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Review Article

Influence of environmental and nutritional factors on the development of lettuce (*Lactuca sativa* L.) microgreens grown in a hydroponic system: A review

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Abstract

Lettuce microgreens are one of the most popular vegetables due to them being perceived as a "healthy food", with high concentrations of nutrients, beneficial vitamins, and minerals. With a short vegetation period, they can be cultivated with minimum investment, and they are increasingly accepted by consumers, as they are healthy and easy to prepare. Lettuce has high ecological plasticity, but, despite this, its phenotypic expression, morphology, physiology, and anatomy are significantly influenced by environmental conditions. Lettuce microgreens contain higher quantities of phytonutrients and minerals and lower quantities of nitrates at the early stage of development than at the completely developed stage. The environmental conditions that influence the development of lettuce microgreens (and their quality) in a hydroponic system are as follows (average ideal values): light (400 W), photoperiodicity (12 h), light intensity (400 μmol m^{-2} s^{-1}), colour spectrum (440-460 nm), temperature (20 ± 2 °C), and humidity (80 ± 5 %). The nutritional solution in a hydroponic system must be carefully monitored, by checking certain essential parameters such as the following (average ideal values): pH (6.3 ± 0.4), electrical conductivity (1.8 ± 0.2 mS), dissolved oxygen (6 mg L^{-1}), and temperature (18 ± 2 °C). The analysis of expert literature reveals that there is a need to establish certain protocols for cultivating microgreens in hydroponic systems, to minimize the factors that can negatively influence the plants, in order to obtain higher concentrations of active substances.

Keywords: hydroponic system; lettuce; microgreens; review

Introduction

Lettuce (*Lactuca sativa* L.), belonging to the Asteraceae family, is one of the most popular vegetables, especially used in salads; it is consumed in ever-increasing quantities, as it is perceived as a healthy food (Dupont *et al.*, 2000). Additionally, lettuce is increasingly accepted by consumers, as it is healthy and easy to prepare (Teng *et al.*, 2021). The most used parts are the leaves, followed by the stems (for juice) and seeds (for seed mixes). Lettuce is cultivated for its head, which is usually consumed fresh, in different salads, and during the last years as microgreens (cotyledon phase) (Turner *et al.*, 2020). Microgreens have become a culinary trend, due to the relative ease of obtaining them and their promotion sites and production equipment, as well as due

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to research indicating that microgreens may have 4-40 times (Xiao *et al.*, 2012) the amounts of some nutrients and vitamins as the mature plants. Lettuce microgreens can provide higher concentrations of phytonutrients (ascorbic acid, β-carotene, α-tocopherol, and phylloquinone) (Kyriacou *et al.*, 2016) and minerals (Ca, Mg, Fe, Mn, Zn, Se, and Mo) than their mature-leaf counterparts (Pinto *et al.*, 2015). It is important in the human diet due to its contents of vitamins, mineral salts and nutrients (Kim *et al.*, 2016). Due to its short vegetation period and ability to withstand cold weather (Ciuta *et al.*, 2020), it can be cultivated in any season, with minimum investment.

Despite all these advantages of lettuce consumption, there are no clear protocols for the high-quality production of lettuce microgreens in hydroponic conditions. Therefore, this literature review is a systematic approach to organizing the information concerning lettuce, with the evaluation of the general environmental and nutritional conditions, particularly for microgreens in hydroponic systems, in order to identify the possibilities for the control and regulation of their quantity and quality. The purpose of this literature review is to determine all the influencing factors, which would allow the elaboration of a protocol for testing platforms for growing lettuce microgreens hydroponically.

Physico-chemical properties of lettuce

Lettuce has a taproot that may reach up to 60-70 cm. Numerous other roots sprout laterally, which can be up to 10-15 cm long. The leaves develop differently, according to type; first, a leaf rosette is developed, and later, certain types also develop a loose, less stuffed head. Lettuce microgreens are harvested when the seed-leaves have fully expanded and before true leaves have emerged (Reed *et al.*, 2018). Leaves are usually coloured vividly, and they vary among greens, reds or yellows. They are short petiolate, corrugated, and the edges can be smooth or serrated. As it develops, the plant forms a head with different shapes and sizes, according to type. In the case of the types with heads, the leaves inside them are etiolated and softer than the ones from the rosette. The head phase lasts 10–15 days, after which the plants develop flowering stems. A flowering stem is highly branched and has a capitulum with yellow flowers on its top. The stem appears 45-65 days after the lettuce is sown and can reach a height of 1-1.2 m (Ciofu *et al.*, 2004). The flowers are grouped in ovoid cylindrical capitula; they are ligulate or tubular, small and yellow. The fruit is small and may have a white-silver, black or brown colour, as well as a pappus.

