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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, fertilisation, 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 antimicrobial 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 need be further examined to establish environments suppressive on respiration but preventive of off-odour development.

Keywords	Genetic resources; Light; Nutrition; Packaging; Quality; Shelf-life
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Dear Editor,

Please consider for publication the attached *Review* manuscript titled "***Micro-scale food production and the rise of microgreens***" by Kyriacou et al. Interest in fresh, functional and nutraceutical foods has been on the rise during the past twenty years, compelled by the growing interest of society in healthy eating. Consumers are questing for new products that support health and longevity combined with gastronomic delight. Microgreens, frequently called 'vegetable confetti', are a new class of specialty crop, defined as a tender immature greens produced from the seeds of vegetables, herbs, or grains, including wild species. The idea of microgreens originated in the late 90's in San Francisco, California, and they have since gained popularity as novel culinary ingredients in the world's finest restaurants and upscale grocery stores. Their popularity stems from their vivid colours, delicate textures, unique flavour 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. However, thorough review of up to date progress on microgreens, highlighting the challenges for prospective research, remains a scarce. Consequently, the present review examined all recent advances on microgreens, particularly the impact of preharvest factors (species selection, fertilisation, biofortification, lighting and growth stage at harvest) 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. The manuscript was prepared in compliance to the *Guide to Authors* provided by *Trends in Food Science & Technology*.

I remain at your disposal for any clarifications pertaining to our submission that might be deemed necessary.

Sincerely,

Youssef Rouphael PhD

Research Highlights

- Pre- and postharvest research advances and prospects on microgreens were examined
- Modular fertilization may fortify bioactive content and sensorial attributes
- Light quality, intensity and period elicit improvements in bioactive content
- Optimal temperature-light-OTR interaction enhances quality and extends shelf-life
- Effective sanitizers and drying methods non-abusive on shelf-life need be developed

Micro-scale food 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, fertilisation, 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 antimicrobial 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 need be further examined to establish environments suppressive on respiration but preventive of off-odour development.

40*Keywords:* Genetic resources; Light; Nutrition; Packaging; Quality; Shelf-life

1. The state of micro-scale food 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 growers, extension specialists and researchers involved in specialty crop production 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, which usually occurs within 7-21 days of the seed germination, depending on the species (Fig. 1) (Sun et al., 2013). The idea of microgreens originated in the late 90'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 from their vivid colours, delicate textures, unique flavour 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 et al., 2012, 2015a). Microgreens are not the same as 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 other greens (i.e. micro and babygreens), has not infrequently been implicated in outbreaks of foodborne epidemics (Ebert, 2012; Xiao, Nou, Luo, & Wang, 2014a). Also,

microgreens have much stronger flavour enhancing properties than sprouts, and a broad range of leaf colour, 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 (Pinto, Almeida, Aguiar, & Ferreira, 2015; Xiao et al., 2012). Nevertheless, the concentration of phytonutrients depends upon both genetics and environment. Accordingly, the interaction of genetic material with ecophysiological, pre- and postharvest conditions against the nutraceutical and organoleptic characteristics of microgreens has aroused great interest among researchers and consumers (Schreiner, 2004). 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 limited scientific information, but growing investment in research which yields 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, fertilisation, biofortification, lighting and growth stage at harvest) 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 systems, harvesting

Seeds constitute a critical component for the production of quality microgreens as they are used in large quantity and represent a major cost of production (Di Gioia, Mininni, & Santamaria, 2015). Foodborne outbreaks have not been associated so far with the consumption of microgreens; however, given their similarities to sprouts, the systemic risk posed by contaminated seeds raises concern regarding seed microbiological quality (Xiao et al., 2014a, 2015b). Seeds intended for microgreens production should be subjected to precautionary sanitary treatments for eliminating pathogenic bacteria, as recommended for the production of sprouts by the U.S. Food and Drug Administration. Effective and sustainable, non-chemical treatments need be identified for seed surface sterilization and antimicrobial action appropriate for production of organic microgreens (Ding, Fu, & Smith, 2013). Use of prime quality, certified seeds is recommended and suppliers should accordingly provide information on seed purity, germinability and mean seed weight, which are essential for estimating optimal seeding rate. 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 and uniform germination, and to standardize and shorten the production cycle (Lee, Pill, Cobb, & Olszewski, 2004). Treatments range from simple water soaking to physiological treatments, such as osmopriming and matrix priming, used to advance the early phases of germination prior to radicle emergence. Lee et al. (2004) observed that matrix priming of table beet and chard seeds in fine exfoliated vermiculite (1:5 seed-to-vermiculite) imbued at 50% d.w. with deionized water and kept in darkness at 12 °C for 6 d, increased the final germination percentage (FGP), and reduced the days to 50% FGP (G50) from 4.8 to 1.8 d and from 6.0 to 2.8 d, for table beet and chard, respectively. It was further observed that soaking seeds in aerated deionized water at 20 °C

