biology and life sciences forum

MDPI

Proceeding Paper

Assessment of Nutritional Properties of *Valerianella locusta* Growing in Indoor Vertical Farms under Different Lighting Conditions †

Niki Mougiou [1], Spyros Didos [1,2], Konstantina Tsotsouli [1,3], Despoina Dafni Avgoustaki [4] and Anagnostis Argiriou [1,2,*]

[1] Institute of Applied Biosciences, Centre for Research and Technology Hellas, Thermi, 57001 Thessaloniki, Greece; nmougiou@certh.gr (N.M.); sdidos@certh.gr (S.D.); konstandina.tsotsoulh@gmail.com (K.T.)
[2] Department of Food Science and Nutrition, University of the Aegean, Myrina, 81400 Lemnos, Greece
[3] School of Science and Technology, Hellenic Open University, Parodos Aristotelous 18, 26335 Patras, Greece
[4] Department of Natural Resources and Agricultural Engineering, Agricultural University of Athens, Iera Odos 75, Athens, Greece; dafni_av@aua.gr
* Correspondence: argiriou@certh.gr; Tel.: +30-2310498475
† Presented at the 4th International Electronic Conference on Foods, 15–30 October 2023; Available online: https://foods2023.sciforum.net/.

Abstract: Vertical Farms (VFs) are controlled indoor growing systems that maximize crop production and land-use efficiency. The major disadvantage of VFs is the high energy demand for lighting, making it necessary to adopt light conditions that offer desirable traits to the plants under minimum energy consumption. The aim of this study was to evaluate nutritional factors of corn salad plants under different lighting conditions. Two different light qualities were tested, white and blue-red-near-infrared parts of the spectrum under three different daily light integrals, DLI, each. The optimal biochemical characteristics of plants were observed under the most energy efficient lighting.

Keywords: vertical farming; biochemical analysis; nutritional properties; nitrate; antioxidants; daily light integral; *Valerianella locusta*

Citation: Mougiou, N.; Didos, S.; Tsotsouli, K.; Avgoustaki, D.D.; Argiriou, A. Assessment of Nutritional Properties of *Valerianella locusta* Growing in Indoor Vertical Farms under Different Lighting Conditions. *Biol. Life Sci. Forum* **2023**, *26*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: date

1. Introduction

Vertical farms (VFs) are fully insulated indoor constructions, that facilitate the cultivation of vegetables stacked in levels, under controlled environmental conditions. VFs allow the production of food in an environmentally friendly way, independent of the climate conditions, potentially without the need for pesticides or fertilizers [1]. VFs were developed as an answer to the problems of traditional open field agriculture and greenhouses, that have large needs for irrigation and arable land, as well as full exposure to unpredictable and severe weather phenomena, that occur more and more often [2]. VFs offer a 70% reduction of water used for irrigation, that may reach 90% for hydroponic cultivation [3]. Moreover, VFs can be installed in the urban areas, were almost 70% of the human population lives, greatly reducing transportation costs and carbon dioxide emissions resulting from transporting [2]. One of the most important features of VFs is that they allow the farmer to fully control the environmental conditions, like temperature, lighting, and humidity, adjusting them according to the needs of each cultivation, achieving maximum yield and product quality [4]. Unfortunately, this leads to the biggest disadvantage of VFs, which is the high energy cost for lighting operation. Researchers estimated that cultivating plants in VFs requires 30 times more energy than cultivating in open field [5]. To address this issue is necessary to find the lighting conditions that are energy efficient and facilitate the growth of plants with desirable nutritional properties to meet the market needs.

