



Quality Evaluation of Indoor-Grown Microgreens Cultivated on Three Different Substrates

Roberta Bulgari ^{1,2,*}, Marco Negri ¹, Piero Santoro ³ and Antonio Ferrante ¹

- Department of Agricultural and Environmental Sciences, University of Milano, Via Celoria 2, 20133 Milano, Italy; marco.negri@guest.unimi.it (M.N.); antonio.ferrante@unimi.it (A.F.)
- Department of Agricultural, Forest and Food Sciences, DISAFA, Vegetable Crops and Medicinal and Aromatic Plants, VEGMAP, University of Torino, 10095 Grugliasco, Italy
- MEG S.r.l., Via Aleardo Aleardi 12, 20154 Milano, Italy; santoro@megscience.com
- Correspondence: roberta.bulgari@unito.it

Abstract: The microgreens are innovative products in the horticultural sector. They are appreciated by consumers thanks to their novelty and health-related benefits, having a high antioxidant concentration. This produce can be adopted for indoor production using hydroponic systems. The aim of the present work was to investigate the influence of three growing media (vermiculite, coconut fiber, and jute fabric) on yield and quality parameters of two basil varieties (Green basil—Ocimum basilicum L., Red basil—Ocimum basilicum var. Purpurecsens) and rocket (Eruca sativa Mill.) as microgreens. Microgreens were grown in floating, in a Micro Experimental Growing (MEG®) system equipped with LED lamps, with modulation of both energy and spectra of the light supplied to plants. Results showed high yield, comprised from 2 to 3 kg $\,\mathrm{m}^{-2}$. Nutritional quality varied among species and higher antioxidant compounds were found in red basil on vermiculite and jute. Coconut fiber allowed the differentiation of crop performance in terms of sucrose and above all nitrate. In particular, our results point out that the choice of the substrate significantly affected the yield, the dry matter percentage and the nitrate concentration of microgreens, while the other qualitative parameters were most influenced by the species.

Keywords: coconut fiber; floating system; LED; microgreens; nitrate; plant antioxidants; vermiculite; jute

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1. Introduction

In recent years, microgreens have received increasing attention by producers and consumers thanks to their characteristics of tenderness and crunchiness, specific flavors, vivid colors, and high nutraceutical value, due to the presence of several bioactive compounds such as antioxidants, vitamins, and macro and micro-minerals [1–8]; for these reasons they are usually considered as functional foods [9,10]. Based on the current literature, there is also a growing interest from researchers, confirmed by the amount of papers published on this topic [11]. Microgreens are immature greens, harvested and marketed as soon as the first leaves are developed and the cotyledons are still tender [12]. They can be obtained from vegetables, herbaceous plants, aromatic herbs, and spontaneous species [2,3,13]. Size varies from species to species, but normally they are between 2.5 and 8 cm in height [14]. The growing cycle is short and varies between 7 and 21 days from the emergence of the seedlings. The shoots are harvested by cutting them just above the roots and are eaten raw either alone or in mixed salads or used as a garnish for dishes. Microgreens can be also commercialized in boxes with substrates, without harvesting. This strategy allows longer shelf life and wide opportunity for the commercialization. One of the major limitation of microgreens is their rapid quality deterioration that occurs soon after harvest, and so restricts their commercialization to local sales [6]. From the point of view of cultivation technique, microgreens are very suited for indoor production [8]; they are often grown in

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hydroponic systems on different substrates [15]. It is important to underline that hydroponic systems could be a sustainable alternative to conventional farming, as they require less water, fertilizer, pesticides, and space for the crops cultivation [16–19]. Moreover, in recent years, vertical farming systems have emerged as a potential solution for urban horticulture, with interesting positive implications in terms of reduced environmental impact [20], thanks to the shortening of the food supply chain, to the reduction of waste and fossil resources for transportation, with a consequent decrease of CO₂ [21]. These systems are also less affected by climate change, being performed in a protected environment [22].

As reported in literature, special attention must be addressed to the choice of growth media, which represents one of the key factors in the production process and could influence microgreens yield and quality [23]. Among common substrates used for the microgreens production, peat-based media are the most utilized, followed by coconut coir and several synthetic media. Recently, natural fiber-based media—such as jute, cotton, cellulose, etc.—have gained increasing popularity since they could represent a sustainable alternative [3,23].

The aim of the present work was to investigate the influence of different growing media (vermiculite, coconut fiber, and jute fabric) on yield and quality traits of microgreens of two basil varieties (Green basil—*Ocimum basilicum* L., Red basil—*Ocimum basilicum* var. Purpurecsens) and rocket (*Eruca sativa* Mill.). Microgreens were cultivated in floating, with a hydroponic nutrient solution, using LED illumination, in a Micro Experimental Growing (MEG[®]) platform. This is an innovative cultivation system that allows the modulation of both energy and spectra of the light supplied to plants.

2. Materials and Methods

2.1. Plant Materials and Sampling

Microgreens of green basil (*Ocimum basilicum* L.), red basil (*Ocimum basilicum* 'Purpurascens'), and rocket (*Eruca sativa* Mill.) were grown at the Faculty of Agricultural and Food Sciences of the University of Milan, in a micro experimental growing chamber ($MEG^{\$}$) (Figure 1). The $MEG^{\$}$ is a system equipped with LED-lamps, open-source software, automated, developed for home indoor growing. This device utilizes a precision illumination system, composed of LED diodes managed by a smart control system which allows the modulation of the light spectrum composition with emission in 454 nm (blue), 663 (red), and 729 (far-red) and light intensity 65 μ mol m⁻² s⁻¹, with 12/12 photoperiod (Figure 2). The temperature inside $MEG^{\$}$ was 20 °C, and the relative humidity was 60–70%.

