ABSTRACT

Title of Document: Nutrition, Sensory, Quality and Safety Evaluation
                          of A New Specialty Produce: Microgreens

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Microgreens are new emerging food products, which are young seedlings of
vegetables and herbs, having two fully developed cotyledons with the first pair of true
leaves emerging or partially expanded. They have gained popularity in upscale
restaurants and grocery stores in recent years. However, little relevant scientific data
is currently available on microgreens. The present research project was dedicated to
explore the nutritional value, sensory attributes, consumer acceptance, postharvest
quality and microbiological safety of microgreens.

In the first part of this project, phytonutrients were determined in 25
commercially available microgreens. Results showed that different microgreens
provided extremely varying amounts of phytonutrients. Among the 25 microgreens
assayed, red cabbage (*Brassica oleracea* L.), cilantro (*Coriandrum sativum* L.),
garnet amaranth (*Amaranthus hypochondriacus* L.) and green daikon radish
(*Raphanus sativus* L.) had the highest concentrations of ascorbic acids, carotenoids,
phylloquinone, and tocopherols, respectively. Compared with the nutrient
concentrations in mature leaves recorded in USDA National Nutrient Database,
microgreens possessed higher nutrient density.

Although microgrees are nutrient-dense, there is little information and data on the consumer acceptability of microgreens; therefore, consumer acceptance test were carried out. Six microgreens were first selected out of 25 varieties of microgreens in the preliminary test and subsequently evaluated by 80 consumer panelists for sensory attributes. Chemical compositions and nutritional values of the taste-panel tested microgreens were also investigated for correlations with sensory attributes. All microgreens evaluated demonstrated "good" to "excellent" consumer acceptance and nutritional profile and overall acceptability of microgreens was significantly correlated with flavor acceptability.

Generally, microgreens are very tender, and thus have a short shelf life. To optimize the postharvest handling conditions, the effects of temperature, modified atmosphere packaging (MAP) and chlorine wash on postharvest quality and shelf life of daikon radish microgreens (*Raphanus sativus L. var. longipinnatus*) were studied. The impacts of light exposure during storage on sensorial quality and bioactive compounds were also investigated. Results showed that 1) one degree Celsius was the optimal temperature for radish microgreens storage; 2) MAP did not significantly affect quality attributes during 28 days of storage at 1°C; 3) chlorine wash treatment reduced microbial populations initially, however, it rebounded to pre-washed levels within 7 days; and 4) light exposure accelerated quality deterioration and increased the amount of ascorbic acid, while dark storage may be profound for quality and carotenoid retention.

The final part of this project was a comparative microbiological study between radish sprouts and radish microgreens produced from artificially contaminated radish

seeds. Starting from seeds with same contamination levels, *E. coli* O157: H7 and *E. coli* O104: H4 populations on harvested radish microgreens were 3-5 logs lower than that on radish sprouts. These results demonstrated that the microbial growth on sprouts were much faster than that on microgreens, which poses great risk of microbiological hazard to sprout-consumers. In contrast, microgreens seem to bear a relatively low food safety risk.

NUTRITION, SENSORY, QUALITY AND SAFETY EVALUATION OF A NEW
SPECIALTY PRODUCE: MICROGREENS

By

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1.1 Introduction of Microgreens

Microgreens are an exotic genre of edible greens appearing in upscale markets
and restaurants which have gained popularity as a new culinary trend over the past
few years. They are tender immature greens produced from the seeds of vegetables
and herbs, having two fully developed cotyledon leaves with or without the
emergence of a rudimentary pair of first true leaves. They are older than sprouts and
much younger than baby greens. Microgreens became popular in the middle of 1990s
in California and the first use of the word "microgreens" was documented in 1998.
Microgreens are usually 1-3 inches in height, harvested at 7-14 days after germination,
depending on the species, and sold with the stem and attached cotyledons (seed
leaves). Although small in size, microgreens can provide a large array of intense
flavors, vivid colors and tender textures. Therefore, microgreens can be served as a
new ingredient in salad, soups and sandwiches enhancing their color, texture, and/or
flavor, and also can be used as edible garnish to brighten up a wide variety of main
dishes (Murphy et al., 2010; Treadwell et al., 2010.; Lee et al., 2009). Although
microgreens have been claimed as nutritionally beneficial, to the best of our
knowledge, little scientific data are available on microgreens.

1.2 Health Benefits of Fruits and Vegetables

Epidemiological studies have shown that consumption fruit and vegetable is
strongly associated with reduced risk in the development of chronic disease, such as
cancer, heart disease, diabetes, hypertension and metabolic syndrome (Bergquist et al.,
2006; Hung et al., 2004). Diets rich in fruit and vegetables provides an abundance of

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human bioactive compounds, such as ascorbic acid, carotenoids, tocopherols, anthocyanins, and isoflavones, which are known to have protective benefits against cancers and cardiovascular disease (Craig, 1997; Rice-Evans et al., 1995). The foods and herbs with the highest anticancer activity include garlic, soybeans, cabbage, ginger, licorice, and the umbelliferous vegetables. Citrus, in addition to providing an ample supply of vitamin C, folic acid, potassium, and pectin, contains a host of active phytochemicals (Craig & Beck, 1999).The new *Dietary Guidelines for Americans* (2010) released by the U.S. Department of Agriculture (USDA) and the Department of Health and Human Services (DHHS) specifically recommends Americans to fill half of their plate with fruits and vegetables because they possess benefits for human health.

1.3 Phytonutrients in Fruits and Vegetables

Phytonutrients play an important role in human growth, development and health maintenance. They are being intensively studied to evaluate their effects on health. These compounds vary widely in chemical structure and function and are grouped accordingly (Kris-Etherton et al., 2002).

1.3.1 Ascorbic Acid

Ascorbic acid (vitamin C) is an essential nutrient for the human body, acting as the most effective water-soluble antioxidant. There are two available forms of vitamin C in plants: reduced form (L-ascorbic acid) and oxidized form (dehydroascorbic acid). When plants are subject to physical or physiological stresses (chilling, irradiation, harvesting injury, etc.), L-ascorbic acid can be oxidized into dehydroascorbic acid (Hodges et al., 2001). In the dietary source, ascorbic acid is also

unstable and easily oxidized under oxygen, alkali and high temperature. It is previously reported that the utilization of dehydroascorbic acid is equivalent to that of free ascorbic acid, although the metabolic turnover time is different (Tsujimura et al., 2008). It is abundantly found in citrus fruits (*Citrus* L.), peppers (*Capsicum annuum* L.), strawberries (*Fragaria × ananassa* D.), tomatoes (*Solanum lycopersicum* L.), broccoli (*Brassica oleracea* L. var. *italica*), Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*), turnips (*Brassica rapa* L.var. *rapa*) and other leafy vegetables. Numbers of studies have provided strong evidence to link dietary vitamin C with protective effects against various oxidative stress-related diseases such as cancers, cardiovascular disease, aging and cataract formation (Steinmetz & Potter, 1996; Iqbal et al., 2004)

1.3.2 Carotenoids

Carotenoids are one of the most widespread groups of naturally occurring pigments. Within the carotenoids are carotenes and xanthophylls and the difference of their structures is that xanthophylls contain one or more oxygen atoms on the basis of carotenes, which are purely hydrocarbons. These compounds are largely responsible for the red, yellow, and orange color of fruits and vegetables, and are also found in many dark green vegetables (Rao & Rao, 2007). The most abundant carotenoids in the North American diet are β-carotene, α-carotene, γ-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin. Several carotenoids are known to exhibit antioxidant activity and some of them such as β-carotene, α-carotene, and cryptoxanthin are recognized as provitamin A, which are turned into vitamin A in the body and, therefore, perform the same functions in the body as vitamin A (Stahl & Sies, 2003).

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Food sources of carotenoids include carrots (*Daucus carota* L.), sweet potatoes (*Ipomoea batatas* (L.) Lam.), spinach (*Spinacia oleracea* L.), kale (*Brassica oleracea* L. var. *acephala*), collard greens (*Brassica oleracea* L. var. *acephala*), bell peppers (*Capsicum annuum* L.), tomatoes (*Solanum lycopersicum* L.) and papayas (*Carica papaya* L.). Numerous observational studies have found that people who include more carotenoids in their diets have a reduced risk of several chronic diseases, including cancer, cardiovascular diseases, cataracts, age-related macular degeneration and other diseases (Mayne, 1996). Some studies have shown that smokers with diets high in carotenoids have a lower rate of lung cancer development than their counterparts whose carotenoid intake is relatively low. Other research efforts have suggested that diets high in carotenoids may also be associated with a decreased risk of breast cancer (Kaur & Kapoor, 2001).