for 48 h, did not improve the FGP, but reduced the G50 to 2.2 and 3.6 d for table beet and chard, respectively. An alternative technique often used to increase the FGP, lower the G50 and advance the microgreens crop is seed pre-germination. As described by Murphy and Pill (2010) for arugula, and by Murphy, Llort, and Pill (2010) for table beet, seeds are mixed with very fine exfoliated vermiculite imbued with deionized water and incubated in darkness at 20°C. Upon radicle emergence, the seed-vermiculite mix is surface broadcasted on the growing media.

Determining optimal sowing rate is of prime importance for commercial production of microgreens. Sowing rate may vary 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). Optimal sowing rate is crop-specific based on average seed weight, germinability and the desired shoot population density. On arugula and table beet, Murphy and Pill (2010), and Murphy et al. (2010) observed a linear increase in fresh yield per unit area with increasing sowing rate, but also a decrease in mean shoot weight. Increasing the sowing rate to maximize yield will reflect on the cost of production, but may also lead to excessive stand density with undesirably elongated shoots and limited air circulation conducive to development of fungal diseases. Seeds are usually broadcast by hand on the surface of growing media, though precision seeding machines are used by large-scale operations.

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 presents a flexible approach, adaptable to micro-scale urban settings as well as large scale commercial operations. Moreover, containers allow 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, bulk density and transport logistics are burdened, and the final growth stage at harvest sets limits analogous to the shelf-life of the cut product. Microgreens may also be produced on growing media placed directly on channels or benches of various materials and sizes (Di Gioia et al., 2015). The growing surface is leveled to allow an even distribution of water or nutrient solution, and to facilitate drainage. In order to maximize space efficiency, growing channels or benches may be arranged in vertical, indoor multi-layer systems furnished with artificial lighting.

The growing medium should have a ratio of macro- and micro-pores to assure optimal water holding capacity (55-70% v/v) and aeration (20-30% v/v) (Abad, Noguera, & Burés, 2001). It should have a pH of 5.5-6.5 and low electrical conductivity (<500 µS/cm). Peat and peat-based mixes represent the most common media used to produce microgreens. Although neither inexpensive, nor derived from renewable resources, peat has optimal physicochemical properties and it is commonly available and suitable for organic production. Coconut coir is a commercially available alternative to peat derived from a renewable resource (Muchjajib, Muchjajib, Suknikom, & Butsai, 2015). However, it has variable physico-chemical properties dependent on particle size, often a high salt content, and high fungal and bacterial counts (Prasad, 1997). Microgreens may be grown on synthetic fibrous materials, such as rockwool or polyethylene terephthalate (PET) media specifically developed for the production of microgreens, which pose however disposal problems after use. Food grade burlap, constituted of recycled jute fibers, has been proposed as a growing medium for microgreens, while other natural fiber media specifically developed for microgreens have been commercialized. 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 et al., 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 may be harvested at the appearance of the first true leaves, when the cotyledons are fully expanded, still turgid, have their typical color, and the seedlings have reached a height of 5 to 10 cm. Harvest is performed by cutting seedlings few millimeters above the growing media surface, either manually, using scissors or a blade, or mechanically, using an electric knife or a semi-automatic harvester. Particular attention should be placed on exclusion of growing media particles, and of seed integuments that, 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

Species exploited for microgreens production belong to the families *Brassicaceae*, *Asteraceae*, *Chenopodiaceae*, *Apiaceae*, *Amarillydaceae*, *Amaranthceae* and *Cucurbitaceae*. In this respect, commercial seed companies offer an array of species, varieties and crop mixtures selected for microgreens production, although available literature reports on a more limited number of taxa (Table 1). 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 et al., 2015a). Genetic variability between and within taxa for traits of interest, the impact of the environment on their expression, and possible 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; Schreiner, 2004).

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 extensive genotypic 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 their contents in flavonoids, carotenoids and α-tocopherol (Janovska et al., 2010). Ebert, Wu, and Yang (2014) 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 while in some cases genotype interaction with harvest stage was observed. It is evident that genetic variability exists between and within taxa for traits of interest for microgreens. Further work is necessary to investigate the extent of genetic variability between and within taxa and to assess the environmental effects on phenotypic attributes. Promising sources of genetic material that warrant examination are the landraces, the underutilized crops and wild edible plants (Ebert, 2014).