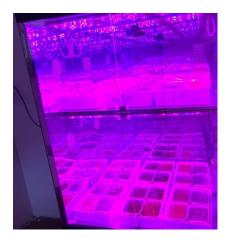




Figure 1. From left to right: Micro Experimental Growing (MEG[®]) chamber and harvesting stage of indoor-grown microgreens.

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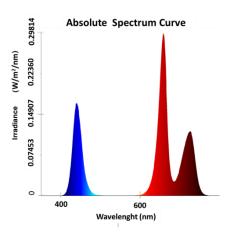


Figure 2. Light spectra utilized for each indoor growing cycle. The specific light spectra was measured by using a portable spectroradiometer (Everfine, PLA 20).

The three growing substrates used for the experiments were: coconut fiber, vermiculite, and jute. Coconut fiber is the mesocarp of *Cocos nucifera* L., containing short, and medium length fibers left from industrial applications. Depending on origin and industrial source, there is a difference in the physical and chemical properties. As reported in literature, coconut fiber possesses remarkable physical and chemical characteristics—such as high water-holding capacity, good drainage, and aeration properties—as well as a high cation exchange capacity. The pH ranged from 5.5 to 7. The porosity was 90–95% v/v. The density was 80–100 kg/m³ [24]. Vermiculite is a silicate mineral, that shows a high water retention and a good aeration. The pH value was 7–8, the cation exchange capacity was high. The porosity was around 85–95% v/v, and the density was 80–150 kg/m³ [24]. Jute was a sustainable substrate obtained from organic fiber. Natural fibers have many advantages, such as biodegradability and low costs. At present, little technical information was available in literature on this substrate. Jute had a density of 1300–1500 kg/m³ [25].

The test was carried out in plastic tanks ($35 \times 27 \times 15$ cm). All plastic tanks contained 2.5 L of half-strength Hoagland's nutrient solution. In each tank was tested a single type of substrate, placed in aluminum trays ($11 \times 9 \times 6.5$ cm). Each aluminum tray contained about 20 g of substrate and 2 g of seeds. The tanks were positioned randomly in the chamber. Growing cycles ranged from 12–16 days from sowing, depending on the species. Two growing cycles were performed. At harvest, yield and dry matter percentage (DM%) were calculated, and some destructive determinations were performed in laboratory, in order to evaluate the produce quality. In particular, nitrate, sucrose, chlorophylls, carotenoids, anthocyanins, and phenols concentrations were measured.

2.2. Yield and Dry Matter Percentage

Yield and dry matter percentage were determined by weighing the whole microgreens obtained in each aluminum tray (cutting them at the base, excluding the substrate), before and after an oven-dry period (4 days, until reaching constant weight) at 75 °C in a ventilated oven.

2.3. Nitrate

Nitrates content was measured with the salicylsulphuric acid method [26]. One g fresh sample was ground in 3 mL of distilled water. The extract was centrifuged at 4000 rpm for 15 min and the supernatant was recovered and used for the colorimetric determination. Twenty μL of sample were added to 80 μL of 5% salicylic acid in sulphuric acid and to 3 mL of NaOH 1.5 N. The samples were cooled at room temperature for 15 min and the spectrophotometer readings were performed at 410 nm. Nitrate concentration was calculated referring to a KNO₃ standard calibration curve (0, 1, 2.5, 5, 7.5, 10 mM KNO₃).

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2.4. Sucrose

For the determination of sucrose, 1 g of fresh sample was ground in 3 mL of distilled water. Homogenate was centrifuged at 4000 rpm for 15 min. After that, 0.2 mL of extract was added to 0.2 mL NaOH 2N and incubated at 100 °C for 10 min; then 1.5 mL of hot resorcinol solution was added and the sample was incubated at 80 °C for 10 min. The resorcinol solution was prepared by adding 35 mg of resorcinol and 90 mg of thiourea in 250 mL HCl 30%, mixed with 25 mL of acetic acid and 10 mL of distilled water. Samples were cooled at room temperature for 15 min and spectrophotometer readings were performed at 500 nm [27]. A calibration curve was built with sucrose standards at 0, 0.5, 1, 1.5, and 2 mM.

2.5. Total Chlorophylls and Carotenoids

Chlorophyll a+b and total carotenoids concentrations were determined spectrophotometrically. Frozen shoot tissue (about 1 g) was extracted using 5 mL of 100% (v/v) methanol, for 24 h at 4 °C in the dark, followed by quantitative determination of pigments. Absorbance readings were measured at 665.2 and 652.4 nm for chlorophylls and 470 nm for total carotenoids. Pigment concentrations were calculated by Lichtenthaler's [28] formula and expressed on the basis of tissue fresh weight (FW).

