


Environmental factors influencing the growth and pathogenicity of microgreens bound for the market: a review

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Review Article

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Abstract

The world is experiencing a global push toward smart agriculture to help feed the burgeoning population by increasing food security while reducing the carbon footprint of food production. The guidelines for healthy eating have increased globally from five to seven servings of vegetables a day and this had led to the quest for a sustainable form of vegetable production that will reduce the carbon footprint and still provide consumers with the required nutrients. Microgreens contain more nutrients than some mature vegetables and can be cultivated on vertical farms, offering a different approach with the potential to resolve environmental and health challenges. Microgreens are young plantlets grown from the seeds of edible leafy vegetables and are usually eaten raw. They contain high levels of bioactive compounds and can be processed into oils to create valuable cosmetic products. Microgreens have become well-known to chefs and are gaining popularity in upmarket grocery outlets. Consequently, growing microgreens are presenting huge market opportunities worldwide. Their nutritional benefits, easy production methods and short production cycle are some of the reasons they are attractive to growers. The most important factors affecting the growth of microgreens are micro and macro-climates. One challenge to producing microgreens is that the growing environment is ideal for microbial organisms to thrive. As such, microgreens are prone to food-borne pathogens such as *E. coli*, *Listeria* and *Salmonella*. Consequently, the microgreens industry is facing various setbacks including product recalls from *Salmonella* and *Listeria* food poisoning outbreaks. In addition, the short shelf-life of microgreens is a serious challenge for getting microgreens to market, this is driving studies in several post-harvest treatments. This review examines the nutrient content and health benefits of microgreens and factors affecting microgreens' growth: temperature, humidity, photoperiod, fertilization, etc. and post-harvest treatments, all of which can potentially impact microbial growth, the phytochemical content and the physical appearance of microgreens bound for the market.

Introduction

Microgreens often called 'vegetable confetti' are vegetable or herb products that have become a new source of food for western countries (Kyriacou *et al.*, 2020). These are the seedlings of edible plants harvested 7–14 days post-sowing (Turner *et al.*, 2020) or 10–20 days after the seedling emerges depending on the species [Lee *et al.*, 2004; Xiao *et al.*, 2012; Choe *et al.*, 2018 (Fig. 1)] and without the roots (Fig. 2). They are usually harvested when they develop their first true leaves (Bergšpica *et al.*, 2020; Turner *et al.*, 2020) or when they have one or two fully developed cotyledons, with or without the emergence of the first true leaves (Xiao *et al.*, 2012) or with only one fully developed cotyledon for monocots (Fry, 2016; Zhang *et al.*, 2021). They can be grown from seeds of a wide variety of plants including both vegetables and herbs (Ebert, 2014) from different plant families: Brassicaceae family—broccoli & cabbage, Asteraceae family—Lettuce, Amaryllidaceae family—Garlic, Apiaceae family—carrot (Kaiser and Ernst, 2018; Turner *et al.*, 2020).

Microgreens were first used by chefs in an up-market restaurant in Los Angeles as an ingredient to enhance the flavor, color and texture of various dishes in the 1980s (Gerovac *et al.*, 2016). They are now found widely in restaurants where they are used in fresh salads, soups and sandwiches (Kaiser and Ernst, 2018; Kyriacou *et al.*, 2021) as well as in grocery and health-food outlets (Yanes-Molina, *et al.*, 2019). They have been used for visual or flavor enhancement and as food supplements for people who focus on a healthy lifestyle (Teng *et al.*, 2021).

Microgreens nutrient content

Vegetables are important in the diet to prevent and control diseases in humans (Vauzour *et al.*, 2010; Sedani *et al.*, 2018; Gonzali and Perata, 2020; Rani *et al.*, 2021). Millions of people

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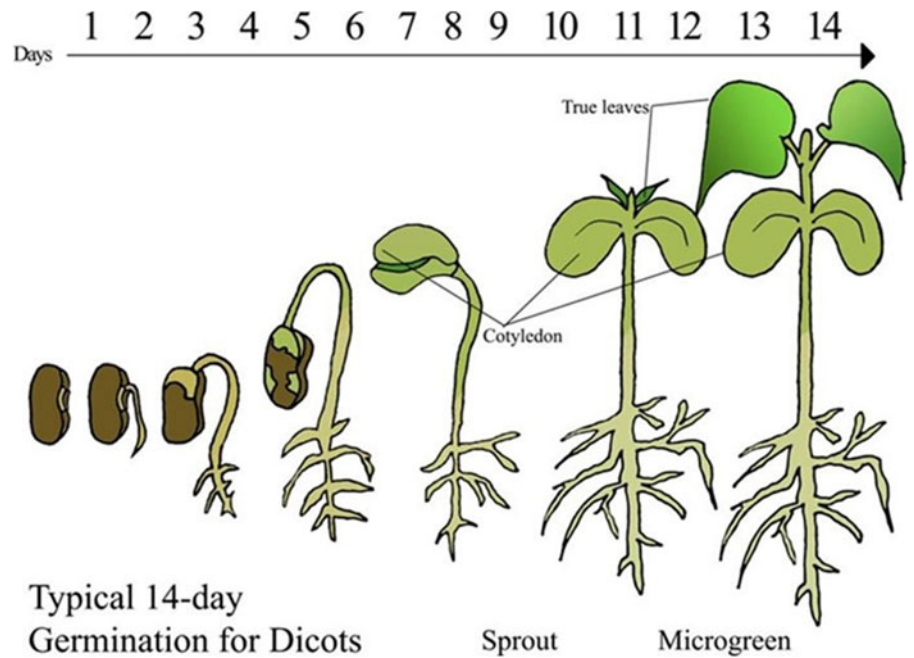


