Feature Article

Microgreen nutrition, food safety, and shelf life: A review

Ellen R. Turner, Yaguang Luo, and Robert L. Buchanan

Abstract: Microgreens have gained increasing popularity as food ingredients in recent years because of their high nutritional value and diverse sensorial characteristics. Microgreens are edible seedlings including vegetables and herbs, which have been used, primarily in the restaurant industry, to embellish cuisine since 1996. The rapidly growing microgreen industry faces many challenges. Microgreens share many characteristics with sprouts, and while they have not been associated with any foodborne illness outbreaks, they have recently been the subject of seven recalls. Thus, the potential to carry foodborne pathogens is there, and steps can and should be taken during production to reduce the likelihood of such incidents. One major limitation to the growth of the microgreen industry is the rapid quality deterioration that occurs soon after harvest, which keeps prices high and restricts commerce to local sales. Once harvested, microgreens easily dehydrate, wilt, decay and rapidly lose certain nutrients. Research has explored preharvest and postharvest interventions, such as calcium treatments, modified atmopsphere packaging, temperature control, and light, to maintain quality, augment nutritional value, and extend shelf life. However, more work is needed to optimize both production and storage conditions to improve the safety, quality, and shelf life of microgreens, thereby expanding potential markets.

Keywords: preharvest, postharvest, shelf life

1. INTRODUCTION

Microgreens are an emerging class of produce that have gained increasing popularity (Kyriacou et al., 2016; Pinto, Almeida, Aguiar, & Ferreira, 2015; Xiao, Lester, Luo, & Wang, 2012). They are the seedlings of edible plants harvested 7–14 days postplanting when the first true leaves start to emerge. Microgreens have been used primarily in the restaurant industry to embellish cuisine and are most commonly consumed fresh in salads, soups, and sandwiches. An assortment of colors, visual textures, aromas, and flavors give appeal to these tender young greens pictured in Fig. 1.

Microgreens are ideally suited for indoor production and are part of the global movement towards controlled environmental agriculture (CEA) (Riggio, Jones, & Gibson, 2019a). This movement is driven by population growth, shrinking arable land, and the need for ensuring food security (Goodman & Minner, 2019; Stoleru, Ionită, & Zamfirache, 2016; Wood, 2019). The short time to harvest for microgreens and high market values makes them important CEA crops (Wood, 2019).

The microgreen market is growing rapidly (Charlebois, 2019; Riggio et al., 2019a; Wood, 2019), but faces many challenges. Microgreens share many characteristics with sprouts, and have been associated with seven recalls in the United States and Canada; three due to *Salmonella* (Canadian Food Inspection Agency [CFIA], 2018a; Clark, 2017; Marler, 2016), and the other four due to *Listeria* contamination (CFIA, 2018b, CFIA, 2019; U.S. Food and Drug Administration [FDA], 2018; Whole Foods Market, 2018).

One major limitation to the growth of the microgreen industry is rapid quality deterioration postharvest. Microgreens are difficult to store, due to their high surface area to volume ratio, high respiration rate, and delicate leaves that easily wilt, and rapid postharvest decay transpiration, leakage of nutrient rich exudates, tissue damage, and early senescence (Berba & Uchanski, 2012; Chandra, Kim, & Kim, 2012; Kou et al., 2013). Some growers sell microgreens as a "living product" so that the customer harvests and washes them as they are needed to serve the freshest quality. Hydroponic pads and soil-less substrates tend to be favored for this practice for ease of transport and perception of cleanliness in a kitchen environment (Renna, Di Gioia, Leoni, Mininni, & Santamaria, 2017). However, these microgreens still need to be used quickly to maintain peak quality.

Most research on microgreens has taken place in the last 7 years by a limited but growing number of international research groups (Brazaitytė et al., 2018; Craver, Gerovac, Lopez, & Kopsell, 2017; Kyriacou et al., 2016; Riggio, Wang, Kniel, & Gibson, 2019b; Xiao et al., 2012). Each group has focused on a narrow subset of microgreens and their issues. The intention of this review is to fit together these pieces and bring attention to the areas that are potential impediments to commercialization.

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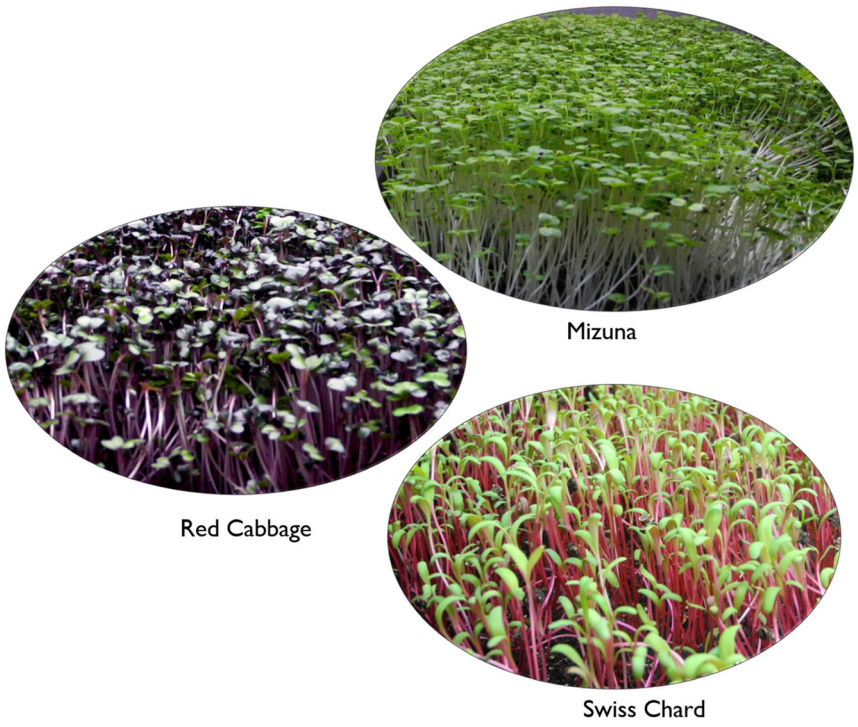

Figure 1–Mizuna, red cabbage, and Swiss chard microgreens grown in peat-based growth medium for 8 days.

Table 1–Taxonomic families of common microgreens.

Family	Commonly grown microgreens
Alliaceae	chives, scallions, shallots, onions, garlic
Amaranthaceae	spinach, amaranth, beets, swiss chard, orach, and magenta spreen
Apiaceae	celery, cilantro, chervil, fennel, parsley, carrot, and dill
Asteraceae	lettuce, endive, sunflower, garland chrysanthemum, shungiku, tagetes (marigold)
Brassicaceae	mustards, cabbages, broccoli, cauliflower, radishes, tatsoi, wasabi, arugula, cresses, kohlrabi, mizuna, turnip, savoy, kale, komatsuna, pak choi, kogane, collard, nasturtium, brussel sprouts, rapini, rutabaga
Cucurbitaceae	cucumber
Fabaceae	sweet pea, alfalfa, fenugreek, adzuki, fava
Lamiaceae	mint, basil, chia, and lemon balm
Oxalidaceae	wood sorrels, clover
Poaceae	corn, lemongrass
Polygonaceae	buckwheat
Portulacaceae	claytonia, purslane

2. MICROGREEN PRODUCTION

Although the focus of this review is postharvest quality and safety, several preharvest practices affect the postharvest nutrition profile, food safety, and shelf life of microgreens.