1.3.3 Tocopherols

Tocopherols and tocotrienols are together summarized as "Vitamin E", known as the main dietary fat soluble antioxidants. Each group has four isomers (α-, β-, γ- and δ-), all of which are naturally occurred and synthesized by plants (Papas, 1999). All chlorophyll-containing tissues contain tocopherols, primarily in the chloroplasts. The most abundant sources of tocopherols are oil seeds, leaves, and other green parts of higher plants. Tocotrienols have been identified in a number of plant tissues, ranging from kale and broccoli to cereal grains and nuts (Piironen et al., 1986). The vitamin E compounds are well recognized for their effective inhibition of lipid oxidation in foods and biological systems. Among these compounds, α-tocopherol has the highest

biological activity, followed by β-, γ-, and δ-tocopherols (Kamal-Eldin & Appelqvist, 1996).

Tocopherols scavenge free radicals by its intermediate a tocopheroxyl radical coupling with lipid peroxyl radicals (Yamauchi, 2007). Evidence exists that tocopherols can prevent atherosclerosis by interfering with the oxidation of LDL, a factor associated with increased risk of heart diseases (Stampfer et al., 1993). It provides vital antioxidant protection for cell membranes, where it works together with both vitamin C and coenzyme Q10. Although vitamin E does not show anticancer activity in animals, a recent clinical chemoprevention study suggests that supplemental vitamin E might decrease risk of prostate cancer, and epidemiological studies support its protective role against colon cancer (Kaur & Kapoor, 2001).

1.3.4 Polyphenols

Polyphenols, including their subcategory, flavonoids, are ubiquitous in all plants. Polyphenols traditionally have been considered antinutrients by animal nutritionists, because of the adverse effect of tannins, one type of polyphenol, on protein digestibility (Bravo, 1998). However, recent interest in food phenolics has increased greatly, owing to their antioxidant capacity (free radical scavenging and metal chelating activities) and their possible beneficial implications in human health. Laboratory studies have shown that specific flavonoids suppress tumor growth, interfere with sexual hormones, prevent blood clots, and have anti-inflammatory properties. Among the important flavonoids are resveratrol, quercetin, and catechin. Evidence suggests that resveratrol (found in red wine, grapes, olive oil) may be extremely potent. In laboratory studies, it increases cell survival and has been shown

to increase the life span of worms and fruit flies. Catechins are the primary flavonoids in tea and may be responsible for its possible beneficial effects. Flavonoids in dark chocolate may also be health protective (Kris-Etherton et al., 2002).

1.3.5 Phytoestrogens

Phytoestrogens, commonly known as isoflavones, have actions that are similar to the female hormone estrogen. A high consumption of soy, which is primarily composed of isoflavones, may reduce symptoms resulting from estrogen depletion during menopause. Various phytoestrogens are present in soy, but also in flaxseed oil, whole grains, fruits, and vegetables. They have antioxidant properties, and some studies demonstrated favorable effects on other CVD risk factors, and in animal and cell culture models of cancer (Kris-Etherton et al., 2002). Lignan is another phytoestrogen and is found in the fiber layers of whole-grains, berries, some seeds, some vegetables, and a few fruits. In laboratory studies, it seems to have anti-cancer properties.

1.3.6 Sulphur-containing Compounds

Organosulfurs are part of the allium family of phytochemicals. Compounds such as allicin may have benefits on the immune system, assist the liver in rendering carcinogens harmless, and reduce production of cholesterol in the liver. These compounds are found in garlic, leeks, onions, chives, scallions, and shallots (Kris-Etherton et al., 2002). Isothiocyanates and related substances, indoles, are also known as mustard oils and are responsible for the sharp taste in cruciferous vegetables. Such vegetables include broccoli, cabbage, Brussels sprouts, cauliflower (*Brassica oleracea* L. var. *botrytis*), collard greens, kale, kohlrabi (*Brassica oleracea* L. var.

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gongylodes), mustard greens (*Brassica juncea* L.), rutabaga (*Brassica napobrassica* (L.) Mill.), turnips, and bok choy (*Brassica rapa* L. var. *chinensis*). Isothiocyanates stimulate enzymes that convert estrogen to a more benign form and may block steroid hormones that promote breast and prostate cancers.

1.4 Sensory Evaluation and Consumer Acceptance

New product development is the driving force and stimulus in the growth of food industry. In this process, new technologies play an important role in sustainable innovation of new product development. However, the advantages that a new processing technology has to offer are not enough to ensure acceptance of these technologies in the market place, because consumer acceptance is a critical factor which considerably affect the marketability of a new food product (Lyndhurst, 2009). Some technologies like organic production are warmly welcomed by many consumers, whereas others like genetic modification and irradiation have been firmly rejected. Thus, we can see that consumers' perception and acceptance to a new product can prevent the application of a technology and delay the marketing promotion of a new product, like in the case of irradiation and genetic modification. "On this background it is very important to know how much consumers will like the new products developed by new technology" (Olsena et al., 2010).

The quality control of food has a significant role in assuming a high quality, safe and nutritious food supply for the public, for their good health and for the economic benefits derived from trade of safe and high quality food. Quality control is applicable throughout the entire processing of food production, continuously involved from raw materials to end products. There are many analytical and instrumental evaluation

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methods in quality control, such as electronic nose to discriminate red wines, gas chromatography-mass spectrometry (GC-MS) to analyze volatiles, colorimeter to estimate color evolution and texture analyzer to evaluate firmness of many kinds of food products (Hansen et al., 2005). Even so, there are still different kinds of limitations resulting from the instruments inevitably. The most reliable way to evaluate the potential human perception is to conduct the human sensory tests. Human sensation is received from an organoleptic system, which can deliver the comprehensive information on the given sensory attributes of the food product, including appearance, color, texture, taste and flavor and overall liking. Therefore, in the modern food industry, human sensory evaluation is becoming more and more important in establishing consumer acceptability, quality controls and new product development.

1.5 Postharvest Storage and Quality Control

1.5.1 Low Temperature Storage

Storage temperature is one of the most important factors affecting the postharvest physiology and storage behavior of produce. In general, low temperature storage can reduce quality loss and extend shelf life by depressing rates of respiration, senescence, and growth of spoilage microorganisms (Spinardi & Ferrante, 2012; Paull, 1999). Optimum storage temperature varies depending on the fruit or vegetable. For some chilling sensitive fruits and vegetables, the use of low temperature storage adversely affects quality attributes and causes deterioration more rapidly (Galvez et al., 2010; Sandhya, 2010). Even though an optimal low temperature is maintained through the storage, transportation and retail, the fruits and

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vegetables can still spoil, as evidenced by fungal attacks and detrimental quality changes. Therefore, low temperature storage should be combined with other postharvest handling methods, like modified atmosphere packaging or UV irradiation.

1.5.2 Modified Atmosphere Packaging

Modified atmosphere packaging is an effective technology for maintaining freshness and prolonging shelf life of produce, which can be created by altering the gas composition in the package, thus to provide an optimum atmosphere for prolonging storage length and maintaining the quality of food. The modified atmosphere can be achieved by using controlled atmosphere (CA) and /or active or passive modified atmosphere packaging (MAP) (Farber et al., 2003). To data, CA and MAP have been successfully applied in fresh and minimally processed produce to increase the quality, such as apple (*Malus × domestica* Borkh.), pear (*Pyrus communis* L.), lettuce (*Lactuca sativa* L.), broccoli, spinach (Spinacia oleracea L.), and mushrooms (Sandhya, 2010). In addition, modification of the atmosphere can help restrict the growth of microorganisms surrounding the product by provide the 'hurdle' of microbial growth. Another 'hurdle' can be provided by storage at low temperatures (< 4 °C). The combination of chill temperatures and MAP generally results in a more effective and safer storage regime and longer shelf-life. There are many factors influencing package atmosphere of products, including product respiration rate, packaging film oxygen transmission rate (OTR), product weight, package surface area, storage temperature and relative humidity (Sandhya, 2010). In food supply chains, package size and product weight are often pre-determined,

therefore, selecting a packaging film with suitable OTR to match the product

respiration rate is the best way to maintain quality and extend shelf life of produce.

1.5.3 Edible Coating

Edible coatings, a new strategy to extend shelf-life and improve food quality of

whole fresh-cut fruits and vegetables, have been applied to many products. On one

hand, edible coatings provide a selective barrier to moisture, oxygen and carbon

dioxide, which retards gas transfer, slows ripening, reduces moisture loss and helps to

maintain fresh aroma and flavor (Olivas & Barbosa-Canovas, 2005). On the other

hand, edible coatings are used as carriers of active ingredients, such as antibrownings,

antimicrobials, texture enhancers, flavors, and nutrients, to improve the quality,

safety, and nutritional value of fresh-cut fruits (Rojas-Graü et al., 2009). Several types

of edible coatings, such as alginate, pectin, gellan, methylcellulose, have been used

for extending shelf life of fresh and fresh-cut commodities like apples, pears, melons,

papayas and pineapples (*Ananas comosus* (L.) Merr.) (Oms-Oliu et al., 2010).

