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Microgreens: Boon for the current Era

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Abstract

Microgreens are an emerging group of vegetables grown when initial leaves have completely grown and just prior to true leaves begin to emerge. These are known to increase the flavour and also possess nutritional value. Few plants which are consumed as microgreens are spinach, cabbage, mustard, buckwheat, radish, lettuce, etc. There is increasing demand for microgreens as they contain essential vitamins, minerals, and antioxidants. This review focuses on the bioactive compounds, nutritional status, crops used for microgreens production, health benefits, preharvest factors shaping physicochemical-functional quality of microgreens, plant nutrition and bio fortification, effects of pre-harvest light conditions: quality, intensity and photoperiod, postharvest quality and storability of microgreens: impediment to a novel food industry, postharvest light exposure, microbial safety of microgreens, future perspective of these microgreens. They are loaded with vitamins (e.g., VC), minerals (e.g., copper and zinc), and phytochemicals, including carotenoids and phenolic compounds, which act as antioxidants in the human body. They are the emerging sector in Horticulture during the last decades and research on microgreens is still at its infant stage. Research studies are required to improve the nutrient content in the pre harvest phase and to enhance the shelf life of the produce and to retain the nutrient status in the post-harvest phase.

Keywords: Microgreens, health benefits, nutritional content, bioactive compounds

Introduction

In the course of 20 years, growing awareness of mass people in healthy meals has encouraged attention in fresh, functional and nutraceutical foods of high end. It is in the favor of micro green crop cultivators, extension experts and scientists to meet upcoming opportunities for relevant products. Micro greens, commonly termed 'Vegetable Confetti', are another form of distinction crop, portrayed as soft juvenile greens raised from the seeds of grains, vegetables, or herbs as well as its wild types. Since, in developed countries attraction towards healthy eating, gourmet cooking and indoor gardening has been increased and thereby it has now attained recognition there. This new form of food has a comparatively small life span even in refrigerator and are utilized in very little amounts as garnishes, toppings, or seasonings (Riggio *et al.*, 2019) [44].

They are identified by various number of colors, tastes, textures and are fresh and tenderly soft vegetables, found from the seeds of abundant varieties (aromatic herbs vegetables, wild edible plants, and herbaceous plants), harvested a few days or weeks after germination during the formation of cotyledons and appearance of the first true leaves (Paradiso *et al.*, 2018) [59]. They have larger concentrations of phenolics, antioxidants, minerals, and vitamins than present in fully developed green or seeds and hence recognized as functional foods consisting of health improving or ailment prevention characteristics apart from their nutritional benefits. These are well recognized as good carriers of biologically active components (Mir *et al.*, 2017) [38].

Unfortunately, commercialization of micro greens is less due to their speedy degradation and a very small storage life, generally 3 to 5 days at encompassing temperature, so these are supposed to be highly decomposable products. As the demand for microgreens rises, consequently their appearance in farmer's markets and specialty on grocery stores also begins, so the improvement of their bundling and post-collect stockpiling circumstances is in this way getting significant for upgraded timeframe of realistic usability (Mir *et al.*, 2017) [38].

Varieties of micro greens

Plant species related to the families Brassicaceae, Asteraceae, Chenopodiaceae, Lamiaceae, Apiaceae, Amarillydaceae, Amaranthceae and Cucurbitaceae are mostly exploited. Bioactive substances are conspicuous in types of rather harsh taste, for example Brassicaceae, the variable adequacy of which warrants distinguishing proof of genotypes that may take into account requests for both taste and wellbeing (Xiao *et al.*, 2012) [55]. Microgreens can be obtained from different sorts of seeds. The well-known species are harvested using seeds from the following plant families (View & Club, 2019).