2.1 Microgreen selection and basic growing practices

The 12 plant families most commonly grown as microgreens are provided in Table 1. Many of these herbs and vegetables are well known for their health benefits. The *Brassica* vegetables, in particular, contain compounds that may protect against cancer (Herr & Büchler, 2010) including glucosinolates (Fuentes, Paredes-Gonzalez, & Kong, 2015), carotenoids (Niranjana et al., 2015; Nishino, Murakoshi, Tokuda, & Satomi, 2009), and selenium (Donaldson, 2004). The hydrolysis products of these glucosinolates have antimicrobial properties (Cavaiuolo & Ferrante, 2014; Delaquis & Mazza, 1995; González-Lamothe et al., 2009). The Amaranthaceae, Apiaceae, and Lamiaceae are also health beneficial, and plants in the Alliaceae and Lamiaceae also produce antimicrobial compounds. Microgreens are usually grown in greenhouses in growing flats containing potting mixes, peat-based mixes, hydroponic growth medium, or even with recycled textile fiber mats (Di Gioia, De Bellis, Mininni, Santamaria, & Serio, 2017).

The cultivation of microgreens requires an ample supply of neutral to slightly acidic water. Seeds of some varieties are soaked overnight enhance germination. Flats may be covered or placed in reduced light condition during germination. After approximately 3 days, the plants are exposed to light and watered daily until the first set of true leaves begin to emerge.

2.2 Lighting systems

Growers often use indoor grow lamps instead of natural lighting. Greenhouse growers often supplement the natural light with "grow lights." Gas-discharge lamps (GDLs) such as high-pressure sodium (HPS) lamps are most commonly used, although researchers are exploring the benefits of light-emitting diode (LED) (Agarwal & Gupta, 2016). LED lighting allows customization of the spectral composition to match plant photoreceptors and optimize production, plant morphology, and nutrient content (Morrow, 2008). LED light systems provide distinct operational advantages and are environmentally friendlier than GDLs (Agarwal & Gupta, 2016; Morrow, 2008). The LEDs also allow more uniform lighting distribution than conventional fluorescent tubes or HPS lamps.

2.3 Effects of light quality

Light quality affects many aspects of plant growth, morphology, color, flavor, and nutrition (Kyriacou et al., 2016). Alrifai, Hao, Marcone, and Tsao (2019) explains that red, blue, and combined red plus blue light are more effective than white light and

other wavelengths for enhancing photosynthesis and regulating plant metabolism. Samuolienė et al. (2011) found that different supplemental LED wavelengths in addition to the basal components of blue (455 nm), red (638 nm), deep red (669 nm), and far red (731 nm) had different effects on the antioxidant compounds in sprouted seeds. Supplementation with green light (510 nm) improved antioxidant properties of lentil and wheat-sprouted seeds (Samuolienė et al., 2011) and improved mineral element content in beet microgreens (Brazaitytė et al., 2018). Carvalho and Folta (2016) found that green LED light produced very different irradiance-dependent effects on anthocyanin production in green and red varieties of the same microgreen species. Lobiuc et al. (2017) found that while blue light enhanced growth, cotyledon area, fresh mass, chlorophyll a, and anthocyanin pigment content of both red and green microgreens, phenolic content and free radical scavenging activity were improved by application of mostly red light in the green cultivar and mostly blue light in the red cultivar. Supplementation with amber light (595 nm) enhanced antioxidant properties of radish sprouts (Samuolienė et al., 2011). Samuolienė et al. (2012) found that supplementing HPS lamps with short-term red LED lighting altered the antioxidant properties of amaranth, basil, mustard, spinach, broccoli, borage, beet, kale, parsley, and pea microgreens. Supplemental light wavelengths resulted in increased metabolic production of different bioactive compounds in different species, presumably to protect the plants from mild photooxidative stress. In most species, total antioxidant activity increased; however, the supplemental wavelengths did not significantly affect the antioxidant content of amaranth, broccoli, and pea, and decreased levels in beet microgreens (Samuolienė et al., 2012). Short-term red LED lighting (638 nm) increased total anthocyanins and total ascorbic acid and decreased nitrate content in purple mint *Perilla frutescens* microgreens (Brazaitytė, Jankauskienė, & Novičkovas, 2013) and also increased P, K, Ca, Mg, S, and Mn, but reduced Na, Fe, Zn, Cu, and B in beet microgreens (Brazaitytė et al., 2018). Total phenolic content (TPC) increased in all microgreens except for amaranth; total ascorbic acid content increased in amaranth, kale, broccoli, mustard, and pea and declined in basil and borage microgreens; total anthocyanins increased in amaranth, kale, broccoli, tatsoi, and pea and declined in mustard, borage, beet ,and parsley microgreens (Samuolienė et al., 2012). Application of blue (470 nm; 41 μmol m^{-2} s^{-1}) LED light 5 days prior to harvest resulted in significant increases in carotenoids, glucosinolates, and micro- and macromineral elements in broccoli microgreens compared to a combination of red (627 nm; 88%) and blue (470 nm; 12%) LED lights with average light intensity of 350 μmol m^{-2} s^{-1} (Kopsell & Sams, 2013). In particular, this treatment increased the levels of β-carotene, violaxanthin, glucoraphanin, K, Mg, and Fe.

Vaštakaitė et al. (2015) reported that application of blue (447 nm) LED lighting in combination with red (638, 665 nm) and far red (731 nm) affected phytonutrient levels differently in red pak choi, tatsoi, and basil microgreens. The balance of the light was made up with 638 nm red light, providing the same total photosynthetic photon flux density (PPFD) for all treatments. Total ascorbic acid levels were highest in tatsoi cultivated under 8% blue light, whereas ascorbic acid levels were maximized in red pak choi and basil cultivated under 16% blue light. Phenols and anthocyanins accumulated more significantly in tatsoi and basil cultivated under 25 and 33% and in red pak choi under 0 and 8% blue light. Flavanols were highest in tatsoi cultivated under 25% blue light, red pak choi cultivated under 33% blue light followed by 16% blue light, and in basil cultivated under 16% blue light. Free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging was highest for 0% followed by 33% blue light in tatsoi and basil and highest at 25% blue light in red pak choi. The authors suggested that photostress could be caused by either insufficient blue light, or an excess of blue light, both resulting in the synthesis of protective antioxidant compounds. All dosages of blue light increased leaf area and decreased hypocotyl length and plant height compared to 0% (Vaštakaitė et al., 2015). Samuolienė et al. (2017) obtained greater accumulation of chlorophylls and carotenoid pigments at blue light intensities of 33%, than at lower intensities. Tocopherols had greater accumulation at 16% blue light (Samuolienė et al., 2017). Brazaitytė et al. (2018) found that supplemental blue LED light 455 nm increased P, K, Ca, Mg, S, and Mn, but did not affect Fe, Zn, Cu, or B in Kohlrabi microgreens.

The antioxidant profile of baby leaf lettuce and sprouted seeds grown under natural lighting and HPS lamps has also been reported to be sensitive to spectral light quality, with different wavelengths of blue and green supplementary LED lighting altering the amounts of phytochemicals produced (Samuolienė et al., 2011, 2012). Increases and decreases in phytochemical synthesis and antioxidant activity under the same supplemental lighting were dependent on variety and season. Seasonal changes in cloud cover, day length, and incident angle of light affect light quantity and quality and cause changes in the requirements for supplemental light. Brazaitytė et al. (2015a) used a set of LED-lighting modules comprising the basal components (447, 638, 665, and 731 nm) with combined PPFD of 285 μmol m^{-2} s^{-1} supplemented with green (520 nm), yellow (595 nm), or orange (622 nm) LEDs, each with PPFD of 15 μmol m^{-2} s^{-1} for a total PPFD of 300 μmol m^{-2} s^{-1} to evaluate the effects of irradiance spectra on carotenoid concentrations in mustard, red pak choi, and tatsoi. All supplemental wavelengths increased total carotenoid content in mustard but decreased it in red pak choi (Brazaitytė et al., 2015a). Supplemental yellow light increased violaxanthin and total carotenoid content in tatsoi (Brazaitytė et al., 2015a). Brazaitytė et al. (2015b) found that UV-A irradiation supplemental to basal LED illumination was generally able to improve antioxidant properties of basil, beet, and pak choi microgreens at 12.4 μmol m^{-2} s^{-1} with some wavelengths benefitting particular antioxidant components. Pak choi microgreens benefitted the most from added UV-A irradiation, with almost all supplemental wavelengths increasing leaf area and fresh weight, DPPH free-radical scavenging activity, total phenols, anthocyanins, total ascorbic acid, and α-tocopherol (Brazaitytė et al., 2015b).