Lettuce has a short vegetation period. The microgreens can be harvested at around 15 days, the heads can be harvested at 45-50 days, and after around 120 days, the seeds can be harvested. Lettuce can be stored in cold rooms, at temperatures of 0-1 °C and 95% humidity, for up to 10-12 days.

Lettuce can be found in a range of colours, sizes and forms, selected upon cultivation according to the tenets desired. Although there have been different systems of classification proposed by different groups of researchers throughout the years (Rodenburg, 1960; Lebeda *et al.*, 2007; Mou, 2008; Güzel *et al.*, 2021), there is no standardized classification system, due to the high genetic and morphological diversity in the types of lettuce (Kim *et al.*, 2016). According to Mou (2008), there are six main lettuce types based upon the leaf shape, size, texture, head formation, and stem type (Kim *et al.*, 2016). They are (1) crisphead lettuce (var. *capitata* L. nidus jaggeri Helm), (2) butterhead lettuce (var. *capitata* L. nidus tenerrima Helm), (3) romaine or cos lettuce (var. *longifolia* Lam., var. *romana* Hort. in Bailey), (4) leaf or cutting lettuce (var. *acephala* Alef., syn. var. *secalina* Alef., syn. var. *crispa* L.), (5) stem or stalk (*Asparagus*) lettuce (var. *angustana* Irish ex Bremer, syn. var. *asparagina* Bailey, syn. L. *angustana* Hort. In Vilm.), and (6) Latin lettuce (no scientific name).

The nutritional qualities and properties of healthy foods are attributed to high contents of antioxidant compounds (vitamins A, C and E, carotenoids, and polyphenols), iron and calcium (Romani *et al.*, 2002), folic acid and fibre (Pepe *et al.*, 2015; Sofo *et al.*, 2016; Van Treuren *et al.*, 2018), and anthocyanins and chlorophylls, with anthocyanins being more abundant in red varieties (Liorach *et al.*, 2008). Lettuce is typically low in calories and packed with fibre (Liorach et al., 2008), and the fresh leaves are an excellent source of vitamin A and β-carotene. Just 100 g of fresh, raw lettuce provides 24% of the daily recommended intake of vitamin A and β-carotene, but high quantities of minerals such as iron, calcium, magnesium, and potassium are also present, which are essential for the human body's metabolism.

However, the nutritional value of lettuce varies among the different varieties (Kim *et al.*, 2016). According to Mou and Ryder (2004), the lower nutritional value of some varieties is due to the high closure of their leaves in the head structure, because most of the edible portion of the head structure includes leaves that are not exposed to light (Ryder, 1997). The contents of beta-carotene (provitamin A), Ca, and Fe were higher in the outer leaves than in the inner leaves of a normal lettuce head, while there was little difference in the vitamin C content between the two classes of leaves. Opening the lettuce head greatly artificially increased the nutrient contents to levels comparable to those for leaf or romaine lettuce. When romaine lettuce leaves were closed, the levels of beta-carotene and vitamin C dropped to a minimum and the contents of Ca and Fe were also largely reduced. These results suggest that the lower nutritional value of crisphead lettuce is due to the enclosure of its leaves in the head structure (Mou and Ryder, 2004). Similar results were also obtained by Park *et al.* (2018), who showed that the total phenolic content was higher in red skirt methanolic extracts, at 64.9 ± 0.3 mg gallic acid g^{-1}, while the phenolic content of green skirt was 49.4 ± 0.3 mg gallic acid g^{-1}.

General ecological requirements

Lettuce has high ecological plasticity, but in spite of this, its phenotypic expression, morphology, physiology, and anatomy are significantly influenced by growing conditions.

