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Review

Micro-scale vegetable production and the rise of microgreens

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ABSTRACT

Background: Interest in fresh, functional foods is on the rise, compelled by the growing interest of consumers for diets that support health and longevity. Microgreens garner immense potential for adapting leafy vegetable production to a micro-scale and for improving nutritional value in human diet. *Scope and approach:* Major preharvest factors of microgreens production, such as species selection, fertilization, biofortification, lighting and growth stage at harvest are addressed with respect to crop physiology and quality, as well as postharvest handling and applications, temperature, atmospheric composition, lighting and packaging technology which influence shelf-life and microbial safety. Key prospects for future research aiming to enhance quality and shelf-life of microgreens are highlighted. *Key findings and conclusions:* Effective non-chemical treatments for seed surface sterilization and anti-microbial action, pre-sowing treatments to standardize and shorten the production cycle and crop-specific information on the interaction of sowing rate with yield and quality deserve further attention. Indigenous landraces, underutilized crops and wild edible plants constitute a vast repository for selection of genetic material for microgreens. Modular fertilization may fortify microgreens bioactive content and augment their sensorial attributes. Pre- and postharvest select-waveband, intensity and photoperiod combinations can elicit compound-specific improvements in functional quality and in shelf-life. Research is needed to identify effective sanitizers and drying methods non-abusive on quality and shelf-life for commercialization of ready-to-eat packaged microgreens. Genotypic variability in postharvest chilling sensitivity and the interactions of temperature, light conditions and packaging gas permeability should be further examined to establish environments suppressive on respiration but preventive of off-odor development.

1. The state of micro-scale vegetable production: sprouts, baby greens, microgreens

Over the past twenty years, interest in fresh, functional and nutraceutical foods has been on the rise, compelled by the growing interest of society in healthy eating (Ebert, 2012). Consumers are questing for new products that support health and longevity combined with gastronomic delight (Drewnowski & Gomez-Carneros, 2000). Accordingly, it is in the best interest of specialty crop growers, extension specialists and researchers to tap upcoming trends and opportunities for niche products. Microgreens, frequently called 'vegetable confetti', are a new class of speciality crop, defined as tender immature greens produced from the seeds of vegetables, herbs, or grains, including wild species (Xiao, Lester, Luo & Wang, 2012). Depending on species and growing conditions, microgreens are generally harvested at the soil level, i.e. at the base of hypocotyls, upon appearance of the first pair of true leaves, when cotyledons are fully expanded and still turgid, usually within 7–21 days from seed germination depending on the species (Fig. 1) (Sun et al., 2013). The idea of microgreens originated in the late 80's in San Francisco, California, and they have since gained popularity as hot novel culinary ingredients in the world's finest restaurants and upscale grocery stores (Treadwell, Hochmuth, Landrum & Laughlin, 2010). Their popularity stems

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Fig. 1. Ready to harvest microgreens of (**A**) red beet (*Beta vulgaris* L.), (**B**) cilantro (*Coriandrum sativum* L.), (**C**) radish (*Raphanus sativus* L.), and (**D**) brassica raab (*Brassica rapa* L., Broccoletto group), grown in trays on a peat mix (A, B and C), or in hydroponic growing channels on a fibrous mat (D). Photos courtesy of Francesco Di Gioia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from their vivid colors, delicate textures, unique flavor enhancing properties as garnishes (e.g. in salads, sandwiches, soups entrées, desserts and drinks), but also from their fortified phytonutrient content and potential bioactive value (Sun et al., 2013; Xiao, Lester, et al., 2015, Xiao et al., 2012). Supply and demand of microgreens is highly influenced by emerging gastronomic trends, and species selection relies on producer interaction with chefs and on consumer familiarization with their particular sensory attributes (Koppertcress, 2016). Microgreens may be distributed as fresh-cut products but also while growing on media, to be harvested by end users. Mostly exploited are species belonging to the families *Brassicaceae*, *Asteraceae*, *Chenopodiaceae*, *Lamiaceae*, *Apiaceae*, *Amarillydaceae*, *Amaranthceae* and *Cucurbitaceae*. Bioactive content is prominent in species of rather acrid taste (e.g. *Brassicaceae*), the variable acceptability of which warrants identification of genotypes that may cater to demands for both taste and health (Xiao, Lester, et al., 2015).

Microgreens are distinct from sprouts even if both *greens* are consumed in an immature state (Treadwell et al., 2010). Sprouts are generally grown in dark, moisture saturated conditions conducive to microbial proliferation, and their consumption, unlike that of micro- and baby-greens, has been implicated in outbreaks of foodborne epidemics (Ebert, 2012; Xiao, Nou, Luo, & Wang, 2014). Also, microgreens have much stronger flavor enhancing properties than sprouts, and a broad range of leaf color, variety and shape (Ebert, 2012). Recent reports demonstrated that microgreens contain higher amounts of phytonutrients (ascorbic acid, β-carotene, α-tocopherol and phylloquinone) and minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo) and lower nitrate content than their mature-leaf counterparts (Table 1) (Pinto, Almeida, Aguiar, & Ferreira, 2015; Xiao et al., 2012). The appeal of microgreens to consumers, coupled to their high price market and short production cycle, has attracted greenhouse growers and many urban and peri-urban farms have invested in their production. On the other hand, microgreens low yield, rapid senescence and very short shelf-life curbs the expansion of their commercial production (Chandra, Kim, & Kim, 2012; Kou et al., 2013).

As a novel crop, microgreens are still in relative infancy, with yet limited available scientific information but expanding research generating insight into their immense potential as *superfood*. The present review focuses on recent advances on microgreens, particularly on the impact of preharvest factors (species selection, fertilization, biofortification, lighting and harvest stage) on their physiology and quality, as well as of postharvest factors (handling and applications, temperature, atmospheric composition, lighting and packaging technology) on their quality, postharvest performance and microbial safety. The review concludes by identifying major prospects for future research aiming to enhance production efficiency, product quality and shelf-life of microgreens.

2. Growing microgreens: seeds, growing media, harvesting

Seeds are demanded in large quantity and represent a major cost for the production of quality microgreens (Di Gioia, Mininni, & Santamaria, 2015). Unlike sprouts, foodborne outbreaks have not been associated so far with the consumption of microgreens; however, the systemic risk posed by contaminated seeds raises requirements for seed microbiological quality (Xiao, Bauchan et al., 2015; Xiao et al., 2014). Seeds should receive precautionary sanitary treatments for eliminating pathogenic bacteria such as those recommended for sprouts production by the U.S. Food and Drug Administration. Effective and sustainable, non-chemical treatments need to be identified for seed surface sterilization and antimicrobial action appropriate for production of organic microgreens (Ding, Fu, & Smith, 2013). Preliminary germination test per seed lot is advisable for adjusting sowing rate (Di Gioia et al., 2015). Many species germinate easily and grow promptly while others are slow and may require pre-sowing treatments to improve, standardize and shorten the production cycle (Lee, Pill, Cobb, & Olszewski, 2004). Treatments used to advance the early stages of germination range from simple water soaking to physiological treatments, such as osmopriming, matrix priming and seed pre-germination (Table 2). Optimal sowing rate is crop-specific, based on average seed weight, germinability and desired shoot population density,

Table 1
Effects of plant growth stage at harvest on vegetable mineral and phytonutrient content.