In addition to wavelength, adjusting the frequency of light pulses can affect plant development and photosynthetic activity (Ani, Ahmad, & Zain, 2014; Vaštakaitė et al., 2017, 2018). Vaštakaitė et al. (2017, 2018) found that phytonutrient contents in red pak choi, mustard, tatsoi, and basil microgreens including phenols, anthocyanins, and total ascorbic acid (in basil), and antiradical activity could be enhanced by supplementing HPS lights with light of a specific wavelengths or by pulsing the LEDs at specific frequencies. TPC was enhanced in both red pak choi and tatsoi at 32 Hz and was maximized at 455 nm for red pak choi and at 627 nm in tatsoi (Vaštakaitė et al., 2017). TPC was enhanced in mustard at 256 and 1024 Hz and was maximized at 470 and 590 nm (Vaštakaitė et al., 2017). TPC and total anthocyanins were maximized in basil for blue (470 nm) and red (627 nm) at a frequency of 1024 Hz, and TPC was also maximized in basil for blue (455 nm) at 256 Hz (Vaštakaitė et al., 2018). The highest DPPH radical scavenging activity occurred in basil at 256 Hz for all wavelengths except 627 nm, while ascorbic

acid content was highest for 32 and 256 Hz frequencies (Vaštakaitė et al., 2018).

2.4 Effects of light quantity

The same light quality at different irradiance levels may have very different effects on plant biochemistry and nutritional quality. Samuolienė et al. (2013) assessed the effect of irradiance level on growth and nutritional quality of *Brassica* microgreens including kohlrabi, mustard, red pak choi, and tatsoi. A system of five lighting modules with 455-, 638-, 665-, and 731-nm LEDs adjusted to 20, 40, 60, 80, and 100% was used to obtain PPFD of 110, 220, 330, 440, and 545 μmol m^{-2} s^{-1}. Optimal microgreen growth was obtained at 330–440 μmol m^{-2} s^{-1}, producing larger leaf surface area, lower nitrate concentrations, and higher total anthocyanins, total phenolics, and DPPH free radical scavenging capacity. However, at the 110 to 220 μmol m^{-2} s^{-1} PPFD, α-tocopherol was higher in all microgreen species and ascorbic acid levels were higher for both tatsoi and red pak choi (Samuolienė et al., 2013). Ying, Kong, Jones-Baumgardt, and Zheng (2020) investigated red-blue LED lighting at 5, 10, 15, 20, 25, and 30% blue light for optimizing the yield and visual quality of cabbage, kale, arugula, and mustard microgreens at PPFD of 300 μmol m^{-2} s^{-1}. Using a 16-hr photoperiod and light /dark temperature of 20/16 °C, they found that 15% blue light was optimal for cabbage, but recommended 5% blue light for kale, arugula, and mustard. Increasing light intensity from 100 to 600 μmol m^{-2} s^{-1}, while keeping the blue: red ratio steady at 15:85, resulted in asymptotic increase in fresh weight and dry weight, but approximately a linear decrease in hypocotyl length and hue angle for all four Brassicaceae species (Jones-Baumgardt, Llewellyn, Ying, & Zheng, 2019). Leaf area was maximal at different light intensities for different species (Jones-Baumgardt et al., 2019). Increasing blue light intensity 3 days prior to harvest at 23 days decreased nitrate content in tatsoi, but reduced ascorbic acid content in plant leaves (Simanavičius & Viršilė, 2018).

High-light conditions result in increased photosynthetic capacity, which was enabled by increased photosystems, electron transport and ATP synthase complexes, and enzymes of the Calvin–Benson cycle (Walters, 2005). Higher concentrations of the photosynthetic "machinery" reduce the susceptibility to photodamage. However, low-light conditions caused plant leaves to undergo an increase in the relative number of light-harvesting complexes and in the stacking of thylakoid membranes to form grana, changes which optimize light utilization (Walters, 2005). High-light treatment of 463 μmol m^{-2} s^{-1} caused a shift in the xanthophyll cycle pigments in broadleaf mustard microgreens (*Brassica juncea* L.), reducing β-carotene levels and increasing zeathanthin levels (Kopsell, Pantanizopoulos, Sams, & Kopsell, 2012). Loedolff, Brooks, Stander, Peters, and Kossmann (2017) were able to increase polyphenolic content in wild rocket microgreens by high-light treatment of 272 μmol photons m^{-2} s^{-1}, and in particular to stimulate synthesis of resveratrol, catechin, and epi-catechin. Lin et al. (2013) recommended 400–600 μmol m^{-2} s^{-1} PPFD to optimize above-ground biomass for hydroponic lettuce production using red, blue, and white LEDs. The same cumulative photosynthetically active radiation can be achieved by using high-intensity light conditions for shorter photoperiods as with low-intensity lighting for a longer photoperiod. Preliminary studies in our laboratory suggest that exposure of plants grown from seed inoculated with *Escherichia coli* to high-intensity fluorescent light hastens bacterial die-off.

While the light intensity clearly makes a difference in plant growth and nutrition, there are few studies on the effects of photoperiod on plant growth and nutrition, with none being found for microgreens. Photoperiod has been shown to play a role in the essential oil produced in plants (Sangwan, Farooqi, Shabih, & Sangwan, 2001), including lemongrass (Herath & Ormrod, 1979) and mint (Farooqi, Sangwan, & Sangwan, 1999). Photoperiod has also been reported to affect the nutrient composition of baby spinach (Lester, Makus, Hodges, & Jifon, 2013).

3. MICROGREEN NUTRIENT CONTENT

3.1 Nutrient profiles

Xiao et al. (2012) showed that red cabbage, cilantro, garnet amaranth, and green daikon radish microgreens had the highest concentrations of ascorbic acid, carotenoids, phylloquinone, and tocopherols, respectively, with the levels of these bioactive components being significantly higher in microgreens compared to data base values for mature vegetable counterparts. One limitation in this early microgreen research was that the growing conditions, postharvest storage conditions, and extraction methods for the mature vegetables were unknown. Considering the substantial effects of light wavelength and intensity, and wavelength–intensity interactions, on phytonutrient contents, comparison of experimental data to database values introduces uncertainties. For example, comparing data from head-forming mature vegetables for which only the outermost leaves are exposed to light is uncertain in relation to the microgreen form of the vegetable.