Light and temperature are considered among the most important environment conditions for the development of lettuce (Ojeda *et al.*, 2012). Solar radiation, especially long-wave red light, is a limiting factor for the germination of lettuce seeds (Hiroshi, 1964; Hegarty and Ross, 2006). Even under conditions of salt stress, seed pretreatment with $CaCl_2$, H_2O_2, and sodium nitroprusside (SNP) led to successful seed germination (Hajihashemi *et al.*, 2020). Temperatures above 24 °C cause significant changes in the endosperm layer, generating a mechanical force that counteracts and may even inhibit germination (Takeba and Matsubara, 1976; Sung *et al.*, 1997). Takaki and Zaia (1984) showed that these two environmental factors could be correlated, as high temperatures also affect the phytochromes, which are phototransmitters acting on blue and red light, causing an expansion of leaves, reduced germination, and problems with the photoperiod.

Lettuce is a plant without high heat requirements, and it resists cold weather well; its temperature tolerance according to development stage is as follows: At 5-10 °C, seeds germinate and plants begin to sprout 6-10 days after sowing. Lettuce resists temperatures ranging between -5 and -6 °C, if it is in the rosette phase with 5-6 leaves, and it can also resist temperatures of -2 and -3 °C in the phase of head formation and stem development. The best temperature at which for leaves to grow and form heads is around 16-20 °C (depending on the light intensity), and a temperature ranging between 20 and 22 °C is needed for the stem and flowers to form (Ciofu *et al.*, 2004). It is important to specify that, at high temperatures, over 25 °C, certain types of lettuce do not sprout, the leaves etiolate, and the head does not form accordingly.

Lettuce needs a lot of light, as sufficient light influences the vegetation period. Lettuce is a long-day plant. Under long-day conditions, it has a short vegetation period and forms flower stems before forming normal heads. Under such conditions, most types form a flowering, branched stem, and flowers are formed on top of them, disposed in calathidium inflorescences. Under short-day conditions (<12 h), they form a rich foliar apparatus and big heads, which do not quickly progress to floral stems. Types that are less influenced by day length (12-14 h) have been created.

Lettuce prefers a pH of 6-7.2; an excessively acid pH must be avoided. Macroelements are important; phosphorus contributes to speeding up head formation, while potassium promotes the formation of a stuffed head. Nitrate stimulates head growth and increases production.

Lettuce needs high humidity, especially when it sprouts and when heads are formed. Thus, when it is cultivated on the ground, the best humidity is 70-80% of the interval of active humidity. Should humidity be low and temperatures high, then the crop yield shall be low. However, excess humidity must also be avoided, as it leads to diseases.

Lettuce must be harvested during cool, but dry, weather because, if the leaves are wet, they can easily become altered.

Main diseases and pests of lettuce

The most serious threat to microgreens appears to be *Pythium* (McGehee *et al.*, 2019). The main diseases and pests that can emerge in the lettuce crop are presented in Table 1 (Ciofu *et al.*, 2004). For microgreens, it is very important to prevent the transmission of bacteria or viruses (Misra, 2020). This is because there is no cooking step to kill the bacteria or viruses in the products before they enter microgreen salads. Washing does not necessarily remove them either, so prevention is essential. Microbial biofungicides can be introduced in nutrient solutions through the nutrient film technique or applied in irrigation to prevent *Pythium* root (McGehee *et al.*, 2019).

Table 1. The main diseases and pests that can damage the lettuce crop

No.	Diseases (1–9) and pests (10–14)	Scientific name	Description
1	Lettuce blight	*Bremia lactucae*	It appears both in field crops and in protected spaces, under high humidity and temperatures ranging between 10 and 21 °C. This disease affects the development of lettuce and the quality of the harvest. The attack can be recognized based on the emergence of yellow angular spots on the upper part of leaves and of fuzz on their lower parts.
2	Grey mould	*Botrytis cinerea*	The attack takes the form of grey-brown to brown-orange spots, with a watery appearance, located on the leaf veins or on the leaves from the base. This disease can rapidly affect the entire plant, even after harvest. If it is not treated in time, plants become discoloured and die.
3	White mould	*Sclerotinia sclerotiorum*	It is the most aggressive ground disease of lettuce. It attacks the stem and the leaves from the base of the plant, and it can lead to the rotting of the collet but also of the leaves from the base, close to the ground.
4	Falling of small plants	*Pythium ultimum*	This disease especially attacks seedlings, and it emerges under conditions of high soil humidity and temperatures over 20 °C. The fungus attacks the seedlings around the collet, towards the root and the stem, leading to their collapse.
5	Lettuce anthracnose	*Microdochium panattonianum*	The signs of the attack include spots with a humid aspect, on both sides of leaves, yellow on the inside and brown on the outside, 2–3 mm long. The spots grow step by step; they become irregular and lead to leaf dryness and necrosis.
6	Lettuce fusarium	*Fusarium oxysporum*	This disease attacks even from the seedling phase, and the wooden vassals of roots become red to brown, while leaves become yellow; they become discoloured and die. Plants affected by fusarium cannot form heads.
7	Lettuce mildew	*Golovinomyces cichoracearum*	This disease especially attacks mature leaves and can be recognized based on the emergence of a white powder on both sides of leaves. Subsequently, affected leaves lose colour and deform.
8	Cercosporiosis	*Cercospora longissima*	The attack can be recognized based on the emergence of brown oval spots, with a white centre, with a diameter of 1 cm, and if temperatures are high (25–35 °C), the spots increase and affect a great part of leaves.

