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Microgreens: Production, shelf life and bioactive components

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ABSTRACT

Microgreens are emerging speciality food products which are gaining popularity and increased attention nowadays. They are young and tender cotyledonary leafy greens that are found in a pleasing palette of colors, textures and flavors. Microgreens are a new class of edible vegetables harvested when first leaves have fully expanded and before true leaves have emerged. They are gaining popularity as a new culinary ingredient. They are used to enhance salads or as edible garnishes to embellish a wide variety of other dishes. Common microgreens are grown mainly from mustard, cabbage, radish, buckwheat, lettuce, spinach etc. The consumption of microgreens has nowadays increased due to higher concentrations of bioactive components such as vitamins, minerals and antioxidants than mature greens, which are important for human health. However, they typically have a short shelf life due to rapid product deterioration. This review aimed to

evaluate the postharvest quality, potential bioactive compounds and shelf life of microgreens for proper management of this specialty produce.

Keywords

Microgreens; shelf life; storage; fresh produce; bioactive components

INTRODUCTION

Microgreens are young and tender cotyledonary leafy greens that are found in a pleasing palette of colors, textures and flavors (Xiao et al., 2012; Pinto et al., 2015). They are very specific type of vegetable which are harvested and consumed in an immature stage (Xiao et al., 2014a). These are an exotic genre of edible greens, appearing in upscale markets, restaurants and have gained popularity as a new culinary trend over the past few years. Microgreens are defined as salad crop shoots harvested for consumption within 10 to 20 days of seedling emergence (Lee et al., 2004; Xiao et al., 2014b). They offer soft texture, color and add a variety of quality attributes which enhances the sensory properties of main dishes (Kou et al., 2014).

Microgreens are specialty leafy green crop harvested shortly after the first true leaves have emerged. They are harvested just above the roots and consumed fresh as salad greens (Kou et al., 2013). They take longer time to grow than sprouts, making them larger leaved and greener (Poorva and Aggarwal, 2013). Harvested at the first true leaf stage and supplied to the consumers with the stem, cotyledons and first true leaves attached, they are among a variety of novel greens available in the market and are typically distinguished categorically by their size and age (Pinto et al., 2015).

Microgreens contain higher concentrations of functional components such as antioxidants, phenolics, vitamins and minerals than found in mature greens or seeds (Janovska et al., 2010). Thus, they are considered as “functional foods” which contain health promoting or disease preventing properties that are additional to their normal nutritional values. They are highly prized for rich source of bioactive components (Xiao et al., 2015).

However, marketing of microgreens are limited due to their rapid senescence and a very short shelf life, usually 3 to 5 days at ambient temperature, so they are characterized to highly perishable products (Chandra et al., 2012). As the demand for microgreens increases, they begin to appear in farmer's markets and specialty grocery stores, so the optimization of their packaging and postharvest storage conditions is therefore becoming important for enhanced shelf life (Poorva and Aggarwal, 2013). Various techniques have been used to extend the shelf life of microgreens. Different sanitizing agents (Chandra et al., 2012; Kou et al., 2013), packaging films, atmospheric and storage conditions (Xiao et al., 2014b) were used for enhancing the shelf life of microgreens to cater their demand in the market.

MICROGREENS VERSUS SPROUTS

Microgreens are different from the sprouts. Sprouts are germinated or partially germinated seeds which eaten with their roots intact. The seeds are washed properly so as to remove dust or any other kind of attached foreign substances. The seed density is high in sprouts and is grown in high moisture, optimum temperature and low light conditions which increase the chances of microbial growth (Poorva and Aggarwal, 2013). The seeds are soaked in water in various time-temperature schedules, depending upon the type and size of seeds. This increases the water content in seeds and eventually germinates after the required time interval (Bergquist et al., 2006; Xiao et al., 2014b).

In case of microgreens, roots are not eaten as these greens are harvested from the soil surface. Also, these greens require sunlight for their efficient growth and are grown in soil or other medium such as peat moss, vermiculite and perlite (Murphy et al., 2010; Xio et al., 2014a). Seed density is low, as we have to eat the young greens which need space to grow. They are

halfway in size between sprouts and salad mix. They also differ from sprouts as they are grown in sunlight and harvested when baby leaves are emerged.