Fig. 1. Microgreens and sprouts differ by age at harvest. Source: Riggio *et al.* (2019). Microgreens are harvested above ground whereas sprouts' roots are consumed.

around the globe suffer from chronic diseases such as diabetes and cardiovascular diseases (Choe *et al.*, 2018; Powell-Wiley *et al.*, 2021). According to WHO (2003a), insufficient fruit and vegetable intake was estimated to cause about 2.7 million deaths annually and was among the top 10 risk factors contributing to the yearly death rate. Consequently, the WHO asked countries to carry out targeted campaigns to increase the consumption of fruits and vegetables (WHO, 2003b). Furthermore, statistics from the Health Service Executive (HSE) (2016), approximately 37% of people in Ireland have normal body weight, 37% are overweight and a further 23% are obese. Therefore, the HSE Healthy

Eating Guidelines for Irish people over the age of five years recommends that Irish people should eat up to seven servings of fruits and vegetables a day (HSE, 2016), and the WHO and FAO suggested that the global vegetable intake be increased to five servings a day (WHO, 2003b). Consequently, Microgreens have been suggested as a vegetable alternative that would help reduce global food insecurity, and complications arising from malnutrition (see Fig. 3 below)

Although the nutrient levels found in some mature vegetables are less than the adult recommended daily allowance (RDA) [Table 1 (Choe *et al.*, 2018)], many studies have reported that



Fig. 2. Microgreens are harvested above the roots, few inches above soil level. Source: Growingjourney.com.

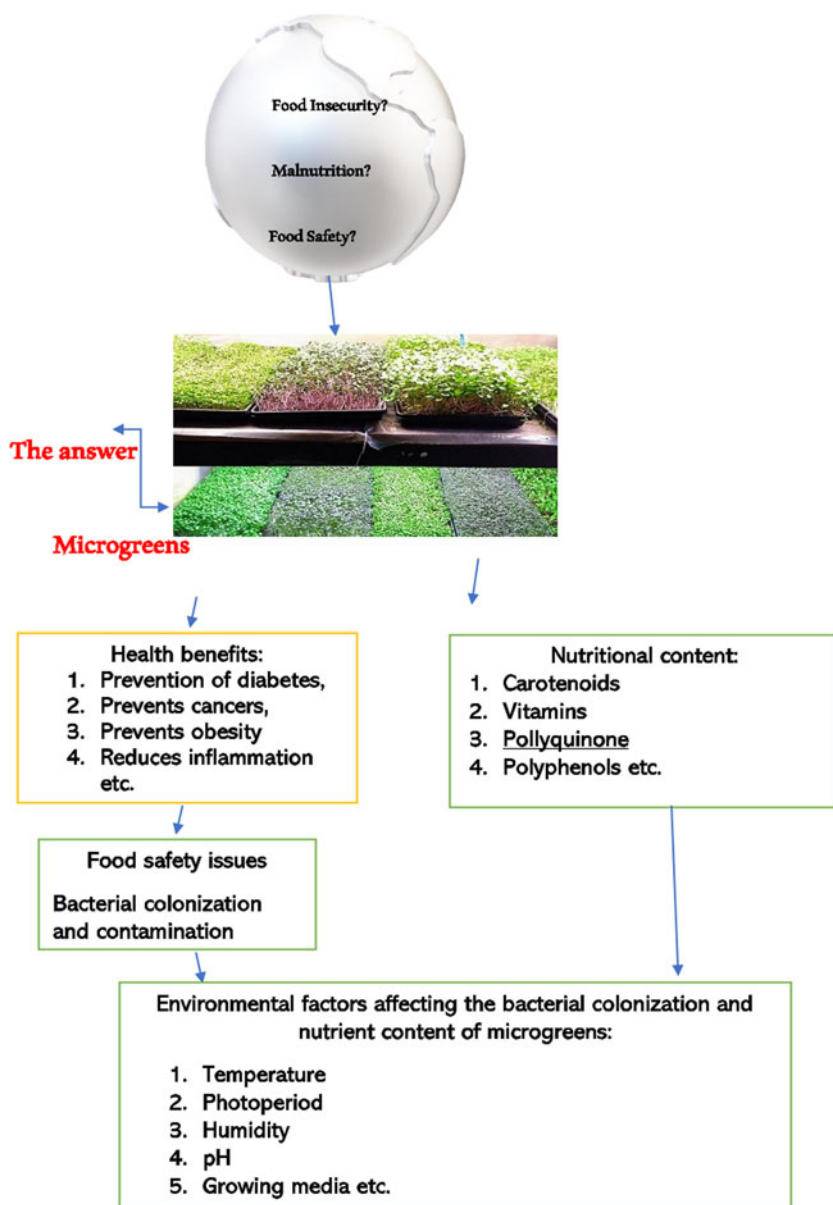


Fig. 3. Microgreens, the Answer to global food insecurity, malnutrition and food safety.

some microgreens contain more of these compounds and other health-promoting micronutrients than their mature counterparts (Johnson *et al.*, 2021; Teng *et al.*, 2021). However, the exact quantity per microgreen serving is not clearly stated. It will be important to ascertain how much of this compound is contained in a serving of microgreens.

The following vitamins and minerals have been identified in some microgreens.