Species	Growing conditions	Treatments	Effect	Reference
Lettuce (*Lactuca sativa* L. var. *capitata*)	Greenhouse	Comparison of microgreens and fully grown lettuce.	Lettuce microgreens had higher content of Ca, Mg, Fe, Mn, Zn, Se, and Mo and a lower content of NO_3^- than their fully grown counterpart. Mature lettuce NO_3^- concentration was four-fold higher than in microgreens.	Pinto et al., 2015
Amaranth *Amaranthus tricolor* (four lines)	Greenhouse	Comparison of sprouts, microgreens and fully grown amaranth.	Fully grown amaranth had higher protein, Fe, vitamin C, β-carotene, violaxanthin and lutein content than sprouts and microgreens. Microgreens had higher content of α-carotene, β-carotene, violaxanthin, lutein, and neoxanthin compared to sprouts. While sprouts had higher protein, Fe, and Zn content than microgreens.	Ebert et al. (2014)
Broccoli (*B. oleracea* L. var. *botrytis*)	Growth chamber	Comparison of sprouts and microgreens	Glucoraphanin content and sulforaphane formation declined with sprout growth from day 3 to day 7 after germination.	Guo, Yang, Wang, Guo, and Gu (2014)
Red Cabbage (*B, oleracea* var. *capitata*), Red and purple mustard (*B. juncea* Czem.), mizuna (*B. rapa* L. var. *nipposinica*), and purple kohlrabi (*B. oleracea* L. var. *gongylodes* L.)	Greenhouse	Comparison of microgreens and fully grown Brassica species	Microgreens had a more complex polyphenol profile and contained a larger variety of polyphenol compounds as compared to their mature plant counterparts	Sun et al. (2013)

Table 2
Effects of seed treatments, sowing rate and growth media on microgreens growth, yield and quality.

Factor	Species	Growing conditions	Treatments	Effect	Reference
Seeds treatment	Table beet and chard (*Beta vulgaris* L.)	Growth chamber in darkness at 12 °C	Matrix priming: for 6 d in vermiculite (1:5 seed-to-vermiculite) imbued at 50% dry weight with deionized water	Increased the final germination percentage (FGP), and reduced days to 50% FGP (G50)	Lee et al. (2004)
		Growth chamber in darkness at 20 °C	Soaking: for 48 h in aerated deionized water	Reduced the G50	
	Arugula (*Eruca vesicaria* subsp. *sativa*) and table beet (*B. vulgaris* L.)	Growth chamber in darkness at 20 °C	Pre-germination: mixing seeds with very fine exfoliated vermiculite imbued with deionized water	Increased shoot fresh weight per m^2	Murphy and Pill (2010); Murphy et al. (2010)
Seed density	Arugula (*E. vesicaria* subsp. *sativa*) and table beet (*B. vulgaris* L.)	Greenhouse	Sowing rate	Increasing sowing rate increased fresh yield per unit area and decreased mean shoot weight	Murphy and Pill (2010); Murphy et al. (2010)
Growing media	Vine Spinach *(Basella alba* Linn.) Kangkong *(Ipomoea aquatica* Forsk.) Krathin *(Leucaena leucocephala* de Wit.) Leaf mustard *(Brassica juncea* Czern. & Coss.) Rat-tailed radish (*Raphanus sativus* var. *caudatus* Linn).	n.a.	Comparison of alternative media: sand, peat, coconut coir dust (CCD), sugarcane filter cake (SFC), vermicompost (VC), CCD + Peat (1:1 v:v), CCD + SFC (1:1 v:v), CCD + VC (1:1 v:v)	The 1:1 mix of CCD and peat provided the maximum yield for vine spinach, while the 1:1 mix CCD + SFC provided maximum yield for kangkong, krathin, leaf mustard and rat-tailed radish	Muchjajib, Muchjajib, Suknikom, and Butsai (2015)
	Rapini (*Brassica rapa* L.)	Greenhouse	Comparison of alternative media: peat mix, Sure to Grow® mat, textile fiber mat, jute-Kenaf fiber mat	Peat, textile fiber and jute-kenaf fiber mat provided higher fresh yield as compared to Sure to Grow®. Microgreens grown on peat had the highest *Enterobacteriaceae* population and presence of *E. coli*, which was not found on microgreens grown on other media	Di Gioia et al., 2016

ranging from 1 seed/cm^2 in large-seeded species such as pea, chickpea and sunflower, up to 4 seeds/cm^2 in small-seeded species like arugula, watercress, mustard (Di Gioia & Santamaria, 2015, p. 118). On arugula and table beet, Murphy and Pill (2010), and Murphy, Llort, and Pill (2010) observed a linear increase in fresh yield per unit area with increasing sowing rate, but also a decrease in mean shoot weight. Increasing sowing rate to maximize yield will reflect on the cost of production, while excessive stand density may produce undesirably elongated shoots and limited air circulation conducive to development of fungal diseases.

Microgreens are produced in a variety of environments (open air, protected environment, indoor) and growing systems (soil, soilless), depending on the scale of production. Containerized production, adaptable both to micro-scale urban and large scale commercial operations, allows for commercialization of the product while growing on the media, to be harvested directly by the end user. This approach bypasses harvesting and many postharvest handling issues, and may ensure freshness and high quality (Di Gioia et al., 2015). However, the product remains subject to environmental conditions, transport logistics are burdened, and the final growth stage at harvest sets limits analogous to the shelf-life of the cut product.

Growing media should have a pH of 5.5–6.5, low electrical conductivity (<500 μS/cm) and optimal water holding capacity (55–70% v/v) and aeration (20–30% v/v) (Abad, Noguera, & Burés, 2001). Peat and peat-based media are the most commonly used for producing microgreens. Coconut coir is an alternative to peat derived from a renewable resource but it has variable physicochemical properties and often high salt content and high fungal and bacterial counts (Prasad, 1997). Synthetic fibrous media specifically developed media for microgreens production, such as rockwool or polyethylene terephthalate (PET), pose disposal problems. Natural fiber based media, such as food grade burlap constituted of recycled jute fibers, have also been developed and currently commercialized for microgreens. Low cost alternatives of natural and renewable origin (e.g. cellulose pulp, cotton, jute, kenaf and sunn hemp fibers) and mixtures of materials combining desirable properties constitute potential growing media for microgreens (Di Gioia, De Bellis, Mininni, Santamaria, and Serio (2016). Such media may be fortified to improve the nutritional value of microgreens (Nyenhuis & Drelich, 2015), or inoculated with beneficial microorganisms to stimulate plant growth or control pathogens (Pill, Collins, Gregory, & Evans, 2011).

Most species are harvested at the appearance of the first true leaves, with cotyledons fully expanded, still turgid, retaining their typical color, and seedlings having a height of 5–10 cm. Harvest is performed by cutting the seedlings manually or mechanically few millimeters above the growing media surface. Particular attention should be placed to exclude growing media particles and seed integuments which in some species remain attached to the cotyledons (Di Gioia et al., 2015).

3. Preharvest factors shaping physicochemical-functional quality of microgreens

3.1. Species selection: commercial cultivars and potential valorization of wild genotypes

Commercial seed companies offer an array of species, varieties and select crop mixtures for microgreens production, although available literature reports on a more limited number of taxa (Table 3). Mostly used in studies were taxa belonging to the *Brassicaceae* family and to lesser extent to the *Chenopodiaceae* family. The most widely used taxa are *Brassica juncea* and *Beta vulgaris*. Traits of interest for promising genotypes constitute the appearance, texture, flavor, phytochemical composition and nutritional value (Xiao, Lester, et al., 2015). Genetic variability between and within taxa for traits of interest, the impact of the environment on their expression, and genotype-environment interaction, remain scarcely investigated topics with respect to microgreens. Variation in the content of bioactive components of vegetables depends upon both genetics and the environment. Accordingly, the effects of genotypic, ecophysiological, preharvest and postharvest conditions on the concentration of bioactive phytochemicals, on flavor quality, and even on textural attributes of vegetables have been reiterated by previous researchers (Jeffery et al., 2003; Kader, 2008).

Extensive variability in the concentration of major phytonutrients found in 25 genotypes of microgreens belonging to 19 different taxa has been demonstrated by Xiao et al. (2012); their results highlighted variability in vitamin and carotenoid content, including intra-specific variability, and even variability within genotypes grown under different conditions. Wide variation was also reported in the macro- and microelements content of 30 microgreens genotypes representing 10 species within 6 genera of the *Brassicaceae* family (Xiao et al., 2016). Similarly, significant differences between and within species were identified among three genotypes of common buckwheat and five genotypes of tartary buckwheat evaluated for antioxidant activity, and flavonoids, carotenoids and α-tocopherol contents (Janovska, Štočkova, & Stehno, 2010). Ebert, Wu, and Yang (2014, pp. 25–27) screened four genotypes of amaranth at sprout, microgreen and fully grown stage for phytonutrients and consumer preference; they found significant differences between genotypes and between harvest stages; moreover, cases of genotype-harvest stage interaction were observed. The extent of genetic variability between and within taxa in traits of interest for microgreens, and the assessment of environmental effects on phenotypic attributes require further investigation.