CULTIVATION, HARVESTING AND MARKETING OF MICROGREENS

Variety of high quality microgreens is grown commercially and sometimes they are grown by individuals at lower scale for home use (Table 1). Mixed cultivation of microgreens is also done by the growers (Poorva and Aggarwal, 2013). Having the appropriate mix of microgreens at the right stage of harvest is one of the most important production strategies for success. The time from seeding to harvest varies greatly from crop to crop (Allende et al., 2004; Pinto et al., 2015). The growers are selecting the crops having a similar growth rate during seeding a mixture of crops, so the entire crop can be harvested at once. Some growers also seeding the various crops singly and mixes them after harvest. Microgreens are also produced in garden bed, in window sill as well as in containers, depending upon the requirement. They are grown in a standard, sterile, loose, soil and many mixes have been used successfully with peat, vermiculite, perlite and bark (Kou et al., 2013).

Seeding for microgreens either done in rows or as a broadcast method. Most growers seeding thickly as possible to maximize its production, but not too thick because crowding encourages elongated stems and increases the risk of various diseases. Most crops require little or no fertilizer, as the seed provides adequate nutrition for the young crop (Xiao et al., 2015). However, the application of various types of fertilizers particularly organic one mostly increases the yield of microgreens (Murphy et al., 2010).

Microgreens are harvested when the desired height is attained and first set of cotyledon leaves and true leaves appear. These are ready for harvest when they reach the first true leaf

stage, usually at about 5 cm tall and also depends on the type of crop. The time from seeding to harvest greatly varied for crops from 1 to 3 weeks (Allende et al., 2004; Xiao et al., 2014b). Harvested microgreens are highly perishable and are washed, cooled as quickly as possible using good handling practices for food safety. These are usually packed in polyethylene packages and cooled to recommended temperatures before supplying to the market or consumers (Kou et al., 2014; Xiao et al., 2014a).

Microgreens are available in vegetable markets and grocery stores in number of packaging materials, storage conditions and usually displayed under light. The effect of light exposure on quality and phytochemical concentrations of different vegetables has been studied extensively. Noichinda et al. (2007) reported that low intensity fluorescent light increased fresh weight loss of Chinese kale (*Brassica oleracea* var. *alboglabra*), but prevented the loss of vitamin C during storage. Lester et al. (2010) observed that continuous light exposure prevented loss of ascorbic acid and was beneficial in enhancing the amounts of carotenoids and tocopherols of baby leaf spinach (*Spinacia oleracea* L.). Literature also reported that light exposure could cause detrimental effects on produce quality, so the proper conditions are also necessary at market outlets to maintain the quality of microgreens (Garrido et al., 2016).

SHELF LIFE

Microgreen consumption has been steadily increasing in recent years due to consumer awareness of their unique color, rich flavor and concentrated bioactive compounds. However, industrial production and marketing is limited due to their short shelf life associated with rapid deterioration in product quality (Bergquist et al., 2006; Xiao et al., 2014b). The delicate and immature tissue structure of microgreens showed a very short shelf life at ambient temperature

(Table 2). The limited shelf life of perishable goods limits their longevity in the market due to rapid post-harvest degradation dictated and controlled by the crop's respiration rate (Artes et al., 2009). They are moving to senescence stage rapidly after harvest and have a very short shelf life due to the sudden disruption of plant growth at a very early stage. Rapid physiological, biochemical and molecular changes have been shown to occur during leaf senescence (Lim et al., 2007; Guo and Gan, 2012). Since microgreens are harvested at the cotyledon stage, this process can result in degradation of the primary product. Slower respiration can be achieved by lowering temperatures which directly correlates with a lower rate of cellular metabolism. This modification of plant metabolic activity has been reported in most fruits, vegetables and cut greens (Loaiza and Cantwell, 1997). Slower metabolic rates induced by low temperatures also impact visual quality and can help to enhance the shelf life.