Microgreens constitute novel culinary ingredients whose spread is dependent on familiarization of consumers with their particular sensory attributes and accordingly on

choice of species and cultivars that garner consumer acceptance most. Using a trained sensory panel, Xiao et al. (2015a) 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, flavour, texture and overall eating quality. Their findings indicated that the astringent, bitter, sour and pungent flavours 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* microgreens, such as beet and amaranth. Studies on consumer behaviour 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 indeed 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

Like their mature counterparts, microgreens require adequate nutrient supply to achieve high yield and premium quality (Murphy & Pill, 2010). Nutrients may be supplied by the

241growing media, by supplemental fertilization before sowing, by post-emergence
242fertigation, or by combining both pre-sowing and post-emergence applications (Murphy &
243Pill, 2010). Comparing different pre-sowing and post-emergence fertilization programs on
244arugula (*Eruca vesicaria* subsp. *sativa*) microgreens grown on peat-lite, Murphy & Pill
245(2010) observed that daily fertigation with a solution of 21-2.2-16.6 (N-P-K) at 150 mg/L
246of N, or at 75 mg/L of N combined with pre-sowing incorporation of 1,000 mg/L of N as
247calcium nitrate, were the most successful applications for increasing fresh yield. In another
248experiment, involving table beet microgreens grown on peat-lite, Murphy et al. (2010)
249found that pre-sowing fertilization with calcium nitrate N at 2,000 mg/L combined with
250daily post-sowing fertigation using a 21-2.2-16.6 N-P-K formula at 150 mg/L of N led to a
251two-fold yield increase, compared to the unfertilized control. Besides rate and application
252method, also fertilizer form may affect the yield and quality of microgreens. Investigating
253the effects of different ammonium:nitrate (NH_4^+:NO_3^-) ratios (0:100; 10:90; 15:85; 25:75)
254on the growth, photosynthetic response, chloroplast ultrastructure and root architecture of
255mini Chinese cabbage (*Brassica pekinensis*), Hu et al. (2015) found that, compared with
256sole nitrate (0:100 NH_4^+:NO_3^-), moderate concentrations of ammonium (15:85 NH_4^+:NO_3^-)
257enhanced plant growth. Di Gioia and Santamaria (2015) observed that, as in the case of
258their mature counterparts, some species of microgreens (e.g. arugula) can accumulate high
259levels of nitrates (> 4,000 mg/kg f.w.) which is considered an anti-nutritional factor. The
260genotypic effect notwithstanding, control of N form and concentration in the nutrient
261solution may allow for production of microgreens with lower nitrate content. Besides
262overhead or sub-irrigation applications of nutrient solutions, foliar application of nutrients
263seems also a promising method to enhance microgreens yield, which warrants further
264attention. Kou et al. (2014) tested the pre-harvest foliar application of calcium chloride
265($CaCl_2$) at different rates (0, 1, 10 and 20 mM) for ten days on broccoli microgreens, and

found that microgreens sprayed with a 10 mM $CaCl_2$ solution attained 50% higher biomass and tripled the calcium content as compared to the untreated control.

By modulating the fertilization program and the nutrient solution composition, biofortification of microgreens is feasible. It is in fact 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 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). Moreover, as a consequence of the germination process, microgreens have relatively low levels of phytate, which ensures high mineral bioavalability (Liang et al., 2009). Przybysz, Wrochna, Małecka-Przybysz, Gawrońska, and Gawroński (2015, 2016) demonstrated that microgreens may be enriched with Mg and Fe; however, it is important to optimize nutrient application rate to avoid yield decrease. 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 allow for increase in the content of specific functional compounds, such as glucosinolates in Brassica species (Yang et al., 2015).