Lichtenthaler's formula using methanol is as follows

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{\rm chlorophyll} \ a = 16.72*{\rm ABS}665.2 - 9.16*{\rm ABS}652.4 {\rm chlorophyll} \ b = 34.09*{\rm ABS}652.4 - 15.28*{\rm ABS}665.2 {\rm carotenoids} = (1000*{\rm ABS}470) - (1.63*{\rm [chl}\ a\ {\rm mg/L}]) - (104.96*{\rm [chl}\ b\ {\rm mg/L}])/221
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Chlorophylls and carotenoids concentration calculation

[chl a or chl b or carotenoids]*volume methanol/mg tissue = μ g/mg FW

2.6. Anthocyanins and Phenols

For anthocyanins determination, samples of frozen shoot tissue (about 1 g) were ground in pre-chilled mortar and extracted into methanolic HCl (1%). Samples were then incubated overnight at 4 $^{\circ}$ C in the dark. The concentration of cyanidin-3-glucoside equivalents was determined spectrophotometrically at 535 nm [29]. Phenols were spectrophotometrically determined in fresh shoot samples (about 1 g) following the direct measure of the methanolic extract absorbance at 320 nm (phenolic index). Phenolic index was expressed as ABS320 nm g $^{-1}$ FW.

2.7. Substrate Analysis

The dried samples of each substrate were ground into powder with a ZM 100 centrifugal mill equipped with a 0.5 mm mesh sieve (Retsch Gmbh & Co., Haan, Germany) to determine the total nitrogen (N) concentration, the carbon (C) concentration, and C/N ratio, by dry combustion, using a ThermoQuest NA1500 elemental analyser (Carlo Erba, Milan, Italy).

3. Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 6 for Windows (GraphPad Software; La Jolla, CA, USA, www.graphpad.com (accessed on 25 April 2021). Data are the result of the average of two different growing cycles, which took place under the same experimental conditions. The reported values are means with standard errors (SE) of n = 6 biological replicates. All data were subjected to two-way ANOVA and differences among means were determined by Tukey's multiple comparison test (p < 0.05).

4. Results

4.1. Yield and Dry Matter Percentage

The ANOVA analysis revealed that the interaction between the two factors (substrate x species) and the two factors (substrate and species) were statistically significant

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(Table 1). The highest yield was observed in rocket microgreens grown on jute substrate (3201.09 g/m^2) . In general, red basil showed the lowest yields (with a minimum of 2008.38 g/m^2 on jute), while green basil showed intermediate values (Figure 3). Based on average data, rocket species showed higher yields (around 3000 g/m^2) compared to the two basil varieties tested. The species with the lowest yields was red basil (average yield around 2200 g/m^2). Also, in the case of the dry matter percentage calculation, the two-way ANOVA highlighted significant differences both for interaction and factors (Table 1). Values ranged from 3.8% (for rocket grown on jute) to 6.6% (in the case of red basil on coconut fiber). Rocket and red basil grown on jute showed the lowest values of dry matter percentage (Figure 4).

Table 1. Summary of the results of the two-way ANOVA for all the analyzes performed. Data were subjected to two-way ANOVA and Tukey's multiple comparison test was used for evaluating the differences among means at (* p < 0.05, *** p < 0.001, **** p < 0.0001). NS = not significant.

Determination	Source of Variation	p Value	p Value Summary
Yield	Interaction (SubxSp)	< 0.0001	****
	Substrate	< 0.0001	****
	Species	< 0.0001	***
Dry matter	Interaction (SubxSp)	< 0.0001	***
	Substrate	< 0.0001	****
	Species	< 0.0001	****
Nitrate	Interaction (SubxSp)	0.759	NS
	Substrate	0.001	**
	Species	0.292	NS
Sucrose	Interaction (SubxSp)	0.183	NS
	Substrate	0.913	NS
	Species	0.032	*
Chlorophyll $a + b$	Interaction (SubxSp)	0.122	NS
	Substrate	0.216	NS
	Species	0.112	NS
Carotenoids	Interaction (SubxSp)	0.483	NS
	Substrate	0.120	NS
	Species	0.019	*
Anthocyanins	Interaction (SubxSp)	0.042	*
	Substrate	0.266	NS
	Species	0.0001	***
Phenolic index	Interaction (SubxSp)	0.136	NS
	Substrate	0.397	NS
	Species	0.018	*

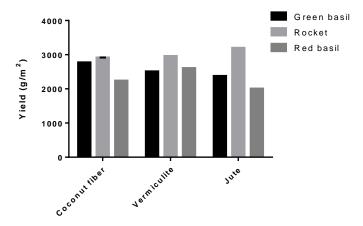


Figure 3. Yield of green basil, rocket, and red basil microgreens grown on three different substrates (coconut fiber, vermiculite, and jute). Values, subjected to two-way ANOVA, are means \pm SE (n = 6).

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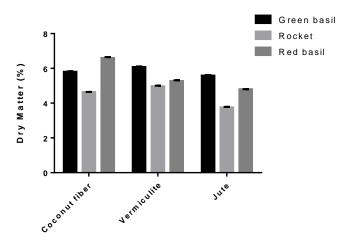


Figure 4. Dry matter of green basil, rocket, and red basil microgreens grown on three different substrates. Values, subjected to two-way ANOVA, are means \pm SE (n = 6).

4.2. Nitrate Concentration

The two-way ANOVA analysis showed that the interaction and the species factor were not significant; on the contrary, the differences among substrates were significant (Table 1). Nitrate concentration was lower in microgreens grown on coconut fiber compared to other substrates; in particular, the lowest value was observed in red basil microgreens (687.37 mg/kg FW). The three species cultivated on vermiculite and jute showed similar levels, ranging between 1191.12 and 1363.13 mg/kg FW (Figure 5).