Promising new sources of genetic material that warrant examination are landraces, underutilized crops and wild edible plants (Ebert, 2014). There is strong evidence of decline in the nutritional value of horticultural crops attributed to changes in agricultural practices and the replacement of landraces with modern varieties and hybrids developed through intensive plant breeding (Davis, 2009; Ekholm et al., 2007). Genotypic and morpho-physiological differences between broccoli landraces and hybrids have been documented by Ciancaleoni, Chiarenza, Raggi, Branca, and Negri (2014). Recent studies have also revealed the importance of wild edible plants in human diet (Romojaro, Botella, Obón, & Pretel, 2013). For example, Faudale, Viladomat, Bastida, Poli, and Codina (2008) found higher radical scavenging activity, total phenolic and total flavonoid contents in wild compared to medicinal and edible fennel, while variation in wild fennel from different geographical areas was also reported. It is evident from the above that landraces, underutilized crops and wild edible plants constitute promising sources of genetic material for microgreens production (Di Gioia & Santamaria, 2015, p. 118; Ebert, 2014). Commercial companies are already active in exploiting such genetic material for microgreens production, although the scientific nomenclature of exploited taxa remains proprietary information (Koppertcress, 2016).

Microgreens constitute novel culinary ingredients whose spread is dependent on familiarization of consumers with their particular sensory attributes and on choice of species and cultivars that garner consumer acceptance most. Xiao, Lester, et al. (2015) assessed six microgreens species for twelve sensory attributes including the intensity of aroma, astringency, bitterness, grassy, heat sourness, sweetness, texture, and the acceptability of appearance, flavor, texture and overall eating quality. Their findings indicated that the

Table 3
Plant taxa examined in studies performed on microgreens production, postharvest handling and storage.

Family	Taxon	Reference
Amaranthaceae	*Amaranthus hypochondriacus*	Xiao et al., 2012
	Amaranthus tricolor	Xiao, Lester, et al., 2015; Ebert et al., 2014
Apiaceae	*Apium graveolens*	Xiao et al., 2012
	Coriandrum sativum	Xiao et al., 2012
Asteraceae	*Lactuca sativa* var. *capitata*	Pinto et al., 2015
Brassicaceae	*Barbarea verna*	Xiao et al., 2016
	Brassica campestris var. *narinosa*	Chandra et al., 2012
	Brassica juncea	Brazaityte, Sakalauskiené, et al., 2015; Kopsell, Pantanizopoulos, Sams, & Kopsell, 2012; Samuolienė et al., 2013; Sun et al., 2013; Xiao et al., 2012; Xiao, Lester, et al., 2015; Xiao et al., 2016
	Brassica narinosa var. *rosularis*	Xiao et al., 2016
	Brassica oleraceae var. *acephala*	Xiao et al., 2016
	Brassica oleraceae var. *alboglabra*	Xiao et al., 2016
	Brassica oleraceae var. *botrytis*	Xiao et al., 2016
	Brassica oleraceae var. *viridis*	Xiao et al., 2016
	Brassica oleraceae var. *capitata*	Xiao et al., 2012; Sun et al., 2013; Xiao et al., 2016
	Brassica oleraceae var. *italica*	Kou, Luo, et al., 2014; Kou, Yang, et al., 2014; Sun et al., 2015; Kopsell and Sams, 2013; Xiao et al., 2016; Kou et al., 2015
	Brassica oleraceae var. *gemmifera*	Xiao et al., 2016
	Brassica oleraceae var. *gongylodes*	Xiao et al., 2012; Samuolienė et al., 2013; Sun et al., 2013; Xiao et al., 2016
	Brassica rapa var. *chinensis*	Brazaityte, Sakalauskiené, et al., 2015; Samuolienė et al., 2013; Brazaityte, Virsile, et al., 2015; Xiao et al., 2016
	Brassica rapa var. *napobrassica*	Xiao et al., 2016
	Brassica rapa ssp *nipposinica*	Xiao et al., 2012; Sun et al., 2013; Xiao et al., 2016
	Brassica rapa var. *pekinensis*	Xiao et al., 2016
	Brassica rapa var. *perviridis*	Xiao et al., 2016
	Brassica rapa var. *rapa*	Xiao et al., 2016
	Brassica rapa var. *ruvo*	Xiao et al., 2016
	Brassica rapa var. *rosularis*	Samuolienė et al., 2013; Brazaityte, Sakalauskiené, et al., 2015
	Eruca sativa	Xiao et al., 2012; Murphy and Pill., 2010; Xiao et al., 2016
	Lepidium bonariense	Xiao et al., 2012; Xiao, Lester, et al., 2015; Xiao et al., 2016
	Nasturtium officinale	Xiao et al., 2016
	Raphanus sativus	Xiao et al., 2012; Xiao, Lester, et al., 2015; Xiao et al., 2016
	Raphanus sativus var. *longipinnatus*	Xiao et al., 2012; Xiao et al., 2014; Xiao, Luo, et al., 2014; Xiao, Lester et al., 2014; Xiao et al., 2016; Xiao, Bauchan et al., 2015
	Wasabia japonica	Xiao et al., 2012; Xiao et al., 2016
Chenopodiaceae	*Artiplex hortensis*	Xiao et al., 2012
	Beta vulgaris	Xiao et al., 2012; Brazaityte, Virsile, et al., 2015; Xiao, Lester, et al., 2015; Murphy et al., 2010; Lee et al., 2004; Pill et al., 2011
	Spinacia oleracea	Xiao et al., 2012
Fabaceae	*Pisum sativum*	Xiao et al., 2012
	Cicer arietinum	Khalil et al., 2007
Lamiaceae	*Ocimum basilicum*	Xiao et al., 2012; Brazaityte, Virsile, et al., 2015; Xiao, Lester, et al., 2015
Poaceae	*Zea mays*	Xiao et al., 2012
Polygonaceae	*Rumex acetosa*	Xiao et al., 2012
	Fagopyrum esculentum	Janovska et al., 2010; Kou et al., 2013
	Fagopyrum tataricum	Janovska et al., 2010

astringent, bitter, sour and pungent flavors commonly encountered among glucosinolate-rich *Brassicaceae* vegetables, such as mustard, radish and cress, garner the lowest acceptability as opposed to sweeter, and preferably colored, *Chenopodiaceae* and *Amaranthaceae* microgreens, such as beet and amaranth. Studies on consumer behavior have demonstrated that functional foods containing increased concentrations of phytonutrients with chemopreventive characteristics tend to be the most aversive in taste and this poses a challenge for future valorization of microgreens since potent phytonutrient content runs counter to consumer preference for less bitter taste (Drewnowski & Gomez-Carneros, 2000). Bioactive content was found prominent in microgreens species of rather acrid taste, such as red cabbage (*Brassica oleracea* L. var. *capitata*), sorrel (*Rumex acetosa* L.), peppercress (*Lepidium bonariense* L.), but also in some species of more agreeable taste such as cilantro (*Coriandrum sativum* L.) and amaranth (*Amaranthus hypochondriacus* L.) (Xiao et al., 2012). Notwithstanding that acceptability of acrid taste varies widely and is subject to inherited taste factors, compounded by sex and age, the identification of microgreen genotypes that may cater to demands for both taste and health remains a challenge (Drewnowski & Gomez-Carneros, 2000).