Numerous studies have shown that the use of edible coatings is a promising approach

for maintaining "fresh" quality of produce and thus contributing to greater consumer

acceptance.

1.6 Microbial Risks and Safety Ensurance

1.6.1 Foodborne Outbreaks Associated with Produce

From the Center of Disease Control and Prevention (CDC) database, it can be

seen that foodborne illness outbreak associated with fresh fruits and vegetables

happened almost every year and most of them were multistate outbreaks. In 2006, the

United States Food and Drug Administration (US FDA) announced a large *E. coli*

O157:H7 outbreak of illness associated with bagged spinach. By the time the outbreak was over, 204 people were reported to have become ill across 26 States and Canada, 104 had been hospitalized, 31 had developed serious complications, and 3 had died. From November of 2010 through February of 2011, the *Salmonella* outbreak linked to eating alfalfa sprouts or spicy sprouts at Jimmy John's restaurants caused 140 individuals to have become infected in 26 states including the District of Columbia. Statistical data showed that since 1996 there have been at least 30 reported outbreaks of foodborne illness associated with raw sprouts. Last year, a *Salmonella* outbreak was linked to cantaloupe: a total of 261 people were infected, 94 people hospitalized and 3 deaths were reported from across 24 states.

These dreadful outbreaks have triggered increasing attention of microbiological safety of produce. More and more studies have been extensively carried out to identify critical control points during preharvest and postharvest process and develop novel technologies to ensure produce safety.

1.6.2 Sanitation Methods on Fresh Produce

1.6.2.1 Washing and Sanitizing

Washing and sanitizing treatments play a crucial role in reducing microbial populations on fresh fruits and vegetables, thereby, improving the quality and safety of fresh or fresh-cut produce.

Chlorine is the most widely used sanitizing agent for fresh produce (Beuchat, 1998). It was shown that the efficacy of chlorine sanitation on produce surface is not sufficient within the range of 1-2 log reduction of microbial population (Sapers et al., 1998). Meanwhile, the reaction of chlorine with organic residues can result in the

formation of potentially mutagenic or carcinogenic reaction products (Hidaka et al., 1992). The use of chlorine in food products has been restricted or prohibited in some countries, such as the European Union (EU) (Johnson, 2011). Therefore, alternatives to chlorine have been studied, and some are in commercial use.

Electrolyzed water (EW) has been received a lot of attention as a new sanitizing agent for produce in recent years. EW has many advantages, such as effective disinfection, easy operation, relatively inexpensive, and environmentally friendly. However, some disadvantages should be considered as well: 1) the initial cost for the equipment purchase is high; and 2) The chlorine gas generated is bothersome to operators. Only recently has EW been tested and used as a disinfectant in the food industry (Huang et al., 2008).

Ozone (O_3) is a strong oxidizing agent with numerous potential applications in the food industry, which has been used for decades in many countries. High reactivity, penetrability, and spontaneous decomposition to a nontoxic product (i.e., O_2) make ozone a viable disinfectant. However, excessive use of ozone may affect food quality due to oxidation of some ingredients on food surface. Caution is needed for workers during operation, since inevitable contact of ozone may affect respiration and cause dizziness and irritation. Ozone has been used with mixed success to inactivate contaminant microflora on meat, poultry, eggs, fish, fruits, vegetables, and dry foods.

Peroxyacetic acid, also known as peracetic acid, is a mixture of the peroxy compound, hydrogen peroxide, and acetic acid. It is usually commercialized as a liquid, like Tsunami®. Peroxyacetic acid is a strong oxidizing agent and tolerant to several factors such as temperature, pH (from 1 to 8), hardness and soil

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contamination; therefore, it is currently applied in the fruit and vegetable processing (Artés et al., 2007). Additionally, the break-down products (acetic acid, O_2, CO_2 and water) of peroxyacetic acid are not particularly harmful for the ecosystem (Artés et al., 2009). However, it is more expensive than chlorine and exposure to peroxyacetic acid can cause irritation to the skin, eyes and respiratory system.

Chlorine dioxide (ClO_2) is a stable dissolved gas in solution, having a higher oxidation and penetration power than NaClO and more effective against spores (EPA, 1999). ClO_2 is a strong bactericide and virucide at levels as low as 0.1 μg/mL. Unlike chlorine, ClO_2 does not ionize to form weak acids in water and remains as dissolved gas in solution, which enables ClO_2 to be effective over a wide pH range. The main drawback is that it has to be generated on-site by reacting sodium chlorite and acid or chlorine (EPA, 1999). Besides, ClO_2 is unstable and can be explosive when the concentration reaches 10% or more in air (Betts & Everis, 2005).

Hydrogen peroxide is a powerful oxidant, which has been demonstrated to be effective in extending shelf-life and reducing native microbial and pathogen populations in fresh and fresh-cut produce products (Sapers & Sites, 2003; Artés et al., 2007). However, such treatments require lengthly application times (i.e., 15-60 min) and can cause injury to some commodities such as mushroom and strawberries (Sapers & Simmons, 1998). Therefore, handling methods and safety issues of hydrogen peroxide are still in discussion (Taormina et al., 1999).

1.6.2.2 Irradiation

Irradiation (increasingly referred to as "cold pasteurization") is a control measure in the production of several types of raw or minimally processed foods such as

poultry, meat and meat products, fish, seafood, and some fruits and vegetables. It has

the potential to eliminate vegetative forms of bacterial pathogens as well as parasites

(Molins et al., 2001). Irradiation is a safe technology, since scientific research has

determined that food irradiation does not make food 'radioactive' and at low to

medium doses, has little negative effect on vitamins and other nutrients humans

obtain from their food supply (Crawford & Ruff, 1996; Lester et al., 2010a). Today,

40 countries permit the irradiation of one or more foodstuffs: 12 countries have

approved its use for pathogen control in poultry, 8 other for use in meats, and 13 in

fish and seafood (Molins et al., 2001). In the USA, there are more than 40 irradiation

plants in operation today, all of which are dedicated to sterilization of certain

industrial products and medical supplies and there is only one commercial food

irradiation plant operating in the USA. To some extent, the slow growth of food

irradiation processing in this country is mainly attributable to consumer perceptions.

Surveys show that Americans know very little about the food irradiation process and

are inclined to answer 'no' when asked if they would purchase irradiated foods.

However, those same surveys indicate that when consumers are told about the

benefits and safety of irradiation, their acceptance level increases (Crawford & Ruff,

1996). While there is very strong support for food irradiation among the informed

scientific community and health organizations, extensive education is needed for

broad public acceptance.

1.6.2.3 Intense light pulses

Intense light pulse (ILP) is an emerging nonthermal technology for

decontamination of food surfaces and food packages, consisting of short time high-

peak pulses of broad spectrum white light. It is considered an alternative to continuous ultraviolet light treatments for solid and liquid foods and has been approved by the US FDA that could be suitable for disinfecting fresh-cut produce. (Oms-Oliu et al., 2010).Intense light pulses treatment kills microorganisms using short time (from 85 ns to 0.3 ms) high frequency pulses (from 0.45 to 15 Hz) and energy per pulse ranging from 3 to 551 J of an intense broad spectrum, rich in UV–C light (Gómez-López et al., 2005). It seems to induce structural changes of microbial DNA, comparable to the effect caused by continuous UV sources, but others mechanisms seem to be involved (Takeshita et al., 2003). Since the ILP decontamination effect seems to be dependent on light absorption by microorganisms, certain food components could also absorb the effective wavelengths and decrease their efficiency. ILP has been used to successfully inactivate *E. coli* O157:H7 on alfalfa seeds (Sharma & Demirci, 2003). Gómez-López et al. (2005) reported that foods rich in carbohydrate, such as fruit and vegetables, seem to be more suitable for decontamination by ILP (Artés et al., 2009). ILP has considerable potential to be implemented in the food industry. However, technological problems need to be solved in order to avoid food overheating as well as to achieve better penetration and treatment homogeneity.

Chapter 2: Assessment of Vitamin and Carotenoid Concentrations of Emerging Food Products: Edible Microgreens

2.1 Abstract

Microgreens have gained popularity as a new culinary trend over the past few years. Although small in size, microgreens can provide surprisingly intense flavors, vivid colors and crisp textures. No scientific data is currently available on the nutritional content of microgreens. The present study was conducted to determine the concentrations of ascorbic acid, carotenoids, phylloquinone and tocopherols in 25 commercially available microgreens. Results showed that different microgreens provided extremely varying amounts of phytonutrients. Total ascorbic acid contents ranged from 20.4 to147.0 mg per 100 g fresh weight (FW), 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. Phylloquinone level varied from 0.6 to 4.1 μg/g FW, meanwhile, 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 assayed, red cabbage, cilantro, garnet amaranth and green daikon radish had the highest concentrations of ascorbic acids, carotenoids, phylloquinone, and tocopherols, respectively. Compared mature leaves nutritional concentrations recorded in USDA National Nutrient Database, microgreen cotyledon leaves possessed higher nutritional densities.