Various techniques are used to enhance the shelf life of microgreens. The two important techniques used for increasing the postharvest shelf life extension are storage temperature and storage atmospheric conditions (Hodges and Toivonen, 2008). Several studies on postharvest shelf life of produce highlighted the importance of temperature control (Brecht, 1995; Watada et al., 1996), which is usually considered as the most significant factor in prolonging shelf life of microgreens (Deza-Durand and Petersen, 2011). Thus, there is room for expansion availability of microgreens in the market if temperature and atmospheric composition favourable for maintain the quality parameters are controlled. Modified atmospheric packaging is one of the techniques used to extend the shelf life of microgreens. This technology effectively increasing the shelf life by decreasing oxygen, increasing carbon dioxide and partial pressures in the package headspace due to the interaction between respiratory oxygen uptake and carbon dioxide evolution of the

packaged plant tissues, and the selective transfer of gases through packaging films (Kim et al., 2004). Proper packaging also reduces the contamination of product by decreasing bacteria, mold spores and other environmental pollutants during storage.

The low storage temperature of fresh vegetables reduces both physiological activities like respiration rate and the activities of microorganisms capable of causing spoilage of the produce (Oliveira et al., 2015). Some packaging techniques help to maintain quality although fresh produce can modify the atmosphere within their packages as a result of respiratory oxygen consumption and carbon dioxide evolution (Pirovani et al., 1998). Consequently, different headspace conditions can occur in the package depending on the interaction between respiratory activity and gas transfer through the polymeric matrix. Different commonly available polymeric films, especially based on polyethylene or polypropylene have been used for the packaging of microgreens (Allende et al., 2004; Bergquist et al., 2006). The selection of suitable packaging film is essential to maintain quality and to assure a longer shelf life of the produce (Martinez-Romero et al., 2003; Chandra et al., 2012).

In addition to storage temperature, shelf life of microgreens depends on many factors including relative humidity, packaging film type and initial microbial load etc. The main problems related with packaged produce are the development of decay, off-odor, discoloration and tissue softening (Hussein et al., 2015). The development of off-flavor as well as the growth and multiplication of microorganisms of packaged produce increase rapidly, particularly at higher storage temperature. In order to achieve fresh like quality of microgreens, high nutritional value and minimum spoilage, the optimized and sustainable techniques or procedures are used for washing and sanitizing the produce. Presently, chlorine is the commonly used sanitizing

agent by the industry mainly due to its antimicrobial activity and low cost. While as, a wide range of diverse agents are available for sanitizing microgreens. Organic acids such as citric acid and ascorbic acid have been applied largely for preserving physicochemical qualities and preventing the microbial growth at levels that did not adversely affect taste and flavor (Rico et al., 2007).

Chandra et al. (2012) studied the effect of citric acid, ascorbic acid, chlorine and ethanol spray on storage qualities of Chinese cabbage (*Brassica campestris* var. *narinosa*) microgreens packaged in polyethylene or polypropylene bags and stored at 5°C for 9 days. The decline of oxygen partial pressure of the packaged headspace and hue angle values were similar in both films. However, the carbon dioxide partial pressure of the packaged headspace and the electrical conductivity were substantially higher in polypropylene packaged samples than that in polyethylene. Off-odor scores were also higher in polypropylene film packaged samples compared to that of polyethylene. The combined effect of citric acid and ethanol spray, citric acid and ascorbic acid treatments showed lower off-odor scale in both films and exhibited better quality on day 7. However, visual quality scores showed similar trends at the beginning of storage in all treatments. Samples washed in chlorine showed lower microbial count numbers on washing day. However, citric acid and ethanol spray treatment exhibited either the lowest or equivalent count numbers in both total aerobic counts and coliforms count to that of chlorine until the end of storage.

Kou et al. (2013) studied the effect of storage temperature, modified atmosphere packaging and washing conditions on quality and shelf life of buckwheat microgreens. Results specified that storage temperature is the most important factor affecting the buckwheat

microgreens followed by washing treatment. The temperature significantly affects the package atmospheric conditions and the quality of product. Microgreens stored at 1, 5 and 10°C showed lesser microbial populations and tissue electrolyte leakage than stored at 15 and 20°C after storage period. Package film oxygen transmission rate (OTR) significantly affected package atmospheres. However, differences in quality and shelf life of microgreens packaged in various OTR films were slight and not evident until day 21 of storage. The buckwheat microgreens packaged in 16.6 pmol/(m² s Pa) OTR package films were observed to have the fresh appearance with lowest tissue electrolyte leakage on 21 day of storage. Initially chlorine (100 mg/L) wash significantly ($p<0.05$) reduced microbial populations. However, after 7 days of storage, all washed microgreens experienced accelerated microbial populations. Buckwheat microgreens stored at 5°C with moderately high oxygen (14.0 to 16.5 kPa) and moderately low carbon dioxide (1.0 to 1.5 kPa) maintaining the highest quality attributes and shelf life.

