRI | 70.8 ± 23.6$^{a}$ | 81.9 ± 28.0$^{a}$ | 92.9 ± 38.4$^{a}$ | 126.8 ± 34.7$^{a}$ | 159.8 ± 32.8$^{b}$ | 137.3 ± 25.6$^{a}$ | 115.6 ± 15.5$^{a}$ | 104.5 ± 7.2$^{a}$ |
| | WB/BRI | 9.5% | 16.0% | 21.1% | −15.8% | −36.8% | −27.7% | −15.4% | −11.3% |
| | WRI/BRI | 26.3% | 42.6% | 55.4% | 11.8% | −14.0% | −4.1% | 9.0% | 17.6% |
| | *p*−value between treatments: 0.3119 | | | | | | | | |
| | *p*−value within treatments: <0.0001 | | | | | | | | |

Means and standard deviations followed by a common letter among treatments within each week, are not significantly different by Tukey's test at the 5% significance level. WB/BRI represents the percentage change of WB treatment from BRI treatment. WRI/BRI represents the percentage change of WRI treatment from BRI treatment.

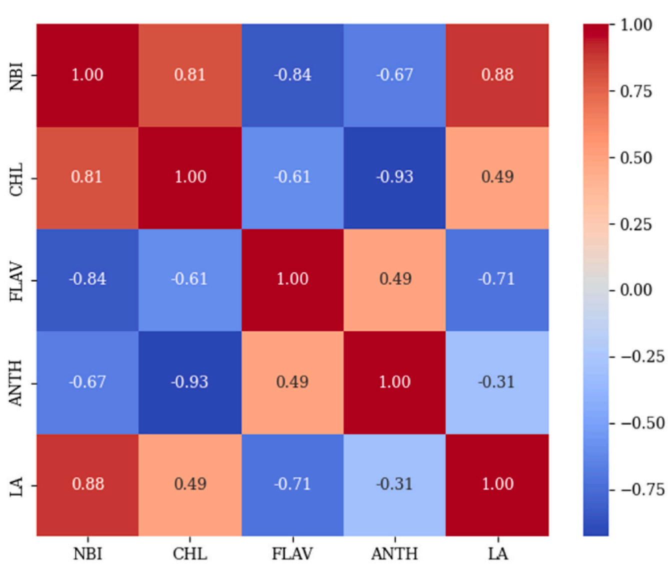


**Fig. 4.** Correlation Matrix for White-Blue (WB) treatment between Nitrogen Balance Index (NBI), Chlorophyll (CHL), Flavonols (FLAV), anthocyanins (ANTH) and Leaf area (LA) variables.

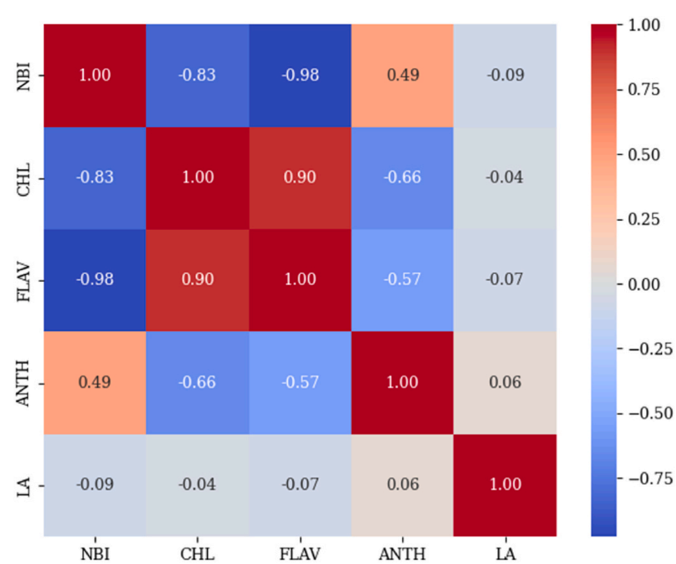


**Fig. 5.** Correlation Matrix for White-Red-Far-red (WRI) treatment between Nitrogen Balance Index (NBI), Chlorophyll (CHL), Flavonols (FLAV), anthocyanins (ANTH) and Leaf area (LA) variables.