2.2 Introduction

Epidemiological studies have shown that fruit and vegetable consumption is associated with reduction in the development of chronic disease, such as cancer and cardiovascular disease (Bergquist et al., 2006; Hung et al., 2004). Diets rich in fruits and vegetables provide an abundance of phytonutrients (Craig & Beck, 1999), such as ascorbic acid (vitamin C), carotenoids (provitamin A compounds), phylloquinone (vitamin K_1) and tocopherols (vitamin E), which are known to have health protective benefits against cancers and cardiovascular disease (Catherine Rice-Evans, 1995). The new *Dietary Guidelines for Americans* (2010) released by the U.S. Department of Agriculture (USDA) and the Department of Health and Human Services (DHHS) specifically recommends Americans to fill half of their plate with fruits and vegetables because they possess benefits for human health.

Microgreens are an exotic genre of edible greens appearing in upscale markets and restaurants which have gained popularity as a new culinary trend over the past few years. Microgreens are tender immature greens produced from the seeds of vegetables and herbs, having two fully developed cotyledon leaves with or without the emergence of a rudimentary pair of first true leaves. Microgreens are usually 2.5-7.6 cm (1-3 in.) in height, harvested at 7-14 days after germination, depending on the species, and sold with the stem and attached cotyledons (seed leaves). Although small in size, microgreens can provide a large array of intense flavors, vivid colors and tender textures. Therefore, microgreens can be served as a new ingredient in salad, soups and sandwiches enhancing their color, texture, and/or flavor, and also can be

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used as edible garnish to brighten up a wide variety of main dishes (Treadwell, 2010; Lee et al., 2004; Lee et al., 2009; Murphy & Pill, 2010).

Although microgreens have been claimed as nutritionally beneficial, to the best of our knowledge, no scientific data are available on the exact phytochemical content of microgreens. Limited studies have shown that some young seedlings may have much higher levels of vitamins, minerals and other health-giving phytonutrients than the mature leaves. In a recent study from Lester et al. (2010a), it was reported that the younger leaves of baby spinach (*Spinacia oleracea* L.) generally had higher levels of phytonutrients: vitamins C, B_9 and K_1, and the carotenoids (lutein, violaxanthin, zeaxanthin and beta-carotene) than the more mature leaves. Oh et al. (2010) also found that young lettuce (*Lactuca sativa*) seedlings, 7 days after germination, had the highest total phenolic concentration and antioxidant capacity in comparison to the older leaves. Therefore, the object of this study was to assess the phytonutrients content of the 25 commercially available microgreens varieties. The phytonutrients assayed include ascorbic acid (total, free and dehydro), carotenoids (beta-carotene, violaxanthin and lutein/zeaxanthin), phylloquinone, and tocopherols (α-tocopherol and γ-tocopherol).

2.3 Materials and Methods

2.3.1 Plant Materials

Twenty-five varieties of microgreens were purchased from Sun Grown Organics Distributors, Inc. (San Diego, CA, USA) from May through July, 2011. They were produced by the grower in an unheated greenhouse and under ambient light except etiolated golden pea tendrils and popcorn shoots, which were grown in the dark. All

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the microgreens were grown in soil and fertilized in a proprietary manner except China rose radish and green daikon radish microgreens, which were grown hydroponically. Samples were harvested without roots, packed in clamshell containers (113.4 g of each) and shipped overnight in a cardboard box which was filled with frozen-ice packs. When received, three grams of fresh tissue were weighed for ascorbic acid analysis. Remaining tissue was frozen in liquid nitrogen and lyophilized for dry weight and other vitamin and carotenoid determinations. It is worth mentioning that golden pea tendrils and green pea tendrils are grown from the same seed source. Golden pea tendrils were grown in dark and green pea tendrils were grown under ambient light. Photographs of the 25 commercially grown microgreens assayed in this study were shown in **Fig. 2.1** and commercial names, scientific names and plant colors are listed in **Table 2.1**.

2.3.2 Dry Weight

Dry matter was determined by freeze-drying according to a previous procedure (Julkunen-Tiitto & Sorsa, 2001). Ten grams of fresh microgreens were weighed into plastic tubes, frozen in liquid nitrogen, and lyophilized for 48 h (VirTis Freezemobile 35 ES Sentry 2.0 freeze dryer, SP Scientific Corp., Warminster, PA, USA), followed by holding at room temperature in a dessicator prior to weighing.

2.3.3 Nutrient Analysis

All chemicals and standards unless otherwise stated were obtained through Sigma-Aldrich Chemical Corp. (St. Louis, MO, USA). Standards of lutein and zeaxanthin were obtained from ChromaDex (Irvine, CA, USA).

Fig. 2.1 Pictures of 25 commercially available microgreens.

Table 2.1 Commercial names, scientific names and plant colors of 25 commercially grown microgreens assayed in the nutrient study.

Commercial name	Scientific Name		Plant color
	Family	**Genus and Species**	
Arugula	Brassicaceae	*Eruca sativa* Mill.	Green
Bull's blood beet	Chenopodiaceae	*Beta vulgaris* L.	Reddish-green
Celery	Apiaceae	*Apium graveolens* L.	Green
China rose radish	Brassicaceae	*Raphanus sativus* L.	Purplish-green
Cilantro	Apiaceae	*Coriandrum sativum* L.	Green
Garnet amaranth	Amaranthaceae	hypochondriacus L.	Red
Golden pea tendrils[*]	Fabaceae	*Pisum sativum* L.	Yellow
Green basil	Lamiaceae	*Ocimum basilicum* L.	Green
Green daikon radish	Brassicaceae	*Raphanus sativus* L.	Green
Magenta spinach	Chenopodiaceae	*Spinacia oleracea* L.	Red
Mizuna	Brassicaceae	*Brassica rapa* L.	Green
Opal basil	Lamiaceae	*Ocimum basilicum* L.	Greenish-purple
Opal radish	Brassicaceae	*Raphanus sativus* L.	Greenish-purple
Pea tendrils[*]	Fabaceae	*Pisum sativum* L.	Green
Peppercress	Brassicaceae	*Lepidium bonariense* L.	Green
Popcorn shoots	Poaceae	*Zea mays* L.	Yellow
Purple kohlrabi	Brassicaceae	*Brassica oleracea* L.	Purplish-green
Purple mustard	Brassicaceae	*Brassica juncea* L.	Purplish-green
Red beet	Chenopodiaceae	*Beta vulgaris* L.	Reddish-green
Red cabbage	Brassicaceae	*Brassica oleracea* L.	Purplish- green
Red mustard	Brassicaceae	*Brassica juncea* L.	Purplish-green
Red orach	Chenopodiaceae	*Atriplex hortensis* L.	Red
Red sorrel	Polygonaceae	*Rumex acetosa* L.	Reddish-green
Sorrel	Polygonaceae	*Rumex acetosa* L.	Green
	Brassicaceae	*Wasabia japonica*	Green
Wasabi		Matsum.	

[*]Golden pea tendrils and pea tendrils are grown from the same seeds. Golden pea tendrils are grown in dark and pea tendrils are grown under light, therefore, the colors of them are different (yellow and green, respectively). All the microgreens were grown organically except China rose radish and green daikon radish microgreens, which were grown hydroponically.

2.3.3.1 Ascorbic Acid

Total ascorbic acid (TAA) and free L-ascorbic acid (AA) were determined spectrophotometrically according to the procedure previously reported by Hodges et al. (2001). Fresh tissue (3 g) was weighed into a 50 mL centrifuge tube, and 10 mL of ice-cold 5% (w/v) meta-phosphoric acid was added, followed by homogenization at the speed of 15,000 rpm for 1 min in ice-water bath using a polytron homogenizer (Brinkman Instruments, Westbury, NY, USA). Homogenized tissue was centrifuged at 7000 g (Beckman J2-MI, Beckman Coulter, Inc., Irving, TX, USA) for 20 min at 4°C, and supernatant was filtered through Whatman Grade 4 filter paper (Millipore Corp. Bedford, MA, USA). Filtrate was used for AA determination and TAA by converting dehydroascorbic acid (DHA) to AA with dithiothreitol. TAA and AA were determined spectrophotometrically (Genesys 20, Thermo Scientific Inc, Logan, UT, USA) at 525 nm. Concentrations of TAA and AA were calculated using an L-ascorbic acid standard curve (all the $R^2 \geq 0.99$), and their difference was equal to the concentration of DHA.