Since 2016, there have been several studies in which mature leaves of nonhead forming vegetables have been demonstrated to have higher levels of certain bioactive compounds than microgreen leaves. Kale microgreens accumulated lower carotenoid contents than has been reported for mature kale; however, broccoli and cauliflower microgreens had higher concentrations than mature florets (Xiao et al., 2019). Klopsch et al. (2018) also found that mature leaves of pea and lupin had higher carotenoid concentrations than pea and lupin microgreens. Niroula et al. (2019) found that carotenoid content increased in wheat and barley microgreens over the course of the 16-day growth period studied. The rate of accumulation slowed between 7 and 10 days in wheat and between 10 and 13 days in barley, but maximum values were obtained on the last harvesting day. Kale and mustard microgreens were noted to have lower ascorbic acid than their mature counterparts (de la Fuente et al., 2019). Some researchers reported that microgreens grown in a hydroponic system had lower concentrations of chlorophylls, carotenoids, phenols, and anthocyanins than in baby leaf or mature leaves of the same species (Bulgari, Baldi, Ferrante, & Lenzi, 2017).

Nevertheless, it is obvious that microgreens are excellent sources of phytonutrients. For example, Sun et al. (2013) profiled polyphenols in five microgreen cultivars of the genus *Brassica* and found 165 phenolic compounds comprising many highly glycosylated and acylated quercetin, kaempferol, cyanadin aglycones, and complex hydroxycinnamic and benzoic acids. They reported more complex polyphenol profiles and a greater variety of polyphenols in the microgreens than in their mature plant counterparts. Analysis of 30 cultivars of microgreens of the family Brassicaceae revealed that *Brassica* microgreens are good sources of the macroelements, K and Ca, and the microelements, Fe and Zn (Xiao et al., 2016). Additionally, microgreens of the family Brassicaceae were found to be moderate to excellent sources of ascorbic acid, phylloquinone, carotenoids, tocopherols, glucosinolates, and

polyphenols (Xiao et al., 2019). Cauliflower, rapini, red radish, China rose radish, and ruby radish microgreens were found to have the greatest contents of total ascorbic acid, phylloquinone, total tocopherols, total glucosinolates, and TPC, respectively. Ruby radish microgreens also had the greatest DPPH radical scavenging capacity. de la Fuente et al. (2019) evaluated the bioaccessibility of several antioxidant bioactive compounds and minerals in broccoli, kale, mustard, and radish microgreens grown hydroponically. Radish and mustard were found to have the highest bioaccessable fraction (BF) for ascorbic acid, total carotenoids, and total isothiocyanates, while broccoli, kale, and radish all had comparable high BF for total polyphenols. Broccoli and mustard showed the lowest and highest BF values, respectively, for potassium and magnesium, while kale had the highest BF value for calcium (de la Fuente et al., 2019). Pinto et al. (2015) showed that microgreen lettuce (*Latuca sativa* var. *capitata*; 2-week old) had higher content of most minerals (Ca, Mg, Fe, Mn, Zn, Se, and Mo) than mature lettuce (10-week old). Kyriacou et al. (2019) reported that basil and swiss chard microgreens were excellent sources of K and Mg, and purple basil was particularly high in ascorbic acid while green basil and coriander were especially good sources of beta-carotene and total polyphenols. Klopsch et al. (2018) added pea and lupin microgreens and mature leaves to bread dough to enhance the nutritional value of bread. In spite of the decline in carotenoids and chlorophylls, flavonoid levels were maintained with low losses during baking and significant pheophytin formation occurred. Lupin microgreen bread retained high levels of genistein which has anticarcinogenic properties, especially in women (Romagnolo, Donovan, Papoutsis, Doetschman, & Selmin, 2017). Polash, Sakil, and Hossain (2018) demonstrated that bioactive components and antioxidant activity in mustard, radish, and cabbage microgreens degraded rapidly after harvest, so that to obtain substantial health benefits from eating microgreens, they should be consumed soon after harvest.

4. OTHER NUTRITIONAL FACETS

4.1 Calcium treatment

While preharvest and postharvest calcium treatments can both affect microgreen phytonutrients, preharvest calcium treatments have a much more significant benefit (Kou et al., 2014). Kou et al. (2014) found that 10 mM calcium chloride treatment applied before harvest delayed decline of overall quality and extended shelf life of broccoli microgreens stored at 5 ˚C from 7–10 days to 14–21 days. The 10 mM calcium chloride treatment stimulated superoxide dismutase and peroxidase activities, reduced tissue electrolyte leakage, and reduced microbial growth during storage (Kou et al., 2014). Sun et al. (2015) found that glucosinolates were the main compounds in broccoli microgreens that were enhanced by 10 mM calcium chloride preharvest treatment. Lu et al. (2018) found that postharvest UV-B radiation after preharvest 10 mM calcium chloride treatment further boosted glucosinolate levels.

4.2 Temperature effect on nutrient retention and content

While overall temperature and nighttime temperature may affect nutrient content of mature plants (Burbott & Loomis, 1967; Steward et al., 1959), no work has been published on the effect of temperature or intermittent temperature treatments on microgreen nutrition. Xiao et al. (2014b) reported that chlorophyll retention in radish microgreens after harvest was dramatically affected by storage temperature. When samples were stored at 1 °C, the radishes retained nearly 100% over 2-week storage period, but when stored at 5 °C retention dropped to approximately 60%, and at 10 °C plummeted to 25%. Microgreen nutrition studies have focused on the Brassicaceae family, but other vegetable families that have received little attention (see Table 1).

4.3 Microgreens as functional foods

Choe, Yu, and Wang (2018) reviewed the use of microgreens as functional foods in diet-based disease prevention, that is, obesity, cardiovascular disease, type 2 diabetes mellitus, and cancer. Huang et al. (2016) found that red cabbage microgreen supplementation had health-promoting effects in mice fed a high fat diet. Supplementation with microgreens attenuated body weight gain, lowered low-density lipoproteins (LDL) cholesterol levels, reduced hepatic cholesterol ester and triglyceride levels, and inflammatory cytokines. Supplementation of high fat diet with mature red cabbage also had beneficial effects but did not reduce triglyceride levels. Interestingly, supplementation of low-fat diet with red cabbage microgreens raised both low-density lipoprotein and high-density lipoprotein cholesterol levels (Huang et al., 2016).

Hydroponic systems have been evaluated as a means of tailoring the optimal nutrients for the cultivar and functional benefits for intended consumers. For example, for patients with impaired kidney function requiring a low potassium diet, the nutrient solution used can be prepared with low or no potassium (Renna, Castellino, Leoni, Paradiso, & Santamaria, 2018). Puccinelli, Malorgio, Rosellini, and Pezzarossa (2019) found that selenium supplementation of the hydroponic nutrient solution for basil microgreens produced selenium-enriched leaves and increased antioxidant capacity. Since rocket microgreens accumulate nitrogen excessively, the ability to meet E.U. vegetable nitrate limitations in rocket greens can be a challenge (Bulgari et al., 2017). Growing rocket microgreens hydroponically, Bulgari et al. (2017) controlled nitrogen content in the microgreens by limiting the nitrogen in the nutrient solution.

5. FOOD SAFETY OF MICROGREENS: RELATION TO LEAFY GREENS AND SPROUTS

Microgreens share many characteristics with leafy greens and sprouts. They are generally consumed raw to retain nutritional benefits and their fresh, crisp appeal. Their cultivation most closely resembles sprout production; they are cultivated in controlled environments, thereby avoiding potential field sources of contamination (Barak & Schroeder, 2012). However, they are immature when consumed and affected by physiological differences that make the young plants more vulnerable to human pathogen colonization and internalization (Warriner, Ibrahim, Dickinson, Wright, & Waites, 2003).

5.1 Vulnerability of young plants to microbial colonization

Warriner et al. (2003) reported that microgreens are more vulnerable to bacterial internalization than mature vegetable plants and described how bacteria present in seeds can become part of the endophytic microflora. During seed germination, the seed releases a mixture of carbohydrates and peptides that can attract surrounding bacteria in the rhizosphere. Access to inner apoplastic space is restricted by protective border cells on the root surface. However, bacteria can enter via germinating radicals or secondary roots and can persist in localized sites (Warriner et al., 2003).