3.3. Light conditions: quality, intensity and photoperiod

Light conditions (quality, intensity and photoperiod) 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; Brazaitytė et al., 2015a; Carvahlo & 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, Brazaitytė et al. (2015a) 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); phenolic concentrations incurred increase ranging from 9.1% in mustard up to 140.8% in tatsoi, whereas the effects on ascorbic acid and total anthocyanin levels were varied and species-dependent. Supplementary red LED (638nm) 3 days before harvest modified the nutritional quality of *Perilla frutescens* microgreens (Brazaitytė, Jankauskiene, & Novickovas, 2013); it increased the contents in main antioxidants (ascorbic acid and anthocyanins) and decreased the levels of unwanted components such as

316nitrates. The activity of nitrate reductase was highly stimulated by red light, which resulted
317in significant decrease of the nitrate concentration in leaf tissue (Ohashi-Kaneko, Takase,
318Kon, Fujiwara, & Kurata, 2007). Both blue and red or a mixture of blue and red lights were
319found more effective than yellow and white lights in reducing nitrate concentrations in
320vegetables (Ohashi-Kaneko et al., 2007; Qi et al., 2007). This could be partly related to
321photosynthetic activity as the increase in carbohydrate levels induced by blue and red light
322provides carbon skeleton and energy for nitrogen metabolism (Champigny, 1995). Beyond
323visible spectra, ultraviolet (UV) radiation is also involved in photo-physiological responses
324of plants, with UV-A (320-400 nm) being the least hazardous quality of UV. The
325phytochemical content of three microgreens (basil, beet and pak choi) incurred species-
326dependent increase under higher basal photon flux density (12.4 µmol m^{-2} s^{-1}) and under
327supplemental UV-A at 366 and 390 nm, which was not detrimental on microgreens growth
328while it increased antioxidant activity, anthocyanins, ascorbic acid and total phenol
329concentrations (Brazaitytė et al., 2015b). Similarly, supplemental greenhouse UV-A LED
330lighting (1, 7 or 14 days before harvest) on purple-leaf and green-leaf basil varieties,
331improved antioxidant properties, although no other positive impact on nutritional quality of
332purple-leaf basil was reported (Vastakaite et al., 2015). Notwithstanding possible
333interaction with genotypic or experimental conditions, these studies demonstrate that by
334managing spectral light quality, the concentrations of targeted phytochemicals can be
335altered. Future research is warranted to identify the molecular, physiological and
336biochemical responses linked to these changes in order to elucidate the mechanism
337mediating induction of secondary metabolites biosynthesis and light signal transduction
338pathways.

339 Optimal management of light intensity may enhance photosynthetic activity and
340phytochemical 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 Brazaitytė et al. (2015b), 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 also 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 compared to the other treatments. Shifting broccoli microgreens, grown under combined red/blue (627/470 nm) LEDs at 350 µmol m^{-2} s^{-1} and 24-h photoperiod, to low intensity (41 µmol m^{-2} s^{-1}) blue (470 nm) LED light for five days before harvest elicited increase in shoot β-carotene, xantophyll 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 et al., 2014a). 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 et al., 2014b). 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

391signalling has been shown to migrate to proximate non-wounded tissue in fresh-cut lettuce
392eliciting phenolic composition and increase in respiratory activity (Choi, Tomás-Barberán,
393& Saltveit, 2005; Saltveit, Choi, Tomás-Barberán, 2005). Nutrient rich exudates from the
394cut stem favour microbial growth, therefore washing the product immediately after harvest
395is desirable and chilled water may be used to effectuate rapid postharvest cooling of
396microgreens (Cantwell & Suslow, 2002). Though washing can be a critical step in the
397cooling and sanitization of microgreens, excess moisture may be picked up during the
398process which may encourage microbial growth and increase sensitivity to mechanical
399damage due to excess turgor. Dewatering is thus an important follow-up step prior to
400packaging which may be facilitated by centrifugation or, in the case of delicate tissues like
401microgreens, by gentle tumbling and forced air along the processing line (Garcia & Barrett,
4022005). The sensitivity of tender microgreens to mechanical damage occurring during the
403washing, spinning and drying steps compromises significantly their shelf-life and
404appropriate technologies must be developed to overcome these limitations and deliver
405ready-to-eat microgreens of superior quality and shelf-life (Kou, Yang, Liu, & Luo, 2015).
406 Time of the day for harvesting (TDH) microgreens is a factor with potentially
407significant implications for their bioactive composition (Hasperué, Guardianelli, Rodoni, &
408Chaves, 2016) and shelf-life (Clarkson, Rothwell, & Taylor, 2005; Garrido, Tudela, & Gil,
4092015). The effect of TDH on quality and postharvest performance seems species-specific
410and accentuated in the spring-summer season, likely due to increased light intensity and
411photoperiod. Shelf-life of baby red chard (*Beta vulgaris* L. var. *flavescens*), lollo rosso
412lettuce (*Lactuca sativa* L. 'Ravita') and leaf roquette (*Eruca vesicaria* ssp. *sativa*), was
413increased by 2-6 days following end of day harvest, which was associated with diurnal
414alterations in leaf sucrose and starch content (Clarkson et al., 2005). In the case of baby
415spinach, harvesting in the early morning during spring, but not during winter, improved

leaf quality and postharvest performance linked to higher leaf water content and color saturation, and lower respiration rate (Garrido et al., 2015). As delicate texture and high transpiration rates constitute negative attributes when selecting species for microgreens production (e.g. lettuce microgreens though palatable are considered prone to postharvest wilting) (Treadwell et al., 2010), improvement in quality, bioactive content and shelf-life through rescheduling the TDH is a topic that merits further research.