3.2. Plant nutrition and biofortification

Adequate nutrients to produce high yield of premium quality microgreens may be supplied by the growing media, by supplemental fertilization before sowing, by post-emergence fertigation, or by combined pre-sowing and post-emergence applications. Reported effects of fertilization and agronomical biofortification on microgreens growth, yield and quality are summarized in Table 4. Pre-sowing application of 1000 mg/L of N as calcium nitrate, combined with daily fertigation using 21-2.2-16.6 (N-P-K) at 150 mg/L of N, or at 75 mg/L of N, were most successful in increasing fresh yield of arugula (*Eruca vesicaria* subsp. *sativa*) microgreens grown on peat-lite (Murphy & Pill, 2010). The same researchers found that pre-sowing fertilization with calcium nitrate N at 2000 mg/L combined with daily post-sowing fertigation using 21-2.2-16.6 (N-P-K) at 150 mg/L of N led to a two-fold yield increase of table beet microgreens grown on peat-lite, compared to the unfertilized control. Besides rate and application method, also fertilizer form, particularly ammonium:nitrate (NH_4^+:NO_3^-) ratio, may affect the yield and quality of microgreens. Hu et al. (2015) found that moderate concentrations of ammonium (15:85 NH_4^+:NO_3^-), compared with sole nitrate (0:100 NH_4^+:NO_3^-),

Table 4
Effects of fertilization and agronomical biofortification on microgreens growth, yield and quality.

Species	Growing conditions	Treatments	Effect	Reference
Arugula (*E. vesicaria* subsp. *sativa*)	Greenhouse	Pre-plant incorporation in the peat-lite medium of 500, 1,000, 2,000, 4000 mg L^{-1} of N as urea, ammonium nitrate, calcium nitrate, ammonium sulfate (solid and liquid form), and/or post-emergence daily fertilization with solutions of 21-2.2-16.6 (N-P-K) at 0, 75, or 150 mg L^{-1} of N.	The most economical and high yielding (fresh weight per m^2) fertilization programs were the post-emergence daily supply of 150 mg L^{-1} of N, or the post-emergence daily fertilization with 75 mg L^{-1} of N combined with a pre-plant media incorporation of 1000 mg L^{-1} of N from calcium nitrate.	Murphy and Pill (2010)
Table beet (*B. vulgaris* L.)	Greenhouse	Pre-plant incorporation in the peat-lite medium of 1000 and 2000 mg L^{-1} of N supplied as calcium nitrate and/or post-emergence daily fertilization with 21-2.2-16.6 (N-P-K) at 75, or 150 mg L^{-1} of N.	Pre-sowing fertilization with calcium nitrate at 2000 mg L^{-1} of N, combined with daily post-sowing fertigation using a 21-2.2-16.6 N-P-K formula at 150 mg L^{-1} of N led to a two-fold yield increase as compared to the unfertilized control.	Murphy et al. (2010)
Broccoli (*Brassica oleracea* L. var. *botrytis*)	Growth chamber	Pre-harvest foliar application of calcium chloride at different rates (0, 1, 10, 20 mM) for ten days.	Broccoli microgreens sprayed with a 10 mM calcium chloride solution produced 50% higher fresh biomass, had three times higher content of calcium, improved overall visual quality, and reduced microbial growth during storage as compared to untreated microgreens.	Kou, Luo, et al., 2014; Kou, Yang, et al., 2014
Chinese cabbage (*B. pekinensis*)	Greenhouse	Nutrient solution with different ammonium:nitrate (NH_4^+:NO_3^-) ratios (0:100; 10:90; 15:85; 25:75).	Moderate concentrations of ammonium (15:85 NH_4^+:NO_3^-) in the nutrient solution enhanced plant growth, photosynthesis and absorption area of root system.	Hu et al. (2015)
Broccoli (*B. oleracea* L. var. *botrytis*), radish (*Raphanus sativus* var. *redicula*), alfalfa (*Medicago sativa* L.), mung bean (*Vigna radiata* L.)	Growth chamber	Distilled water with 0, 50, 100, 200 and 300 mg L^{-1} of Mg prepared using magnesium sulfate. Distilled water with 0, 6, 12, 24 and 36 mg L^{-1} of Fe prepared using Ferric EDTA.	Enrichment of sprouts with Mg and Fe led to significant increase in Mg (23–152%) and Fe (50–130%) concentration, respectively, especially in alfalfa, without depletion of other ions. Higher Mg concentration had minor effects on microgreens biomass accumulation, while higher Fe concentrations slightly decreased fresh biomass, especially in brassica species.	Przybysz et al. (2015, 2016)
Broccoli (*B. oleracea* L. var. *botrytis*)	Growth chamber	Distilled water with 2 mmol L^{-1} of zinc sulfate ($ZnSO_4$), potassium sulfate (K_2SO_4), methionine (Met) and without a S-source	The use of zinc sulfate as an S-source stimulated the sulforaphane formation in broccoli microgreens by enhancing myrosinase activity and gene expression related to glucoraphanin biosynthesis.	Yang et al. (2015)

enhanced plant growth, photosynthetic response, chloroplast ultrastructure and root architecture of mini Chinese cabbage (*Brassica pekinensis*). Like their mature counterparts, some species of microgreens (e.g. arugula) can accumulate high levels of nitrates (>4000 mg/kg f.w.), considered an anti-nutritional factor, but lower nitrate content may be achieved by controlling N form and concentration in nutrient applications (Di Gioia & Santamaria, 2015, p. 118). Besides overhead or sub-irrigation applications of nutrient solutions, foliar application seems also a promising method for enhancing microgreens yield. Kou, Yang, Luo, Liu, and Huang (2014) tested the pre-harvest foliar application at 0, 1, 10 and 20 mM of calcium chloride ($CaCl_2$) for ten days on broccoli microgreens, and found that microgreens sprayed with 10 mM $CaCl_2$ attained 50% higher biomass and triple the calcium content compared to the untreated control.

As in sprouts and other vegetable categories, microgreens may be biofortified by increasing the concentration of essential mineral elements often lacking in the human diet (White & Broadley, 2009). Biofortification of microgreens is feasible by modulating the fertilization program and the nutrient solution composition

(Table 4). It is possible to lower or increase the content of specific minerals (Tomasi et al., 2015), reduce the concentration of anti-nutrients, increase that of beneficial compounds, enhance the sensorial properties, and extend the shelf-life of microgreens. As a consequence of the germination process, microgreens have relatively low levels of phytate, which ensures high mineral bioavalability (Liang, Han, Nout, & Hamer, 2009). Przybysz, Wrochna, Małecka-Przybysz, Gawrońska, and Gawroński (2015, 2016) demonstrated that microgreens may be enriched with Mg and Fe. The same authors reported that mineral accumulation capacity is species-dependent, which highlights the importance of genotype selection. Appropriate management of the nutrient solution composition may also increase the levels of specific functional compounds, such as glucosinolates in *Brassica* species (Yang et al., 2015).

3.3. *Effects of pre-harvest light conditions: quality, intensity and photoperiod*

Light conditions are highly influential on the morpho-physiology of microgreens, and the biosynthesis and accumulation of phytochemicals, especially in controlled growth environments (Delian, Chira, Badulescu, & Chira, 2015). Supplemental light sources frequently used in vegetable production include metal halide, fluorescent, incandescent and high-pressure sodium (HPS) lamps (Bian, Yang, & Liu, 2015). In the last decade, however, advanced light-emitting diode (LED) technology has become increasingly feasible for providing optimal management of light conditions: high photon flux (intensity) and spectral quality (wavelength) that elicit selective activation of photoreceptors and increase of phytochemical contents in vegetables, including microgreens (Bian et al., 2015; Brazaityte, Sakalauskienė, et al., 2015; Carvalho & Folta, 2016).