- **Brassicaceae family:** Broccoli, cauliflower, watercress, cabbage, arugula and radish
- **Asteraceae family:** Endive, lettuce, radicchio and chicory
- **Apiaceae family:** Carrot, dill, celery and fennel
- **Amaryllidaceae family:** Onion, leek and garlic
- **Amaranthaceae family:** Quinoa swiss chard, amaranth, spinach and beet
- **Cucurbitaceae family:** Cucumber, squash and melon
- **Cereals:** Rice, oats, wheat, corn and barley
- **Legumes:** Chickpeas, beans and lentils

Micro greens may differ in flavor that can vary from plain to spiced, tangy or even bitter, considering the type of green. Basically, their flavor is supposed to be strong and concentrated (View and Club, 2019) [52]. They are rich in bioactive compounds, macro and micro nutrients.

Bioactive compounds

Bio active amount is usually described in less edible microgreens varieties like sorrel (*Rumex acetosa* L.), peppercress (*Lepidium bonariense* L.), red cabbage (*Brassica oleracea* L. var. *capitata*) and also in a few varieties of more acceptable flavor like amaranth (*Amaranthus hypochondriacus* L.) and cilantro (*Coriandrum sativum* L.). The list of verified human bio active compounds present in the microgreens are carotenoids (violaxanthin, β-carotene and lutein/zeaxanthin), ascorbic acid (free, total and dehydro), tocopherols (α- and γ-tocopherol), and phylloquinone (Xiao *et al.*, 2012) [55].

Macro vs Micro nutrients

Microgreens are full of nutritional sources. While their concentration may vary in less amounts, many types are rich in K, Fe, Zn, Mg and Cu (Xiao *et al.*, 2012) [55]. They are a good resource of significant plant compounds like antioxidants. In addition to this, their nutritional value is concentrated, indicating higher vitamins, minerals and antioxidants than the same quantity of mature greens (Xiao *et al.*, 2016) [26]. Researchers have shown that levels of nutrients in micro greens are up to nine times greater than those found in mature greens (Pinto *et al.*, 2015) [49].

The higher concentrations of ascorbic acid, carotenoids, phylloquinone, and tocopherols are found in red cabbage, cilantro, garnet amaranth, and green daikon radish microgreens respectively along with various bioactive components while comparing with database values for fully grown vegetable counterparts (Xiao *et al.*, 2012) [55]. However, this early small-scale green research was carried out with restriction because the developing conditions, post cultivation conditions, and extraction techniques for the fully grown vegetables were unclear. As compared to database values, experimental data introduces uncertainties if we consider significant impacts of light wavelength and intensity on phytonutrients content. For instance, looking at information from fully grown vegetables for which just the peripheral leaves are accessible to light is questionable according to the micro green type of the vegetable (Xiao *et al.*, 2012) [55]. Researchers also reported that micro greens possess antioxidants and a number of polyphenols as contrast to their fully grown vegetable counterparts (Bull, 2008) [5]. According to one report, in 25 microgreen varieties which are commercially available, vitamins and antioxidant concentrations were found. While comparing these values with the USDA National Nutrient Database for fully grown vegetable leaves, vitamin and antioxidant values varied and it was approximated that values measured in microgreens were up to 40 times more than those reported for fully grown vegetable leaves (Xiao *et al.*, 2012) [55].

Microgreens or Sprouts?

Microgreens might be generally misconstrued for grown seeds (sprouts), which have been regularly concerned in food-borne disease although, microgreens possess some characteristic similarities with freshly herbs (e.g. basil, thyme, and cilantro), petite greens (e.g. baby spinach and spring mix) and sprouts. Many research studies discussed nutrition and physiological properties of microgreens but since 2009 a very few have particularly examined the food safety hazards of microgreens whereas worldwide studies have been carried out in order to explore leafy green and sprout safety (Riggio *et al.*, 2019) [44].

Microgreens and sprouts are consumed in immature condition however they are distinct from each other (Treadwell *et al.*, 2013) [50]. Sprouts are mainly grown-up in a dark environment of moisture where ready to microbial proliferation and their use different from of micro- and baby-greens has been applied in outbreaks of food borne epidemics. Also, micro greens have a wide range of leaf color, shape and varieties and greater taste increasing properties than sprouts. Many recent reports suggested that the nitrate content in microgreens is lower than that in fully grown vegetable leaves, further they also have higher amounts of minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo) and phytonutrients (ascorbic acid, b-carotene, a-tocopherol and phylloquinone) (Xiao *et al.*, 2012) [55].