Phylloquinone

Phylloquinone is vital for blood coagulation and bone restoration (Palermo *et al.*, 2017; Beato *et al.*, 2020), and has been identified in some microgreen species (Xiao *et al.*, 2019). A study from the USDA National Nutrient Database (2011), reported that red cabbage microgreens had a 6-fold higher concentration of vitamin C than previously published data for mature red cabbage (24.4 mg 100 g FW⁻¹) 17 and 2.6 times greater (57.0 mg 100 g FW⁻¹) than that recorded in the USDA National Nutrient Database for

Standard Reference, Release 24. In addition, garnet amaranth had a much higher total ascorbic acid (TAA) concentration than their mature counterparts, (131.6 mg 100 g FW⁻¹ compared to 11.6–45.3 mg 100 g FW⁻¹) (Xiao *et al.*, 2012). Vitamin E in the α and γ forms were also found to be more abundant in the green daikon radish, cilantro, opal radish and pepper cress microgreens than in their mature counterparts in Xiao *et al.* (2012).

Carotenoids

The antioxidant β -carotene, known for its critical roles including vision development (Sies and Stahl, 1995), abounds in some species of microgreens (Kyriacou *et al.*, 2016). Twenty-five cabbage microgreens were studied, in which wide ranges of β -carotene concentrations were detected and compared with that of their mature counterpart (Xiao *et al.*, 2012). Red cabbage microgreens had approximately 260-fold more β -carotene concentration than mature red cabbage (Xiao *et al.*, 2012). Another study by Xiao *et al.* (2019) reported that broccoli and cauliflower microgreens

Table 1. Five mature vegetables and their microgreens counterparts were assessed for vitamin concentrations (Choe *et al.*, 2018)

Variety	Vitamin K		Vitamin C		Vitamin A		Vitamin E	
	Mature	Microgreen	Mature	Microgreen	Mature	Microgreen	Mature	Microgreen
Red cabbage	3.82	280 ± 10	57	45.8 ± 3.0	0.056	7.5 ± 0.4	0.11	9.1 ± 4.3
Cilantro	310	250 ± 10	27	147 ± 3.6	0.337	11.5 ± 1.2	2.5	24.1 ± 5.5
Garnet amaranth	1140	410 ± 0.0	43.3	131.6 ± 2.9	0.146	8.6 ± 0.3	0	17.1 ± 2.1
Green daikon radish	1.3	190 ± 10	14.8	70.7 ± 2.7	0	6.1 ± 0.1	0	7.4 ± 15.9

Measurements are in micrograms/100 g fresh weight (FW) for Vitamin K and milligrams/100 grams fresh weight (FW) for all other vitamins. Microgreens show higher concentrations than their mature counterparts.

had higher β -carotene concentrations (6-fold) than the mature florets.

Furthermore, it has been documented that, two of the seven carotenoids found in human, lutein and zeaxanthin found in the blood (Watson and Preedy, 2010), and the only carotenoids present in the retina and lens of the eye that protects the eye from the UV rays and works as an antioxidant (Bone *et al.*, 1997), abound in microgreens (Xiao *et al.*, 2019). In the latter study, which examined the concentration of lutein and zeaxanthin in some microgreens and their mature counterparts, microgreens had 11.2-fold and 28.6-fold higher lutein/zeaxanthin concentrations than the mature plants. In contrast, Klopsch *et al.* (2018) reported that mature leaves of pea and lupin had higher carotenoid concentrations than pea and lupin microgreens. In another study, Kale and mustard microgreens were noted to have lower ascorbic acid than their mature counterparts (de la Fuente *et al.*, 2019). These contrasting results suggest that the abundance of bioactive compounds in a specific plant life stage is species dependent.

Polyphenol and GSL

Polyphenol and glucosinolates (GSL) which reduce the risk of certain cancer developments (Odongo *et al.*, 2017) have recently been reported in kale and broccoli microgreens (Liu *et al.*, 2021). The protection offered by brassica vegetables against cancer is partly related to their relatively high content of GSL (Odongo *et al.*, 2017). However, Liu *et al.* (2021) did not compare the polyphenol and GSL content of kale and broccoli microgreens to their mature counterpart. Though these studies show that some microgreens contain more of certain nutrients than their mature counterparts, it is not documented whether the environmental growing conditions of the microgreens or the nutrient extraction methods were taken into consideration. According to Giménez *et al.* (2021) and El-Nakhel *et al.* (2021), environmental conditions, as well as cultivation methods (Liu *et al.*, 2021), play a pivotal role in nutrient availability in plants including microgreens. In the latter study, the phytochemical contents of kale and broccoli microgreens grown under windowsill conditions were significantly lower compared to those grown under chamber conditions.

Health benefits of microgreens

Foods are necessary to sustain human growth, development and survival by supplying necessary nutrients to the body including vitamins and minerals to enable it to ward off diseases (De Filippo *et al.*, 2021). Many people suffer from chronic diseases such as diabetes, high blood pressure and arteritis (Anderson

and Durstine, 2019). A plethora of studies including Choe *et al.* (2018) and Martinon *et al.* (2021) suggest that the consumption of vegetables can significantly reduce the risk of many chronic diseases. However, the nutrients most influential in improving human health are not always adequately found in regular mature vegetables (Choe *et al.*, 2018). Providentially, many microgreens have been found to contain an abundance of these nutrients and health-promoting micronutrients compared to their mature counterparts (Sies and Stahl, 1995; Kyriacou *et al.*, 2016; Choe *et al.*, 2018; Johnson *et al.*, 2021; Teng *et al.*, 2021).