The aim of this study was to evaluate nutritional factors of corn salad (*Valerianella locusta*) plants, like ascorbic acid, polyphenol content, antioxidant activity and nitrate concentration under different lighting conditions, to find the lighting conditions that offer the optimal nutritional values.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The experiments were conducted in a pilot VF located at the campus of Agricultural University of Athens, Greece. The VF was installed inside a 40 ft cube with external dimensions of 12 m (L) × 2.44 m (W) × 3 m (H). The VF was equipped with 2 growing towers (T1, T2) and 3 growing layers (L1, L2, L3), per tower. Corn salad (*Valerianella locusta*) plants of 'Elixir' cultivar (HM Clause S.A., Portes les Valence, France) were transferred in the VF on rockwool 14 days after germination. In the VF, plants were cultivated at 25 °C, 500–600 ppm CO_2 and 50–55% relative humidity for 30 days, using a close loop Ebb & flow hydroponic system. All growing layers were irrigated simultaneously twice a day, for 10 min each time, with water enriched with nutrient solution at pH 5.6, that was then returned to the tank of the tower. Two different light qualities were applied, white and blue-red-near-infrared (B-R-NIR) parts of the spectrum, one at each tower using LED lamps. Three treatments (in the three growing layers, Table 1) were performed under each light quality resulting in 6 different conditions. The plants were harvested on the 30th day of the light treatment.

Table 1. The conditions applied under each light quality.

Condition	Light Quality	PPFD (μmol $m^{-2}s^{-1}$)	Daylight	DLI (mol $m^{-2}d^{-1}$)
T1L1	White	150	16 h	8.6
T1L2		250		14.4
T1L3		350		20.2
T2L1	B-R-NIR	150		8.6
T2L2		250		14.4
T2L3		350		20.2

2.2. Reagents and Chemicals

Methanol, Folin–Ciocalteu reagent, DPPH and H_2SO_4 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid, potassium nitrate, salicylic acid and oxalic acid were obtained from Penta (Prague, Czech). Sodium hydroxide was purchased from Applichem (Darmstadt, Germany) and 2,6-dichloroindophenol sodium salt hydrate from Carbosynth (Compton, UK).

2.3. Determination of Ascorbic Acid

The content of ascorbic acid (vitamin C) was determined using Tillman's method [6,7]. Freeze-dried plant material (1 g) was homogenized with 50 mL of oxalic acid solution (2%) and titrated with 0.1% 2,6-dichloroindophenol sodium salt hydrate, until the presence of a rosy color for more than 5 s. The results were expressed as mg of ascorbic acid per 100 g of dry weight (dw).

2.4. Extraction of Phenolic Compounds

Using 80% methanol as solvent, phenolic compounds were extracted from freeze-dried plant material in accordance to Fukumoto and Mazza, 2000 method [8] with some modifications. Samples of 0.2 g of plant material were homogenized with 5 mL of 80% methanol and sonicated for 10 min in an ultrasonic water bath. After centrifugation at 3000× *g* for 15 min, the supernatant was collected. The extraction procedure was repeated five times. The supernatants were combined and evaporated to dryness under air nitrogen

flow. The residue was dissolved in 5 mL of 100% methanol. These extracts were used for the determination of total phenolic content and antioxidant capacity.

2.5. Total Phenolic Content

Total phenolic content (TPC) was determined using Folin-Ciocalteu reagent as described by Petkovsek et al., 2010 [9]. Gallic acid was used as standard and TPC was expressed in mg of gallic acid equivalents (GAE) per 100 g of dry weight.

2.6. Determination of Antioxidant Activity

The ability of the extracts to react with radical of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) was used to determine their antioxidant activity. In the DPPH assay, the reaction mixture consisted of 25 μL of the extract or standard solution and 975 μL of a 0.1 mM solution of DPPH. After incubation in the dark at room temperature for 30 min, a reduction in optical density compared to the control was observed at 517 nm [10]. The absorbance was measured using a Shimadzu UV-2600 spectrophotometer (Kyoto, Japan). Results were expressed as the percentage of DPPH neutralization.