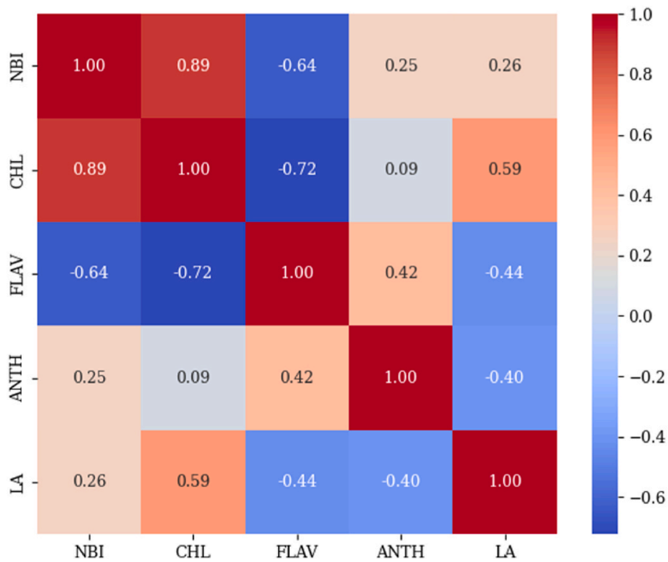


**Fig. 6.** Correlation Matrix for Blue-Red-Far-red (BRI) treatment between Nitrogen Balance Index (NBI), Chlorophyll (CHL), Flavonols (FLAV), anthocyanins (ANTH) and Leaf area (LA) variables.

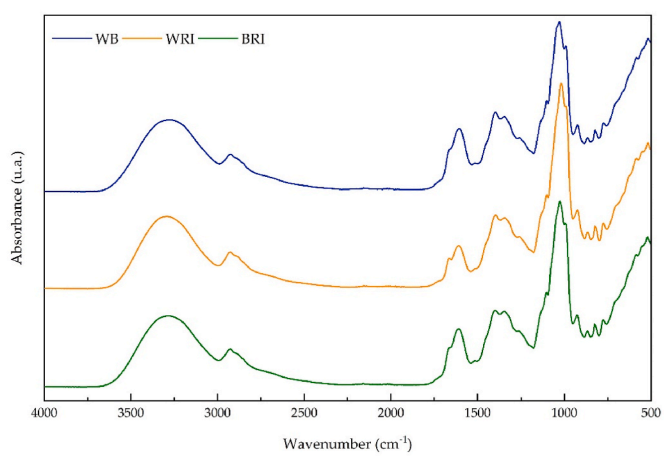


**Fig. 7.** Detail of the ATR-FTIR spectra of freeze-dried lettuce extracts in the range 400-4000 $cm^{-1}$.

bonded, alcohols and phenols.

### 3.3. GC–MS chromatograms

The analysis of GC-MS chromatograms for the three extracts studied, along with the ranking of identified species by percentages and the selection of the ten most abundant, is presented in Table 3. The three extracts share six of the selected species, while two of them, WRI and BRI, overlap in eight species, including 1,3-dioxyacetone dimer, D-glucitol and 2,5-dimethylhydroxy-3(2H)-furanone.

When comparing the GC-MS profiles of the extracts, the presence of a specific component in each of them is noted among the most significant species in percentage terms: 2-imidazolidinethione (3.5%) for WRI, guanazine (3.1%) for BRI and 3-ethyl-2,2-dimethyl-oxazolidine (3.3%) for WB.

**Table 2**
Main bands of the infrared spectra of lyophilized extracts of lettuce subjected to different irradiation conditions. Wave numbers are expressed in $cm^{-1}$.