In mature plants, bacteria localized in apoplastic fluid surrounding root cells cannot enter the xylem because of the Casparian strip: a thickened cell wall containing the water-insoluble substance, suberin. However, in immature plants protective structures are not fully formed, enabling entry of bacteria into xylem (Warriner et al., 2003). Dong, Iniguez, Ahmer, and Triplett (2003) inoculated the roots of 1–2-day old alfalfa seedlings with a low inoculum level of 10^2 cfu per plant *Salmonella* Typhimurium and found that the pathogen colonized the interiors of 6–9-day old seedlings in high numbers. They observed significant colonization of lateral root cracks, suggesting that this may be the site of entry for these bacteria. Gyaneshwar et al. (2001) also noted that higher concentrations of *Serratia marcescens* were seen at 3 days after inoculation near emerging lateral roots of rice seedlings, again indicating a potential site of ingress for bacteria into plants. At 6 days, the bacteria were found in stems and leaves. Using microscopy, large numbers of bacteria were observed within intracellular spaces, senescing root cortical cells, arenchyma, and xylem vessels (Gyaneshwar et al., 2001). Jablasone, Warriner, and Griffiths (2005) found *E. coli* O157:H7 in the internal tissues of cress, lettuce, radish, and spinach seedlings, but not within the tissues of mature plants. The pathogen preferentially colonized epidermal root junctions, sites of exudate release (Jablasone et al., 2005; Solomon, Yaron, & Matthews, 2002).

5.2 Food safety of microgreens versus sprouts

The greater vulnerability of sprouts to pathogen contamination may be primarily the result of sprout production practices. Sprout seeds are soaked in water to enhance germination and are generally sprouted in jars, bins, or rotating drums without light, in very high humidity and warm temperatures, often with recirculating water. If microbial contamination is present on seeds or production equipment, or introduced by insects or lack of hygienic practices by workers, rapid growth of microbes will ensue, resulting in contamination of the entire batch. Seeds have been found to be the main source of pathogens responsible for sprout-related foodborne illness outbreaks (Fett, 2006; Yang et al., 2013). Therefore, obtaining seeds that have been certified for sprouting and produced using good agricultural practices is an important first step for sprout growers. Seed decontamination is an important second step. The U.S. FDA (1999) recommended that all seed destined for sprout production should be treated with one or more treatments (such as 20,000 ppm calcium hypochlorite) that have been approved for reduction of pathogens in seeds or sprouts. Although this decontamination process has improved the safety of sprouted seeds, occasional foodborne illness outbreaks and product recalls associated with sprouts continue to occur (Erdozain, Allen, Morley, & Powell, 2013). More scientific knowledge is needed to develop technologies to eliminate seeds as a source of human pathogen contamination. Some of the seeds for sprouting, for example, alfalfa and clover, are not typically grown for microgreens. However, others, like radish and broccoli seeds, have been implicated in microgreen recalls. Despite widely varying practices, microgreen production generally has several key differences from sprout production. For microgreen production (1) plants are generally anchored by rooting them in some type of solid medium; (2) plants are generally exposed to light and moving air after germination and elongation, which cause evaporation of water from the growth matrix, reducing humidity; (3) water is not usually recirculated in solid medium-based production systems, although frequently is in hydroponic systems; and (4) plants are generally harvested by cutting stems above the root at the emergence of the first pair of true leaves when the cotyledons are fully developed (Berba & Uchanski, 2012). These conditions may lead to safer product than those associated with sprout production. Xiao, Nou, Luo, and Wang (2014c) compared the survival and proliferation of *E. coli* O157:H7 and O104:H4 on radish sprouts and microgreens cultured in a BSL-2 growth chamber under sprout and microgreen production conditions. Although the pathogens were able to proliferate in both production systems, they were significantly (3–5 log cfu/g) higher on sprouts than on microgreens. However, hydroponically grown microgreens may result in safety concerns similar to those of sprouts due to the humid conditions and constant warm temperatures. Furthermore, use of recirculated water may allow pathogens to proliferate as in sprout production (Riggio et al., 2019b).

Wright and Holden (2018) found that Shiga toxin-producing *E. coli* (STEC) inoculated onto seeds and into irrigation water were able to proliferate on eight different species of microgreens grown on hydroponic mats. Even at their lower inoculation level (3 log cfu/mL,) they observed colonization to high levels on all microgreen species exposed to contaminated irrigation water. Seeds were sanitized in bleach solution prior to inoculating with STEC at 7 log cfu/g. Most seed contamination resulted in colonization of the surrounding water. The authors suggested that the same seed pregermination treatments required for growing sprouted seeds should be taken for microgreens, at least those grown in hydroponic systems. Wright and Holden (2018) did not test STEC growth/survival on microgreens grown in soil substitute. Reed, Ferreira, Bell, Brown, and Zheng (2018) found that *Salmonella enterica* was at least as successful at surviving and growing on Swiss chard microgreens as on alfalfa sprouts and successful colonization was inoculum level dependent. However, *S. enterica* growth on seed-inoculated alfalfa sprouts was also affected by seed storage time, while on Swiss chard microgreens it was dependant on serovar. Although studies on the survival and growth of pathogens on microgreens are limited, such studies are abundant for sprouts (Phua, Neo, Khoo, & Yuk, 2014; Taormina, Beuchat, & Slutsker, 1999; Waje et al., 2009; Wilderdyke, Smith, & Brashears, 2004).

Since microgreens are cut to harvest and sprouts are not, the increased need for worker handling could be another potential source of contamination for microgreens. Mechanization of the harvesting can reduce the need to handle microgreens, and education can improve worker hygiene practices. Riggio et al. (2019b) suggested that the interaction of the harvesting implement with the cut edge of the stem may be another source of contamination that is not shared by sprouts. It should be possible to manage such potential sources in a controlled environment using good agricultural practices. On the other hand, lessons learned from sprouts indicate that effective seed decontamination without hampering seed viability can be challenging.

In the new Food Safety Modernization Act's Produce Safety Rule, several previous recommendations for sprouts are now requirements (U.S. FDA, 2015). These include treating seeds for decontamination, taking measures to prevent introduction of pathogens into or onto seeds used for sprouting, testing spent sprout irrigation water, testing the growing, harvesting, packing, and holding environment, documenting all treatments and testing, and taking corrective actions if samples are found positive (U.S. FDA, 2015). Microgreens are not subject to these requirements. The U.S. FDA has not defined commodity specific guidelines for microgreens (Wang, 2016). However, part of the distinction between microgreens and sprouts is that microgreens are typically

grown in soil or substrate and harvested above the soil or substrate line. A category of sprouts referred to as "green sprouts," includes wheatgrass, is grown in soil or substrate, harvested above the soil or substrate line, and exposed to light (Bari, Enomoto, Nei, & Kawamoto, 2011). The U.S. FDA distinguishes these from true sprouts and treats them similarly to microgreens (U.S. FDA, 2017).

Since microgreens are young and tender plants harvested by cutting the stems, they are highly susceptible to dehydration and quality deterioration. Refrigeration and packaging are essential to maintain quality.