2.7. Determination of Nitrate (NO_3^-) Contents in Leaf Tissues

The concentration of NO_3^- in the samples was determined according to the method described by Fatkullin et al., 2021 [11] with some modifications. One gram of fresh sample was extracted with 5 mL deionized water and boiled at 100 °C for 20 min. The samples were centrifuged at 15.871× *g* for 10 min, and 0.1 mL of the supernatant was mixed with 0.4 mL of 5% (*w*/*v*) salicylic acid in concentrated H_2SO_4. After 20 min incubation at room temperature, 9.5 mL of 8% (*w*/*v*) NaOH solution were added. The samples were cooled until room temperature (about 20–30 min) and NO_3^- concentration was determined spectrophotometrically, by measuring the absorbance at 410 nm. The NO_3^- concentration was calculated by linear regression analysis using standard solutions of KNO_3 (10–120 mg/L). The results were expressed as mg per 100 g of fresh weight (fw).

2.8. Statistical Analysis

For each biochemical analysis, triplicate measurements were conducted, and data were expressed as mean value ± standard deviation (n = 3). Statistical analysis was performed using unpaired *t*-test (GraphPad, San Diego, CA, USA), while *p*-value significance threshold was 0.05 ($p \leq 0.05$).

3. Results

Biochemical analysis revealed the nutritional properties of the plants under each different light treatment. Vitamin C levels differentiated between the white light treatments, exhibiting maximum levels at the second layer (DLI 14.4 mol $m^{-2}d^{-1}$) and minimum at the other two, while the maximum value was observed at all treatments under B-R-NIR light (Figure 1). Polyphenol levels were significantly higher at the first layer of each tower, at DLI 8.6 mol $m^{-2}d^{-1}$, dropping by 11% approximately at the other layers with higher DLI values (Figure 2).

The estimation of the plants' antioxidant activity revealed an opposite pattern for the two light qualities. Under white light, antioxidant activity was raising as DLI increased, reaching the highest value at the maximum DLI of 20.2 mol $m^{-2}d^{-1}$. On the other hand, under B-R-NIR light, antioxidant activity was dropping as DLI increased, exhibiting the highest value at the minimum DLI of 8.6 mol $m^{-2}d^{-1}$ (Figure 3). Nitrate concentration was at lowest levels at the first layer of each tower, meaning at DLI 8.6 mol $m^{-2}d^{-1}$ under both light spectra. More specifically, it was measured at the lowest value at 3995 mg/kg fw in T1L1 and then at 4926 mg/kg fw in T2L1. Increase of the DLI led to an increase of nitrates under both light qualities (Figure 4).

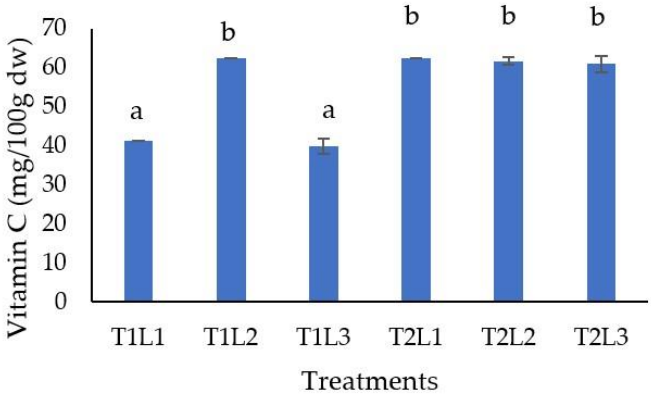

Figure 1. Concentration of Vitamin C under each light treatment. Different letters indicate statistically important difference ($p \leq 0.05$).