2.3.3.2 Carotenoids and Tocopherols

Carotenoids and tocopherols were extracted under yellow light according to the modified method described by Lester et al. (2010a). Briefly, 0.05 g of lyophilized samples were weighed into 15 mL screw cap glass vials, and then 7.5 mL of 1% butylated hydroxytoluene (BHT) in ethanol and 500 µL of internal standard (86.82 µM trans-β-apo-8 carotenal) were added, followed by ultrasonic homogenization for 15 s using a Fisher Scientific Model 300 Sonic Dismembrator (Pittsburg, PA, USA). The vials were capped under a stream of N_2 and placed in a 70°C dry bath for 15 min,

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after which 180 µL of 80% KOH was added. After mixing and flushing with flow N_2, vials were capped again and placed in a 70°C dry bath for 30 min. Vials were then removed and cooled for 5 min in ice, and then transferred into 15 mL centrifuge tubes, after which 3.0 mL of deionized water and 3.0 mL of hexane/toluene solution (10:8 v/v) were added. The mixture was vortexed for 1 min, and then centrifuged at 1000 g (Clay Adams Dynac II Centrifuge, Block Scientific, Inc., Bohemia, NY, USA) for 5 min. The top organic layer was collected into an 8 mL glass culture tube, and immediately placed into a nitrogen evaporator (Organomation Associates, Inc., Berlin, MA, USA) set at 30°C and flushed with stream of N_2. The bottom layer was extracted again with 3.0 mL of hexane/toluene solution (10:8 v/v) for further partition. This extraction was repeated at least four times until the top layer was colorless, and all the supernatants were combined into a glass culture tube. After evaporation, the residue was reconstituted in 500 µL of mobile phase acetonitrile/ethanol (1:1 v/v), filtered into an HPLC amber vial through 0.22 µm nylon filter (Milliopore Corp., Bedford, MA, USA) with a glass syringe and 20 µL was injected for HPLC analysis. Carotenoids and tocopherols concentrations were simultaneously determined using an isocratic reverse phase high performance liquid chromatography (RP-HPLC), which were separated on a C18 column (Adsorbosphere C18-UHS, 5µm, 150×4.6mm, Grace, Deerfield, IL, USA) with a photo diode array detector (DAD) (G1315C, Agilent, Santa Clara, CA, USA), using isocratic mobile phase acetonitrile/ethanol (1:1 v/v). The flow rate was 1.2 mL/min and the running time was 20 min. Absorbance was measured at 290 and 450 nm simultaneously for

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tocopherols and carotenoids, respectively. Quantification was based on a standard curve for each compound.

2.3.3.3 Phylloquione

Phylloquinone was extracted from 25 microgreens under dim light and determined by reversed-phase high performance liquid chromatography (RP-HPLC), as described by Booth et al. (1994). Each sample (0.1 gram of freeze-dried tissue) was homogenized (Brinkman Instruments, Westbury, NY, USA) with 10 mL of H_2O and 0.4 mL of 200 µg/mL menaquinone (internal standard) at the speed of 15000 rmp for 1min, after which 15 mL of 2-propanol/hexane (3:2 v/v) was added. The sample was then vortexed for 1 min and centrifuged (Beckman J2-MI, Beckman Coulter, Inc., Irving, TX, USA) for 5 min at 1500g, 21°C. The upper (hexane) layer was transferred into a glass culture tube and dried under a stream of N_2. The residue was dissolved in 4 mL of hexane. The sample extract was purified by loading 1 mL of redissolved extract onto preconditioned silica gel columns (4mL of 3.5% ethyl ether in hexane, followed by 4 mL of 100% hexane), and then washing column with 2 mL of hexane. Phylloquinone was eluted with 8 mL of 3.5% ethyl ether in hexane, and the eluate was evaporated on a water-jacketed heating block (Pierce Reacti-Therm, Pierce Chemical Company, Rockford, IL, USA) at 40°C under N_2 flow, and then reconstituted in 2 mL of mobile phase (99% Methanol, 1% 0.05M sodium acetate buffer, pH=3.0) and filtered through a 0.22 µm nylon syringe filter (Millipore Corp., Bedford, MA, USA). Detection of phylloquinone was with a photodiode array detector (DAD) (G1315C, Agilent, Santa Clara, CA, USA) on Agilent 1200 Series HPLC system and absorbance wavelength was 270 nm. The extract (20 µL) was

injected into HPLC and run through a C18 column (201TP, 5µm, 150 × 4.6 mm, Grace, Deerfield, IL, USA) using an isocratic mobile phase (described above) flowing at the rate of 1 mL/min. The phylloquinone content of the samples was quantified according to the internal standard method based on the peak areas.

2.3.4 Statistical Analysis

Dry weight analysis and all assays were performed on three replicates. All phytonutrient analysis was conducted through one extraction of each replicate from each sample. All the data was reported as the mean of three replicates ± standard error. Statistical separation of phytonutrient values per species is based by Coefficient of Variability (CV) and this variability is in relation to the mean of the population from mature leaf data. A combined population of microgreens for each phytonutrient CV is listed in tables.

2.4 Results and Discussion

2.4.1 Dry Weight Percentage

Dry weight percentage of the 25 commercially available microgreens ranged from 4.6% to 10.2%, as shown in **Table 2.2**. Among them, pea tendrils had the highest dry weight percentage and red beet possessed the lowest one. The overall average dry weight percentage of the 25 varieties of microgreens was 6.9 ± 0.1 %.

2.4.2 Phylloquinone Concentrations

Phylloquinone (vitamin K_1) is required for blood coagulation and most abundant in photosynthetic tissues of dark-green vegetables, such as spinach (*Spinacia oleracea* L.), kale (*Brassica oleracea* L. var. *acephala*) and broccoli (*Brassica oleracea* var. *italica*) (Bolton-Smith et al., 2000). The HPLC chromatograph of

Vitamin K standards and extract from microgreens was shown in **Fig. 2.2**. Among the 25 microgreens assayed, there was considerable variation in phylloquinone concentration, ranging from 0.6 to 4.1 µg/g fresh weight (FW) as shown in **Table 2.2**. Among them, the most concentrated in phylloquinone was garnet amaranth (4.1 µg/g FW), red sorrel (3.3 µg/g FW), green basil (3.2 µg/g FW), pea tendrils (3.1 µg/g FW) and red cabbage (2.8 µg/g FW) microgreens. In contrast, magenta spinach, golden pea tendrils, red orach microgreens and popcorn shoots had vitamin K_1 concentration ranging from 0.6 to 0.9 µg/g FW. Samples identified as rich in phylloquinone were generally green (e.g. pea tendrils) and bright red in color (e.g. garnet amaranth microgreens), while yellow-colored microgreens, such as popcorn shoots and golden pea tendrils, had relatively low concentration of vitamin K_1, which is in agreement with a previous report (Bolton-Smith et al., 2000). Surprisingly, magenta spinach, which has a similar appearance to the leading vitamin K_1 microgreens source, garnet amaranth (4.1 µg/g FW), had among the lowest vitamin K_1 concentration. Comparison of fully-grown and cotyledon leaves demonstrated that growth stage affected vitamin K_1 concentration, and for some of the varieties, the effect was obvious. For example, according to the USDA National Nutrient Database (2011), phylloquinone concentration in mature amaranth, basil and red cabbage were 1.14, 0.41 and 0.04 µg/g FW, respectively, which were much lower than their corresponding microgreens (4.09, 3.20 and 2.77 µg/g FW, respectively). Four of the 25 microgreen varieties assayed in this study had comparable amount of phylloquinone to mature leaf spinach, which is generally considered as an excellent

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source of vitamin K_1; and 18 out of 25 exhibited vitamin K_1 densities equal to or higher than that of broccoli, the most commonly consumed vegetable in US (Bolton-Smith et al., 2000; USDA, 2011); demonstrating that most of the 25 microgreens can serve as good natural sources of vitamin K_1.

Table 2.2 Dry weight percentage and phylloquinone concentrations in 25 commercially grown microgreens[a].