Health benefits of micro greens

Bazzano *et al.* (2002) [1] and Carter *et al.* (2010) [1, 7] reported that the level of vitamins, minerals and beneficial plant compounds are high in microgreens so eating green vegetables is associated with a decreased danger of many diseases. They are also blessed with such vital nutrients to protect us from diseases.

Heart disease

It has great content of antioxidants e.g. polyphenols which can reduce the risk heart disease. As per different animal studies it is clear that microgreens may lower down the level of triglyceride and "bad" LDL cholesterol (Huang *et al.*, 2016; Tangney and Rasmussen, 2013) [16, 48].

Alzheimer's disease

Antioxidant-rich foods, including polyphenols, can decrease probability of memory related disease such as Alzheimer (Guest and Grant, 2016) [24].

Diabetes: Presence of antioxidants can facilitate to lower risk of type 2 diabetes. In laboratory experiments, fenugreek microgreens are supposed to increase cellular sugar uptake by 25–44% (Wadhawan *et al.*, 2018) [53].

Certain type of cancers: Antioxidant-rich fruits and vegetables particularly containing polyphenols, may decrease danger of different kinds of cancer (Zhou *et al.*, 2016) [58].

Preharvest factors shaping physicochemical-functional quality of microgreens

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 taxanomy.

Table 1: Show the Family Botanical name

Family	Botanical name	Reference
Amaranthaceae	*Amaranthus hypochondriacus*	Xiao *et al.*, 2012 [55]
	Amaranthus tricolor	
Apiaceae	*Apium graveolens*	Xiao *et al.*, 2012 [55]
	Coriandrum sativum	
Asteraceae	*Lactuca sativa* var *capitata*	Pinto *et al.*, 2015 [49]
Brassicaceae	*Barbarea verna*	Xiao *et al.*, 2016 [26]
	Brassica campestris var. Narinosa	Chandra *et al.*, 2012 [8]
	Brassica juncea	Xiao *et al.*, 2016 [26]
	Brassica narinosa var. Rosularis	Xiao *et al.*, 2016 [26]
	Brassica oleraceae var. Acephala	Xiao *et al.*, 2016 [26]
	Brassica oleraceae var. Alboglabra	Xiao *et al.*, 2016 [26]
	Brassica oleraceae var. Botrytis	Xiao *et al.*, 2016 [26]
	Brassica oleraceae var. Viridis	Xiao *et al.*, 2016 [26]
	Brassica oleraceae var. Capitata	Xiao *et al.*, 2016 [26]
	Brassica oleraceae var. Italica	Xiao *et al.*, 2016 [26]
	Brassica oleraceae var. Gemmifera	Xiao *et al.*, 2016 [26]
	Brassica oleraceae var. Gongylodes	Xiao *et al.*, 2016 [26]
	Brassica rapa var. Chinensis	Xiao *et al.*, 2016 [26]
	Brassica rapa var. Napobrassica	Xiao *et al.*, 2016 [26]
	Brassica rapa ssp Nipposinica	Xiao *et al.*, 2016 [26]
	Brassica rapa var. Pekinensis	Xiao *et al.*, 2016 [26]
	Brassica rapa var. Perviridis	Xiao *et al.*, 2016 [26]
	Brassica rapa var. Rapa	Xiao *et al.*, 2016 [26]
	Brassica rapa var. Ruvo	Xiao *et al.*, 2016 [26]
	Brassica rapa var. Rosularis	Brazaityte, Sakalauskien *et al.*, 2015 [3]
	Eruca sativa	Xiao *et al.*, 2016 [26]
	Lepidium bonariense	Xiao *et al.*, 2016 [26]
	Nasturtium officinale	Xiao *et al.*, 2016 [26]
	Raphanus sativus	Xiao *et al.*, 2016 [26]
	Raphanus sativus var. Longipinnatus	Xiao *et al.*, 2016 [26]
Chenopodiaceae	*Wasabia japonica*	Xiao *et al.*, 2016 [26]
	Artiplex hortensis	
	Beta vulgaris	
Fabaceae	*Spinacia oleracea*	Xiao *et al.*, 2012 [55]
	Pisum sativum	
	Cicer arietinum	
Lamiaceae	*Ocimum basilicum*	Xiao *et al.*, 2012 [55]
Poaceae	*Zea mays*	Xiao *et al.*, 2012 [55]
Polygonaceae	*Rumex acetosa*	Xiao *et al.*, 2012 [55]
	Fagopyrum esculentum	
	Fagopyrum tataricum	