Light quality demonstrates far more complex effects than light intensity and photoperiod in regulating growth processes and physiology (Bian et al., 2015). In this respect, Brazaityte, Sakalauskienė, et al. (2015); demonstrated the species-dependent enhancement of various oxygenated (lutein, neoxanthin, violaxanthin and zeaxanthin) and hydrocarbon (α- and β-carotene) carotenoids in *Brassicaceae* microgreens by altering LED spectral quality. Supplemental green light (520 nm) increased the lutein/zeaxanthin ratio and β-carotene content in mustard microgreens, whereas tatsoi and red pak choi accumulated higher levels of carotenoids under standard blue/red/far red (447/638 and 665/731 nm) LED illumination. Application of blue, red and white LED lighting improved the soluble solids and vitamin C contents of buckwheat microgreens as compared to control dark treatment (Choi, Chang, Eom, Min, & Kang, 2015). Further to basal HPS lighting, supplementary red LED for 3 days before harvest influenced the antioxidant properties of amaranth, basil, mustard, spinach, broccoli, borage, beet, kale, parsley and pea microgreens (Samuolienė et al., 2012); increase in phenolic concentrations ranged from 9.1% in mustard to 40.8% in tatsoi, whereas the effects on ascorbic acid and total anthocyanin levels were varied and species-dependent. Supplementary red LED (638 nm) 3 days before harvest modified the nutritional quality of *Perilla frutescens* microgreens (Brazaitytė, Jankauskiene, & Novickovas, 2013); it increased the main antioxidants (ascorbic acid and anthocyanins) and decreased unwanted components such as nitrates. The activity of nitrate reductase was highly stimulated by red light, which resulted in significant decrease of nitrate concentration in leaf tissue (Ohashi-Kaneko, Takase, Kon, Fujiwara, & Kurata, 2007). Both blue and red or a mixture of blue and red lights were found more effective than yellow and white lights in reducing nitrate concentrations in vegetables (Ohashi-Kaneko et al., 2007; Qi et al., 2007). This could be partly related to photosynthetic activity as the increase in carbohydrate levels induced by blue and red light provides carbon skeleton and energy for nitrogen metabolism (Champigny, 1995). Beyond visible spectra, ultraviolet (UV) radiation is also involved in photo-physiological responses of plants, with UV-A (320–400 nm) quality being the least hazardous. The phytochemical content of basil, beet and pak choi microgreens receiving 12.4 μmol m^{-2} s^{-1} basal photon flux density incurred species-dependent increase when supplemented with UV-A at 366 and 390 nm, which was not detrimental on microgreens growth while it increased antioxidant activity, anthocyanins, ascorbic acid and total phenol concentrations (Brazaityte, Virsile, et al., 2015). Similarly, supplemental greenhouse UV-A LED lighting (1, 7 or 14 days before harvest) on purple-leaf and green-leaf basil varieties, improved antioxidant properties, although no other positive impact on nutritional quality of purple-leaf basil was reported (Vastakaite et al., 2015). Notwithstanding possible interaction with genotypic or experimental conditions, these studies demonstrate that by managing spectral light quality, the concentrations of targeted phytochemicals can be altered. Future research is warranted to identify the molecular, physiological and biochemical responses linked to these changes in order to elucidate the mechanism mediating induction of secondary metabolites biosynthesis and light signal transduction pathways.

Optimal management of light intensity may enhance photosynthetic activity and phytochemical content in vegetables, whereas excessive irradiance can provoke photo-damage with detrimental effects on plant growth and product quality (Bian et al., 2015). The effects of five LED irradiation levels (545, 440, 330, 220 and 110 μmol m^{-2} s^{-1}) on nutritional quality of *Brassica* microgreens (kohlrabi, mustard, red pak choi and tatsoi) were investigated by Samuolienė et al. (2013) and Brazaityte, Virsile, et al. (2015), who found that applications of 330–440 μmol m^{-2} s^{-1} resulted in notable but species-specific increase in carotenoids, total phenols and antioxidant activity, while they also lowered nitrate levels. Moreover, limited light intensity (110 μmol m^{-2} s^{-1}) negatively affected growth and nutritional quality, whereas high intensity (545 μmol m^{-2} s^{-1}) had no positive impact on most of the examined parameters. Additionally, in 2012 Kopsell, Pantanizopoulos, Sams, and Kopsell had demonstrated that application of high light (cool white and incandescent) intensity (463 μmol m^{-2} s^{-1}) for 36 h cumulative duration under 14 h photoperiod, resulted in biochemical shifts in the xanthophylls cycle pigment concentrations of 'Florida Broadleaf' mustard microgreens, mostly due to a significant increase (by 133%) of zeaxanthin concentrations.

Photoperiod can affect phytochemical accumulation in microgreens and potentially interact with light quality and intensity. Wu et al. (2007) investigated the effects of continuous 96-h illumination using blue, red and white LEDs on biosynthesis and accumulation of phytochemicals in pea seedlings. Their data revealed that continuous red light considerably increased carotenoids concentration and antioxidant capacity. Shifting broccoli microgreens from combined red/blue (627/470 nm) LEDs at 350 μmol m^{-2} s^{-1} to low intensity (41 μmol m^{-2} s^{-1}) blue (470 nm) LED under 24-h photoperiod for five days before harvest elicited increase in shoot β-carotene, xanthophyll cycle pigments, glucoraphanin, epiprogoitrin, aliphatic glucosinolates, and essential macronutrients (P, K, Ca and Mg) and micronutrients (B, Mn, Mo and Zn) (Kopsell & Sams, 2013). The effects of continuous blue light on stomatal opening and membrane transport activity through variations in H^+, K^+ and Ca^{2+} could be the main cause behind nutrient accumulation in broccoli shoot tissue.

4. Postharvest quality and storability of microgreens: impediment to a novel food industry

4.1. Postharvest handling and pre-storage applications on microgreens

Postharvest perishability is arguably the most limiting factor for the expansion of commercial microgreens production (Kou, Yang, et al., 2014). Comprised of young tissues respiring substantially higher than their mature counterparts, microgreens are characterized by limited shelf-life and high sensitivity to harvest and postharvest handling practices (Cantwell & Suslow, 2002). They require careful, often tedious harvesting, and quick cooling to remove vital heat and suppress the rate of respiration, spoilage and senescence. Harvesting microgreens is labor intensive and can have a direct impact on the cost of production, especially when production is implemented in trays that require harvesting with scissors. Use of loose substrates in trays slows down the harvesting process, whereas seeding on synthetic fiber, food-grade plastic or burlap-type mats can facilitate easier handling, and faster harvesting and cooling of the product (Treadwell et al., 2010). Microgreens behave similarly to fresh-cut produce as they are prone to follow patterns of stress-induced rather than natural senescence, consequent to mechanical trauma incurred by cutting and handling at harvest, and also by postharvest processing, temperature abuse, desiccation and abusive package headspace composition, all of which may accelerate loss of quality and limit their shelf-life (Hodges & Toivonen, 2008; Kou, Luo, et al., 2014). Use of blunt blades has been shown to reduce storage life of fresh-cut leafy vegetables and harvesting microgreens must likewise be performed with sharp blades to avoid bruising and damage to stem cells adjacent to the cut (Portella & Cantwell, 2001). Wound-induced signalling has been shown to migrate to proximate non-wounded tissue in fresh-cut lettuce eliciting phenolic composition and increase in respiratory activity (Saltveit, Choi, & Tomás-Barberán, 2005). Nutrient rich exudates from the cut stem favor microbial growth, therefore washing the product immediately after harvest is desirable and chilled water may be used to effectuate rapid postharvest cooling of microgreens (Cantwell & Suslow, 2002). Though washing can be a critical step in the cooling and sanitization of microgreens, excess moisture may be picked up during the process which may encourage microbial growth and increase sensitivity to mechanical damage due to excess turgor. Dewatering is thus an important follow-up step prior to packaging which may be facilitated by centrifugation or, in the case of delicate tissues like microgreens, by gentle tumbling and forced air along the processing line (Garcia & Barrett, 2005). The sensitivity of tender microgreens to mechanical damage occurring during the washing, spinning and drying steps compromises significantly their shelf-life and appropriate technologies must be developed to overcome these limitations and deliver ready-to-eat microgreens of superior quality and shelf-life (Kou, Yang, Liu, & Luo, 2015).