Although temperature and package atmosphere are undoubtedly the most critical factors for extending the shelf life of microgreens, preharvest and prestorage treatments can be effective in improving quality and storage performance. Preharvest spray applications and postharvest dip treatments using calcium based solutions have been demonstrated to improve quality and shelf-life of broccoli microgreens (Kou et al., 2014a, 2015; Sun et al., 2015). Preharvest, daily 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). Calcium chloride preharvest spray treatments were further shown to increase 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 et al., 2014a; Sun, 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 the mechanical damage incurred on microgreens 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). In view of the above reports, preharvest calcium spray applications present an efficient means for improving productivity and enhancing quality and shelf-life of microgreens, which deserves to be examined on a wider range of species utilized for microgreens production.

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; Jacxsens, Devlieghere, & Debevere, 2002; Kou et al., 2014b). Temperature exerts a direct impact on microgreen postharvest physiology and storage performance by regulating the rate of respiratory activity and of metabolic activity related to the process of senescence (Xiao, et al., 2014b). The limited shelf-life of microgreens, which at ambient temperature spans 2-4 d, and at 5 °C may extend up to 10-14 d, limits their industrial production and consumption (Chandra et al., 2012; Kou et al., 2013; 2014a; 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 in the package atmosphere, 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).

The optimal storage temperature for most leafy vegetables and fresh cut products is 0 °C, although short-term storage, transport and display are usually performed in the range of 5-10 °C (Kader, 2002; Hodges & Toivonen, 2008). Highly respiring greens, such as microgreens, benefit most from rapid cooling and storage at temperature near genotypic chilling tolerance (Kader, 2002). Genotypic variability in microgreens chilling sensitivity is likely compounded by growth stage, storage duration and atmospheric modification (Kou et al., 2013; Xiao et al., 2014c). 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 which reflects 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 characterised 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 et al., 2014c). Provided a favourable 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 et al., 2014c).

The effect of package film OTR on shelf-life and tissue integrity of buckwheat and daikon radish microgreens proved significant only after prolonged (21-28 d) storage (Kou et al., 2013; Xiao et al., 2014c). Buckwheat microgreens stored for 14 d at 5 °C maintained highest quality and tissue integrity 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 package film OTR and 29.5 pmol/(m^2 s Pa) OTR film maintained better overall quality during 28 d storage (Xiao et al., 2014c. 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 concomitant higher off-odor scores. Development of off-odours 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 favoured 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 oxygen concentration throughout 16 d storage at 5 °C, was reported to cause rapid yellowing,

tissue senescence and chlorophyll degradation manifested in the drop of CIELAB hue angle (h°) values (Xiao et al., 2014c). Visual quality was thus better maintained under high OTR [29.5 pmol/(m^2 s Pa)] film, than under LMP film, while high OTR film also preserved a higher ratio of reduced/ oxidized form of ascorbic acid (ascorbate/dehydroascorbate). Nevertheless, the unhindered gas exchange through LMP film was more effective in retarding off-odour development inside the radish microgreens package.

Microgreens are highly respiring products that require fast postharvest handling and precooling. Though their storage performance may benefit from MAP conditions of high OTR, it remains nevertheless primarily temperature-dependant, while 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 already commenced degradative processes such as cell wall disassembly (Kou et al., 2014). Shelf-life of highly respiring commodities, such as microgreens, 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-odour development (Kader, 2002).