9	Verticillium	*Verticillium dahliae*	The attack appears at the plant base, and leaves become yellow and fade, but they remain attached to the head. The root tissues can turn different colours (brown, black, and green), appearing as stripes.
10	Gray limax	*Deroceras agreste*	This pest mostly attacks at night or in rainy weather, and it generally takes shelter in the ground. The attack can be recognized based on the presence of holes in the centre and on the leaf edges.
11	Black flea	*Phyllotreta atra*	It attacks leaves but also flowers and seeds. In a massive attack, poorly developed plants can be entirely destroyed.
12	Wireworms	*Agriotes spp.*	They attack lettuce crops both in the field and in greenhouses, and they have a generation every 4 or 5 years.
13	Cabbage fly	*Aleyrodes brassicae*	This pest attacks the lettuce leaves; the plants affected turn yellow, and their development is halted.
14	Cucumber beetle	*Aphis gossypii*	They suck the cell sap, and affected leaves lose colour, deform, or become dry.

Hydroponic environmental conditions

Under hydroponic conditions, microgreens reach harvest maturity fast, which allows many crop cycles per year. The study of plants in controlled environments facilitates the understanding of factors that influence their development, especially the active principles of their vegetative organs. The complexity of these types of studies results from the fact that hydroponic microgreens are influenced by a great number of environment and nutritional factors, and these must therefore be correlated and analysed in detail in order to develop appropriate cultivation protocols (Zheljazkov *et al.*, 2012; Mbarki *et al.*, 2020). This leads to the improvement of the quality indices, purity, consistency, bioactivity, and biomass of products, on a commercial scale (Hayden, 2006). A hydroponic crop offers greater control and flexibility for the crop environment than a field crop, through the specifications of the system used (Sheikh, 2006; Cruz *et al.*, 2012; Van Treuren *et al.*, 2018).

The biochemical profile of plants is an indicator of their development conditions. The primary metabolism of plants is responsible for their survival and produces metabolites such as sugars, amino acids, vitamins, etc. (Giurgiu, 2016). The secondary metabolism of plants is not vital for survival, but the resulting metabolites impart colour, taste and smell to products, play defensive roles, etc., and they are valued in the pharmaceutical, food and cosmetic industries (Isah *et al.*, 2018). The concentrations of these substances fluctuate a lot because of factors that stimulate secondary metabolism. At the same time, the metabolites differ from one species to another and even within the same species (based on the development phase). The factors that influence the accumulation of bioactive substances are wide-ranging, but they can be grouped in two categories: factors of biotic and abiotic stress (Giurgiu, 2016; Lenzi *et al.*, 2019).

The results obtained highlight the possibility of exploiting genetic biodiversity in order to obtain tailored microgreens with the desired nutritional profiles, with particular regard to mineral nutrients and bioactive compounds (Paradiso *et al.*, 2018; Bian *et al.*, 2020; Ghoora *et al.*, 2020). Functionally, phytonutrient-packed and principally raw food is in high demand (Treadwell *et al.*, 2011). Microgreens constitute such a novel functional food that combines high sensory and bioactive value (El-Nakhel *et al.*, 2020) but also a dense phytochemical content.

Influence of environmental conditions on the quantity and quality of microgreen production in hydroponic system

The environmental conditions that influence the development of plants in a hydroponic system are light (photoperiodicity, light intensity, colour spectrum, and position), temperature, and humidity.