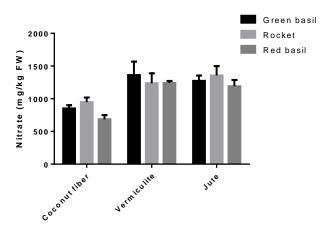


Figure 5. Nitrate concentration of green basil, rocket, and red basil microgreens grown on three different substrates. Values, subjected to two-way ANOVA, are means \pm SE (n = 6).

4.3. Sucrose Levels

The analysis of sucrose concentration allowed highlighting that the species factor was significant (Table 1). Values ranged from 320 to around 500 mg/kg FW. Green basil microgreens contained the lowest levels. The highest concentration was found in red basil microgreens grown on coconut fiber (484.35 mg/kg FW). The coconut fiber induced different crop performance. Rocket microgreens showed the same sucrose concentrations, around 400 mg/kg FW (Figure 6).

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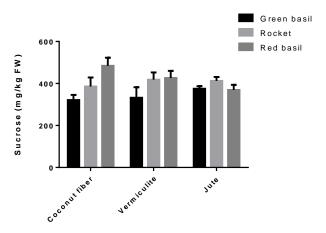


Figure 6. Sucrose concentration of green basil, rocket, and red basil microgreens grown on three different substrates. Values, subjected to two-way ANOVA, are means \pm SE (n = 6).

4.4. Total Chlorophylls and Carotenoids

With regard to chlorophylls a + b concentration, the two-way ANOVA analysis showed that interaction and factors were not statistically significant (Table 1), with values ranging from 202.51 to 316.42 μ g/g FW (Figure 7). In general, lower chlorophyll concentrations were observed in red basil, without difference among the substrates. The higher values were found in green basil microgreens (Figure 8), although the highest concentration was obtained for rocket grown on coconut fiber. Regarding carotenoids, it is possible to observe a significant effect deriving from the species (Table 1); the lowest concentrations were measured in red basil microgreens, while the other two species showed similar values (Figure 9).

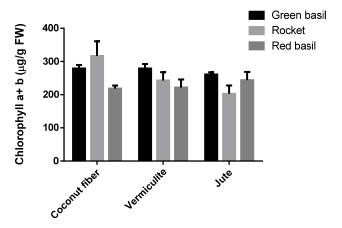


Figure 7. Chlorophyll a + b concentration of green basil, rocket, and red basil microgreens grown on three different substrates. Values, subjected to two-way ANOVA, are means \pm SE (n = 6).



Figure 8. From left to right: green basil microgreens grown on jute, vermiculite and coconut fiber. This species showed high concentrations of chlorophyll a + b.

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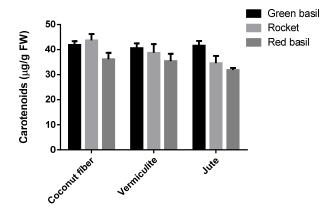


Figure 9. Carotenoid concentrations of green basil, rocket, and red basil microgreens grown on three different substrates. Values, subjected to two-way ANOVA, are means \pm SE (n = 6).

4.5. Anthocyanins and Phenolic Index

Statistical analysis showed that for anthocyanins the interaction between substrate and species was significant for p < 0.05 (Table 1). The species factor had a significant effect for p < 0.0001. As could be expected, red basil microgreens showed higher anthocyanins levels (up to 27.16 mg/100 g FW) compared to rocket and green basil, in particular this species grown on vermiculite and jute showed the highest values. The lowest concentration can be found in green basil on jute (9.89 mg/100 g FW) (Figure 10). The species factor was significant also in the case of phenolic index (Table 1). Red basil microgreens grown on vermiculite (Figures 11 and 12) and jute reached the highest levels (6.44 and 7.37 ABS_{320 nm}/g respectively); green basil showed in general lower values compared to the other species considered.

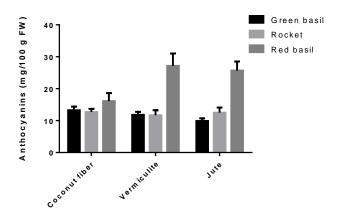


Figure 10. Anthocyanin concentrations of green basil, rocket, and red basil microgreens grown on three different substrates. Values, subjected to two-way ANOVA, are means \pm SE (n = 6).

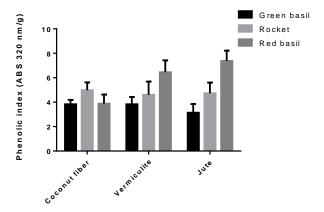


Figure 11. Phenolic index values of green basil, rocket, and red basil microgreens grown on three different substrates. Values, subjected to two-way ANOVA, are means \pm SE (n = 6).

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Figure 12. Red basil microgreens grown on vermiculite substrate. This species reached the highest levels of phenolic compounds.

4.6. Substrate Analysis

The total N was higher in coconut fiber, with 0.49%, followed by vermiculite, with 0.31%, while no nitrogen was detected in jute (Table 2). The C concentration was higher in vermiculite and coconut fiber, with 44.25 and 41.31% respectively, while 0.28% was found in jute. The highest C/N ratio was observed in vermiculite substrate, followed by jute (Table 2).

Table 2. Total N and C percentage, and C/N ratio in the different substrates utilized for the trial. Values are means (n = 2).