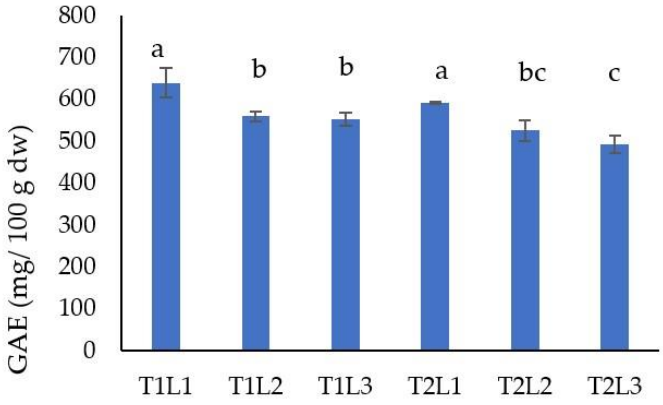

Figure 2. Concentration of gallic acid equivalents under each light treatment. Different letters indicate statistically important difference ($p \leq 0.05$).

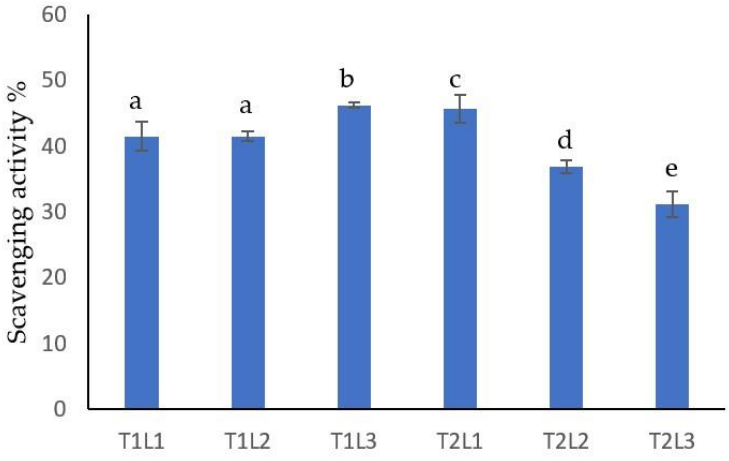

Figure 3. Scavenging activity of each sample under each light treatment. Different letters indicate statistically important difference ($p \leq 0.05$).

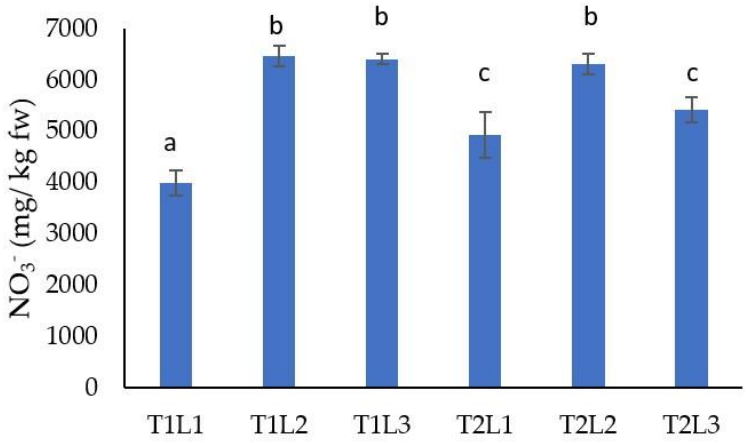

Figure 4. Concentration of NO_3^- under each light treatment. Different letters indicate statistically important difference ($p \leq 0.05$).

As a result from all measurements, it can be assumed that the lighting condition that offered the most desirable traits, which are high concentration of ascorbic acid, polyphenols and antioxidants but low concentration of nitrates, was DLI of 8.6 mol $m^{-2}d^{-1}$ under B-R-NIR lighting. This condition was also the most energy efficient, since it used the lowest PPFD values, as it was confirmed by the estimation of the kWh (kilowatt hour) consumed (data now shown).