Even though research on microgreens is still in its infancy, their health benefits have been published through research in microgreen nutrition. The consumption of microgreens can prevent or alleviate some health problems including inflammation of the immune system pathways, obesity (Castelão-Baptista *et al.*, 2021), cardiovascular disease, type 2 diabetes, cancers and modulation of the gut microbiome (Machlin and Bendich, 1987). Other studies have reported that their abundance in vitamins C, E, K, carotenoids and polyphenols, has the potential to regulate cellular pathways, modulating inflammation (Choe *et al.*, 2018; Begum *et al.*, 2021; Sirajunnisa *et al.*, 2021), as well as the generation and scavenging of reactive oxygen species (Finley *et al.*, 2011).

Exhibits indicate that microgreen supplementation of 0.4 mL 20 g (kg body weight)^{-k} broccoli microgreens juice to male rats that had been fed a high-fat diet could reduce weight gain associated with a high-fat diet (Li *et al.*, 2021a and 2021b). In an earlier study, microgreen supplementation significantly lowered low-density lipoprotein cholesterol levels in animals fed with a high-fat diet and reduced hepatic cholesterol ester, triacylglycerol levels and expression of inflammatory cytokines in the liver (Huang *et al.*, 2016). It is therefore suggested that microgreens may reduce weight gain and cholesterol metabolism, thereby preventing hypercholesterolemia and subsequent cardiovascular diseases in humans (Huang *et al.*, 2016). More recent studies also found that microgreens' flavonoid content, such as quercetin, modulates weight gain to prevent obesity and other complications (Rayalam *et al.*, 2008; Castelão-Baptista *et al.*, 2021).

Another facet of the health benefit of microgreens is their ability to aid in the prevention of certain cancers (Maina *et al.*, 2021; Truzzi *et al.*, 2021). It was estimated that over 43,000 people were diagnosed with cancer in Ireland in 2019 (Irish cancer society, 2020). Compared with other diseases, the cause of cancer is not yet well understood and there are few effective treatments (Irish cancer society, 2020). Therefore, research into cancer prevention would be of vital importance. It is suggested that a third of all cancers could be prevented by eating a diet rich in fruits and vegetables and low in fat and calories (Donaldson, 2004; Bertoia *et al.*, 2016).

Although the preventative mechanism is not yet clear (Liu, 2004), studies suggest that bioactive compounds may have protective effects against a variety of cancers, such as breast (Liu and Lv, 2013), prostate (Liu *et al.*, 2012) and colon cancers (Wu *et al.*, 2013). According to Kopsell and Sams (2013) and Poiroux-Gonord *et al.* (2010), brassica vegetables (such as broccoli, cabbage and radish) contain high levels of cancer-fighting glycosylates, carotenoids, phytochemicals, vitamins and minerals. Bradfield and Bjeldanes (1987) and Minich and Bland, 2007 also found that cruciferous microgreens are rich in indole-3-carbinol (I3C), indole-3-acetonitrile (IAN) and 3,3'-diindolylmethane (I3M) which possess anticarcinogenic properties. They further explained that the activation of Phase I, and II xenobiotic 14 metabolizing enzymes may be a potential mechanism by which microgreens protect against cancers. Microgreens may also aid in the regulation of the sex-steroid hormone-mediated pathway by protecting against hormone-dependent cancers such as breast and prostate cancer (Bradfield and Bjeldanes, 1987).

According to Ni *et al.* (2015) and Li *et al.* (2021a and 2021b), plants contain 400 compounds, including kaempferol, quercetin and apigenin, which are associated with 609 microbial targets in the gut suggesting that the consumption of microgreens can also modulate the gut microbiome. All these health benefits have been associated with the consumption of microgreens, but because the benefits are still species-dependent and, in addition, the gut pH and the impact of other external factors can reduce their effects (Kong and Singh, 2008; Li *et al.*, 2011), a study to ascertain the synergetic effects of the above parameters on the bioavailability of these nutrients would be of importance.

Furthermore, some nutrients may not be bioavailable due to the presence in microgreens of antinutrient factors such as phytates, tannins, trypsin inhibitors and lectins, which have been reported in chickpea, legumes, cotton seeds and leaves and seeds of other species (Francis *et al.*, 2001) and amaranth species (Nana *et al.*, 2012). Moreover, these anti-nutrient chemicals that have been reported to be harmful to some organisms, including humans (Friedman and Brandon, 2001; Gilani *et al.*, 2012), have not yet been fully profiled in microgreens. This notwithstanding studies on how to decrease these antinutrient factors in microgreens are being conducted. Yang *et al.* (2015) in their study used zinc sulfate ($ZnSO_4$) as a sulfur (S)-source and found that it stimulated the sulforaphane formation in broccoli microgreens by enhancing myrosinase activity and gene expression related to glucoraphanin biosynthesis. More research into technologies to enhance nutrient availability in microgreens is very important.

Most grown as microgreens species

Although these young vegetables are nutritive (Ghoora *et al.*, 2020; Teng *et al.*, 2021) not all species should be eaten as it has been reported that some species such as potatoes, tomatoes, eggplants, peppers and potentially rhubarb are toxic if eaten when immature (Jagatheeswari, 2014). The consumption of these plants has been associated with symptoms including diarrhea, fever or hypothermia, headache, breathing disorders, abdominal pain, vomiting and nervous problems (Jagatheeswari, 2014). This area of research in microgreens should be considered to identify those phytochemicals capable of causing allergenic reactions in some consumers. Fortunately, several species have been identified to be safe for consumption as microgreens (Table 2).