Mostly used in studies were taxonomy 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.*, 2016) [26]. 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, eco-physiological, 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) [28]. 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) [55], 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) [26]. 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 a-tocopherol contents (Janovska, Stockova, & Stehno, 2010) [27]. Ebert *et al.* (2014) [17] 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) [17]. 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) [13].

Genotypic and morpho-physiological differences between broccoli landraces and hybrids have been documented by Ciancaleoni *et al.* (2014) [9]. Recent studies have also revealed the importance of wild edible plants in human diet (Romojaro *et al.*, 2013) [45]. For example, Faudale *et al.* (2008) [18] 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 (Ebert, 2014) [17].

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) [29]. 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, *et al.* (2016) [26] 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 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 chemo-preventive 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) [16].

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 hypo- chondriacus* L.) (Xiao *et al.*, 2012) [55]. Notwithstanding that acceptability of acrid taste varies widely and is subjected to inherit 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) [16].

Plant nutrition and bio fortification

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 bio fortification on microgreens growth, yield and quality.

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 *et al.*, 2010) [39]. 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, 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 NH4:NO_3), compared with sole nitrate (0:100 NH_4: NO_3), 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 and Santamaria, 2015) [15].

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) [26] 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) [60]. Biofortification of microgreens is feasible by modulating the fertilization program and the nutrient solution composition

It is possible to lower or increase the content of specific minerals (Tomasi *et al.*, 2015) [49], 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 bioavailability Przybysz *et al.* (2015, 2016) [43] reported that microgreens may be enriched with Mg and Fe. 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 Amaranthus species (Yang *et al.*, 2014) [57].

Table 2: Species growing conditions

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 postemergence daily fertilization with solutions of 21-2.2-16.6 (N-P-K) at 0, 75, or 150 mg L1 of N.	The most economical and high yielding (fresh weight per m^2) fertilization programs were the post-emergence daily supply of 150 mg L1 of N, or the post-emergence daily fertilization with 75 mg L1 of N combined with a pre-plant media incorporation of 1000 mg L1 of N from calcium nitrate.	Murphy *et al.* (2010) [39]
Table beet (*B. vulgaris* L.)	Greenhouse	Pre-plant incorporation in the peat-lite medium of 1000 and 2000 mg L1 of N supplied as calcium nitrate and/or postemergence daily fertilization with 21-2.2-16.6 (N-P-K) at 75, or 150 mg L1 of N.	Pre-sowing fertilization with calcium nitrate at 2000 mg L1 of N, combined with daily post-sowing fertigation using a 21-2.2-16.6 (N-P-K) at 150 mg L1 of N led to a two-fold yield increase as compared to the unfertilized control.	Murphy *et al.* (2010) [39]
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 *et al.*, 2014 [31]
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 L1 of Mg prepared using magnesium sulfate. Distilled water with 0, 6, 12, 24 and 36 mg L1 of Fe prepared using Ferric EDTA.	Enrichment of sprouts with Mg and Fe led to significant increase in Mg (23e152%) and Fe (50e130%) concentrations 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) [43]
Broccoli (*B. oleracea* L. var. Botrytis)	Growth chamber	Distilled water with 2 mmol L1 of zinc sulfate ($ZnSO_4$), potassium sulfate (K_2SO_4), methionine (Met) and without a S-source	The use of zinc sulfate as an source stimulated the sulforaphane formation in broccoli microgreens by enhancing myrosinase activity	Yang *et al.* (2014) [57]

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 *et al.*, 2015) [14]. Supplemental light sources frequently used in vegetable production include metal halide, fluorescent, incandescent and high-pressure sodium (HPS) lamps (Bian *et al.*, 2015) [2].