4. Discussion

Antioxidants, polyphenols and ascorbic acid are of great interest to food industry as they offer high- quality products with elevated market value [12]. Thus, these traits were evaluated in this study, in order to find the lighting conditions that are energy efficient and offer desirable values. Studies in other cultivars of corn salad have shown high values of ascorbic acid and polyphenols, under blue and red lighting at PPFD value of 200 μmol $m^{-2}s^{-1}$. Our study is in accordance with that, since we reported optimal PPFD value at 150 μmol $m^{-2}s^{-1}$ under B-R-NIR light [6]. More studies have highlighted the beneficial effects of red and blue LED light at PPFD value of 200 μmol $m^{-2}s^{-1}$ on various aspects of corn salad, like carotenoids, yield, ascorbic acid and antioxidant activity [13].

Nitrate concentration is also crucial for green leafy vegetables, since elevated levels are dangerous for human consumption. European Union has set the acceptable upper limits for nitrate content in green leafy vegetables, like lettuce, spinach, rucola but not corn salad [14]. *V. locusta* is considered a plant that accumulates large quantities of nitrates and some countries have set limits at 3500 mg/kg fw in Belgium or 2500 mg/kg fw in Germany [13]. In our study we monitored a range of 3995–6457 mg/kg fw, where the lower values are in accordance with studies reporting a range of 3118–4169 mg/kg fw [15], or 3878–4695 mg/kg fw [16]. Other researchers showed that LED light at percentages of 80% red and 20% blue, at PPFD value of 200 μmol $m^{-2}s^{-1}$, reduced nitrate levels in corn salad of two other cultivars [6,13]. In our study the lowest levels under B-R-NIR lighting were observed at a PPFD value of 150 μmol $m^{-2}s^{-1}$. Optimization of the conditions is required to further reduce nitrate concentration down to acceptable levels.

Additional lighting in layers 2 and 3 did not seem to have a positive effect in any of the biochemical properties studied in the current research. This is a positive outcome, since it is desirable to have the best nutritional properties under the minimum energy cost, which is accomplished by using the minimum value of PPFD and was further confirmed by calculating the kWh consumed by each layer.