The light conditions have a strong influence on the development of lettuce microgreens, as well as on the biosynthesis and accumulation of chemical compounds (Lin *et al.*, 2013). Light systems with LEDs (light-emitting diodes) have important advantages over traditional ones due to their spectral composition, durability, wavelength specificity, low radiant heat, and energetic efficiency (Urrestarazu *et al.*, 2016). LEDs are important lamp types because the concentration of the light spectrum they emit can be changed to provide plants at various developmental stages with the required light spectrum (Olle and Alsiòa, 2019). The quality effects of the light spectrum on the production features and the influence on the contents of bioactive compounds in lettuce are well-known (Ohashi-Kaneko *et al.*, 2007; Samuolienė *et al.*, 2017). The photoresponses of antioxidant compounds suggest that the photoprotective mechanism is stimulated by both light-dose-dependent and wavelength-dependent reactions (Carvalho and Folta, 2016; Samuoliene *et al.*, 2016) Lettuce growth is influenced by light colour (Table 2; Olle and Alsiòa, 2019). Far-red lighting increases the number of leaves, elongates the leaves, and promotes the growth of the lettuce, resulting in taller plants (Yuyao and Krishna, 2021).

Table 2. The effect of light colour on growth of lettuce

No.	*Lactuca sativa* L.	Reference	Light colour	Effect on growth
1	Lettuce-Green Salad Bowl	Legendre and van Iersel (2021)	Far-red, 700-800 nm	Increasing supplemental far-red light increased leaf length and width, which was associated with increased projected canopy size
2	Green leaf lettuce (Lobjoits green cos) and red leaf lettuce	Viršilė *et al.* (2020)	Green, 510 nm	Green light had reasonable impact on the contents of nutritive primary metabolites in red and green leaf lettuce
3	Lettuce-var. *foliosum* cv. 'Dubacek' and cv. 'Michalina'	Sergejeva *et al.* (2018)	Blue, 440 nm	Compact plant morphology; impact of illumination source on the dry matter content significantly depended on cultivar and sampling time
4	Lettuce-Frillice Crisp	Pinho *et al.* (2017)	Far red, 700-850 nm	Addition of far-red light increased leaf area index; faster growth may have caused decrease in dry weight content
5	Lettuce-Sunmang seedlings from 16 days old	Lee *et al.* (2016)	Far red, 700-850 nm	Improved shoot and root growth; far-red LEDs improved lettuce growth and bioactive compound content in a closed-type plant production system
6	Red lettuce-Sunmang	Lee *et al.* (2015)	Far red, 700-850 nm	The number of leaves increased, and the leaves were longer; the results of this study suggest that the supplementation with far-red LEDs should be considered when designing artificial lighting systems for closed-type plant factories
7	Lettuce-cultivars, red leaf Sunmang and green leaf Grand Rapid TBR, 18-day seedlings for 4 weeks	Son and Oh, 2015	Green, 490-550 nm	The substitution of blue with green LEDs in the presence of a fixed proportion of red enhanced growth of lettuce
8	Red leaf lettuce-cv. 'Banchu Ref Fire'	Johkan *et al.* (2012)	Green, 490-550 nm	High-intensity (300 μmol m^{-2}s^{-1}) green LED light promoted lettuce growth; 510 nm light had the greatest effect on plant growth
9	Red leaf lettuce seedlings-cv. 'Banchu Red Fire'	Johkan *et al.* (2010)	Blue, 425-490 nm	Resulted in compact lettuce seedling morphology; promoted the growth of lettuce after transplanting
10	Baby leaf lettuce-Red Cross	Li and Kubota, 2009	Far red, 700-850 nm	The fresh weight, dry weight, stem length, leaf length, and leaf width significantly increased by 28%, 15%, 14%, 44%, and 15%, respectively, with supplemental FR light compared to white light
11	Red leaf lettuce-Outeredgeous	Stutte *et al.* (2009)	Far red, 700-850 nm	Leaf elongation; total dry weight of plants grown under red LEDs alone was ≈20% lower than that of plants with blue or far red added
12	Red leaf lettuce-cv. 'Outeredgeous'	Stutte *et al.* (2009)	Blue, 425-490 nm	Leaf expansion; the addition of blue light allowed full development of anthocyanin to occur