| WB | WRI | BRI | Assignment |
|---|---|---|---|
| | 3629.434 | | O-H stretch, H bonded, Alcohols, phenols |
| 3625.095 | 3624.131 | 3625.095 | O-H stretch, H bonded, Alcohols, phenols |
| | 3609.667 | 3309.785 | O-H stretch, H bonded, Alcohols, phenols |
| 3327.142 | | | O-H stretch, H bonded, Alcohols, phenols |
| 3319.91 | 3319.428 | | O-H stretch, H bonded, Alcohols, phenols |
| 3311.714 | 3312.196 | | O-H stretch, H bonded, Alcohols, phenols |
| 2998.815 | | | C-H stretch, Alkanes, chlorophylls and carotenoids |
| 1660.919 | | 1664.294 | stretching ν(C=O) phenolic compounds and flavonoids |
| 1652.241 | | 1654.17 | C=O stretching |
| | | 1527.853 | N-O stretching, Nitro compound |
| | 1522.55 | 1523.996 | (N-H bending), chlorophylls and carotenoids |
| 1401.054 | | | stretching ν(C-C) aromatic compounds |
| 1272.809 | 1275.22 | 1276.666 | NH bending, C-N stretching |
| 1177.83 | 1178.313 | 1179.759 | stretching -C-C-O, phenolic components |
| 1100.691 | 1100.691 | 1102.137 | C-O stretch, Alcohols, carboxylic acids, esters, and ethers |
| 1027.408 | | 1024.997 | C-O stretch, Alcohols, carboxylic acids, esters, and ethers |
| | 1019.211 | | C-O stretch, Alcohols, carboxylic acids, esters, and ethers |
| 923.2687 | 923.7508 | 925.1971 | C=C bending, Alkene |
| 743.4362 | | | aromatic ring vibration, phenolic components and flavonoids |

**Table 3**
Major compounds of three layers of lettuce irradiated with different light treatments.

| WB | WRI | BRI |
|---|---|---|
| 5-Hydroxymethylfurfural | 5-Hydroxymethylfurfural | 5-Hydroxymethylfurfural |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 2-Hydroxy-gamma-butyrolactone | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- |
| 1,2-Epoxy-3-propyl acetate | 1,3-Dihydroxyacetone dimer | 1,2-Epoxy-3-propyl acetate |
| 1,2-Cyclopentanedione | 1,2-Epoxy-3-propyl acetate | 2-Hydroxy-gamma-butyrolactone |
| 2-Propanamine, N-methyl-N-nitroso- | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 1,2-Cyclopentanedione |
| Oxazolidine, 3-ethyl-2,2-dimethyl- | 1,2-Cyclopentanedione | 2-Propanamine, N-methyl-N-nitroso- |
| 2-Hydroxy-gamma-butyrolactone | 2-Imidazolidinethione | Guanazine |
| | D-Glucitol, 1,4:3,6-dianhydro-, diacetate | 1,3-Dihydroxyacetone dimer |
| | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | D-Glucitol, 1,4:3,6-dianhydro-, diacetate |
| | | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone |

## 4. Discussion

### 4.1. Impact of light spectra on phytochemicals

Different light irradiations exert distinct influences on NBI, CHL, ANTH, and lettuce LA. However, despite previous research suggesting that higher phytonutrients levels, such as FLAV, are achieved under blue and red light (Lycoskoufis et al., 2022; Matysiak et al., 2021; Ouzounis et al., 2015), this research demonstrates that light spectra did not significantly affect these compounds. This finding is consistent with other research reporting no significant differences in the amounts of phenolic acids, FLAV, and pigments under blue LED lighting exposure (Ouzounis et al., 2015). This phenomenon may be explained by the influence of plant nutritional status, specifically nitrogen, on FLAV biosynthesis, as elevated FLAV concentrations are typically associated with low nitrogen availability (Matysiak et al., 2021; Song et al., 2020; Wan et al., 2015). It is plausible that the nutrient solution employed in the aeroponic system contained either high nitrogen levels or a low carbon-to-nitrogen (C/N) ratio. The consistent composition of the solution throughout the experiment likely limited FLAV production, with the plant responding more sensitively to nitrogen concentration in the water than the variations in light conditions.

The results reveal a negative correlation between the physiological content of NBI and FLAV in lettuce under the three light treatments, also observed by other investigations (Kaniszewski et al., 2021; Matysiak et al., 2021) which suggests that increasing NBI concentration, indicative of higher nitrogen availability, correspond with a reduction in flavonoid accumulation. Furthermore, a negative correlation was observed between CHL and FLAV, under WB and BRI treatments. CHL levels, closely associated with nitrogen status, generally increase with nitrogen availability, enhancing photosynthetic capacity (Matysiak et al., 2021). In contrast, FLV accumulation typically signals nitrogen deficiency or stress conditions. The observed inverse relationship between CHL and FLAV under WB and BRI suggests that increased nitrogen supports active growth and reduces the need for flavonoid-based protective mechanisms (Kaniszewski et al., 2021).