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 when OTR-specific films are used to establish a modified equilibrium headspace composition (Xiao et al., 2014c). 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, and Ayaki, 2003). Likewise, postharvest exposure of baby spinach leaves to light conditions was found to interfere with passive package atmosphere modification and affected the quality of baby spinach mainly because of the generated high *p*O2 under light and high *p*CO2 under dark storage conditions (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}) was reported to accelerate yellowing, loss of fresh weight and decline of overall visual quality, though yellowing was not directly linked to chlorophyll degradation (Xiao et al., 2014c). Continuous low light intensity (25-30 µmol s^{-1} m^{-2}) unequivocally seems to promote 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 potentially be alleviated by suppression of transpiration through NIR-induced stomatal closure mediated by ROS accumulation, as demonstrated by Kozuki et al., (2015) on young lettuce (*Lactuca sativa* L.) 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 (Grozeff, Chaves, & Bartoli, 2013; Glowacz, Mogren, Reade, Cobb, & Monaghan, 2014). Continuous light exposure, compared to dark storage, was also reported to increase off-odour 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 et al., 2014c). Resolving the problem off-odour 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 et al., 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 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 concomitant increase in the availability of soluble carbohydrates, especially of D-glucose which serves as a precursor for ascorbate synthesis (Grozeff et al., 2013; Zhan, Li, Hu, Pang & Fan, 2012; Xiao et al., 2014c). 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 et al., 2014c). The dynamic xanthophyll cycle of violaxanthin-zeaxanthin interconversion, employed for dissipation of excessive light energy, remains active during postharvest storage, as 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 need be further examined with respect to the light compensation point under temperature-controlled storage, where at 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-flavour 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 2h 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 allow more efficient use of supplemental lighting energy by directing LED to select-wavebands (Massa et al., 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 foremost the storage temperature. 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 et al., 2004). It has been hypothesised that the delicate, soft textured hypocotyls of microgreens may favour 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

643shelf-life of broccoli microgreens but also inhibited the proliferation of AMB and yeast and
644mould (Y&M) populations at 5 °C (Kou et al., 2015). This effect was further characterized
645by dosage specificity and proved most effective at 10 mM concentration in controlling
646postharvest AMB proliferation (Kou et al., 2014). On the other hand, postharvest dip
647treatments in calcium lactate, which is firming agent not impacting negatively the flavour
648of fresh-cut products, also showed promising results on suppressing microbial proliferation
649on stored broccoli microgreens; however, mechanical damage incurred in the wash and
650drying processes poses an impediment to their wide application (Kou et al., 2015; Yang &
651Lawsless, 2005).

652 Package film OTR and gas composition did not affect the growth of AMB and Y&M of
653radish microgreens stored at 1°C, which reinforces the predominant role of temperature on
654the proliferation of microbial populations (Xiao et al., 2014b). Changes in AMB and Y&M
655populations are highly responsive to storage temperature of microgreens. In radish
656microgreens stored for 14 d at 1, 5 and 10 °C, AMB populations increased by 0.8, 0.2, and
6570.1 log CFU/g, respectively. However, microbial growth may be encouraged also by
658suboptimal storage temperatures causing chilling injury, which impairs cellular membrane
659function, increases electrolyte leakage, and sets off a series of senescence related reactions,
660including increase in respiratory activity and ethylene production. Chilling injury related
661microbial proliferation has been reported for buckwheat microgreens stored in 16.6 pmol/
662(m^2 s Pa) OTR film at 1°C beyond 10 d (Kou et al., 2013).

663 Washing microgreens prior to packaging, especially in chlorinated water, can
664effectively reduce AMB populations (Chandra et al., 2012). Initial, pre-storage AMB
665counts on buckwheat microgreens were reduced by 0.3, 0.9, and 1.3 log CFU/g following
666water, 50 mg/L and 100 mg/L chlorinated wash treatments, respectively (Kou et al., 2013),
667whereas the same chlorinated treatments on radish microgreens proved not as effective

668(Xiao et al., 2014b). Moreover, the effectiveness of wash treatments was limited to the first
6697 d of storage at 5 °C, after which bacterial populations rebounded, reaching 10.3 log
670CFU/g by 21 d in the water washed buckwheat microgreens (Kou et al., 2013). Similar
671rebounding behaviour was also reported for Y&M during storage of washed broccoli
672microgreens (Kou et al., 2015). Rebounding microbial growth on radish, buckwheat,
673broccoli, and Chinese cabbage microgreens was associated with increase in electrolyte
674leakage and water-soaking of hypocotyls, and it was associated with excess moisture
675residue due to insufficient drying after wash treatments (Chandra et al., 2012; Kou et al.,
6762013, 2015; Lee at al., 2009). In fact unwashed microgreens in the above studies supported
677the lowest microbial populations throughout storage. This highlights the dilemma facing
678microgreens postharvest handling: the initial benefits of wash treatments are counteracted
679by excess residual moisture, whereas the wash and particularly the drying processes are
680likely to aggravate mechanical damage and reduce shelf life.