Xiao et al. (2014b) studied the postharvest quality of radish microgreens. The quality and shelf life of radish microgreens were impacted by three major postharvest treatment factors viz., storage temperature, packaging film OTR and chlorine wash treatment. Storage temperature significantly affected package atmosphere, product quality and shelf life. The temperature of 1°C was found optimal for storage of radish microgreens with no chill injury. Film OTR significantly affected headspace gas composition. Chlorine wash treatment (100 mg/L) significantly reduced initial microbial population by 0.5 log cfu/g. However, microbial population rebound after 7 days of storage period.

Garrido et al. (2016) studied the effect of light exposure on leaf quality and senescence parameters of baby spinach. Minimally processed baby spinach was stored in passive modified

atmosphere packaging and in controlled atmosphere under different light conditions. In passive modified atmosphere packaging, three different headspace gas compositions within the bags due to photosynthesis and respiration reactions were generated that strongly affected the quality properties. To isolate the light effect from the atmosphere composition influence and understand the mechanisms causing the quality changes, baby spinach was stored under two controlled atmosphere of 0.5 kPa oxygen + 10 kPa carbon dioxide (low oxygen + high carbon dioxide levels) and air combined with 2 light conditions (continuous light and darkness). The changes observed under the different light conditions were mainly caused by the differences in gas composition. Under light, modified atmosphere packaging with high oxygen partial pressure and low carbon dioxide partial pressure was detrimental because of the growth of *Pseudomonas* spp. and the progress in tissue senescence due to oxidative stress, increasing cell damage, lipid peroxidation and chlorophyll degradation. Under darkness, modified atmosphere packaging with low oxygen partial pressure and high carbon dioxide partial pressure was also detrimental because of the intense off-odor developments, the increase in pH and electrolyte leakage and the reduction in chlorophyll fluorescence. The results showed that modified atmosphere generated with exposure to the different light conditions affected the quality of baby spinach mainly due to high oxygen partial pressure under light and high carbon dioxide partial pressure under darkness.

Allende et al. (2004) evaluated the quality changes in minimally processed baby spinach leaves under modified atmospheric conditions. Packages prepared with the barrier film of an initial oxygen level at 21% accumulated carbon dioxide during storage exhibited a significant reduction in aerobic mesophilic bacterial growth compared to the perforated film package, but induced off-odor and loss of tissue integrity. The addition of super atmospheric oxygen to

packages decrease tissue injury in addition to reducing microbial growth and was beneficial in maintaining quality of fresh cut baby spinach.

Kou et al. (2014) investigated the effect of pre-harvest calcium application on the post-harvest quality attributes and shelf life of broccoli microgreens. Broccoli microgreen seedlings were sprayed daily with calcium chloride at a concentration of 1, 10 and 20 mM including water (control) for 10 days. The fresh cut microgreens were packed in polyethylene film bags. Package headspace atmospheric conditions, overall visual quality and tissue membrane integrity were evaluated on 0, 7, 14, and 21 day of storage at 5°C. Results indicated that 10 mM calcium chloride treatment increased the biomass more than 50% and tripled the calcium content as compared to the water treated controls. Microgreens treated with 10 mM calcium chloride spray exhibited higher superoxide dismutase and peroxidase activities, lower tissue electrolyte leakage, improved overall visual quality and reduced microbial growth during storage. Calcium application significantly increases microgreen yield and calcium content, reduces tissue electrolyte leakage, inhibit microbial growth during storage and improve overall visual quality during storage. Calcium treated microgreens exhibit higher antioxidant activities and increased expression of the senescence associated genes as compared to water spray (control). The study demonstrates that calcium is important to enhance both broccoli microgreens productivity and improve postharvest quality and shelf life.