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 (Brazaityte *et al.*, 2015) [3].

Light quality demonstrates far more complex effects than light intensity and photoperiod in regulating growth processes and physiology (Bian *et al.*, 2015) [2]. In this respect, Brazaityte *et al.* (2015) [3]; demonstrated the species dependent enhancement of various oxygenated (lutein, neoxanthin, violaxanthin and zeaxanthin) and hydrocarbon (a- and b-carotene) carotenoids in Brassicaceae microgreens by altering LED spectral quality.

Supplemental green light (520 nm) increased the lutein/zeaxanthin ratio and b-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.

Further to basal HPS lighting, supplementary red LED for three days before harvest influenced the antioxidant properties of amaranth, basil, mustard, spinach, broccoli, borage, beet, kale, parsley and pea microgreens (Samuoliene *et al.*, 2012) [46]; 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) three days before harvest modified the nutritional quality of Perilla frutescens microgreens; 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 *et al.,* 2007) [40].

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) [40]. 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. Beyond visible spectra, ultraviolet (UV) radiation is also involved in photo-physiological responses of plants, with UV-A (320e400 nm) quality being the least hazardous. The phytochemical content of basil, beet and pak choi microgreens receiving 12.4 mmol 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 *et al.*, 2015) [3]. 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.

Notwithstanding possible interaction with genotypic or experimental conditions, these studies demonstrated that by managing spectral light quality, the concentrations of targeted phytochemicals can be altered. 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) [2]. The effects of five LED irradiation levels (545,440, 330, 220 and 110 mmol m^{-2} s^{-1}) on nutritional quality of Brassica microgreens (kohlrabi, mustard, red pak choi and tatsoi) were investigated by Samuoliene *et al.* (2013) and Brazaityte *et al.* (2015) [3], who found that applications of 330e440 mmol 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 mmol $m^{-2}s^{-1}$) negatively affected growth and nutritional quality, whereas high intensity (545 mmol m^{-2} s^{-1}) had no positive impact on most of the examined parameters.

Additionally, in 2013 Kopsell and Sams had demonstrated that application of high light (cool white and incandescent) intensity (463 mmol 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 mmol m^{-2} s^{-1} to low intensity (41 mmol m^{-2} s^{-1}) blue (470 nm) LED under 24-h photoperiod for five days before harvest elicited increase in shoot b-carotene, xanthophyll cycle pigments, glucoraphanin, epi-progoitrin, aliphatic glucosinolates, and essential macronutrients (P, K, Ca and Mg) and micronutrients (B, Mn, Mo and Zn) (Kopsell and Sams, 2013). The effects of continuous blue light on stomatal opening and membrane transport activity through variations in Hþ, Kþ and Ca2þ could be the main cause behind nutrient accumulation in broccoli shoot tissue.

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.*, 2014) [31]. Comprising 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 and Suslow, 2002) [6]. 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) [50]. 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 (Kou, Luo, *et al.*, 2014) [31].

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. Wound induced signaling has been shown to migrate to proximate non wounded tissue in fresh-cut lettuce eliciting phenolic composition and increase in respiratory activity (Saltveit *et al.*, 2005) [47]. 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 and Suslow, 2002) [6]. 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 increased 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 and Barrett, 2005) [19]. 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 *et al.*, 2015) [33]. Time of the day for harvesting may have significant implications for the bioactive composition and shelf-life of microgreens (Clarkson *et al.*, 2005[10]; Garrido *et al.*, 2015) [20]. 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 26 days following end of day harvest, associated with diurnal alterations in leaf sucrose and starch content (Clarkson *et al.*, 2005) [10]. 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) [20]. 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.*, 2013) [50], 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 pre-storage calcium applications may enhance microgreens quality and storage performance.

Preharvest, spray applications (z200 mL) of calcium amino acid chelate (1e20 mM), calcium lactate (1e20 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 5^0 Celsius (Kou *et al.*, 2015) [33]. 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 *et al.*, 2014).