681 Sanitation remains a critical process for the establishment of ready-to-eat packaged
682microgreens, and the expansion of industrial microgreens production. Further research is
683needed to examine the effectiveness of various sanitation solutions as well as the impact of
684drying methods on quality and shelf-life. There is a pressing need for effective sanitizers
685alternative to sodium hypochlorite (CAS number: 7681-52-9), which is currently under
686review for the European Biocidal Products Directive 98/8/EC due to the human health and
687environmental hazards it poses (EUR-lex, 2014; Gil, Selma, López-Gálvez, & Allende,
6882009). Encouraging results in this direction have been reported by Chandra et al. (2012),
689who demonstrated that a 2 min dip treatment in 0.5 % (w/v) citric acid solution combined
690with a 50% ethanol spray treatment were as effective as a standard industrial sodium
691hypochlorite disinfection treatment (2 min dip in 100 µl/L, pH 7.0) in controlling
692proliferation of AMB and coliform populations on Chinese cabbage microgreens stored for

6939 d at 5 °C in darkness. Future studies should also entail both mesophilic bacteria, which 694grow best at 20-45 °C, as well as psychrotrophic bacteria, which grow best at 7°C or lower, 695in order to have a complete picture of microbial growth against the range of microgreens 696temperature exposure (Kou et al., 2013; 2015).

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698**5. Concluding remarks and the challenges ahead**

699 Microgreens gather an immense potential for adapting leafy vegetable production to a 700micro-scale, for improving nutritional value in human diet and for influencing 701gastronomical trends. Progress in the understanding of preharvest factors affecting their 702production and quality, and postharvest factors commanding shelf-life have been examined 703in the current review along with challenges lying ahead. Effective and sustainable, non-704chemical treatments for seed surface sterilization and antimicrobial action, pre-sowing 705treatments and seed pre-germination to standardize and shorten the production cycle, as 706well as crop-specific information on the interaction of sowing rate or growing media with 707yield and quality deserve further attention. Selection of genetic material must valorize 708indigenous resources, such as landraces, underutilized crops and wild edible plants, and 709quest for a balance between phytonutrient content and organoleptic appeal, as bioactive 710value tends to run counter to consumer preference for less bitter taste.

711 Modulating the fertilization program for microgreens can be a means to fortify the 712content of essential minerals often lacking in the human diet and the content of bioactive 713functional compounds, to reduce the concentration of anti-nutrients, increase that of 714beneficial compounds and enhance their sensorial properties. Improvement in quality and 715bioactive content through preharvest spray applications, rescheduling of the time of day for 716harvest, and the impact of growth stage at harvest on microgreens composition are topics 717that 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 need also be evaluated to establish O_2/CO_2 balance suppressive on respiration but preventive of off-odour development.

Author contribution statement

MK and YR set up and defined the contents of the review. MK wrote the entire section devoted to postharvest conditions, the abstract and conclusion sections and critically

revised, edited and merged the whole manuscript. YR and SDP wrote the introduction and the light section. AK and MK wrote the part relative to species selection. FDG, FS, MR and PS developed the part relative to plant nutrition and biofortification. FDG defined and wrote the part relative to growing microgreens. YR was responsible for final approval of the version to be published.

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Table 1

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 et al., 2015a; 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	Xiao et al., 2012; Xiao et al., 2015a; Samuoliene et al., 2013; Sun et al., 2013; Kopsell et al., 2012; Brazaityte et al., 2015a; 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 et al., 2014; Sun et al., 2015; Kopsell et al., 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; Samuoliene et al., 2013; Sun et al., 2013; Xiao et al., 2016
	Brassica rapa var. *chinensis*	Brazaityte et al., 2015a; Samuoliene et al., 2013; Brazaityte et al., 2015b; 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*	Samuoliene et al., 2013; Brazaityte et al., 2015a
	Eruca sativa	Xiao et al., 2012; Murphy and Pill., 2010; Xiao et al., 2016
	Lepidium bonariense	Xiao et al., 2012; Xiao et al., 2015a; Xiao et al., 2016
	Nasturtium officinale	Xiao et al., 2016
	Raphanus sativus	Xiao et al., 2012; Xiao et al., 2015a; Xiao et al., 2016
	Raphanus sativus var. *longipinnatus*	Xiao et al., 2012; Xiao et al., 2014a; Xiao et al., 2014b; Xiao et al., 2014c; Xiao et al., 2016; Xiao et al., 2015b
	Wasabia japonica	Xiao et al., 2012; Xiao et al., 2016
Chenopodiaceae	*Artiplex hortensis*	Xiao et al., 2012
	Beta vulgaris	Xiao et al., 2012; Brazaityte et al., 2015b; Xiao et al., 2015a; 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 et al., 2015b; Xiao et al., 2015a
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