Author Contributions: Conceptualization, D.D.A. and A.A.; methodology, S.D. and K.T.; formal analysis, N.M.; investigation, N.M. and S.D.; data curation, N.M. and S.D.; writing—original draft preparation, N.M. and S.D.; writing—review and editing, N.M.; supervision, D.D.A. and A.A.; project administration, D.D.A. and A.A.; funding acquisition, D.D.A. and A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T2EDK-VF2FARM).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request due to restrictions eg privacy.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Van Gerrewey, T.; Boon, N.; Geelen, D. Vertical Farming: The Only Way Is Up? *Agronomy* **2022**, *12*, 2. https://doi.org/10.3390/agronomy12010002.
2. Vatistas, C.; Avgoustaki, D.D.; Bartzanas, T. A Systematic Literature Review on Controlled-Environment Agriculture: How Vertical Farms and Greenhouses Can Influence the Sustainability and Footprint of Urban Microclimate with Local Food Production. *Atmosphere* **2022**, *13*, 1258. https://doi.org/10.3390/atmos13081258.
3. Al-Kodmany, K. The Vertical Farm: A Review of Developments and Implications for the Vertical City. *Buildings* **2018**, *8*, 24. https://doi.org/10.3390/buildings8020024.
4. van Delden, S.H.; SharathKumar, M.; Butturini, M.; Graamans, L.J.A.; Heuvelink, E.; Kacira, M.; Kaiser, E.; Klamer, R.S.; Klerkx, L.; Kootstra, G.; et al. Current Status and Future Challenges in Implementing and Upscaling Vertical Farming Systems. *Nat. Food* **2021**, *2*, 944–956. https://doi.org/10.1038/s43016-021-00402-w.
5. Van Ginkel, S.W.; Igou, T.; Chen, Y. Energy, Water and Nutrient Impacts of California-Grown Vegetables Compared to Controlled Environmental Agriculture Systems in Atlanta, GA. *Resour. Conserv. Recycl.* **2017**, *122*, 319–325. https://doi.org/10.1016/j.resconrec.2017.03.003.
6. Długosz-Grochowska, O.; Wojciechowska, R.; Kruczek, M.; Habela, A. Supplemental Lighting with LEDs Improves the Biochemical Composition of Two *Valerianella locusta* (L.) Cultivars. *Hortic. Environ. Biotechnol.* **2017**, *58*, 441–449. https://doi.org/10.1007/s13580-017-0300-4.
7. Tauber, H.; Kleiner, I.S.; Mishkind, D. Ascorbic Acid (Vitamin C) Oxidase. *J. Biol. Chem.* **1935**, *110*, 211–218. https://doi.org/10.1016/s0021-9258(18)75168-5.
8. Fukumoto, L.R.; Mazza, G. Assessing Antioxidant and Prooxidant Activities of Phenolic Compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597–3604. https://doi.org/10.1021/jf000220w.
9. Petkovsek, M.M.; Slatnar, A.; Stampar, F.; Veberic, R. The Influence of Organic/Integrated Production on the Content of Phenolic Compounds in Apple Leaves and Fruits in Four Different Varieties over a 2-Year Period. *J. Sci. Food Agric.* **2010**, *90*, 2366–2378. https://doi.org/10.1002/jsfa.4093.
10. Feduraev, P.; Skrypnik, L.; Nebreeva, S.; Dzhobadze, G.; Vatagina, A.; Kalinina, E.; Pungin, A.; Maslennikov, P.; Riabova, A.; Krol, O.; et al. Variability of Phenolic Compound Accumulation and Antioxidant Activity in Wild Plants of Some Rumex Species (Polygonaceae). *Antioxidants* **2022**, *11*, 311. https://doi.org/10.3390/antiox11020311.
11. Cataldo, D.A.; Haroon, M.H.; Schrader, L.E.; Youngs, V.L. Rapid Colorimetric Determination of Nitrate in Plant Tissue by Nitration of Salicylic Acid. *Commun. Soil Sci. Plant Anal.* **1975**, *6*, 71–80. https://doi.org/10.1080/00103627509366547.
12. Fatkullin, R.; Naumenko, N.; Popova, N.; Ruskina, A.; Kalinina, I.; Potoroko, I. Explaining Consumer Intentions for Foods with Antioxidant Properties: Predictors of Choice and Purchase Barriers. *Int. J. Food Sci.* **2021**, *2021*, 9971425. https://doi.org/10.1155/2021/9971425.
13. Wojciechowska, R.; Kołton, A.; Długosz-Grochowska, O.; Knop, E. Nitrate Content in *Valerianella locusta* L. Plants Is Affected by Supplemental LED Lighting. *Sci. Hortic.* **2016**, *211*, 179–186. https://doi.org/10.1016/J.SCIENTA.2016.08.021.
14. European Union COMMISSION REGULATION (EU) No 1258/2011. Available online: https://www.fao.org/faolex/results/details/en/c/LEX-FAOC108181/ (accessed on).
15. Iacuzzo, F.; Gottardi, S.; Tomasi, N.; Savoia, E.; Tommasi, R.; Cortella, G.; Terzano, R.; Pinton, R.; Dalla Costa, L.; Cesco, S. Corn Salad (*Valerianella locusta* (L.) Laterr.) Growth in a Water-Saving Floating System as Affected by Iron and Sulfate Availability. *J. Sci. Food Agric.* **2011**, *91*, 344–354. https://doi.org/10.1002/JSFA.4192.
16. Manzocco, L.; Foschia, M.; Tomasi, N.; Maifreni, M.; Dalla Costa, L.; Marino, M.; Cortella, G.; Cesco, S. Influence of Hydroponic and Soil Cultivation on Quality and Shelf Life of Ready-to-Eat Lamb's Lettuce (*Valerianella locusta* L. Laterr). *J. Sci. Food Agric.* **2011**, *91*, 1373–1380. https://doi.org/10.1002/JSFA.4313.