5.3 Food safety of hydroponic versus potting soil systems and other substrates

Recently, studies have demonstrated that microgreen growing systems, especially hydroponic systems, are vulnerable to pathogen proliferation when seeds are contaminated, highlighting the importance of seed sanitation (Reed et al., 2018; Wright & Holden, 2018; Xiao et al., 2015). Xiao et al. (2015) showed that *E. coli* O157:H7 were able to survive and proliferate significantly on radish microgreens in both soil-substitute and hydroponic production systems, with higher populations reported in the hydroponic production system. Di Gioia et al. (2017) reported lower microbial populations in recycled fiber mats and on microgreens growing on them than in peat-based mixes and microgreens grown in peat. They suggested that recycled fiber mats may be safer growth media than peat. However, this is likely dependent on the prior use of the recycled mats and the conditions to which the mats are exposed during the recycling process, rather than the inherent inability of the textile fiber mats to support microbes. Reed et al. (2018) reported that the type of growth medium played an important role in serovar-dependant *Salmonella* survival and growth on microgreens irrigated with contaminated water. Of the different growth media tested, hydroponic pads resulted in the highest percentage of *Salmonella*-positive samples and the highest *Salmonella* population level on microgreens. Wang, Luo, and Nou (2015) examined the survival and proliferation of seed-borne *L. monocytogenes* and other members of the seeds microbiota on microgreen plants grown in soil substitute and hydroponic production systems. During microgreen growth for 10 days, *L. monocytogenes* counts on the seed coats increased by 0.7 and 1.3 log, respectively, for soil and hydroponic systems. Similar increases were observed on the edible portion of the microgreens. Seed coats, roots, and cotyledons were most heavily contaminated. Wang and Kniel (2016) evaluated the capability of the human norovirus surrogate, murine norovirus (MNV), to internalize from roots to edible tissues of kale and mustard microgreens, as well as virus survival in recirculated water without disinfection. They found constant high levels of viral RNA in edible tissues. MNV remained infectious in previously contaminated hydroponic systems for up to 12 days and was translocated in edible tissues via roots (Wang & Kniel, 2016). Examination of the spatial distribution of bacterial cells on different parts of microgreen plants showed that contaminated seeds led to systematic contamination of whole plants, including both aerial parts and roots.

There is a potential for LED light in the UV and blue ranges to enhance food safety of hydroponically grown microgreens by treating the water as it circulates. Light in blue and UV wavelengths is able to kill bacteria (Kim, Mikš-Krajnik, Kumar, & Yuk, 2016; Maclean, MacGregor, Anderson, & Woolsey, 2009; McKenzie, Maclean, Timoshkin, MacGregor, & Anderson, 2014).

Sanitization of harvested product is not likely to be an effective control strategy. Once contaminated, it is almost impossible to eliminate pathogens from living plant tissues. Microgreens are very delicate and can be easily damaged by harsh sanitizing treatments. Surface morphology is a key factor limiting the effectiveness of sanitizer treatments. Park, Kushad, and Feng (2013) examined the survival characteristics of *E. coli* O157:H7 on arugula, kale, lettuce, and mizuna microgreen surfaces stored in a refrigerator and compared the surface morphology of microgreens and mature greens. Most of the inoculated *E. coli* cells survived on the microgreen surfaces, showing only a slight decline from initial inoculum levels after 7 days of storage. For all varieties examined by SEM, microgreen true leaves were more wrinkled than the mature leaves. The hills and valleys on the microgreens were deeper than for the mature produce, and the stomata of the microgreens were slightly longer than those of the mature leaves (Park et al., 2013). Since microgreen tissues are more delicate than mature tissues, the wrinkling observed may be damage caused by radiation exposure under SEM. In our laboratory, we observed that older microgreens have more wrinkling and deeper hills and valleys and more root hairs than the younger microgreens, and that *E. coli* inoculated onto seeds survive subsequent sanitizing treatments in greater numbers on older microgreens plants (10 to 14 days) than on younger microgreens (4 to 8 days).

6. PROBLEMS RELATED TO STORAGE OF MICROGREENS

When microgreens are harvested, they a have high respiration rate (Chandra et al., 2012). Mir, Shah, and Mir (2017) stated that shelf life of microgreens is 3 to 5 days at ambient temperature. However, it would be unwise to store any harvested leafy green at ambient temperature. The greatest "shelf life" of microgreens is achieved by selling them still rooted in the growth medium. Harvested microgreens must be kept cold to maintain quality. Depending on cultivar and storage conditions, quality may be maintained for over 14 days. There are no food code requirements for microgreens, but preliminary studies suggest that microgreens should be stored at temperature's of ≤5 °C (Kou et al., 2013; Xiao et al., 2014b). Microgreens resistant to chilling injury can be held as low as 1 °C (Berba & Uchanski, 2012). Microgreens freeze rapidly if held below 0 °C, causing substantial physical damage. Although high humidity is necessary to prevent dehydration, it also promotes microbial growth and decay (Zagory & Kader, 1988). Thus, a combination of adequate cold chain and suitable modified atmosphere packaging (MAP) are essential to reduce respiration rates, prevent moisture loss, reduce environmental contamination, and inhibit growth of spoilage and pathogenic microorganisms (Berba & Uchanski, 2012; Zagory & Kader, 1988).

Microgreens may be washed after harvest to remove soil particles and provide a clean product for packaging. Washing greens prior to packaging reduces initial bacterial load, but creates a humid environment which promotes microbial growth and necessitates removal of excess water to discourage such growth. Many growers choose not to wash them, as the additional handling that washing and dewatering entail can damage the delicate greens, making them more susceptible to microbial growth. Removing excess moisture after washing without causing damage is a challenge. Thus, a delicate balance is required to maintain temperature, moisture, and atmosphere that optimize the quality retention and shelf life of microgreens, while discouraging growth of spoilage microbes and human pathogens.

While microgreens are different in many aspects from other types of produce, many of the lessons learned for other produce types also apply to microgreens. Many of the factors that decrease risks associated with foodborne pathogens on fresh and fresh-cut produce may also improve quality and shelf life. Important measures to maintain postharvest quality include harvesting at optimal maturity, minimizing injury due to handling, reducing microbial infection through proper sanitation, and maintaining optimal temperature and humidity (Mir et al., 2017; Zagory & Kader, 1988). Most of these factors are also important in maintaining produce safety. However, specific data on the optimal prevention and intervention steps needed for the various microgreens are generally not available. Acquisition of needed research is likely hampered by the small scale, local nature of microgreen cultivation.

6.1 Harvesting at optimal maturity

Berba and Uchanski (2012) suggest that microgreen shelf life may be influenced by the age of the seedlings at harvest. Different crops are harvested at different ages according to industry standards and to achieve marketable hypocotyl length and leaf area, for example, radish is harvested at 7 days, arugula at 9 days, and red cabbage at 11 days (Berba & Uchanski, 2012). Radish microgreens had the lowest respiration rate after the first week, which corresponded with best visual quality. Shelf life at 4 °C, based on visual quality, averaged 21 days for radish, and 14 days for arugula and red cabbage at 4 °C (Berba & Uchanski, 2012). No formal studies in the literature were found on harvest age effects on shelf life of microgreens.

6.2 Minimizing injury

Minimizing injury of produce is important, because injured fruits and vegetables likely to spoil faster and are more likely to harbor pathogens. Wells and Butterfield (1997) found that *Salmonella* was about twice as likely to be isolated from fruits and vegetables that were affected by soft rot than from healthy samples. They also found that potato, carrot and pepper disks coinoculated with *S. enterica* serovar Typhimurium and *Erwinia carotovora* (since renamed *Pectobacterium carotovorum)* supported 10-fold higher *Salmonella* populations than produce inoculated with *Salmonella* alone. Additionally, Seo and Frank (1999) demonstrated by confocal laser scanning microscopy that *E. coli* O157:H7 attached preferentially to cut edges over intact lettuce tissue. Aruscavage, Miller, Lewis Ivey, Lee, and LeJeune (2008) found that populations of *E. coli* O157:H7 remained higher in traumatically injured leaves than on healthy plants. Since microgreens are very delicate and more susceptible to physical damage, it can be assumed that preventing physical injury during harvesting and subsequent handling, distribution, and marketing is critical. Potentially effective methods for microgreen harvest are application of a very sharp shear force that cauterizes the wound simultaneously such as a laser or heated wire. Another possibility would be application of an edible coating that aids in wound healing.