Time of the day for harvesting may have significant implications for the bioactive composition (Hasperué, Guardianelli, Rodoni, & Chaves, 2016) and shelf-life of microgreens (Clarkson, Rothwell, & Taylor, 2005; Garrido, Tudela, & Gil, 2015). This effect seems species-specific and accentuated in the spring-summer season, likely due to increased light intensity and photoperiod. Shelf-life of baby red chard (*Beta vulgaris* L. var. *flavescens*), lollo rosso lettuce (*Lactuca sativa* L. 'Ravita') and leaf roquette (*Eruca vesicaria* ssp. *sativa*), was increased by 2–6 days following end of day harvest, associated with diurnal alterations in leaf sucrose and starch content (Clarkson et al., 2005). Harvesting baby spinach in the early morning improved leaf quality and storability linked to higher leaf water content, color saturation, and lower respiration rate (Garrido et al., 2015). As delicate texture and high transpiration rates constitute undesirable attributes when selecting species for microgreens production (e.g. lettuce microgreens though palatable are considered prone to postharvest wilting) (Treadwell et al., 2010), potential improvement in quality, bioactive content and shelf-life through rescheduling the time of day for harvesting microgreens merits further research.

Although temperature and package atmosphere are undoubtedly the most critical factors for extending shelf-life, preharvest and prestorage calcium applications may enhance microgreens quality and storage performance. Preharvest, spray applications (≈200 mL) of calcium amino acid chelate (1–20 mM), calcium lactate (1–20 mM) and especially calcium chloride (10 mM at pH 6.5) had a positive effect on postharvest overall quality and shelf life of broccoli microgreens underlined by a sharp reduction in electrolyte leakage during storage at 5 °C (Kou et al., 2015). Moreover, preharvest calcium chloride spray treatments increased broccoli microgreens yield by 50%, linked to stem elongation; they increased calcium and bioactive glucosinolates content, and also increased the activities of important ROS detoxification enzymes thereby protecting membranes against senescence-associated lipid peroxidation (Kou, Yang, et al., 2014; Sun et al., 2015; Supapvanich, Arkajak, & Yalai, 2012). Whereas shelf-life of untreated microgreens was limited to 7 d, preharvest calcium treatments prolonged shelf-life to over 14 d (Kou et al., 2015). In the same study, broccoli microgreens having received a 30 s postharvest dip in 50 mM calcium lactate maintained the highest overall quality and lowest electrolyte leakage during 14 d storage. However, the benefits of postharvest dip treatments on quality and shelf-life were significantly compromised by tissue mechanical damage incurred during the spinning and drying steps. Previous studies on buckwheat microgreens have in fact demonstrated the improved visual quality and postharvest performance of unwashed samples (Kou et al., 2013). Overall, preharvest calcium spray applications present an efficient means for improving quality and shelf-life of microgreens, which deserves to be examined on a wider range of utilized species.

4.2. Storage temperature, atmospheric composition and packaging technology

Temperature is unequivocally the most critical factor influencing the rate of microgreens postharvest deterioration, while it also interacts with the effects of ethylene and of reduced pO_2 and elevated pCO_2 in the product environment (Kader, 2002, p. 3311; Kou, Luo, et al., 2014). Temperature impacts directly microgreens storage performance by regulating the rate of respiratory and metabolic activities related to the process of senescence (Xiao, Luo, et al., 2014). Microgreens' limited shelf-life, which spans 2–4 d at ambient temperature and may extend up to 10–14 d at 5 °C, limits their broad commercialization (Chandra et al., 2012; Kou et al., 2013; Kou, Yang, et al., 2014; 2015). In the case of packaged ready-to-use microgreens, temperature effect on respiratory activity may further complicate the products postharvest performance by passively modifying pO_2/pCO_2 balance, given that packaging material oxygen transmission rate (OTR) is temperature-specific. Although microgreens benefit from a 90–95% relative humidity, severe temperature fluctuation during handling and transport of packaged microgreens may result in significant changes in the relative humidity inside the package, thereby leading to condensation with potentially detrimental effects on product appearance and microbial build up (Kou et al., 2013). Optimal storage temperature for most leafy vegetables and fresh-cut products is 0 °C, although short-term storage, transport and display are conventionally performed in the range of 5–10 °C (Hodges & Toivonen, 2008; Kader, 2002, p. 3311). Highly respiring

greens, such as microgreens, benefit most from rapid cooling and storage at temperature near genotypic chilling tolerance (Kader, 2002, p. 3311). Genotypic variability in microgreens chilling sensitivity is likely compounded by growth stage, storage duration and atmospheric modification (Kou et al., 2013; Xiao, Lester et al., 2014). Thus cultivar-specific chilling sensitivity and respiration rate constitute essential information for optimizing postharvest handling of microgreens and expanding their commercial production.

Deterioration of cellular membranes due to lipid degradation and consequent increase in electrolyte leakage is a consistent feature of senescence (Paliyath, Tiweari, Yuan, & Whitaker, 2008). Electrolyte leakage is a common index of senescence reflecting physiological tissue damage induced by abiotic factors such as temperature extremes (e.g. chilling injury) and mechanical damage (Kou et al., 2013; Kyriacou, Gerasopoulos, Siomos, & Ioannides, 2008); it has been applied in monitoring the shelf life of fresh-cut fruits and vegetables, including microgreens (Kim, Luo, & Gross, 2004; Kou et al., 2013; Luo, McEvoy, Wachtel, Kim, & Huang, 2004; Petrou, Soteriou, Schouten, & Kyriacou, 2013). Shelf-life and quality of buckwheat (*Fagopyrum esculentum* Moench cv. Manner) microgreens, packaged in 16.6 pmol/(m^2 s Pa) OTR film, was best at 5 °C, as storage beyond 10 d at 1 °C was characterized by hike in electrolyte leakage, CO_2 concentration and aerobic mesophillic bacterial count, possibly originating from tissue chilling injury (Kou et al., 2013). However, in the case of daikon radish (*Raphanus sativus* var. *longipinnatus*) microgreens stored for 14 d under the same MAP conditions, 1 °C was the optimal storage temperature (Xiao, Lester et al., 2014). Provided a favorable O_2/CO_2 equilibrium and the absence of anaerobic conditions causing physiological tissue damage, the effect of temperature on shelf-life of both buckwheat and daikon radish microgreens proved more critical than that of package film gas permeability (Kou et al., 2013; Xiao, Lester et al., 2014).

Package film OTR proved significant for shelf-life and tissue integrity of buckwheat and daikon radish microgreens only after prolonged (21–28 d) storage (Kou et al., 2013; Xiao, Lester et al., 2014). Buckwheat microgreens maintained highest quality and tissue integrity for 14 d at 5 °C when packaged in either 16.6 pmol/(m^2 s Pa) OTR film, which equilibrated at moderately low pO_2 (14.0–16.5 kPa) and moderately high pCO_2 (1.0–1.5 kPa), or in 29.5 pmol/(m^2 s Pa) OTR film, which equilibrated at higher pO_2 (16.3–16.8 kPa) and lower pCO_2 (0.8–1.2 kPa) (Kou et al., 2013). Similarly, the effect of different OTR films on daikon radish microgreens kept at 1 °C was limited; nevertheless, off-odor development and electrolyte leakage, associated with loss of cell membrane integrity, increased with decreasing OTR, and 29.5 pmol/(m^2 s Pa) OTR film maintained better overall quality during 28 d storage (Xiao, Lester et al., 2014). Likewise, Chandra et al. (2012) looked at the postharvest performance at 5 °C of 'Tah Tasai' Chinese cabbage (*Brassica campestris* var. narinosa) packaged in PE and PP films of higher and lower gas permeability, respectively, and found that PP films, owing to higher build up of CO_2, caused faster and irreversible membrane damage inferred by increased electrolyte leakage and off-odor scores. Development of off-odors is usually linked to increase in acetaldehyde and ethanol concentrations, indicative of a shift from aerobic to anaerobic metabolism (Cantwell & Suslow, 2002). These findings suggest that microgreen postharvest performance is favored by relatively high O_2 atmosphere equilibrated under MAP packaging with high OTR films and possibly by conventional perforated films used for salad crops. However, packaging of radish microgreens in laser microperforated oriented polypropylene film (LMP) that facilitated high pO_2 throughout 16 d storage at 5 °C, was reported to cause rapid yellowing, tissue senescence and chlorophyll degradation (Xiao, Lester et al., 2014). Visual quality was better maintained under high OTR [29.5 pmol/(m^2 s Pa)] film, than under LMP film, while high OTR also preserved a higher ratio of reduced to oxidized ascorbic acid (ascorbate/dehydroascorbate). Nevertheless, the unhindered gas exchange through LMP film was more effective in retarding off-odor development inside the radish microgreens package.