Substrate	N (%)	C (%)	C/N
Coconut fiber	0.49	41.31	85.10
Vermiculite	0.31	44.25	144.15
Jute	0	0.28	/

5. Discussion

The growing medium plays a very important role in determining the microgreens' yield and quality [23], and the sustainability of the production process. Associated with this, in the last few years, an increased number of scientific papers reported the beneficial effects of LED light, in controlled environment agriculture, on plant growth and quality traits, including the accumulation of molecules of interest, such as carotenoids, phenolics, and glucosinolates [7,8,30–33]. In the present work, different growing media usable for indoor microgreens cultivation were evaluated: vermiculite (inorganic material), coconut fiber (a natural, organic fiber), and jute (organic by-product and discarded material from several industrial processes). At present, peat-based substrates are the main growing media used for microgreens cultivation, but they are expensive and non-renewable. An alternative to peat may be coconut coir, an organic and renewable resource. However, coir has some disadvantages, in terms of possible high concentration of salts, as well as high fungal and bacterial counts [23,34]. A low-cost and renewable alternative could be the use of fibrous materials—such as polyester, cotton, or jute fiber. Other available inorganic media are for example perlite, vermiculite, and rockwool but these media are expensive, their production is energy demanding, and they are not easily disposable at the end of the production. In our experiment, the analysis of the performance of the three different substrates has also the aim of identifying alternatives that could be more environmentally friendly and cost effective, as by-products from industrial processes.

Certainly, the fresh yield is a key factor in the cultivation of microgreens, if we consider that they are typically sold on a "per-FW" basis [35]. A low yield continues to be a limiting factor for microgreen industry [1]. Data related to fresh yield were similar or

slightly higher than that reported in literature for microgreens [1,8,13,35–37]. Specifically, Bulgari et al. [1] obtained a yield of $1 \, \text{kg/m}^2$ for basil microgreens and around $1.5 \, \text{kg/m}^2$ for rocket, and Kyriacou et al. [38] produced $1.6 \, \text{kg/m}^2$ of green basil microgreens and $3 \, \text{kg/m}^2$ of red basil. In our trial, good results were especially obtained for rocket, on all the three substrates tested, with the highest data observed on jute. The dry matter percentage showed an opposite behavior; in fact, rocket microgreens had the lowest DM% in all the three substrates tested. A significant effect, resulting from species and substrates and their interaction, was therefore noticed in our experiment on these quantitative parameters, and the results underline the importance of growth media.

Regarding qualitative parameters, nitrates are among the main compounds that determine foods healthiness. Vegetables can accumulate different concentrations of nitrate (NO₃⁻) and this compound could consequently cause health problems in human [39]. Microgreens can be considered a good source of minerals and their NO₃⁻ concentration is generally very low. Several studies reported that the nitrate in microgreens had lower levels than in mature salads [8,13,40,41]. Thus, microgreens can be safely consumed for a healthy diet, even in the case of children, avoiding harmful phenomena such as methemoglobinemia. Our findings showed that nitrate concentrations ranged from 687.37 to 1363.13 mg/kg FW; the substrate factor had a significant effect on the concentration. A marked reduction of nitrate was in particular observed on coconut fiber. Microgreens are confirmed as non-nitrate accumulators [11]. As reported by Di Gioia et al. [23], the choice of the substrate can limit microgreens NO₃⁻ accumulation thus influencing the produce quality. Regarding green and red basil, we have found lower levels, and in some cases halved, compared to the data reported in the experiment of Kyriacou et al. [38], who studied 13 microgreens species grown in a growth chamber, on a commercial peat-based substrate. They observed that nitrate levels can vary considerably across species; however, the species factor did not significantly affect our results.

The C/N is usually considered a parameter that can affect the nitrate leaf accumulation. However, the obtained results cannot be justified by the C/N, due to the limited growing period. The light type could also induce different responses in plants and influence the nitrate content [8]; with regards to this, an accurate choice of the substrate combined with LED illumination can be a good strategy to get produce rich in molecules of interest and with lower amounts of nitrate. The sucrose concentration allowed highlighting that the species factor was relevant; the highest concentration was found in red basil grown on coconut fiber. Sucrose is important for microgreens preservation during the distribution chain and storage. In the case of microgreens, differences in the sugars level can be attributed to variations in the genotype [11]. Furthermore, different lighting conditions during the growing cycle can also affect the content of carbohydrates, resulting from a stimulation of the photosynthetic process [30]. It is interesting to note that sucrose levels are moderate and lower compared to baby leaf and adult vegetable leaves [42]. Such low sugars concentration can explain the very short shelf life (1–2 days) of microgreens [43,44]. However, we found higher values than those described by Bulgari et al. [1] related to microgreens cultivated outdoor, on vermiculite; this denotes a possible effect derived by the light quality. As previously reported by Lin et al. [45], a specific LED light can be used to enhance the sucrose concentration and the nutritional profile of vegetables.