6.3 Sanitation and handling skill

Sanitation of equipment used for handling and transporting produce prevents cross-contamination and is equally important for preventing infection from both spoilage and pathogenic microorganisms. McEvoy, Luo, Conway, Zhou, and Feng (2009) found that a single contaminated coring knife successively inoculated at least 19 lettuce heads. Yang, Luo, Millner, Turner, and Feng (2012) further investigated the impact of cutting method, cutting blade, and coring blade on pathogen transference in different soil types and at different inoculum levels. Worker training and skill could greatly impact the safety of harvested produce (Yang et al., 2012). Special skills are required for proper harvesting, handling, grading, and packaging of vegetables to ensure optimum produce quality (Wagner, Dainello, & Parsons, 2009) and minimize microbial contamination. While studies have not been conducted on the spread of microbes during microgreen cultivation and harvest, some simple precautions would be expected to minimize spread of plant and human pathogens. Containers or flats used to grow microgreens should be sanitized prior to reuse. Cutting implements used to harvest microgreens should also be sanitized between flats, and care should be taken to avoid their contact with growth medium.

6.4 Temperature control

Temperature is the most important environmental factor that influences quality and shelf life of harvested produce (Kader & Rolle, 2004). When microgreens were stored at 10 °C instead of 4 °C, shelf life was reduced from 14 to 7 days for arugula and red cabbage microgreens and from 21 to 14 days for radish microgreens (Berba & Uchanski, 2012). Xiao et al. (2014b) found that 1 °C was preferable to 5 °C for maintaining quality of radish microgreens. Packaging film permeability decreases with lower temperature (Zagory & Kader, 1988). Refrigerated temperatures also slow respiration rate, which is a key factor in delaying senescence. The rate of deterioration of perishables increases two- to threefold with every 10 °C increase in temperature (Kader & Rolle, 2004). Most perishable horticultural commodities have an optimal shelf life at temperature of approximately 0 °C. However, some commodities are sensitive to cold temperatures and experience tissue damage, increased respiration rate, and more rapid senescence if their lower temperature limit is surpassed. For example, buckwheat microgreens had lowest electrolyte leakage at 10 °C during the second week of storage, while samples stored at 1 °C had an increase in electrolyte leakage at the end of storage that corresponded to increases in aerobic mesophilic bacteria (Kou et al., 2013). Chilling sensitivity, while genetic, is affected by other factors including growth stage and package atmosphere composition (Kyriacou et al., 2016). Temperature has a dramatic effect on spore germination and pathogen growth, with maintenance of optimal temperature throughout the marketing chain benefitting both the safety and quality of fresh and fresh-cut produce (Arteca, 2015). Use of blue LEDs during cold storage of microgreens may be a strategy worthwhile investigating, because low temperatures have been found to aid in bacterial inactivation (Ghate et al., 2013; Kumar et al., 2015). Light in this wavelength range generates little heat and may help to delay chlorophyll degradation (D'Souza, Yuk, Khoo, & Zhou, 2015).

6.5 Relative humidity

Relative humidity (RH) is another factor influencing quality and safety of fresh-cut produce. While dehydration primarily is detrimental to quality rather than safety of produce, excessive humidity is a problem for both produce quality and safety. Condensation of moisture on the commodity ("sweating") over long periods of time stimulates microbial growth and decay more than a high RH of the ambient air alone (Wagner et al., 2009). Humidity is discussed in greater depth in the discussion of washing treatments below.

6.6 Modified atmosphere packaging storage

Microgreens are harvested by cutting the stem above the root and are consequently highly perishable depending on the species

(Berba & Uchanski, 2012). MAP has been successfully used to extend shelf life of many fruits and vegetables. However, there are insufficient studies on the use of MAP for microgreens. The benefit of packaging film to reduce moisture loss and protect the plants from environmental contaminants such as mold spores is undeniable. Yet, some researchers have not found significant differences among films of different oxygen transmission rates in their ability to maintain quality of microgreens until late in shelf life (21–28 days) (Kou et al., 2013; Xiao et al., 2014b). MAP must be optimized for individual commodities since use of inappropriate modified atmospheres can induce physiological disorders, prevent wound healing, hasten senescence and increase susceptibility to pathogen growth and decay (Wagner et al., 2009). High CO_2 levels may cause tissue injury, and low O_2 levels may result in anaerobic conditions fostering off-odors and off-flavors due to formation of ethanol and acetaldehyde (Allende, Luo, McEvoy, Artes & Wang, 2004; Zagory & Kader, 1988). High-respiration rates of microgreens are reduced drastically at low temperatures, which are key to maintaining sufficient oxygen in the package to prevent damage due to anaerobic conditions (Chandra et al., 2012; Xiao et al., 2014b). Since temperature can affect the permeability of the film, the optimal MAP at one temperature may not be optimal at another temperature (Zagory & Kader, 1988). Typically, MAP is only effective when the integrity of the cold chain can be maintained at <8 °C. Active packaging and intelligent packaging refer to packaging technologies that help extend shelf life, improve safety, monitor freshness, and display information on quality and/or safety (Dainelli, Gontard, Spyropoulos, Zondervan-van den Beuken, & Tobback, 2008). Some types of active packaging include antimicrobial polymers and films that inhibit growth of spoilage and pathogenic microorganisms (Rooney, 1995). Others contain indicators which react with toxins to signal their presence or indicate when a package is leaking, when quality deterioration occurs, or when temperatures rise above a threshold value for a given length of time (Yuan, 2002). There have not been any published studies to date on the use of active packaging technologies for storage of microgreens.

6.7 Postharvest light treatments

Light treatments may also benefit both quality and safety, but the few studies on the effect of light on harvested produce have reported conflicting results. Garrido, Tudela, Hernández, and Gil (2016) found that MAP during both continuous light and dark conditions caused physiological degradation of spinach leaves. During light conditions, photosynthesis elevated O_2 and lowered CO_2 partial pressures which encouraged oxidative damage (discoloration) and microbiological growth. On the contrary, in packages held in darkness, respiration depleted O_2 and raised CO_2 levels, causing the accumulation of alkaline compounds which increased pH, off-odors and leaf tissue CO_2 injury (Garrido et al., 2016). Martínez-Sánchez, Tudela, Luna, Allende, and Gil (2011) found similar results with continuous light exposure resulting in high oxygen levels leading to browning of Romaine lettuce, while continuous darkness led to CO_2 injury and anaerobic conditions in lettuce packages stored for 3 days at 4 °C and then for 7 days at 7 °C. A 12-hr photoperiod treatment resulted in less discoloration than the constant light treatment and less tissue injury than the constant dark treatment, but still did not score well on quality. Lester, Makus, and Hodges (2010) found that spinach leaves exposed to continuous light at 4 °C in clear plastic containers had higher levels of most bioactive compounds, but were more prone to wilting than leaves stored in continuous darkness. Zhan, Hu, Li, and Pang (2012) measured nutritional quality associated with pigments, antioxidant power (AP), total phenols (TP), reduced ascorbic acid (AA), and fresh weight loss of fresh-cut broccoli exposed to continuous 24 μmol m^{-2} s^{-1} light or held in darkness (control) during storage at 7 °C temperature during 10 days shelf life. The light treatment preserved higher levels of chlorophyll a, chlorophyll b, total chlorophyll, AP, TP, and AA than did darkness. However, it accelerated fresh weight loss after 5 days storage. Jin, Yao, Xu, Wang, and Zheng (2015) found that light treatments significantly extended shelf life and delayed chlorophyll degradation of broccoli florets, with green LED light being more effective than fluorescent light at maintaining high levels of bioactive compounds including phenols and glucosinolates. On the contrary, Xiao et al. (2014a) found that light exposure accelerated deterioration of radish microgreeens, while dark-storage maintained quality. They reported that light exposure during storage increased the amount of ascorbic acid and had no effect on α-tocopherol or total phenolic concentrations. Dark storage resulted in higher hydroxyl radical scavenging capacity and carotenoid retention. No significant differences were found for relative DPPH radical scavenging capacity between light and dark treatments (Xiao et al., 2014a).