Microgreen name	Dry Weight (%)	Phylloquinone ($\mu g/g$ FW)
Arugula	5.5 ± 0.0	1.6 ± 0.1
Bull's blood beet	6.2 ± 0.1	2.0 ± 0.1
Celery	6.8 ± 0.1	2.2 ± 0.1
China rose radish	8.1 ± 0.1	1.8 ± 0.1
Cilantro	8.3 ± 0.1	2.5 ± 0.1
Garnet amaranth	9.3 ± 0.1	4.1 ± 0.0
Golden pea tendrils	9.8 ± 0.2	0.7 ± 0.0
Green basil	7.3 ± 0.0	3.2 ± 0.1
Green daikon radish	7.8 ± 0.1	1.9 ± 0.1
Magenta spinach	5.1 ± 0.2	0.6 ± 0.0
Mizuna	5.3 ± 0.0	2.0 ± 0.0
Opal basil	6.8 ± 0.1	2.0 ± 0.1
Opal radish	7.8 ± 0.1	2.2 ± 0.2
Pea tendrils	10.2 ± 0.2	3.1 ± 0.2
Peppercress	7.3 ± 0.1	2.4 ± 0.2
Popcorn shoots	7.0 ± 0.1	0.9 ± 0.0
Purple kohlrabi	6.1 ± 0.0	2.3 ± 0.1
Purple mustard	5.7 ± 0.1	1.3 ± 0.1
Red beet	4.6 ± 0.1	1.9 ± 0.1
Red cabbage	7.7 ± 0.1	2.8 ± 0.1
Red mustard	5.6 ± 0.1	1.9 ± 0.1
Red orach	6.2 ± 0.2	0.7 ± 0.0
Red sorrel	7.0 ± 0.1	3.3 ± 0.0
Sorrel	4.9 ± 0.0	1.7 ± 0.1
Wasabi	5.6 ± 0.0	1.9 ± 0.1
Coefficient of variation		15%

[a] Values are expressed as means $\pm$ standard error (n=3).
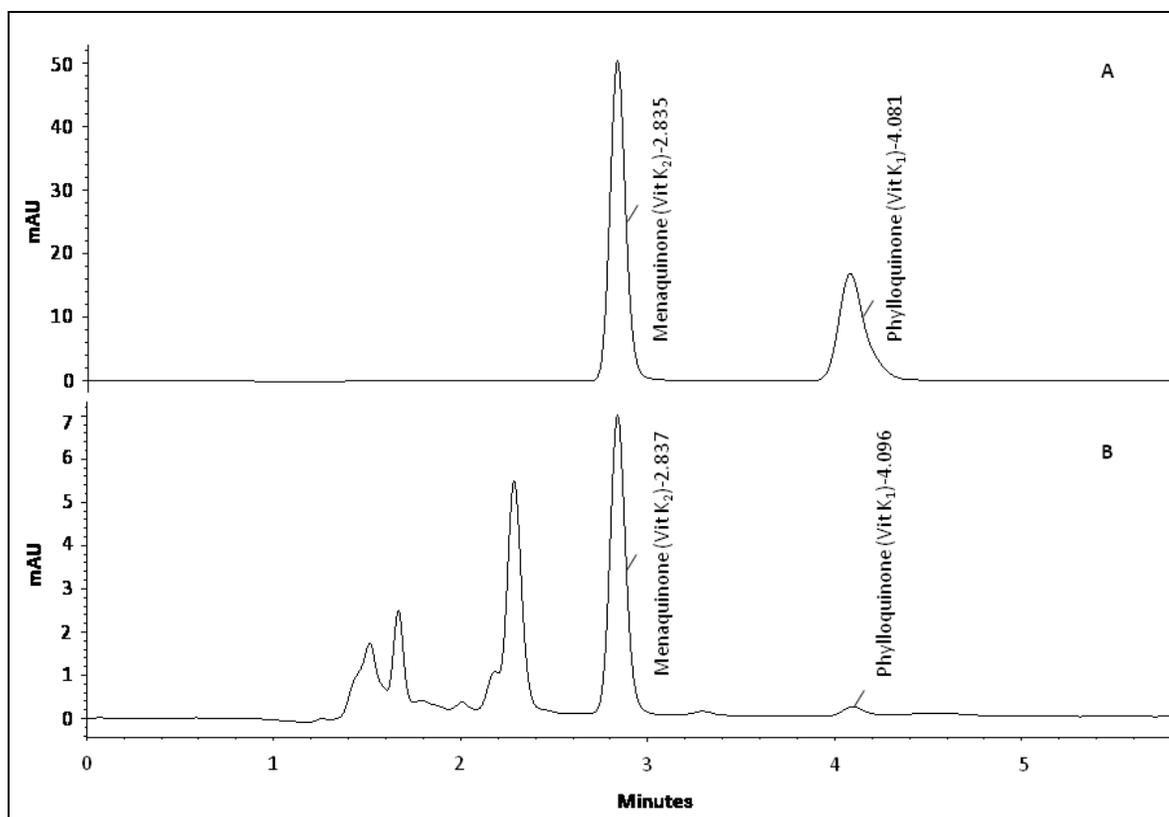
Fig. 2.2 HPLC chromatograms of vitamin K standards (A) and extraction of garnet amaranth microgreens (B).
Menaquinone (Vit K_2) is the internal standard. HPLC conditions are described in the materials and methods.

2.4.3 Ascorbic Acid Concentrations

Ascorbic acid (vitamin C) is an essential nutrient for the human body, acting as an antioxidant. When the plant is subject to physical or physiological stress (harvesting injury, chilling, irradiation, etc.), the AA can be oxidized into DHA (Hodges et al., 2001). It is previously reported that the utilization of DHA is equivalent to that of AA, although the metabolic turnover time is different (Tsujimura et al., 2008). In this study, TAA, AA and DHA concentration were determined and listed in **Table 2.3**.

The 25 microgreens exhibited TAA concentration ranging from 20.4 to 147.0 mg/100 g FW. Among samples tested, red cabbage and garnet amaranth microgreens

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had the highest TAA contents, followed by China rose radish, opal basil and opal radish. The vitamin C content of red cabbage microgreens (147.0 mg/100 g FW) was 6-fold higher than previously published data of the mature red cabbage (24.4 mg/100 g FW) (Singh et al., 2006) and 2.6 times greater than that (57.0 mg/100 g FW) recorded in USDA National Nutrient Database for Standard Reference, Release 24 (USDA, 2011), and was determined to be 2.4 times greater than estimated average requirement (EAR) for ascorbic acid. Garnet amaranth (131.6 mg/100g FW) had much higher ascorbic acid content than reported concentration of mature leaf (11.6-45.3 mg/100 g FW) (Mensah et al., 2008; Punia et al., 2004). China rose radish, opal basil and opal radish microgreens also were relatively abundant sources of vitamin C with more than 90.0 mg/100 g FW, equal to 1.5 times of the recommended dietary allowance (RDA).These microgreen varieties had higher ascorbic acid concentration than does broccoli (89.2 mg/100 g FW) (USDA, 2011), which is generally recognized as an excellent source of vitamin C. Even though some of the 25 microgreens tested had relatively low levels of total AA, such as golden pea tendrils (25.1 mg/100 g FW) and sorrel microgreens (20.4 mg/100 g FW), they were comparable to spinach (28.1 mg/100 g FW) (USDA, 2011), which is one of the most commonly consumed leaf-vegetables in US. Therefore, it was suggested that fresh microgreens are generally good to excellent sources of ascorbic acid and likely more concentrated with TAA than their mature plant counterparts, which is in accordance with Bergquist's (2006) findings on baby spinach: that younger plants had higher ascorbic acid content than older harvested leaves.

Table 2.3 Total ascorbic acid (TAA), free ascorbic acid (AA), and dehydroascorbic acid (DHA) concentrations in 25 commercially grown microgreens [a].

Microgreen name	TAA (mg/100 g FW)	AA (mg/100 g FW)	DHA (mg/100 g FW)
Arugula	45.8 ± 3.0	32.7 ± 1.3	13.2 ± 2.8
Bull's blood beet	46.4 ± 3.0	46.0 ± 3.3	0.5 ± 0.3
Celery	45.8 ± 3.1	32.6 ± 1.3	13.2 ± 2.8
China rose radish	95.8 ± 10.3	73.2 ± 3.4	22.6 ± 7.4
Cilantro	40.6 ± 2.4	24.5 ± 1.8	16.1 ± 2.2
Garnet amaranth	131.6 ± 2.9	105.1 ± 3.1	26.5 ± 1.4
Golden pea tendrils	25.1 ± 0.7	15.3 ± 1.7	9.8 ± 1.2
Green basil	71.0 ± 2.7	59.0 ± 1.8	12.0 ± 1.1
Green daikon radish	70.7 ± 2.7	58.8 ± 1.7	11.9 ± 1.1
Magenta spinach	41.6 ± 0.8	36.0 ± 0.8	5.6 ± 0.2
Mizuna	42.9 ± 1.6	32.3 ± 1.0	10.6 ± 0.7
Opal basil	90.8 ± 2.7	81.8 ± 1.6	9.0 ± 2.0
Opal radish	90.1 ± 2.7	81.1 ± 1.7	9.0 ± 1.9
Pea tendrils	50.5 ± 0.9	27.9 ± 1.1	22.5 ± 0.3
Peppercress	57.2 ± 1.6	33.0 ± 0.7	24.2 ± 1.8
Pop corn shoots	31.8 ± 0.7	21.4 ± 2.5	10.4 ± 3.0
Purple kohlrabi	62.8 ± 7.3	48.1 ± 3.7	14.7 ± 3.7
Purple mustard	72.1 ± 4.6	53.6 ± 2.6	18.5 ± 4.4
Red beet	28.8 ± 0.4	27.5 ± 0.3	1.3 ± 0.5
Red cabbage	147.0 ± 3.6	103.3 ± 9.0	43.7 ± 5.4
Red mustard	62.2 ± 2.6	40.8 ± 1.4	21.4 ± 1.3
Red orach	45.4 ± 0.9	43.7 ± 0.9	1.7 ± 0.2
Red sorrel	56.7 ± 1.4	41.9 ± 1.9	14.9 ± 0.7
Sorrel	20.4 ± 0.5	17.9 ± 0.3	2.6 ± 0.2
Wasabi	44.8 ± 1.9	35.0 ± 2.0	9.8 ± 0.1
Coefficient of variation	12%	18%	35%

[a] Values are expressed as mean ± standard error (n=3).