Moving to the chlorophylls, the content of these pigments in vegetables is moreover important for the visual appearance of the produce. Color and appearance determine if a product is accepted or rejected by the consumer, and these aspects are even more relevant in a product like microgreens, highly appreciated also for their colors [46]. Chlorophylls play a significant role in photosynthesis, since they represent part of light-harvesting complex. As reported in literature, significant genotypic variations were observed for chlorophylls, and the levels can be also influenced by the light conditions [11]. Considering that microgreens are mainly composed by cotyledons, it is evident that lower concentrations of chlorophylls, carotenoids, phenols, and anthocyanins were detected as opposed to baby leaf or adult vegetables of the same species [47,48]. Red basil microgreens reached, in general, the

highest levels of phenolic compounds compared to the other species. Purple varieties are characterized by the accumulation of anthocyanins in leaves and flowers, mostly at adult stage [49]. Total phenolics are higher in the purple basils than in the green cultivars [50] and this was confirmed also at microgreens stage, as shown by our data. Purple basil is a very good natural source of anthocyanins, which are correlated with prevention of diverse human diseases. The obtained results for red basil microgreens grown on vermiculite and jute look promising to get a product with a high nutraceutical value.

6. Conclusions

As we have witnessed over the past few years, the interest in microgreens has increased [11]. They are appreciated by consumers thanks to their novelty and health-related benefits, being rich in antioxidant compounds. Moreover, microgreens can be easily grown by people for home use, or be produced at a larger scale, with indoor grow systems. Indoor plant cultivation systems are emerging because they allow to produce fresh food in urban environments and unfavorable climatic contexts [18,51]. The sustainability of the cultivation system is a critical point of indoor production, which can be improved by the optimization of the production factors, such as water, substrate, and energy intake [51]. In particular, energy consumption mainly depends on the lighting, since the optimization of light emission—in terms of quality and intensity—can improve the system sustainability [52,53]. The choice of the substrate is no less important, as we have seen. In addition to the economic and environmental impact, the substrate can influence the produce quality [23]. According to this, we included different substrates in the present work, with different characteristics, in order to suggest valid alternatives in the choice of the growing media, maintaining at the same time a good final quality of the product. Our results point out that the choice of the substrate significantly affected the yield, the dry matter percentage and the nitrate concentration of microgreens. On the contrary, the other qualitative parameters were most influenced by the species.

Certainly, additional studies may be needed to evaluate the effect of these substrates (vermiculite, coconut fiber, and jute fabric) on the production and quality traits (nutritional value, color, texture, taste, etc.) of microgreens, also in relation to the determination of the optimal protocol for LED management, to identify the best cultivation conditions. Moreover, this study has provided interesting preliminary data on the use of red basil for the production of microgreens, a species that has been little studied to date for this type of production.

Author Contributions: R.B., substantial contribution to the experimental work, interpretation of data, and drafting of the manuscript; M.N., participated in the experimental work, drafting, and revision of the manuscript; P.S., experimental setup of the Micro Experimental Growing (MEG[®]) platform for microgreens cultivation; A.F., experimental design and coordination of the work, interpretation of data, drafting and revision of the manuscript. All authors read and approved the final version of the manuscript.

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References

1. Bulgari, R.; Baldi, A.; Ferrante, A.; Lenzi, A. Yield and quality of basil, Swiss chard, and rocket microgreens grown in a hydroponic system. *N. Z. J. Crop. Hortic. Sci.* **2017**, *45*, 119–129. [CrossRef]

2. de la Fuente, B.; López-García, G.; Máñez, V.; Alegría, A.; Barberá, R.; Cilla, A. Evaluation of the bioaccessibility of anti-oxidant bioactive compounds and minerals of four genotypes of Brassicaceae microgreens. *Foods* **2019**, *8*, 250. [CrossRef] [PubMed]

3. Kyriacou, M.C.; El-Nakhel, C.; Pannico, A.; Graziani, G.; Soteriou, G.A.; Giordano, M.; Palladino, M.; Ritieni, A.; De Pascale, S.; Rouphael, Y.; et al. Phenolic constitution, phytochemical and macronutrient content in three species of microgreens as modulated by natural fiber and synthetic substrates. *Antioxidants* **2020**, *9*, 252. [CrossRef] [PubMed]