Microgreens production

Because of their high nutritional content, microgreens are a highly sought-after and highly-priced commodity (Folta, 2019). This together with their short production cycle (Xiao *et al.*, 2012; Folta, 2019) is attracting many growers (Kyriacou *et al.*, 2016). They can be produced indoors in a protected environment, such as a greenhouse or high tunnel (Singh, 2018) using the vertical agricultural technique which is a significant part of the global push towards climate-controlled agriculture (Benke and Tomkins, 2017) provided the growing conditions are right and the facility meets the local standards and regulations for food safety as stipulated in the factsheet for Commercial Microgreens: Food Safety and Third-party Certifications, Agdex 268/089-1 [Alberta Ag-Info Center, 268/18-1 (2018)].

Most commercial growers according to Alberta Ag-Info Center, 268/18-1 (2018), use either of the following methods to grow microgreens:

- Soil-based (garden beds, raised planters).
- Various forms of hydroponic crop production; substrate culture (a pH-neutral media) solution culture, (nutrient solutions) and Nutrient Film Technique, (where the roots are grown in a constant flow of nutrient-rich water).
- Aeroponics.

Considering the various factors that may affect microgreens production, a good knowledge of the environment and requirements is important to understand what the seeds need to germinate, and what seedlings to grow to maturity. Consequently, optimizing the environmental factors necessary for microgreens production and in addition, those which would discourage the growth of food borne-disease-causing pathogens, and improve the self-life of microgreens bound for the markets is pivotal

Fertilizer application rate

Generally, microgreens do not require fertilizers (Treadwell *et al.*, 2020). Nevertheless, to improve yield and to stimulate growth in slow-growing species, seedlings can be fertigated with water containing 20N-8.7P-16.6 K (Peters® Professional 20-20-20 General Purpose; ICL Specialty Fertilizers containing (wt/wt) 0.05% Mg, 0.05% Fe, 0.025% Mn, 0.013% boron (B), 0.013% copper (Cu), 0.005%; 16 molybdenum (Mo) and 0.025% Zn,) at a rate of 100 mg L^{-1} N four days after planting. Li *et al.* (2021a and 2021b) and Kou *et al.* (2014) also documented the increase of microgreen biomass, and calcium content, and reduced microbial growth during storage by spraying the crop with calcium chloride preharvest. Microorganisms have also been used for the same purpose. For example, in a study carried out by Briatia *et al.* (2017), where seed and soil were inoculated with *Herbaspirillum* sp. ST-B2, sprouts, and microgreen buckwheat species yields were increased. In a more recent study by Dembele (2021), bio-stimulants were shown to increase the biomass of some microgreen species.

However, the use of fertilizer has been linked to bacterial colonization of microgreens as well as soils (Jechalke *et al.*, 2019). Another study by Reed *et al.* (2018) also noted that high levels of *E. coli* were heavily persistent in manure-fertilized soils for over three months. As such, microgreens grown in fertilized media are at a higher risk of microbial contamination compared to those grown in non-fertilized media. As a preventative

Table 2. Some commercially grown microgreens (Xiao *et al.*, 2012)

Scientific name			
Commercial name	Family	Genus and species	Plant color
Arugula	Brassicaceae	<i>Eruca sativa</i> Mill.	Green
Bull's blood	Chenopodiaceae	<i>Beta vulgaris</i> L.	Reddish-
Beet			Green
Celery	Apiaceae	<i>Apium graveolens</i> L.	Green
China rose	Brassicaceae	<i>Raphanus sativus</i> L.	Purplish-
Radish			Green
Cilantro	Apiaceae	<i>Coriandrum sativum</i> L.	Green
Garnet	Amaranthaceae	<i>Amaranthus</i>	Red
Amaranth		<i>hypochondriacus</i> L.	
Golden pea	Fabaceae	<i>Pisum sativum</i> L.	Yellow
Tendrils			
Green basil	Lamiaceae	<i>Ocimum basilicum</i> L.	Green
Green daikon	Brassicaceae	<i>Raphanus sativus</i> L.var.	Green
Radish		<i>longipinnatus</i>	
Magenta	Chenopodiaceae	<i>Spinacia oleracea</i> L.	Red
Spinach			
Mizuna	Brassicaceae	<i>Brassica rapa</i> L. ssp.	Green
		<i>nipposinica</i>	
Opal basil	Lamiaceae	<i>Ocimum basilicum</i> L.	Greenish-
			Purple
Opal radish	Brassicaceae	<i>Raphanus sativus</i> L.	Greenish-
			Purple
Pea tendrils	Fabaceae	<i>Pisum sativum</i> L.	Green
Peppergrass	Brassicaceae	<i>Lepidium bonariense</i> L.	Green
Popcorn shoots	Poaceae	<i>Zea mays</i> L.	Yellow
Nutrient purple	Brassicaceae	<i>Brassica oleracea</i> L. var.	Purplish-
Kohlrabi		<i>Gongyloides</i>	Green
Purple mustard	Brassicaceae	<i>Brassica juncea</i> (L.)	Purplish-
		<i>Czern.</i>	Green
Red beet	Chenopodiaceae	<i>Beta vulgaris</i> L.	Reddish-
			Green
Red cabbage	Brassicaceae	<i>Brassica oleracea</i> L. var.	Purplish-
		<i>capitata</i>	Green

Fast-growing vegetables (7 to 14 days): cabbage, corn, cress, kale, kohlrabi, mustard, radish Slow-growing vegetables (15 to 25 days): amaranth, arugula, beet, carrot, Swiss chard, scallion
Slow-growing herbs (15 to 30 days): anise, basil, cilantro, dill, fennel, parsley, saltwort, shiso and sorrel.

measure, the FAO recommends the harvesting of organic crops whose edible portions are exposed to soil be done at least 120 days post-application of non-composted manure (Reed *et al.*, 2018).