In an experiment conducted by Kim *et al.* (2021), 14 days after sowing, the combined effects of light intensity (photosynthetic photon flux density, PPFD: 100, 250, and 500 μmol $m^{-2}s^{-1}$) and electrical conductivity (EC level: 0.8, 1.4, and 2.0 dS m^{-1}) meant that the photosynthesis rate, stomatal conductance, transpiration rate, and water use efficiency of Indian lettuce increased as the light intensity increased. Considering the growth and functional material contents, the appropriate light intensity and EC level for the hydroponic cultivation of Indian lettuce are PPFD 500 with EC 2.0, and PPFD 100 and 250, which are low-light conditions, under which EC 0.8 is suitable for growth. LED light with a blue light (B) and red light (R) combination (BR) has been widely used for horticultural crop production in controlled environments (Goto, 2012). BR (containing 5% to 15% B) at a PPFD of ≈300 to 400 μmol $m^{-2}s^{-1}$ and a photoperiod of ≈16 h appears to be optimal for the indoor production of microgreens based on crop yield and appearance quality (Ying *et al.*, 2020). However, the impact of varying the proportions of these light qualities on the composition of diverse phytochemicals in indoor-grown microgreens is unclear (Ying *et al.*, 2021).

Various studies show that lettuce microgreens contain higher quantities of phytonutrients and minerals and lower quantities of nitrates at the early stages of development than at the completely developed stage (Lester *et al.*, 2010; Pinto *et al.*, 2015). Thus, according to Ferron-Carrillo *et al.* (2021), consuming lettuce as microgreen lettuce may be preferable to consuming it at the baby leaf stage. Lettuce contains significant concentrations of carotenoids at the microgreen stage. The amounts of nitrate were lower at the microgreen stage than at the baby leaf stage, and the nitrate content was inversely correlated with the carotenoid content, which, in tissues, was higher at the microgreen stage and influenced by LEDs. Three different LED lamps were tested (Ferron-Carrillo *et al.*, 2021): artificial white light, continuous light-emitting diodes with a longer blue wavelength, and continuous light-emitting diodes with a longer red wavelength. The accumulation of nitrates in plant tissues was clearly lower during the initial stages than during the final stages of crop development, ranging from 50.2 to 73.4 mg 100 g^{-1} fresh weight for continuous light-emitting diodes with a longer red wavelength.

According to Zha *et al.* (2020), ascorbate tends to be present at higher levels in lettuce cultivated under LEDs supplying 75% blue and 25% red light relative to those delivering 25% blue and 75% red, and 50% blue and 50% red light. Pre-harvest supplemental lighting using UV-A and blue or red/blue LED lighting can increase the growth and nutritional quality of lettuce grown hydroponically (Brazaitytė *et al.*, 2015; Verdaguer *et al.*, 2017; Hooks *et al.*, 2021). We can conclude that, by manipulating the colour of the lamps used, we can better support and direct the yield, growth, and nutrition of plants.

The photoperiodicity has a significant impact on the development of the lettuce crop, but for microgreens, good results have been obtained over very long periods of time: 12/12 h (day/night) (Paradiso *et al.*, 2018; El-Nakhel *et al.*, 2020) and 16/8 h (Ferron-Carrillo *et al.*, 2021).

Lettuce microgreens are less demanding regarding temperature, requiring 20° C (Paradiso *et al.*, 2018; Ferron-Carrillo *et al.*, 2021) or a differentiated 24/18 °C (light/dark) cycle (El-Nakhel *et al.*, 2020).

A low relative air humidity reduces the risk of disease. For lettuce microgreens, the ideal humidity is 80% (Paradiso *et al.*, 2018), 80-85% (Ferron-Carrillo *et al.*, 2021), or an alternating 65%/80% (light/dark) cycle (El-Nakhel *et al.*, 2020).

Nutritional solution in the hydroponic system

The nutritional solution in the hydroponic system must be carefully monitored, by checking certain essential parameters such as the pH, electrical conductivity, dissolved oxygen, and temperature. The nutritional solution plays an important role in the growth and development of plants. We can note its composition for obtaining lettuce microgreens from the literature (Table 3).