- 4. Galieni, A.; Falcinelli, B.; Stagnari, F.; Datti, A.; Benincasa, P. Sprouts and microgreens: Trends, opportunities, and horizons for novel research. *Agronomy* **2020**, *10*, 1424. [CrossRef]
- 5. Caracciolo, F.; El-Nakhel, C.; Raimondo, M.; Kyriacou, M.C.; Cembalo, L.; De Pascale, S.; Rouphael, Y. Sensory attributes and consumer acceptability of 12 microgreens species. *Agronomy* **2020**, *10*, 1043. [CrossRef]
- 6. Turner, E.R.; Luo, Y.; Buchanan, R.L. Microgreen nutrition, food safety, and shelf life: A review. *J. Food Sci.* **2020**, *85*, 870–882. [CrossRef]
- 7. Zhang, X.; Bian, Z.; Yuan, X.; Chen, X.; Lu, C. A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. *Trends Food Sci. Technol.* **2020**, *99*, 203–216. [CrossRef]
- 8. Ferrón-Carrillo, F.; Guil-Guerrero, J.L.; González-Fernández, M.J.; Lyashenko, S.; Battafarano, F.; da Cunha-Chiamolera, T.P.L.; Urrestarazu, M. Led Enhances Plant Performance and Both Carotenoids and Nitrates Profiles in Lettuce. *Plant Foods Hum. Nutr.* **2021**, 1–9. [CrossRef]
- 9. Sun, J.; Xiao, Z.; Lin, L.-Z.; Lester, G.E.; Wang, Q.; Harnly, J.M.; Chen, P. Profiling polyphenols in five brassica species microgreens by uhplc-pda-esi/hrmsn. *J. Agric. Food Chem.* **2013**, *61*, 10960–10970. [CrossRef]
- 10. Le, T.N.; Chiu, C.H.; Hsieh, P.C. Bioactive compounds and bioactivities of brassica oleracea l. var. italica sprouts and microgreens: An updated overview from a nutraceutical perspective. *Plants* **2020**, *9*, 946. [CrossRef]
- 11. Teng, J.; Liao, P.; Wang, M. The role of emerging micro-scale vegetables in human diet and health benefits—An updated review based on microgreens. *Food Funct.* **2021**, *12*, 1914–1932. [CrossRef]
- 12. Treadwell, D.; Hochmuth, R.; Landrum, L.; Laughlin, W. *Microgreens: A New Specialty Crop*; IFAS: Gainesville, FL, USA, 2020. [CrossRef]
- 13. Lenzi, A.; Orlandini, A.; Bulgari, R.; Ferrante, A.; Bruschi, P. Antioxidant and mineral composition of three wild leafy species: A comparison between microgreens and baby greens. *Foods* **2019**, *8*, 487. [CrossRef]
- 14. Bliss, R.M. Specialty greens pack a nutritional punch. Agric. Res. 2014, 62, 10–11.
- 15. Negri, M.; Bulgari, R.; Santoro, P.; Ferrante, A. Evaluation of different growing substrates for microgreens production. *Acta Hortic.* **2021**, 109–114. [CrossRef]
- 16. AlShrouf, A. Hydroponics, aeroponic and aquaponic as compared with conventional farming. *Am. Sci. Res. J. Eng. Technol. Sci.* **2017**, 27, 247–255.
- 17. Sharma, N.; Acharya, S.; Kumar, K.; Singh, N.; Chaurasia, O. Hydroponics as an advanced technique for vegetable production: An overview. *J. Soil Water Conserv.* **2018**, 17, 364. [CrossRef]
- 18. Nicola, S.; Pignata, G.; Ferrante, A.; Bulgari, R.; Cocetta, G.; Ertani, A. Water use efficiency in greenhouse systems and its application in horticulture. *AgroLife Sci. J.* **2020**, *9*, 248–262.
- 19. Khan, S.; Purohit, A.; Vadsaria, N. Hydroponics: Current and future state of the art in farming. J. Plant Nutr. 2020, 1–24. [CrossRef]
- 20. Martin, M.; Molin, E. Environmental assessment of an urban vertical hydroponic farming system in Sweden. *Sustainability* **2019**, 11, 4124. [CrossRef]
- 21. Romeo, D.; Vea, E.B.; Thomsen, M. Environmental impacts of urban hydroponics in Europe: A case study in Lyon. *Procedia CIRP* **2018**, *69*, 540–545. [CrossRef]
- 22. Kozai, T.; Niu, G.; Takagaki, M. (Eds.) *Plant Factory: An Indoor Vertical Farming System for Efficient Quality Food Production*; Academic Press: Cambridge, MA, USA, 2019.
- 23. Di Gioia, F.; De Bellis, P.; Mininni, C.; Santamaria, P.; Serio, F. Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (*Brassica Rapa* L.) microgreens. *J. Sci. Food Agric.* **2016**, 97, 1212–1219. [CrossRef]
- 24. Pardossi, A.; Gianquinto, G.P.; Pietro, S.; Incrocci, L. *Orticoltura*. *Principi e Pratica*; Edagricole-New Business Media: Bologna, Italy, 2018.
- 25. Venkateshwaran, N.; ElayaPerumal, A. Mechanical and water absorption properties of woven jute/banana hybrid composites. *Fibers Polym.* **2012**, *13*, 907–914. [CrossRef]
- 26. Cataldo, D.A.; Maroon, M.; 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. [CrossRef]
- 27. Rorem, E.S.; Walker, H.G.; McCready, R.M. Biosynthesis of sucrose and sucrose-phosphate by sugar beet leaf extracts. *Plant Physiol.* **1960**, *35*, 269–272. [CrossRef] [PubMed]
- 28. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1987; Volume 148, pp. 350–382.
- 29. Klein, A.O.; Hagen, C.W. Anthocyanin production in detached petals of impatiens *Balsamina* L. *Plant Physiol.* **1961**, *36*, 1–9. [CrossRef] [PubMed]
- 30. Cocetta, G.; Casciani, D.; Bulgari, R.; Musante, F.; Kołton, A.; Rossi, M.; Ferrante, A. Light use efficiency for vegetables production in protected and indoor environments. *Eur. Phys. J. Plus* **2017**, *132*, 132. [CrossRef]
- 31. Alrifai, O.; Hao, X.; Marcone, M.F.; Tsao, R. Current review of the modulatory effects of led lights on photosynthesis of secondary metabolites and future perspectives of microgreen vegetables. *J. Agric. Food Chem.* **2019**, *67*, 6075–6090. [CrossRef]

32. Loconsole, D.; Cocetta, G.; Santoro, P.; Ferrante, A. Optimization of led lighting and quality evaluation of romaine lettuce grown in an innovative indoor cultivation system. *Sustainability* **2019**, *11*, 841. [CrossRef]