Photoperiods

In the past, growers supplemented natural lights with gas-discharged lamps (GDL) but these days, light-emitting diodes

(LEDs) are becoming crucial in the horticulture industry as technologies to replace natural light are advancing (Ajdanian *et al.*, 2019). The LED light is a better option because it gives a more uniform light distribution, is more flexible, and is more eco-friendly than GDLs (Morrow, 2008).

The quality of light affects various aspects of the plant's growth and phytochemical content (Brazaitytė *et al.*, 2018; Ajdanian *et al.*, 2019; Misra, 2020). Studies have shown that red/blue light may have a better effect on certain crops. For example,

Ajdanian *et al.* (2019) found that cress exposed to red/blue light yielded more than the plants exposed to natural light. Red/blue light has also been reported to promote elongation for cabbage, kale, arugula and mustard without affecting crops' yield and quality (Kong, and Zheng, 2019). Further, Lim and Harrison (2016) and Alrifai *et al.* (2019), reported that red/blue and combined spectrums of light are better than white light in aiding photosynthesis and plant metabolism regulation.

In the research by Alrifai *et al.* (2019), it was noted that different wavelengths had varying effects on the antioxidant components of the product which concurs with the findings of a study by Misra, (2020). The latter found that higher light conditions increased photosynthetic capacity which was enabled by increased photosynthesis and electron transport; while low light conditions, on the other hand, resulted in the plant leaves experiencing harvesting complexities and weaker thylakoid membranes which shifted the xanthophyll pigments to improve photosynthesis. This can result in easier *Salmonella* and *E. Coli* spread (Misra, 2020). Moreover, the study by Lim and Harrison (2016), in which UV light's efficacy in reducing salmonella on food contact surfaces was evaluated, found that coupons that were exposed to UV light had greater salmonella population reduction compared to control coupons. This points to light as a factor that influences microbial colonization of plants including microgreens. Therefore, studies to optimize the photoperiodism of growing microgreens void of disease-causing pathogens are important.

Relative humidity, pH and air circulation

There is a dearth of literature identifying the optimal levels of relative humidity and pH for microgreens production. A recent study reported that most microgreens flourish with humidity levels between 40 and 60% (Li *et al.*, 2021a and 2021b). In addition, the growth of bacteria is affected by the pH of the environment. Bacteria are divided into three groups according to their response to pH. The acidophiles are acidic lovers, some can thrive in pH as low as pH 1 e.g., *Thiobacillus thiooxidans*, (Booth, 1985). The neutrophils (*Escherichia coli*, *Rhizobium* and *Bradyrhizobium*) grow best at pH 6.5–7, and the alkalophilic, e.g., *Bacillus alkalophilus* grows at pH 10.5 or 0.15 mS cm⁻¹ (Li *et al.*, 2021a and 2021b). The optimal conditions for microgreen growth are a pH between 6.56 and 7.54, and electrical conductivity of 0.41 mS cm⁻¹ (Kyriacou *et al.*, 2020).

Good air circulation is vital for plant growth and disease prevention (Sharma *et al.*, 2016). Air circulation regulates the temperature and humidity throughout the growing area (Chakraborty *et al.*, 2014). This is usually achieved using horizontal airflow fans along with forced air or natural air vents to mix and exchange air within the growing space (Wolverton *et al.*, 1984). This affects the overall temperature of the growing area (Wolverton *et al.*, 1984). Studies to optimize air circulation in growth chambers will be vital to the microgreens industry in that mold-causing microorganisms on red maranta and other mildew-prone species could be prevented.

Temperature

The optimal environment for microgreen production can be variety-specific, but a favorable temperature range of 18–25°C has been documented, and temperatures above this have been shown to encourage microbial growth (Li *et al.*, 2021a and 2021b). Several studies have reported the growth of foodborne

bacteria on plant extracts during the production process including work by Posada-Izquierdo *et al.* (2016); the growth potential for *E.coli* O157:H7 has been evaluated in water, where maximum growth rates in plant extracts were significantly influenced by temperature (Vital *et al.*, 2010; Merget *et al.*, 2019). Other factors were also influential including the plant tissue type and species, as well as the plant age, defense response, growth conditions and associated microbiomes (Merget *et al.*, 2019).

Effect of growing medium on bacterial colonization

Soil temperature and humidity alone do not influence microbial colonization of plants; the type of medium used also contributes to the overall environmental factors that influence the growth of pathogens and the colonization of microgreens as well as the nutrient content (Fig. 3). Peat-based mixes and synthetic mats are the main growing media used for microgreens production (Işik *et al.*, 2020; Le *et al.*, 2020). However, both are expensive and non-renewable (Di Gioia *et al.*, 2017). Recycled textile-fiber (polyester, cotton and polyurethane traces) and jute-kenaf-fiber have been proposed as low-cost and renewable alternative substrates; in addition, they both deliver a good yield, low nitrate content, and a similar microbiological quality (Di Gioia *et al.*, 2017).

Similarly, Misra and Gibson (2020) pointed out that a growing medium with the highest carbon and micronutrient content would support bacterial growth and persistence. In their study, Biostrate and hemp growing mats, coconut coir and peat were evaluated, and Biostrate® and hemp growing mats were found to have supported the growth of *S. javiana* and *L. monocytogenes* R2–574, while coconut coir and peat did not. This also ties in with reports from Di Gioia *et al.* (2017) and Reed *et al.* (2018). Although growing media have been identified as one of the means through which bacteria contaminate and colonize microgreens, many other contamination routes have been documented.