2.4.4 Carotenoid Concentrations

2.4.4.1 β-Carotene

β-Carotene (provitamin A) is an important fat-soluble antioxidant and can protect cellular membranes by scavenging free radicals (Singh et al., 2006). Together with other carotenoids, the HPLC chromatogram of β-carotene standard and extraction of

cilantro microgreens was shown in **Fig. 2.3**. As shown in **Table 2.4**, the β-carotene

levels ranged from 0.6-12.1 mg/100 g FW. Among the tested microgreens, red sorrel

had the highest β-carotene concentration (12.1 mg/100 g FW), followed by cilantro,

red cabbage and peppercress (11.7, 11.5, and 11.1 mg/ 100 g FW, respectively). The

lowest β-carotene concentration was found in golden pea tendrils and popcorn shoots

(around 0.6 mg/100 g FW) with the other microgreens at intermediate values (5.2 to

8.6 mg/100 g FW). Compared with fully-developed cilantro leaves, cilantro seedlings

contained 3-fold more β-Carotene. Red cabbage microgreens contained an average of

11.5 mg/100 g FW which is approximately 260-fold more than the value (0.044

mg/100 g FW) reported for mature red cabbage leaves (Singh et al., 2006). Wasabi,

green basil, pea tendrils and garnet amaranth microgreens are also abundantly

concentrated with β-carotene. The β-carotene concentration in these microgreens is

comparable to that of carrot (*Daucus carota* L.) and sweet potato (*Ipomoea*

batatas (L.) *Lam*) which are well known β-carotene-rich vegetables (Punia et al.,

2004; USDA, 2011). In summary, all the microgreens tested can be considered

as excellent sources of β-carotene, with the exceptions of popcorn shoots and golden

pea tendrils.

2.4.4.2 Lutein/zeaxanthin

Lutein and zeaxanthin are xanthophyll carotenoids, accumulating in the macula of

human eyes. Numerous epidemiological studies have shown lutein and zeaxanthin

play a critical role in the prevention of age-related macular degeneration and cataract

Table 2.4 β-Carotene, violaxanthin and lutein/zeaxanthin concentrations in 25 commercially grown microgreens[a].

Microgreen name	β-Carotene (mg/100 g FW)	Lutein/zeaxanthin (mg/100 g FW)	Violaxanthin (mg/100 g FW)
Arugula	7.5 ± 0.4	5.4 ± 0.2	2.6 ± 0.1
Bull's blood beet	5.3 ± 0.8	4.3 ± 0.7	2.3 ± 0.1
Celery	5.6 ± 0.1	5.0 ± 0.1	2.6± 0.1
China rose radish	5.4 ± 0.5	4.9 ± 0.4	1.9 ± 0.1
Cilantro	11.7 ± 1.1	10.1 ± 0.3	7.7 ± 0.6
Garnet amaranth	8.6 ± 0.3	8.4 ± 0.1	4.4 ± 0.1
Golden pea tendrils	0.6 ± 0.0	2.7 ± 0.0	1.0 ± 0.1
Green basil	8.4 ± 0.4	6.6 ± 0.3	2.7 ± 0.2
Green daikon radish	6.1 ± 0.1	4.5 ± 0.1	1.7 ± 0.0
Magenta spinach	5.3 ± 0.3	3.2 ± 0.2	3.7 ± 0.5
Mizuna	7.6 ± 0.4	5.2 ± 0.3	2.4 ± 0.1
Opal basil	6.1 ± 0.4	5.3 ± 0.3	2.0 ± 0.0
Opal radish	6.3 ± 1.0	5.5 ± 0.9	2.3 ± 0.4
Pea tendrils	8.2 ± 1.1	7.3 ± 1.2	3.9 ± 1.4
Peppercress	11.1 ± 0.6	7.7 ± 0.4	3.1 ± 0.2
Popcorn shoots	0.6 ± 0.1	1.3 ± 0.1	0.9 ± 0.1
Purple kohlrabi	5.7 ± 0.2	4.0 ± 0.1	1.5 ± 0.0
Purple mustard	5.6 ± 0.4	6.4 ± 1.9	1.0 ± 0.2
Red beet	7.7 ± 0.1	5.5 ± 0.0	3.7 ± 0.0
Red cabbage	11.5 ± 1.2	8.6 ± 1.0	2.9 ± 0.3
Red mustard	6.5 ± 0.4	4.9 ± 0.3	1.7 ± 0.1
Red orach	6.3 ± 0.3	3.9 ± 0.2	3.2 ± 0.2
Red sorrel	12.1 ± 0.6	8.8 ± 0.2	3.6 ± 0.1
Sorrel	5.2 ± 1.0	4.2 ± 0.8	1.3 ± 0.3
Wasabi	8.5 ± 0.2	6.6 ± 0.3	2.2 ± 0.2
Coefficient of Variation	31%	18%	18%

[a] Values are expressed as mean ± standard error (n=3).

(Ma & Lin, 2010). In the analysis of lutein and zeaxanthin, these two isomers were co-eluted in HPLC system, so all the values were calculated based on the area under the curve of lutein standard and expressed in lutein equivalents but represented as the sum of lutein and zeaxanthin. While all 25 microgreens assayed in this study contained lutein and zeaxanthin (**Table 2.4**), cilantro had the highest lutein/zeaxanthin levels with 10.1 mg/100g FW. Red sorrel, red cabbage and garnet

amaranth microgreens followed with lutein/zeaxanthin concentrations of 8.8, 8.6 and 8.4 mg/100g FW, respectively. These values were higher than that of mature spinach (7.2 mg/100g FW) , which contains high quantities of lutein/zeaxanthin (Perry et al., 2009). The lowest concentration of lutein/zeaxanthin, 1.3 mg/100g FW was found in popcorn shoots. According to the USDA National Nutrient Database (2011), it was determined that the values of lutein/zeaxanthin in raw mature cilantro and red cabbage were 0.9 and 0.3 mg/100g FW, respectively, which contrasted with the more abundant concentrations in their microgreens counterparts which had 11.2 times and 28.6 times greater lutein/zeaxanthin concentrations, respectively. These findings suggest that these immature leaves of the microgreens tend to possess higher lutein/zeaxanthin concentration than their fully-grown plant counterparts (USDA, 2011).