On the other hand, FLAV also exhibits an inverse relationship with the LA across all treatments, with a more pronounced negative correlation observed under WB and BRI treatments. This can be attributed to the higher proportion of blue light, which slows plant growth while increasing the concentration of bioactive compounds such as FLAV (Azad et al., 2020; Matysiak et al., 2021).

The positive correlation between LA and NBI reflects the role of NBI in supporting robust plant growth and development. This correlation is most prominent under the WB treatment, likely due to the strong influence of blue and white light on physiological processes related to leaf expansion and CHL synthesis. Notably, even in the other two treatments, where blue and white light are combined with red and far-red light, the positive correlation between LA and NBI remains, underscoring the overall impact of nitrogen availability on plant health (Matysiak et al., 2021, 2023; Viršilė et al., 2019).

The WB and BRI treatments exhibit similar positive and negative correlations among the NBI, CHL, FLAV, ANTH, and LA variables, largely due to the impact of blue light on plant development. Blue light is critical for regulating NBI and CHL by supporting photosynthetic activity and nitrogen assimilation, which in turn drives healthy growth and optimizes LA expansion (Pennisi et al., 2019; Sawatdee et al., 2023). Blue light also stimulates secondary metabolite accumulation, specifically FLAV and ANTH, as a protective response to stress, indicating that its presence in WB and BRI likely contributes to increased resilience (Matysiak & Kowalski, 2019). However, in the BRI treatment, the addition of red and far-red light alongside blue further enhances these physiological responses. Red light has a direct effect on CHL synthesis, photosynthetic efficiency, and leaf expansion, which promotes biomass accumulation and maintains a stable NBI by ensuring efficient energy transfer in the photosystems (Azad et al., 2020; Samuolienė et al., 2021). Far-red light, meanwhile, modulates growth-related hormones, particularly phytochromes, that influence stem elongation and leaf area, creating more expansive foliage under BRI conditions (Ouzounis et al., 2015; Podsędek et al., 2023).

Regarding gas chromatography, no significant differences were observed among treatments, although the species with the highest percentage for each treatment are highlighted. For WRI, 2-imidazolidinethione was identified, an organosulfur compound with applications in creating carbon dots that exhibit multiple photoluminescence emissions, potentially serving as zero-dimensional nanomaterials for bioimaging and energy-based applications (Velusamy et al., 2018). Additionally, 2-imidazolidinethione functions as a precursor in the production of gel-polymer electrolytes based on cross-linked polyepichlorohydrin terpolymer (GECO), useful in lithium-sulfur batteries (Choudhury et al., 2017). Furthermore, it acts as a vulcanization accelerator in the manufacture of styrene-butadiene rubber (SBR) and rubber compounds (Zhong et al., 2015). As a deuterated form, 2-imidazolidinethione-4,5-d4 is also used as a reagent in the synthesis of pharmaceuticals and agricultural chemicals. It has been investigated for potential therapeutic applications due to its antimicrobial and antifungal properties, especially for skin conditions and infections (Clearsynth).

For BRI, Guanazine (4H-1,2,4-Triazole-3,4,5-triamine) is identified. This is a useful compound and starting material in the preparation of bicyclic heterocycles (Emilsson, 1989). The free base and its monohydrochloride or monohydrobromide salts are obtained through various synthetic methods (Emilsson, 1989). It has also been identified as a non-predominant component in methanolic extracts of the normal flower part of *Crataeva religiosa* using GC-MC analysis (Sharma et al., 2018). As part of the chemical compound of Triazoles, it has been extensively researched for their diverse range of biological effects, encompassing antibacterial, antineoplastic, antitubercular, anti-HIV, and antiphytopathogenic activities (Hosseini et al., 2020). Triazole-based energetic materials possess favorable attributes, including a high nitrogen content and low susceptibility to external forces such as impact and friction, rendering them reliable and safe as energetic materials (Bichay et al., 2006; Hosseini et al., 2020; Xue et al., 2010).