As highly respiring products microgreens require fast postharvest handling and precooling. Though their storage performance may benefit from MAP conditions under high OTR, it remains nevertheless primarily temperature-dependant, and temperature abuse may lead to fast CO_2 build up, tissue damage and off-odor development (Chandra et al., 2012). Cold chain continuity is critical, as temperature abuse occurring at later shelf-life stages, usually associated with retail display, can accelerate senescence because it impacts on products with already partially depleted carbohydrate reserves and commenced degradative processes such as cell wall disassembly (Kou, Luo, et al., 2014; Kou, Yang, et al., 2014). Microgreens shelf-life is generally much more temperature-dependent than MAP conditioned, and their high rates of respiration demand packaging of sufficient O_2 permeability to prevent anaerobic conditions and off-odor development (Kader, 2002, p. 3311).

4.3. Postharvest light exposure

Postharvest exposure to light is common in retail display of fresh horticultural products including microgreens, and has increasingly come under investigation as a storage application with respect to its effect on sensorial quality, phytonutrient composition and on shelf-life at large (D'Souza, Yuk, Khoo, & Zhou, 2015; Garrido et al., 2015; Lester, Makus, & Hodges, 2010). Work on packaged daikon radish (*Raphanus sativus* var. *longipinnatus*) microgreens has revealed significant interaction between light exposure and package atmosphere composition established under OTR-specific films (Xiao, Lester et al., 2014). Light interference with pO_2/pCO_2 balance is related on one hand to light-induced stomatal opening causing increase in respiratory activity and transpiration rate, which encourage CO_2 increase, O_2 depletion, fresh weight loss and often condensation inside packages; on the other hand, exposure to light seems to sustain some photosynthetic activity, dependant on light intensity and photoperiod, that consumes CO_2 and releases O_2 within the packages (Kozuki et al., 2015; Sanz, Olarte, Ayala, & Echavarri, 2008; Toledo, Ueda, Imahori, & Ayaki, 2003). Likewise, postharvest exposure of baby spinach leaves to light conditions interfered with passive package atmosphere modification and affected the quality of baby spinach mainly because of high *p*O2 and high *p*CO2 generated under light and under dark storage conditions, respectively (Garrido, Tudela, Hernández, & Gil, 2016).

Exposure of daikon radish microgreens kept at 5 °C to continuous low intensity fluorescent light (≈30 μmol s^{-1} m^{-2}) accelerated yellowing, loss of fresh weight and decline of overall visual quality, though yellowing was not directly linked to chlorophyll degradation (Xiao, Lester et al., 2014). Continuous low light intensity (25–30 μmol s^{-1} m^{-2}) unequivocally promotes decline of leaf turgidity as a result of sustained photosynthesis and stomatal opening, as shown in packaged baby and mature spinach leaves (Lester et al., 2010; Toledo et al., 2003). The negative effects of light on microgreens texture and visual quality may be alleviated potentially by suppression of transpiration through NIR-induced stomatal closure mediated by ROS accumulation, as demonstrated by Kozuki et al. (2015) on young lettuce leaves: short duration (10–60 min) pre-storage applications of low intensity NIR (100 μmol m^{-2} s^{-1} at λ > 850 nm) reduced transpiration rates

during subsequent storage under both dark and fluorescent light conditions (140 μmol m^{-2} s^{-1}). On the other hand, the effect of postharvest light exposure on chlorophyll content of leafy greens remains controversial with reports of positive effect, on greens such as kale and basil (Costa, Montano, Carrión, Rolny, & Guiamet, 2013; Noichinda, Bodhipadma, Mahamontri, Narongruk, & Ketsa, 2007), but both positive and negative effects on spinach (Glowacz, Mogren, Reade, Cobb, & Monaghan, 2014; Grozeff, Chaves, & Bartoli, 2013). Continuous light exposure, compared to dark storage, was also reported to increase off-odor development and reduce overall sensorial quality in packaged radish microgreens after 8 d at 5 °C, though these side-effects subsided provided higher film permeability (Xiao, Lester et al., 2014). Resolving the problem of off-odor development under light storage conditions was possible by increasing film permeability also on fresh-cut chard (*Beta vulgaris* L. var. *vulgaris*) and Romaine lettuce leaves (Martínez-Sánchez, Tudela, Luna, Allende, & Gil, 2011; Sanz, Olarte, Ayala, & Echa, 2008). Recent work on packaged fresh-cut baby spinach has further shown that postharvest light-induced changes in quality, with the exception of increased transpiration, were mainly effected indirectly as a result of modified gas composition (Garrido et al., 2016).

Although, postharvest performance of fresh microgreens has been reported to benefit from dark storage, and light exposure has been postulated to accelerate deterioration of sensorial quality, this topic warrants further investigation. The mechanisms behind light-induced changes on sensorial and phytochemical components of microgreens quality need to be elucidated, particularly as they appear highly compound-specific. Enhancement of ascorbic acid levels in radish microgreens by postharvest light exposure has been interpreted as derivative of ongoing photosynthetic activity and increase in the availability of soluble carbohydrates, especially of D-glucose which serves as a precursor for ascorbate synthesis (Grozeff et al., 2013; Xiao, Lester et al., 2014; Zhan, Li, Hu, Pang, & Fan, 2012). Similar increase in ascorbate levels has been reported for fresh-packaged spinach leaves under simulated retail conditions of continuous low intensity fluorescent light, suggesting that this effect is independent of leaf maturity (Lester et al., 2010; Toledo et al., 2003). On the contrary, light exposure accelerated the degradation of carotenoid compounds (β-carotene and violaxanthin), and reduced the hydroxyl radical scavenging capacity of cold-stored radish microgreens (Xiao, Lester et al., 2014). The dynamic xanthophyll cycle of violaxanthin-zeaxanthin interconversion, employed for dissipation of excessive light energy, remains active during postharvest storage, indicated by violaxanthin accumulation under dark storage. In young spinach leaves, however, exposed to continuous PPFD of 26.9 μmol m^{-2} s^{-1}, the concentrations of xanthophylls (lutein, zeaxanthin, and violaxanthin) and β-carotene did not differ from those under dark storage, despite concomitant light-induced increase in phylloquinone (Vitamin K1); which corroborates that either carotenogenesis is light-independent or it is stimulated at higher light intensity (Lester et al., 2010). The role of postharvest light intensity on microgreens quality and shelf-life needs to be further examined with respect to the light compensation point under temperature-controlled storage, whereas the rate of photosynthesis is equal to the rate of respiration (D'Souza et al., 2015). Optimal light intensity putatively lies near compensation point where moderate MA is effected and pO_2 is neither low enough to induce off-flavor development nor high enough to cause oxidative stress and accelerate spoilage (Garrido et al., 2016).

The role of postharvest photoperiod on the other hand deserves also particular attention. Low irradiance pulses seem a promising, alternative application for extending microgreens shelf-life. Application of light pulses near compensation point PPFD (≈30 μmol m^{-2} s^{-1}) in 7 min cycles every 2 h for 3 d on spinach leaves suppressed leaf senescence parameters, such as chlorophyll and ascorbate degradation and hydrogen peroxide production, during subsequent 4 °C dark storage (Grozeff et al., 2013). Applications focusing on light spectral quality using LED light sources constitute another novel area for research on the preservation of microgreens and greens in general. For instance, blue (470 nm) LED light at 30 μmol s^{-1} m^{-2} was effective in reducing the bitter-tasting, undesirable gluconapin content in shoots of seven-day old Chinese kale sprouts while enhancing the levels of total phenolics, anthocyanins and antioxidant capacity; whereas white (440–660 nm) LED light induced higher levels of vitamin C (Qian et al., 2016). Kozuki et al. (2015) demonstrated the potential for suppressing postharvest transpiration on fresh-cut young lettuce leaves through stomatal closure induced by applications of short duration low intensity NIR. The main objective remains to identify species-specific and even cultivar-specific optimal spectral, intensity and photoperiod combinations that can be strategically applied for improving the functional quality of microgreens and to allow more efficient use of supplemental lighting energy by directing LED to select-wavebands (Massa, Kim, & Wheeler, 2008).