Calcium plays a pivotal role in plant growth, development and response to external and internal signals. Calcium treatment was also found to have a beneficial effect on the storage of vegetables by retarding leaf senescence (Martin-Diana et al., 2007). This chemical has the capability to retarding ripening and senescence by cross linking with pectin polymers in cell wall

(Liu et al., 2009) and protecting cell membrane integrity (Guimaraes et al., 2011). Gras et al. (2003) studied that calcium application can significantly increased the broccoli microgreen yield, improve postharvest quality and extend the shelf life. In addition, the calcium content in microgreens has been significantly increased and enhanced the consumer appreciation due to the benefits of calcium to human health.

Lester and Hallman (2010) used gamma irradiation to prolonging the shelf life of baby spinach leaf. Baby leaf spinach was packaged under air or N₂ atmosphere and then exposed to cesium-137 γ -radiation at 0.0, 0.5, 1.0, 1.5 and 2.0 kGy. The atmospheres by irradiation treatment had little effect, but N₂ versus air was associated with elevated dihydroascorbic acid levels. Phytonutrients (vitamin B₉, E, K and neoxanthin) exhibited little or no change in concentration with increasing of an irradiation dose. However, total ascorbic acid content, lutein/zeaxanthin, violaxanthin and β -carotene were significantly reduced at 2.0 kGy.

BIOACTIVE COMPONENTS

In recent years due to change of lifestyle pattern and health conscious among consumers, consumption of microgreens have increased and appreciation for their concentrated functional components such as phenolics, vitamins and minerals etc., as compared to mature leafy greens (Chandra et al., 2012; Xiao et al., 2012; Kou et al., 2013). Demand for these products is growing rapidly due to the recent attention of consumers towards functional foods. Microgreens have been found to contain higher levels of concentrated active compounds than found in mature plants or seeds. They are highly valued for their health benefits, as they are rich in vitamins, trace elements, amino acids, antioxidants etc. (Finley et al., 2001; Han et al., 2006).

Microgreens are grown from wide variety of crops like cabbage, table beet, kale, mustard, radish, amaranth, lettuce etc. (Xiao et al., 2012; Kou et al., 2014). They generally contain higher concentrations of functional components like vitamins, carotenoids and minerals than their mature counterparts. Red cabbage microgreens, for instance, had the highest concentration of vitamin C, while green daikon radish microgreens had the most vitamin E. Broccoli microgreens contain about 50 times more sulfurophane by weight than mature broccoli (Mewis et al., 2012; Xiao et al., 2015).

Studies have shown that microgreens may have much higher levels of vitamins, minerals and other health beneficial phytonutrients than the mature leaves. Lester and Hallman (2010) reported that the younger leaves of baby spinach (*Spinacia oleracea* L.) generally contain higher levels of phytonutrients: vitamins C, B₉, K₁ and carotenoids (lutein, violaxanthin, zeaxanthin and β -carotene) than the mature leaves. Oh et al. (2010) also found that young lettuce (*Lactuca sativa*) seedlings after 7 days of germination had the highest total phenolic concentration and antioxidant capacity in comparison to the mature leaves.

Xiao et al. (2012) studied the concentrations of ascorbic acid, carotenoids, phyloquinone and tocopherols in 25 commercially available microgreens. The results revealed that different microgreens provided extremely varying amounts of vitamins and carotenoids. Total ascorbic acid contents ranged from 20.4 to 147.0 mg/100 g fresh weight (FW), while β -carotene, lutein/zeaxanthin and violaxanthin concentrations ranged from 0.6 to 12.1, 1.3 to 10.1 and 0.9 to 7.7 mg/100 g FW, respectively. Phyloquinone level varied from 0.6 to 4.1 μ g/g FW; while as, α -tocopherol and γ -tocopherol ranged from 4.9 to 87.4 and 3.0 to 39.4 mg/100 g FW, respectively. Among the 25 microgreens analysed, red cabbage, cilantro, garnet amaranth and green daikon

radish had the highest concentrations of ascorbic acids, carotenoids, phyloquinone and tocopherols, respectively.