- 33. Paradiso, R.; Proietti, S. Light-Quality Manipulation to Control Plant Growth and Photomorphogenesis in Greenhouse Horticulture: The State of the Art and the Opportunities of Modern Led Systems. *J. Plant Growth Regul.* **2021**, 1–39. [CrossRef]
- 34. Prasad, M. Physical, chemical and biological properties of coir dust. Acta Hortic. 1997, 450, 21–30. [CrossRef]
- 35. Jones-Baumgardt, C.; Llewellyn, D.; Ying, Q.; Zheng, Y. Intensity of sole-source light-emitting diodes affects growth, yield, and quality of brassicaceae microgreens. *HortScience* **2019**, *54*, 1168–1174. [CrossRef]
- 36. Paradiso, V.M.; Castellino, M.; Renna, M.; Gattullo, C.E.; Calasso, M.; Terzano, R.; Allegretta, I.; Leoni, B.; Caponio, F.; Santamaria, P. Nutritional characterization and shelf-life of packaged microgreens. *Food Funct.* **2018**, *9*, 5629–5640. [CrossRef] [PubMed]
- 37. Renna, M.; Castellino, M.; Leoni, B.; Paradiso, V.M.; Santamaria, P. Microgreens production with low potassium content for patients with impaired kidney function. *Nutrients* **2018**, *10*, 675. [CrossRef] [PubMed]
- 38. Kyriacou, M.C.; El-Nakhel, C.; Graziani, G.; Pannico, A.; Soteriou, G.A.; Giordano, M.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chem.* **2019**, 277, 107–118. [CrossRef]
- 39. Cavaiuolo, M.; Ferrante, A. Nitrates and glucosinolates as strong determinants of the nutritional quality in rocket leafy salads. *Nutrients* **2014**, *6*, 1519–1538. [CrossRef]
- Xiao, Z.; Lester, G.E.; Luo, Y.; Wang, Q. Assessment of vitamin and carotenoid concentrations of emerging food products: Edible microgreens. J. Agric. Food Chem. 2012, 60, 7644–7651. [CrossRef]
- 41. Pinto, E.; Almeida, A.A.; Aguiar, A.A.; Ferreira, I.M. Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. *J. Food Compos. Anal.* **2015**, *37*, 38–43. [CrossRef]
- 42. Cavaiuolo, M.; Cocetta, G.; Bulgari, R.; Spinardi, A.; Ferrante, A. Identification of innovative potential quality markers in rocket and melon fresh-cut produce. *Food Chem.* **2015**, *188*, 225–233. [CrossRef]
- 43. Chandra, D.; Kim, J.G.; Kim, Y.P. Changes in microbial population and quality of microgreens treated with different sanitizers and packaging films. *Hortic. Environ. Biotechnol.* **2012**, *53*, 32–40. [CrossRef]
- 44. Xiao, Z.; Luo, Y.; Lester, G.E.; Kou, L.; Yang, T.; Wang, Q. Postharvest quality and shelf life of radish microgreens as impacted by storage temperature, packaging film, and chlorine wash treatment. LWT Food Sci. Technol. 2014, 55, 551–558. [CrossRef]
- 45. Lin, K.-H.; Huang, M.-Y.; Huang, W.-D.; Hsu, M.-H.; Yang, Z.-W.; Yang, C.-M. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. capitata). *Sci. Hortic.* 2013, 150, 86–91. [CrossRef]
- 46. Barrett, D.M.; Beaulieu, J.C.; Shewfelt, R. Color, Flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: Desirable levels, instrumental and sensory measurement, and the effects of processing. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 369–389. [CrossRef] [PubMed]
- 47. Ferrante, A.; Incrocci, L.; Maggini, R.; Serra, G.; Tognoni, F. Colour changes of fresh-cut leafy vegetables during storage. *J. Food Agric. Environ.* **2004**, *2* (*3 and 4*), 40–44.
- 48. Spinardi, A.; Ferrante, A. Effect of storage temperature on quality changes of minimally processed baby lettuce. *J. Food Agric. Environ.* **2012**, *10*, 38–42.
- 49. Prinsi, B.; Morgutti, S.; Negrini, N.; Faoro, F.; Espen, L. Insight into composition of bioactive phenolic compounds in leaves and flowers of green and purple basil. *Plants* **2019**, *9*, 22. [CrossRef] [PubMed]
- 50. Juliani, H.R.; Simon, J.E. *Trends in New Crops and New Uses. Antioxidant Activity of Basil*; ASHS Press: Alexandria, VA, USA, 2002; pp. 575–579.
- 51. Pennisi, G.; Pistillo, A.; Orsini, F.; Cellini, A.; Spinelli, F.; Nicola, S.; Fernandez, J.A.; Crepaldi, A.; Gianquinto, G.; Marcelis, L.F. Optimal light intensity for sustainable water and energy use in indoor cultivation of lettuce and basil under red and blue LEDs. *Sci. Hortic.* **2020**, 272, 109508. [CrossRef]
- 52. Piromalis, D.; Arvanitis, K.G.; Papageorgas, P.; Ferentinos, K.P. Smart precision lighting for urban and landscape closed controlled horticultural environments. In *Molecular Breeding for Sustainable Crop Improvement*; Metzler, J.B., Ed.; Springer: Berlin/Heidelberg, Germany, 2018; pp. 107–140.
- 53. Durmus, D. Real-time sensing and control of integrative horticultural lighting systems. J. Multidiscip. Sci. J. 2020, 3, 20. [CrossRef]