Whereas shelf-life of untreated microgreens was limited to 7 days, preharvest calcium treatments prolonged shelf-life to over 14 days (Kou *et al.*, 2015) [33]. 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 days for 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.

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 *et al.*, 2015). Work on packaged daikon radish (*Raphanus sativus* var. *longipinnatus*) microgreens have revealed significant interaction between light exposure and package atmosphere composition established under OTR-specific films (Xiao *et al.*, 2014).

Light interference with pO2/pCO2 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, dependent on light intensity and photoperiod, that consumes CO_2 and releases O_2 within the packages (Kozuki *et al.*, 2015).

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 pO2 and high pCO2 generated under light and under dark storage conditions, respectively (Garrido *et al.*, 2016). Exposure of daikon radish microgreens kept at 5^0 Celsius to continuous low intensity fluorescent light (z30 mmol 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 *et al.*, 2014). Continuous low light intensity (25e30 mmol $^{s-1}$ 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) [35].

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 (10e60 min) pre-storage applications of low intensity NIR (100 mmol m^{-2} s^{-1} at l > 850 nm) reduced transpiration rates during subsequent storage under both dark and fluorescent light conditions (140 mmol 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 *et al.*, 2013), but both positive and negative effects on spinach (Glowacz *et al.*, 2014; Grozeff *et al.*, 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 days at 5 degree Celsius, though these side-effects subsided provided higher film permeability (Xiao *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 *et al.*, 2011). 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 (Xiao *et al.*, 2014).

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). On the contrary, light exposure accelerated the degradation of carotenoid compounds (b-carotene and violaxanthin), and reduced the hydroxyl radical scavenging capacity of cold-stored radish microgreens (Xiao *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 mmol m^{-2} s^{-1}, the concentrations of xanthophylls (lutein, zeaxanthin, and violaxanthin) and b-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 the compensation point where moderate MA is affected and pO2 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 (z30 mmol m^2 s^1) in 7-minute cycles every 2 hours for 3 days on spinach leaves suppressed leaf senescence parameters, such as chlorophyll and ascorbate degradation and hydrogen peroxide production, during subsequent 4^0 Celsius 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 mmol 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 (440e660 nm) LED light induced higher levels of vitamin C (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 *et al.*, 2008).

Microbial safety of microgreens

Several postharvest factors may interact with microbial build up on microgreens including, proximity to the soil (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 (Chandra *et al.*, 2012 [8]; Kou *et al.*, 2014) [31].

It has been hypothesized that the delicate, soft textured hypocotyls of microgreens may favor more microbial growth compared to their mature counterparts (Chandra *et al.*, 2012) [8]. Preharvest spray applications (z200 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 5^0 C but also inhibited the proliferation of AMB and yeast and mould (Y&M) populations (Kou *et al.*, 2014) [31].

This effect was characterized by dosage specificity and proved most effective at 10 mM concentration in controlling AMB proliferation (Kou *et al.*, 2014) [31]. On the other hand, post-harvest 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.*, 2014) [31].

Future Perspective of these microgreens

Most of the microgreen analysis and studies are carried out at comparatively small level and is limited to only a few numbers of researchers with limited targeted areas. There is a broad range of areas yet to be explored. Moreover, some of the varieties of microgreens have been studied and analyzed, but many of them have not been put for commercialization. The influence of sunlight on microgreens development and nutrition has been precisely taken care of whereas the effect of low night temperatures on plant development, nutritional level, and food risks of microgreens has not been analyzed.

Prevention and treatment methods should be identified for microgreens because they are beneficial but maintaining quality and safety of microgreens is still in its earliest stages. It has been established that post-harvest light treatments can increase the formation of bioactive elements, but this was not properly analyzed to apply on a broad range of microgreens. It is an issue of discussion that phytonutrient substances could give innate protection from quality and wellbeing issues. Identification of many post cultivation treatments have been carried out from time to time to keep quality and to extend the lifespan of microgreens.

For the production of ready-to-eat microgreen products, washing and drying methods should be more focused. It is especially significant to put more and more research into ensuring the safety and quality of this new addition to healthy diets so that the food industry could resolve some of the problems that have created challenges for fully grown vegetables.

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