Contamination routes

The seed sprouting process presents an environment for microorganisms (Gu *et al.*, 2018). Fertilization is also a contamination route (Reed *et al.*, 2018; Jechalke *et al.*, 2019) as well as a growing medium (Di Gioia *et al.*, 2017; Misra and Gibson, 2020). Contamination risks from pre- or post-harvest are also presented in the harvesting process. The most significant limitation to the growth of the microgreens industry is the short shelf-life (Kaiser and Ernst, 2018). Microgreens are hard to store because their quality deteriorates very quickly after harvest Turner *et al.* (2020). Kaiser and Ernst, (2018) suggested the product be used as soon as possible after harvest to maintain quality. Turner *et al.* (2020) also highlighted that post-harvest decay and transpiration make the product leak nutrients and its tissues become damaged by bacterial contamination and subsequent colonization. As a result, advances are being made to combat these limitations by exploring post-harvest treatments that can increase the shelf lives of microgreens.

Post-harvest

After harvesting, microgreens will last longer if they are maintained cold in a bag or container devoid of humidity which ensures that they do not wilt or dry during transport or storage (Turner *et al.*, 2020). Various technologies are being researched to prolong the shelf life of microgreens.

Harvesting time

Many studies have reported several approaches to extend the shelf-life of microgreens. Berba and Uchanski (2012), suggested that microgreens' shelf life may be influenced by the age of the seedlings at harvest. In a study carried out by Clarkson *et al.* (2005), baby salad leaves of arugula (*Eruca vesicaria* ssp. *sativa*) harvested at the end of the day compared with leaves harvested at the start of the day had an increased postharvest shelf-life of 2 to 6 days, while lollo rosso lettuce (*Lactuca sativa* L. 'Ravita') and red chard (*Beta vulgaris* L. var. *flavescens* (Lam.) baby salad leave's shelf-life was increased by 1 to 2 days. In another study by Garrido *et al.* (2016), it was shown that baby spinach can be harvested at any time in winter but needs to be harvested in the early morning in spring, to ensure the product retains a higher water content, firm texture and good visual quality.

Minimizing injury to produce

Another aspect of post-harvest treatment that could positively affect the shelf-life of microgreens is minimizing injuries. Because microgreens are young and delicate, they are vulnerable to physical damage compared to mature green leaves. Fresh-cut microgreens are very tender and subject to handling stress, leading to rapid senescence (Kou *et al.*, 2014). Therefore, preventing physical injury during harvesting and subsequent handling, distribution and marketing is critical.

Presently, growers produce microgreens on mats because it facilitates harvesting (Treadwell *et al.*, 2010). They also reported that some chefs also ask them to deliver trays or mats without harvesting. In this way, produce will last longer since there would be no cut which may lead to dehydration/transpiration or infections. They can be easily harvested with electrical knives allowing microgreens to fall from the mat into a clean harvest container (Treadwell *et al.*, 2020). The sharper the blades of the knife, the better. Although there is no study yet on the effect of the sharpness of the blades on the shelf-life of microgreens, Portela and Cantwell (2001); Sapers *et al.* (2001) reported the shelf-life of melons cut with sharp blades lasted longer than those cut using blunt blades, suggesting that the sharpness of harvesting knives can affect the shelf-life of microgreens. Research to develop technologies for handling with minimal injuries would be of vital importance to the microgreen industry.

Modified atmospheric storage (MAP)

MAP has also been exploited for its ability to protect plants from environmental contaminants such as fungi and other pathogens to prolong the shelf-life of fresh fruits and vegetables (Wagner *et al.*, 2009). However, there has been little MAP research undertaken on microgreens. Kou *et al.* (2013) reported no significant differences among films of different oxygen transmission rates in their ability to maintain the quality of microgreens for 21–28 days in shelf-life, meaning that the use of inappropriate MAP can induce physiological disorders, prevent wound healing, hasten senescence and increase susceptibility to pathogen growth and decay (Wagner *et al.*, 2009).

As a result, packaging technologies that help extend shelf-life, improve safety, ensure freshness and display information on quality and/or safety such as active and intelligent packaging have been proposed (Dainelli *et al.*, 2008). These have been found to inhibit spoilage and growth of pathogenic microorganisms

(Rooney, 1995) and signal when temperatures rise above a threshold value for a given time (Yuan, 2002). A study to explore this technology for microgreen's shelf-life would be valuable to the microgreen industry.

1-Methylcyclopropene (1-MCP)

1-Methylcyclopropene has also received little attention for its ability to extend the shelf-life of microgreens despite its success with fresh fruits (Blankenship and Dole, 2003), vegetables (Watkins, 2006) and edible flowers (Able *et al.*, 2003), potatoes (Foukaraki *et al.*, 2012). However, while 1-MCP reduces sprouting in potatoes, it reduces the sugar quantity and subsequent microbial growth (Foukaraki *et al.*, 2012). 1-MCP should be evaluated for its ability to prolong the shelf-life of microgreens.

Pathogenicity of microgreens

Most pathogens especially those that are pathogenic to humans such as *E. coli*, *Salmonella* and *Listeria* can adapt and thrive in a wide variety of environments (Gandhi and Chikindas, 2007). About 15% of cases of Salmonellosis in humans have been connected to the consumption of Salmonella-contaminated vegetables and/or fruits (Batz *et al.*, 2011). *Listeria* was responsible for the deaths of 10 people in 2010 and 30 people due to contaminated melon in 2011 (Gaul *et al.*, 2010) and 14 people were reported dead from the infection of enterohaemorrhagic *E. coli* (EHEC) from consuming romaine cabbage in 2011 (Buchholz *et al.*, 2011). In addition, the CDC has recently announced a recall and disposal of romaine salads due to an outbreak of *E. coli* in 5 states in America (CDC, 2020).