Table 3. The composition of the solution used to obtain lettuce microgreens

No.	Authors	Description
1	Hooks *et al*. (2021)	100 N, 20 P, 129 K, 90 Ca, 26 Mg, 35 S, 1.3 Fe, 0.21 B, 0.14 Mn, 0.08 Zn, 0.04 Cu, and 0.03 Mo mg L^{-1}; electrical conductivity of 1.5 dS m^{-1} and a pH of 6.0
2	Ferron-Carrillo *et al*. (2021)	Standard nutrient solution, at pH 5.8 and EC 2.2 dS m^{-1}
3	El-Nakhel *et al*. (2020)	9.0 mM N-NO^{3-}, 2.0 mM S, 1.0 mM P, 4.0 mM K, 4.0 mM Ca, 1.0 mM Mg, 1.0 mM NH4+, 15 M Fe, 9 M Mn, 0.3 M Cu, 1.6 M Zn, 20 M B, and 0.3 M Mo; resulting in an electrical conductivity of 1.5 dS m^{-1} and a pH of 6.0
4	Renna *et al*. (2018)	119 N, 16 P, 24 Mg, 116 Ca, 54 S, 1.12 Fe, 0.27 Mn, 0.13 Zn, 0.27 B, 0.03 Cu, and 0.01 Momg L^{-1}; which resulted in an electrical conductivity of 1.8 dS m^{-1} and pH of 6.3
5	Paradiso *et al*. (2018)	105 N, 15 P, 117 K, 100 Ca, 24 Mg, 0.25 B, 0.01 Cu, 2.5 Fe, 0.25 Mn, 0.025 Zn, and 0.005 Mo mg L^{-1}

A concentration of oxygen dissolved in water over 6 mg O_2 L^{-1} min^{-1} prevents the development of disease in the nutritional solution (Msayleb, 2014). The nutritional solution must be maintained at a low temperature (under 20 °C) in order to maintain the quantity of oxygen dissolved in the water under optimal parameters (6 mg L^{-1}).

Based on the literature analysed, Table 4 shows the average values for the growth and development of lettuce microgreens.

Table 4. Needs of lettuce microgreens grown in a hydroponic system

No.	Parameter	Unit of measurement	Average value of parameter
1	Light	W	400
1.1	Photoperiodicity	h	07:00–20:00 (12 h)
1.2	Light intensity	$\mu molm^{-2}s^{-1}$	400
1.3	Colour spectrum	nm	440-460
1.4	Position	cm	150-Lamps, HPS (high-pressure sodium) 40-Lamps, LED
2	Temperature	°C	20 ± 2
3	Humidity	%	80 ± 5
4	Nutrients	N–P–K: 3:2:3 (5:1:5)	Changed every 10 days
5	pH	pH units	6.3 ± 0.4
6	Electrical conductivity	mS	1.8 ± 0.2
7	Dissolved oxygen	mgL^{-1}	6
8	Temperature	°C	18 ± 2

Nutrient solution and production quality

The mineral composition of microgreens can be modified through the solution used. Thus, for example, for people with impaired kidney function, small plants low in potassium can be obtained (Alvarado-Camarillo *et al.*, 2020). Renna *et al.* (2018) used lettuce (cultivar Bionda da taglio) grown using a hydroponic system with different potassium (K) levels (0, 29.1, 58.4, and 117 mg L^{-1}) in order to produce microgreens with a low potassium content. The K content in the microgreens was successfully reduced by using a nutrient solution, without K or with 29.1 mg K L^{-1}, which supplied between 103 and 129 mg of K 100 g^{-1} shoot fresh weight (about 7.7-8.6% of the daily K intake that was recommended for patients affected by chronic kidney disease).

The contents of nitrates in lettuce and other vegetables depends on several factors: the (i) variety (Sotelo *et al.*, 2007), (ii) growing season (Ferron-Carrillo *et al.*, 2021), (iii) wavelength of light received by the plant, (iv) crop system, and (v) fertigation (Urrestarazu *et al.*, 1998). In 2011, the European Union regulated the maximum allowable concentrations as 500 and 400 mg NO_3^- 100 g^{-1} fresh weight for lettuce harvested in winter and spring, respectively, and 250 mg NO_3^- 100 g^{-1} fresh weight for iceberg lettuce (ECR, 2011).

By comparing the mineral, phytochemical, and antioxidant capacity attributes of two pigmented lettuce *capitata* cultivars (green and red Salanova) harvested at the microgreen and mature-leaf stages, El-Nakhel *et al.* (2020) obtained the following results:

- The calcium and magnesium were higher in microgreens irrespective of the cultivar; conversely, phosphorous, potassium, and nitrate were higher in mature leaves;
- All the pigments including chlorophyll, lutein, and beta-carotene increased at the advanced maturity stage and were more concentrated in the red-pigmented cultivar at both stages;
- The total polyphenols accumulated more densely in red Salanova, particularly in the microgreen stage, whereas, in green Salanova, the accumulation was significant but less pronounced in the microgreen stage;
- Chlorogenic acid, quercetin malonyl glucoside, rutin, and coumaroyl quinic acid were the most concentrated phenolic acids in microgreens, while feruloyl tartaric acid was predominant in mature leaves.