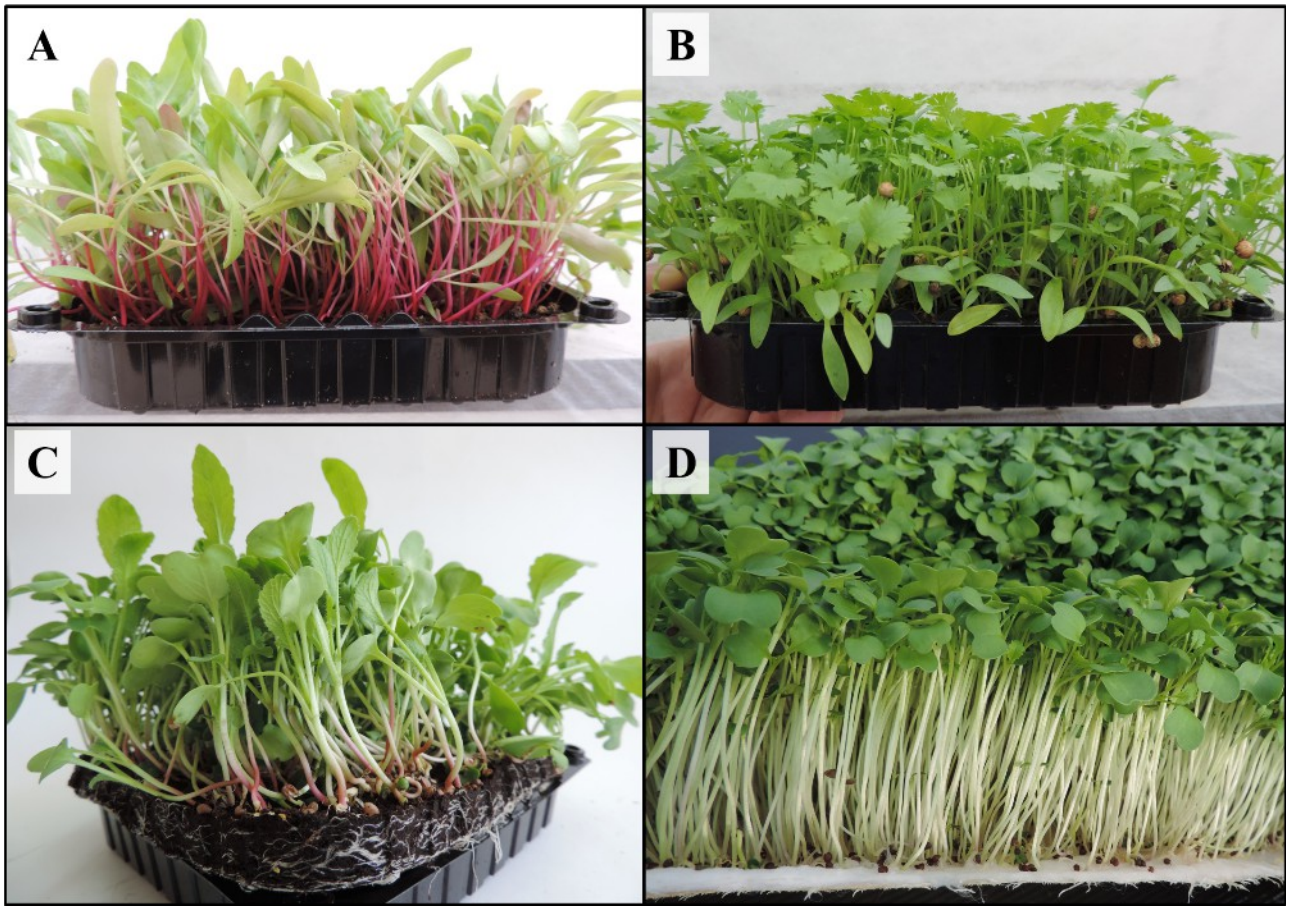

Fig. 1. Ready to harvest microgreens of (**A**) red beet (*Beta vulgaris*L.), (**B**) cilantro (*Coriandrum sativum* L.), (**C**) radish (*Raphanus sativus* L.), and (**D**) brassica raab (*Brassica rapa* L., Broccoletto group), grown in trays on a peat mix (A, B and C), or in hydroponic growing channels on a fibrous mat (D). Photos courtesy of Francesco Di Gioia.