4.4. Microbial safety of microgreens

Several postharvest factors may interact with microbial build up on microgreens including, proximity to the soil (i.e. plant height) at harvest, residual humidity following pre-packaging wash treatments, and storage temperature foremost. Initial total aerobic mesophilic bacteria (AMB) plate count for unwashed radish, buckwheat and Chinese cabbage microgreens were 7.1, 7.2 and 7.8 log CFU/g, respectively, which is considerably high and comparable to that reported for cilantro and baby spinach (Allende, Luo, McEvoy, Artés, & Wang, 2004; Chandra et al., 2012; Kou et al., 2013; Wang, Feng, & Luo, 2004). It has been hypothesised that the delicate, soft textured hypocotyls of microgreens may favor more microbial growth compared to their mature counterparts (Chandra et al., 2012). Preharvest spray applications (≈200 mL) of calcium amino acid chelate, calcium lactate and especially calcium chloride (10 mM at pH 6.5) improved the overall quality and shelf-life of broccoli microgreens at 5 °C but also inhibited the proliferation of AMB and yeast and mould (Y&M) populations (Kou et al., 2015). This effect was characterized by dosage specificity and proved most effective at 10 mM concentration in controlling AMB proliferation (Kou, Yang, et al., 2014). On the other hand, postharvest dip treatments in calcium lactate, a firming agent not impacting flavor of fresh-cut products, also showed promising results in suppressing microbial proliferation on stored broccoli microgreens; however, mechanical damage incurred in the wash and drying processes poses an impediment to their wide application (Kou et al., 2015; Yang & Lawless, 2005).

Package film OTR and gas composition did not affect the growth of AMB and Y&M of radish microgreens stored at 1 °C, which reinforces the predominant role of temperature in the proliferation of microbial populations (Xiao, Luo, et al., 2014). Changes in microgreens AMB and Y&M populations are highly responsive to storage temperature. In radish microgreens stored for 14 d at 1, 5 and 10 °C, AMB populations increased by 0.8, 0.2, and 0.1 log CFU/g, respectively. However, microbial growth may be encouraged also by suboptimal storage temperatures causing chilling injury, which impairs cellular membrane function, increases electrolyte leakage, and sets off a series of senescence related reactions, including increase in respiratory activity and ethylene production. Chilling injury related microbial proliferation has been reported for buckwheat microgreens stored in 16.6 pmol/(m^2 s Pa) OTR film at 1 °C beyond 10 d (Kou et al., 2013).

Washing microgreens prior to packaging, especially in

chlorinated water, can effectively reduce AMB populations (Chandra et al., 2012). Initial, pre-storage AMB counts on buckwheat microgreens were reduced by 0.3, 0.9, and 1.3 log CFU/g following water, 50 mg/L and 100 mg/L chlorinated wash treatments, respectively (Kou et al., 2013), whereas the same chlorinated treatments on radish microgreens proved not as effective (Xiao, Luo, et al., 2014). Moreover, the effectiveness of wash treatments was limited to the first 7 d of storage at 5 °C, after which bacterial populations rebounded, reaching 10.3 log CFU/g by 21 d in the water washed buckwheat microgreens (Kou et al., 2013). Similar rebounding behavior was also reported for Y&M during storage of washed broccoli microgreens (Kou et al., 2015). Rebounding microbial growth on radish, buckwheat, broccoli, and Chinese cabbage microgreens was associated with increase in electrolyte leakage and water-soaking of hypocotyls, and it was associated with excess moisture residue due to insufficient drying after wash treatments (Chandra et al., 2012; Kou et al., 2015, 2013; Lee, Kim, & Park, 2009). In fact, unwashed microgreens in the above studies supported the lowest microbial populations throughout storage. This highlights the dilemma facing microgreens postharvest handling: the initial benefits of wash treatments are counteracted by excess residual moisture, whereas the wash and particularly the drying processes are likely to aggravate mechanical damage and reduce shelf life.

Sanitation remains a critical process for the establishment of ready-to-eat packaged microgreens, and the expansion of industrial microgreens production. Further research is needed to examine the effectiveness of various sanitation solutions as well as the impact of drying methods on quality and shelf-life. There is a pressing need for effective sanitizers alternative to sodium hypochlorite (CAS number: 7681-52-9), which is currently under review for the European Biocidal Products Directive 98/8/EC due to the human health and environmental hazards it poses (EUR-lex, 2014; Gil, Selma, López-Gálvez, & Allende, 2009). Encouraging results in this direction have been reported by Chandra et al. (2012), who demonstrated that a 2 min dip treatment in 0.5% (w/v) citric acid solution combined with a 50% ethanol spray treatment were as effective as a standard industrial sodium hypochlorite disinfection treatment (2 min dip in 100 μl/L, pH 7.0) in controlling proliferation of AMB and coliform populations on Chinese cabbage microgreens stored for 9 d at 5 °C in darkness. Future studies should also entail both mesophilic bacteria, which grow best at 20–45 °C, as well as psychrotrophic bacteria, which grow best at 7 °C or lower, in order to have a complete picture of microbial growth against the range of microgreens temperature exposure (Kou et al., 2013; 2015).

5. Concluding remarks and the challenges ahead

Microgreens gather an immense potential for adapting leafy vegetable production to a micro-scale, for improving nutritional value in human diet and for influencing gastronomical trends. Progress in the understanding of preharvest factors affecting their production and quality, and postharvest factors commanding shelf-life have been examined in the current review along with challenges lying ahead. Effective and sustainable, non-chemical treatments for seed surface sterilization and antimicrobial action, presowing treatments and seed pre-germination to standardize and shorten the production cycle, as well as crop-specific information on the interaction of sowing rate or growing media with yield and quality deserve further attention. Selection of genetic material must valorize indigenous resources, such as landraces, underutilized crops and wild edible plants, and quest for a balance between phytonutrient content and organoleptic appeal, as bioactive value tends to run counter to consumer preference for less bitter taste.

Modulating the fertilization program for microgreens can be a means to fortify the content of essential minerals often lacking in the human diet and the content of bioactive functional compounds, to reduce the concentration of anti-nutrients, increase that of beneficial compounds and enhance their sensorial properties. Improvement in quality and bioactive content through preharvest spray applications, rescheduling of the time of day for harvest, and the impact of growth stage at harvest on microgreens composition are topics that demand further research. The mechanisms behind light-induced changes on sensorial and phytochemical components of microgreens quality appear highly compound-specific, and narrow-bandwidth LED sources open wide possibilities for eliciting specific pre- and postharvest responses at the species and even cultivar level. Future research is warranted to identify the molecular, physiological and biochemical responses linked to these changes and elucidate the mechanism mediating induction of secondary metabolites biosynthesis and light signal transduction pathways, while the objective remains to identify optimal spectral, intensity and photoperiod combinations that can be strategically applied for improving the functional quality of microgreens and allow more efficient use of supplemental lighting energy directed to select wavebands.

Mechanical damage occurring during the washing, spinning and drying steps compromises microgreens shelf-life and appropriate technologies must be developed to overcome these limitations. Sanitation remains a critical process for the establishment of ready-to-eat packaged microgreens, and the expansion of industrial microgreens production. Further research is needed to examine the effectiveness of various sanitation solutions as well as the impact of drying methods on quality and shelf-life, while there is a pressing need for effective sanitizers alternative to sodium hypochlorite. Genotypic variability in chilling sensitivity and interaction with growth stage, storage duration and atmospheric composition, constitute essential information for optimizing postharvest handling and developing ready-to-eat products of superior quality. Postharvest temperature-light-OTR interactions on microgreens must also be evaluated to establish O_2/CO_2 balance suppressive on respiration but preventive of off-odor development.

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