From the literature, controlling the growing environmental conditions and postharvest storage of microgreens has been aimed mostly at pathogen prevention. However, the pathways or factors influencing the survival and proliferation of pathogens on plants including microgreens have not been well documented. The persistence of pathogens on fresh produce is influenced by the organism, species and pre-/ post-harvest environmental conditions (Harris *et al.*, 2003). Pathogenic organisms may survive but not persist on the uninjured outer surface of fresh fruits or vegetables, partly because of the plant's protective structures (Harris *et al.*, 2003). Unfortunately, these structures are underdeveloped in young plants (Warriner *et al.*, 2003). This suggests that young plants such as microgreens are more vulnerable to bacterial internalization than mature plants. They further stated that bacteria present in seeds can become part of the endophytic microflora. Many other studies have also reported the internalization of pathogens in young plants including Dong *et al.* (2003) who reported significant pathogen colonization of the interiors of 6–9-day old seedlings of *Medicago sativa* (alfalfa).

Plant pathogens have developed many ways to get nutrients from their host, and plants in turn have also developed physical and chemical properties to fight against pathogen attacks (Zhang *et al.*, 2013). Studies have reported that *Salmonella* strains not having SPI-1, SPI-2, 97 SPI-3, SPI-4 and SPI-5 can colonize tomato and cantaloupe fruits (de Moraes *et al.*, 2017); suggesting *Salmonella* may not only infect plants by the translocation of SPI-encoded T3SS effectors. This raises the question of understanding the requirement of the corresponding genes during plant-host interaction which influences plant colonization and in addition the genetic functions with which these pathogens colonize microgreens. Therefore, a study on the phenotypic

characterization for the persistence of these pathogens within microgreens in relation to their growing environmental conditions; particularly, pH, temperature and relative humidity is imperative.

Foodborne contamination associated with microgreens consumption

Vegetables that are eaten raw such as microgreens carry foodborne diseases (Riggio *et al.*, 2019). Various outbreaks have been reported in Europe and North America with the increased production of microgreen products.

Microgreens are defined as the first 2 or 3 inches of shoots from germinating vegetable seeds mostly grown in greenhouses in soil mixes, hydroponic growth mediums and peat mixes as well as recycled fiber mats (Jechalke *et al.*, 2019). Since microgreens are harvested very close to the soil matrices, it is very easy to get them contaminated with microorganisms from their growing matrices. Furthermore, a study by Wang and Kniel (2015) indicated that, microgreens can become contaminated by microorganism from poorly disinfected recirculated water.

Their growing and environmental conditions are also the optimum conditions for growth for most pathogens to thrive (Gu *et al.*, 2018).

Even though various contamination routes are documented, contamination risks from pre-or post-harvest are present from the farm to the marketing process; where many attempts including postharvest treatments (Wells and Butterfield, 1997; Berba and Uchanski, 2012; Garrido *et al.*, 2016; Wright and Holden, 2018) have been made to minimize the risk of contamination and consequently prolong the shelf-life of these farm produces. These risks in association with microgreens have not been adequately evaluated.

Conclusion

While several studies have been conducted into microgreen nutrition and microbial contamination, there is still a vast territory to be researched; for instance, the effect of cool nighttime temperatures on plant growth, nutrition and food safety of microgreens has been overlooked. 1-Methylcyclopropene has received little attention for its effects on the shelf-life of microgreens despite its success with other fresh produce (Able *et al.*, 2003; Blankenship and Dole, 2003; Watkins, 2006).

Studies to optimize nutrient content in a full range of potential microgreens such as pre-and post-harvest light treatment effects to enhance their bioactive compounds' formation have also received little attention. Also, some plant species contain bioactive compounds that are associated with symptoms like those presented by foodborne diseases, yet there have been few studies to identify which plants are safe to be eaten as microgreens.

Plants contain anti-nutritional factors that can limit the digestion of the protein quantity absorbed by an organism about the consumed amount, exemplified by phytates, tannins, trypsin inhibitors and lectins (Francis *et al.*, 2001). These chemicals are said to be identified in some microgreen species (Gilani *et al.*, 2012). We suggest a study to profile these chemicals in more microgreen species.

Fertilization has been used to improve the nutrient content of microgreens. However, little information on how fertilization may improve the taste of microgreens can be impaired by antinutritional factors. Research to optimize the digestibility of protein

from plant sources, particularly microgreens, is limited. More insights into such studies would provide useful information on the reduction of the concentration of anti-nutrients and increase the concentration of beneficial compounds and enhance their sensorial properties, especially taste.

Mechanical damage occurring during the harvesting, washing, spinning and drying steps affect the shelf-life of microgreens. Research to develop technologies for handling minimal injuries would be of vital importance to the microgreen industry.

Some authors have reported the successful control of pathogenic bacteria from growing plants with the use of other bacteria-biological control technology; despite these advances, no data is suggesting that plant disease control against bacterial colonization of microgreens has been conducted. Finally, it would be important to investigate biological means of controlling the colonization of microgreens by important pests. Also, the interactive effects of the environmental factors affecting the growth of microgreens should be evaluated in relation to the degree of bacterial colonization.

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Conflict of interest. We declare that there is no conflict of interest in this work.

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