6.8 1-Methylcyclopropene

While 1-methylcyclopropene (1-MCP) has been found to be an effective treatment to prolong shelf life of a wide variety of fruits and vegetables and edible flowers, it has not, to our knowledge, been tested on microgreens. The plant hormone ethylene induces a wide range of physiological responses in horticultural crops including abscission, ripening and senescence, chlorophyll loss softening, physiological disorders, discoloration, decay and stimulation of defense systems (Saltveit, 1999). 1-MCP inhibits ethylene perception by binding competitively to ethylene receptors (Blankenship & Dole, 2003). In addition to its widespread use on fruits, 1-MCP is also registered for use on several vegetables including broccoli, cauliflower, Brussel sprouts, cabbage, and carrot (Watkins, 2006). Bower and Mitcham (2001) reported that 1-MCP delayed senescence including leaf yellowing, abscission, and decay in several vegetables including cultivars of *Brassica oleracea*. Able, Wong, Prasad, and O'Hare (2003) evaluated the effect of 1-MCP on shelf life of six leafy Asian vegetables (Chinese mustard, choy sum, garland chrysanthemum, mibuna, mizuna, and tatsoi). In the absence of ethylene, 1-MCP treatment only increased shelf life for mizuna and tatsoi (21 and 67% increase, respectively). In contrast, 1-MCP treatment in the presence of ethylene significantly protected Chinese mustard, choy sum, garland chrysanthemum, and tatsoi (Able et al., 2003).

6.9 Washing treatments

Washing treatments in some instances may prolong shelf life by rinsing away exudates that would otherwise provide nutrients for microbes, reducing microbial load, and providing moisture for greens that dehydrate easily. In our previous unpublished research, Ruby radish microgreens washed in 100 ppm chlorine and dried centrifugally at 300 rpm maintained better visual quality and lower electrolyte leakage until day 12 than unwashed ruby radish microgreens. However, washing often results in excess moisture in the package which stimulates microbial growth and decay and damage to delicate greens. Removing excess moisture is necessary to prevent microbial proliferation, but most drying methods for fresh minimally processed greens result in additional damage and quality loss. Improved washing and drying technologies are needed to

provide ready-to-eat microgreens with better quality and longer shelf life. Chandra et al. (2012) reported that quality scores for "Tah Tasai" Chinese cabbage (*Brassica rapa* subsp. *narinosa*) microgreens washed with chlorine or tap water and stored in polyethylene bags at 5 ˚C had declined to the limit of acceptability by day 5, while quality scores for microgreens washed with 0.5% citric acid followed by 50% ethanol spray, or with 0.25% citric acid plus 0.25% ascorbic acid were still above the limit of acceptability on day 7, but not day 9. Washed samples were dried centrifugally for 1 min and then air dried for 30 min prior to packaging, but were not compared to unwashed controls. Washed radish (Xiao et al., 2014b) and buckwheat microgreens (Kou et al., 2013) deteriorated much faster than unwashed microgreens, due in part to damage incurred during washing and dewatering, but primarily due to the excess moisture in packages of washed microgreens. Microbial counts on microgreens washed in 50–100 mg/L chlorine declined initially but rebounded and far exceeded unwashed microgreens by the end of the 21 day storage. For buckwheat microgreens (Kou et al., 2013), water washed samples had the highest microbial counts, while for radish microgreens (Xiao et al., 2014b) 100 mg/L chlorine washed samples had the highest microbial counts followed by 50 mg/L chlorine. The chlorine treatment may have altered the natural microflora on the radish microgreens allowing chlorine-resistant microbes to thrive that had previously been checked by competing microbes. If spoilage or human pathogens are among the chlorine-resistant species, this scenario could create problems for quality or safety of microgreens, respectively. Calcium lactate (50 mM) postharvest dip was somewhat of an improvement over chlorine dip in terms of maintaining overall quality of broccoli microgreens and reducing electrolyte leakage and microbial growth (Kou, Yang, Liu, & Luo, 2015). However, all postharvest wash treatments caused quality degradation compared to samples receiving preharvest calcium chloride treatment only (Kou et al., 2015).

7. FUTURE RESEARCH DIRECTIONS

Most microgreen research has been conducted by a small number of researchers in conjunction with relatively narrow focus areas. There is a vast amount of territory yet to be explored. Few species of microgreens have been studied and have not necessarily correlated with the varieties most likely to be commercialized. The effect of photoperiod on microgreen growth and nutrition has been largely overlooked. Similarly, the effect of cool nighttime temperatures on plant growth, nutrition, and food safety of microgreens has not been assessed. Identifying prevention and intervention treatments that are beneficial for maintaining both quality and safety of microgreens is still in its infancy. It is certainly clear that postharvest light treatments can enhance the formation bioactive components, but this has not been systematically studied to optimize nutrient content in a full range of potential microgreens. Augmenting phytonutrient content could provide inherent resistance to quality and safety issues. There are many postharvest treatments that have been explored for other produce items that may help to maintain quality and extend shelf life of microgreens. Optimizing washing and drying techniques for delicate greens or finding alternative technologies would be of great value to produce ready-to-eat microgreen products. It is particularly important that the fundamental research into ensuring the safety and quality of this new addition to healthy diets is done so that the produce industry can avoid some of the problems that have challenged the mature produce and sprout industries during the past several decades.

NASA scientists have begun to explore the challenges and benefits of growing microgreens in space. Plants are highly valued in space to regenerate oxygen, fix nitrogen, provide vital nutrients and fresh ingredients, and to enhance morale of astronauts during extended stays away from Earth (Kyriacou, De Pascale, Kyratzis, & Rouphael, 2017). Microgreens are ideally suited because of their low space, nutrient and growth medium requirements, and short growing period. Growing food in space holds many challenges with regard to microgravity including seed germination, watering plants, and anchoring plant roots, as well as limited resources. Padgett (2018) describes the production and testing of films to hold seeds in place during cultivation. Vanderbrink and Kiss (2017) suggest that microgravity may even affect epigenetic processes and consequently gene expression in plants. Additionally, food safety concerns are paramount for astronauts who have limited access to medical treatment during space travel and are confined together in a small space. Seeds must be sanitized to ensure that they do not harbor human pathogens (Padgett, 2018). A final field of research that has not been specifically explored is new uses, e.g. foods or ingredients from wasted microgreens or microgreens at shelf life end. Even though shelf life extension of microgreens is critical, and has been summarized in this review to reduce waste, novel processing and reformulating of wasted microgreens into new products is a future research direction.

AUTHOR CONTRIBUTIONS

Ellen Turner wrote the manuscript, and Yaguang Luo and Robert Buchanan reviewed it as mentors, edited it, made suggestions, and requested changes.

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