Pinto *et al.* (2015) and Weber (2016) obtained similar results by comparing the mature leaves of lettuce with lettuce microgreens and their respective mineral profiles. According to Pinto *et al.* (2015), microgreens possess higher contents of most minerals (Ca, Mg, Fe, Mn, Zn, Se, and Mo) and a lower NO_3^- content than mature lettuce. Consequently, microgreens can be considered a good source of minerals in the human diet (Elena and Ernst, 2015), and their consumption could be an important strategy for meeting children's dietary mineral requirements without exposing them to harmful NO_3^-. By using existing information on microgreens and the available technology, which is continually evolving, the range of natural products used for the treatment of many diseases can be enriched (Hossain, 2011).

Lettuce microgreens can be considered good sources of Ca and K, and they also contain the highest levels of carotenoids. The microgreens also present higher amounts of tocopherol and carotenoids than mature vegetables (Kyriacou *et al.*, 2016; Paradiso *et al.*, 2018). In addition, the nutraceutical value of microgreens can be further improved through Se biofortification, delivering Se-enriched foods and, potentially, an enhanced content of bioactive compounds (Pannico *et al.*, 2020). Improving the availability of Se to plants is, therefore, a potential pathway to overcoming human Se deficiencies (Hossain *et al.*, 2021).

However, lettuce microgreens typically have a short shelf life due to rapid product deterioration (Mir *et al.*, 2017). Due to their delicate tissues, fresh cut microgreens showed a shelf life not exceeding 10 days at 5 °C (Paradiso *et al.*, 2018). Harvesting with the roots can help to retain the freshness of hydroponic lettuce (Suo *et al.*, 2021); the optimal length of root retention for hydroponic lettuce was found to be 9 cm. Changing the lettuce's characteristics through certain treatments such as light and allicin treatment can extend its shelf life (Peng *et al.*, 2015; Ufuk and Rezzan, 2017).

The control of pathogens is a major issue for the lettuce microgreen crop. Hydrogen peroxide (H_2O_2) is an oxidizing agent used to disinfect recirculated irrigation water during production. According to Eicher-Sodo *et al.* (2019), the maximum quantity that can be applied, without reducing the quality, aspect, and physiological growth is 125 mgL^{-1} (Othilie), 75 mg L^{-1} (Rouxai), and 125 mg L^{-1} (Xandra). The lettuce microgreen hydroponic system uses less water due to the constant reuse of nutrient solution, and it also reduces the risk of soilborne diseases (Bevly *et al.*, 2016). As there is the potential for phytopathogens to develop, it is very important to take measures during production to eliminate them (Aruscavage *et al.*, 2008; D'Souza *et al.*, 2015). Recent research shows the possibility of intervening before and after harvest, in the form of calcium treatments, active food packaging, and the control of temperature and light, in order to maintain the quality, increase the nutritional value, and extend the retail period (Dainelli *et al.*, 2008; Chandra *et al.*, 2012; Turner *et al.*, 2020).

Conclusions

Lettuce microgreens have interesting nutritional properties, mainly regarding their contents of mineral nutrients and bioactive compounds, and they have notable potential for improving the nutritional content of the human diet. Appropriate pre- and postharvest strategies should be developed, to allow lettuce microgreens to retain their nutritional value for as long as possible.

Hydroponic lettuce microgreens require the application of chemical nutritional solutions, which should meet the nutritional demands of plants according to their stages of development. In order to control any risk, the nutritional solution must be carefully monitored by checking essential parameters such as the pH, electrical conductivity, temperature, and oxygen dissolved in water, as well as the environmental conditions of light (photoperiodicity, light intensity, colour spectrum, and position), temperature, and humidity. The optimal intervals for these parameters for hydroponic growth are different from those for classical soil growth.

Planting crops in protected environments together with hydroponic systems could lead to the establishment of protocols for cultivating microgreens that minimize factors that negatively influence plants, in order to obtain higher concentrations of active substances.

Authors' Contributions

TR: Writing - original draft; Conceptualization; Project administration; PIM: Writing - review and editing; Data curation; Investigation; OSM: Writing - review and editing; Data curation; Investigation;

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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