For WB, Oxazolidine, 3-ethyl-2,2-dimethyl-, is finally identified. While some derivatives of oxazolidine can be found naturally, others are synthetically produced as a drug against a broad spectrum of multiresistant gram-positive bacteria (GPB), for surgical infections, drug-resistant lung infections, multiresistant tuberculosis infections, skin infections, allowing inhibition of bacterial protein biosynthesis, and effective against Linezolid (LNZ)-resistant strains (Cordero et al., 2021; Foti et al., 2021; Redasani & Bari, 2015).

### *4.2. Limitations and future lines of research*

The experimentation involved one variety of lettuce. For future studies, broadening the scope to encompass additional varieties could yield valuable insights into their responses to a diverse spectral photon flux.

The primary focus of the research is analyzing and understanding the phytochemical profiles and promoting compounds of lettuce rather than on the biomass or yield itself. LA analysis could be considered the closely related morphological parameters to biomass, and it can be used to predict each other. Also, as a non-invasive measurement of LA, it is possible to have the approximate growth behavior of the plant cycle. In this order of ideas, the BRI treatment stands out as the optimal option by showing higher values in the LA. However, limiting treatment to a maximum of 5 weeks is recommended to avoid possible biomass losses due to prolonged exposure.

While all treatments can produce widely used medicinal components in pharmacology, WB treatment includes a specific component effective against various infections. To improve the value of lettuce by increasing secondary metabolites, WRI treatment emerges as an ideal solution as it offers a higher amount of ANTH throughout the study period. ANTH is mainly recognized for its antioxidant properties. However, it is reiterated that the treatment should be applied for a maximum of 4 weeks since the content tends to decrease after this period. Given the considerations, it is important for future investigations to explore additional variables that align directly with commercial needs. Recognizing the dynamic nature of the field, incorporating such parameters could enhance the practical relevance of the findings.

Around the 6th to 7th week of lettuce irradiation, most variables showed decreasing development, likely due to constant illumination in terms of intensity and duration. Authors suggest changes in lighting to achieve better and more consistent results, including the design of more suitable lighting structures (L. F. Lozano-Castellanos, Navas-Gracia, & Correa-Guimaraes, 2023), reducing exposure time to light (Shao et al., 2020), optimizing light intensity for specific crop species and cultivars in plant factories (Miao et al., 2023), alternating frequency of two or more light qualities (Shao et al., 2022), and controlling the Daily Light Integral (DLI), Photosynthetic Photon Flux Density (PPFD), and photoperiod (Kelly et al., 2020; Palmer & van Iersel, 2020).

Finally, for future research endeavors, it will be considered the impact of irrigation cycles and the water composition used in the aeroponic system, the exploration of extended and different photoperiods and/or intermittent lighting, and the replication of the lighting combinations used in this study but with varying intensities throughout the crop growth cycle.

## 5. Conclusions

The research findings indicate that, despite expectations based on previous studies, light's spectral composition does not significantly influence phytonutrients, such as flavonoids, in lettuce plants. Although the reason cannot be asserted with certainty, it can be associated with the nitrogen content in the liquid solution used in the aeroponic system.

The strength and direction of correlations between variables vary, suggesting unique patterns for each light treatment. In this context, the BRI treatment shows the highest number of positive correlations between physiological variables, attributed to the well-known and effective impact of blue, red, and far-red light on crops.

Regarding gas chromatography, by disregarding the number of species and focusing on the relative positions that, in terms of percentage, are present in different extracts, it is inferred that the composition of the BRI extract represents an intermediate variety between the WRI and WB extracts, but closer to WRI.

Specific compounds are identified for each treatment, revealing properties and applications in different fields.

## CRediT authorship contribution statement

**Luisa Fernanda Lozano-Castellanos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Eva Sánchez-Hernández:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Luis Manuel Navas-Gracia:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Pablo Martín-Ramos:** Validation, Software, Formal analysis, Data curation, Conceptualization. **Adriana Correa-Guimaraes:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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## 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: The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Luisa Fernanda Lozano Castellanos reports financial support was provided by European Union. Eva Sanchez Hernandez reports financial support was provided by European Union. Luis Manuel Navas Gracia reports financial support was provided by European Uinon. Pablo Martin Ramos reports financial support was provided by European Union. Adriana Correa Guimaraes reports financial support was provided by European Union. Luisa Fernanda Lozano Castellanos reports a relationship with European Social Fund that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

The data that has been used is confidential.

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