Bergquist et al. (2006) studied the variation in nutritional quality of spinach with growth stage and postharvest storage sown on three different occasions. For each occasion, the spinach was harvested at three growth stages at 6 day intervals. The second stage corresponded to a growth period used for baby spinach by commercial growers. The harvested leaves were stored in polypropylene bags at 2 and 10°C. The highest ascorbic acid content in fresh material was found at the stage first. However, during storage period ascorbic acid content decreased significantly, while as, the dehydroascorbic acid ratio increased. The baby leaves stored at 2°C gave a smaller reduction in ascorbic acid content than at 10°C. The total carotenoid content increased or remained stable during storage. Lutein was the major carotenoid, making up about 39% of the total carotenoid content followed by violaxanthin, β -carotene and neoxanthin. Visual quality decreased during storage in most cases and was correlated to initial ascorbic acid and dry matter contents. Results also indicate that harvesting the baby spinach a few days earlier than commercial stage of harvest improved the postharvest visual and nutritional quality.

Pinto et al. (2015) compared the mineral profile and nitrate content of microgreens and mature lettuce. Microgreens had higher content of most minerals including calcium, magnesium, iron, manganese, zinc, selenium, molybdenum and lower nitrate content than mature lettuces. Santos et al. (2014) quantified the several minerals like phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc and copper in ready to eat baby leaf vegetables. The younger leaves of baby spinach (*Spinacia oleracea* L.) possessed higher levels of phytonutrients

(ascorbic acid, carotenoids, folate, α -tocopherol and phyloquinone) than mature spinach leaves (Lester et al., 2010).

Sun et al. (2013) profiled the five *Brassica* microgreens species using ultrahigh performance chromatography photodiode array high resolution multistage mass spectrometry. A total of 164 polyphenols including 30 anthocyanins, 105 flavonol glycosides and 29 hydroxycinnamic acid and hydroxybenzoic acid were identified. The results showed that the *Brassica* species microgreens tended to have more complex polyphenol profiles and contain more varieties of polyphenols compared to mature plant counterparts.

Six species of microgreens, including Dijon mustard (*Brassica juncea* L. Czern.), opal basil (*Ocimum basilicum* L.), bull's blood beet (*Beta vulgaris* L.), red amaranth (*Amaranthus tricolor* L.), peppergrass (*Lepidium bonariense* L.) and China rose radish (*Raphanus sativus* L.), were evaluated for their sensory attributes and chemical compositions by Xiao et al. (2015). Results showed that bull's blood beet had the highest rating on acceptability of flavor and overall eating quality while peppergrass as the lowest one. Chemical compositions also differed significantly among the six species of microgreens. China rose radish had the highest titratable acidity and total sugars, while red amaranth had the highest pH value and lowest total sugars. Regarding the phytonutrient concentrations, the highest concentrations of total ascorbic acid, phyloquinone, carotenoids, tocopherols and total phenolics were found in China rose radish, opal basil, red amaranth, China rose radish, and opal basil, respectively. The relationships between sensory attributes and chemical compositions showed that overall eating quality of microgreens was best correlated with flavor score and microgreen's pH value. The total phenolic content was strongly correlated with flavor attributes, e.g., sourness, astringency and bitterness.

FUTURE RESEARCH PROSPECTIVE

There is a great potential for growing microgreens form variety of crops which will cater their demand in the market. Combined with the great advances from past years, the future development will contribute to deeper understating of microgreen production. Microgreen industry is the growing field and there is a good scope for future research in this area due to increase of consumer demand. The achievement of appropriate type of crop, harvesting and marketability will act as vehicle for this industry. Progress towards establishment of shelf life stable techniques will also likely to accelerate the demand of this crop in the coming years. The physiological and biochemical changes occurring during microgreen storage deserves more attention. Advancements of postharvest processing techniques and packaging technology will help to maintain the quality for longer periods of time and extend their shelf life. In addition to quality parameters, the functional information of microgreens will help to select the specific crop and harvest at appropriate stage of growth.

CONCLUSION

Nowadays microgreens are gaining popularity in the market due to change of life style pattern and health consciousness among consumers. Microgreens provides the high concentration of antioxidant, vitamins and minerals which are linked with the promotion of good human health. A thorough understanding of crop management of microgreens and their postharvest and storage characteristics is crucial for extending their shelf life and for expanding these products into larger markets. This review provides insight which will add to the knowledge base about the management and processing of this new specialty crop.

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