2.4.4.3 Violaxanthin

Violaxanthin is a natural orange-colored carotenoid found in photosynthetic organs of plants. The concentrations of violaxanthin in the 25 microgreens varied considerably with cilantro microgreens containing 7.7 mg/100 g FW violaxanthin while popcorn shoots and golden pea tendrils only containing 0.9 and 1.0 mg/100 g FW violaxanthin, respectively (**Table 2.4**). The rest of the microgreens had violaxanthin ranged from 1.3 to 4.3 mg/100 g FW. The maximum concentration of violaxanthin in cilantro microgreens was more than 5-fold than that of mature cilantro leaves (1.4 mg/100 g FW) and 2.8 times than that of mature spinach (2.7 mg/100 g FW), both of which are considered as good sources of violaxanthin (Bunea et al., 2008; Kobori & Arnaya, 2008). Twenty-two out of the 25 microgreens assayed possessed violaxanthin concentration higher than mature cilantro, and 40% of them were at levels equal to or higher than commonly consumed mature-leaf spinach and baby-leaf spinach (Lester et al., 2010a).
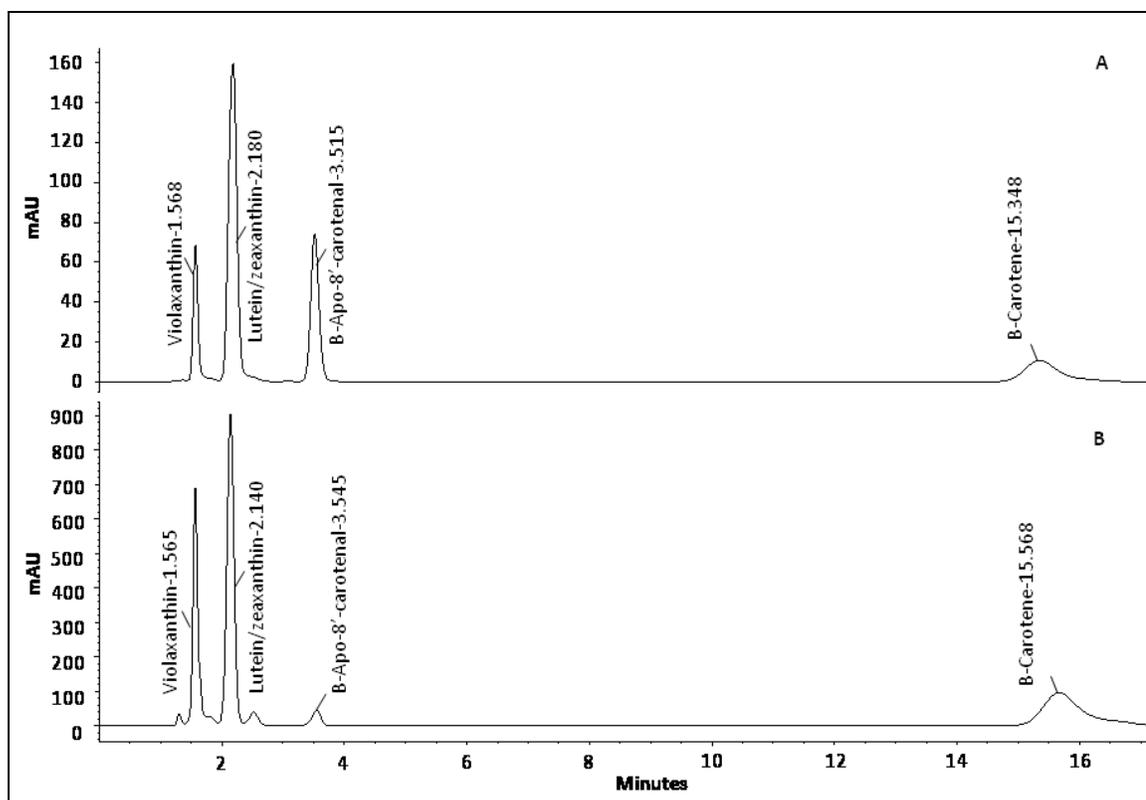
Fig. 2.3 HPLC chromatograms of carotenoid standards (A) and extraction of cilantro microgreens (B).
β-Apo-8'-carotenal is the internal standard and lutein and zeaxanthin are co-eluted. HPLC conditions are described in the materials and methods.

2.4.5 Tocopherol Concentrations

Tocopherols and tocotrienols are together summarized as "vitamin E", known as fat soluble antioxidants. Each group has four isomers (α-, β-, γ- and δ-). The most active form of all the tocopherols is α-tocopherol, while γ-tocopherol is the most abundant one in plants (Schwartz et al., 2008). In this study, α-and γ-tocopherol contents for the 25 different microgreens varieties are summarized (**Table 2.5**) and the HPLC chromatogram of tocopherol standards and extraction of green daikon radish microgreens was shown in **Fig. 2.4**. Green daikon radish has extremely high α-tocopherol and γ-tocopherol contents of 87.4 and 39.4 mg/100 g FW, respectively. In

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addition, cilantro, opal radish and peppercress microgreens are also excellent sources of α-tocopherol and γ-tocopherol, with the α-tocopherol concentrations ranging from 41.2 to 53.1 mg/100 g FW, and γ-tocopherol values from 12.5 to 16.7 mg/100 g FW. Even though the values of α-tocopherol (4.9 mg/100g FW) and γ-tocopherol (3.0 mg/100g FW) in golden pea tendrils were among the lowest of the 25 microgreens, their values were still markedly higher than those for more mature spinach leaves (2.0 and 0.2 mg/100g FW, respectively) (USDA, 2011). Red cabbage microgreens contained over 40 times the vitamin E content of that in its mature counterpart (0.06 mg/100 g FW) reported by Podsedek et al. (2006).
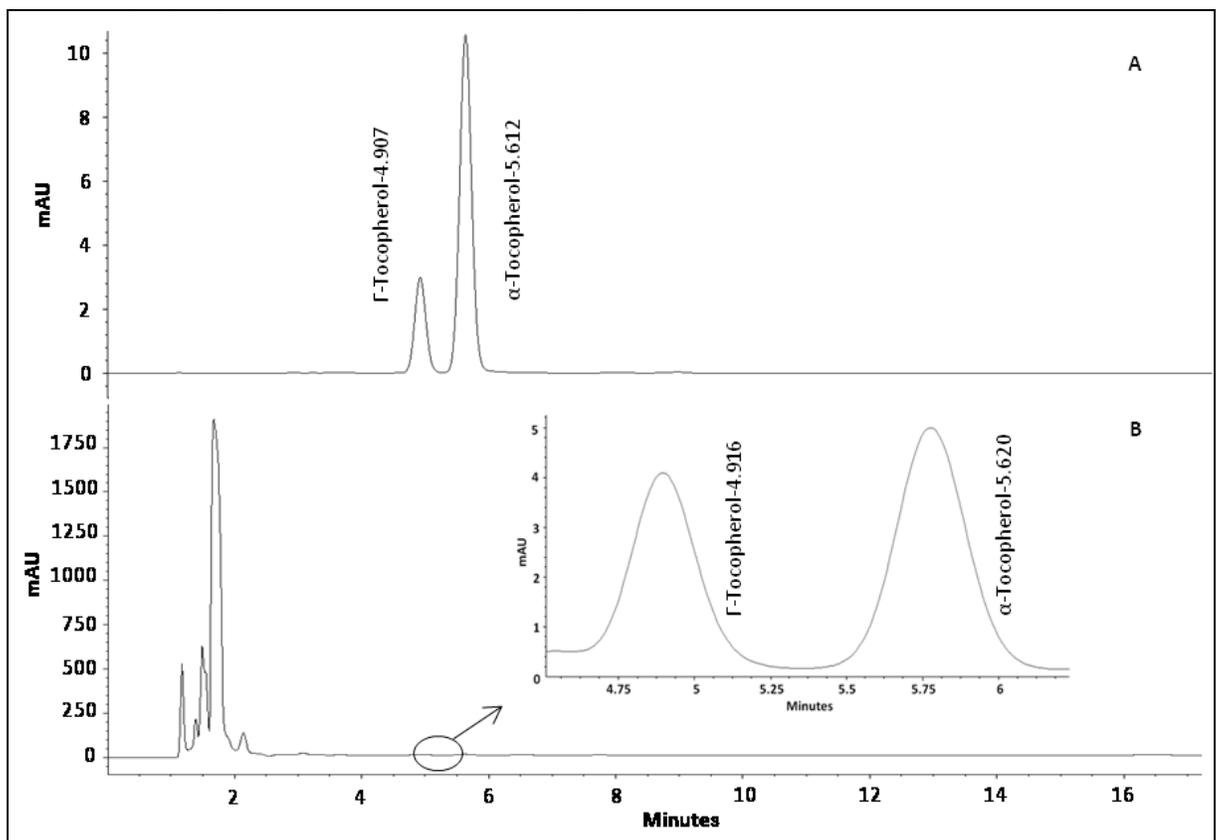
Fig. 2.4 HPLC chromatogram of tocopherol standards (A) and extraction of green daikon radish microgreens (B).

Table 2.5 α-Tocopherol and γ-tocopherol concentrations in 25 commercially grown microgreens[a].

Microgreens name	α-Tocopherol (mg/ 100 g FW)	γ-Tocopherol (mg/ 100 g FW)
Arugula	19.1 ± 4.3	7.1 ± 2.4
Bull's blood beet	18.5 ± 2.5	5.0 ± 0.7
Celery	18.7 ± 5.1	6.1 ± 1.4
China rose radish	19.7 ± 3.1	7.5 ± 1.1
Cilantro	53.0 ± 13.5	12.5 ± 2.0
Garnet amaranth	17.1 ± 2.1	11.2 ± 1.3
Golden pea tendrils	4.9 ± 0.3	3.0 ± 0.2
Green basil	19.9 ± 0.3	6.0 ± 0.4
Green daikon radish	87.4 ± 15.9	39.4 ± 7.8
Magenta spinach	14.2 ± 3.3	5.1 ± 0.8
Mizuna	25.0 ± 3.7	9.6 ± 1.4
Opal basil	24.0 ± 2.1	8.3 ± 0.8
Opal radish	47.7 ± 14.6	16.7 ± 5.3
Pea tendrils	35.0 ± 6.8	8.3 ± 2.0
Peppercress	41.2 ± 3.7	14.5 ± 1.4
Popcorn shoots	7.8 ± 0.1	3.5 ± 0.0
Purple kohlrabi	13.8 ± 1.0	5.6 ± 0.4
Purple mustard	18.6 ± 1.3	7.0 ± 0.7
Red beet	34.5 ± 2.3	8.3 ± 0.6
Red cabbage	24.1 ± 5.5	10.3 ± 3.1
Red mustard	22.1 ± 1.9	8.2 ± 0.7
Red orach	18.3 ± 2.8	7.0 ± 0.9
Red sorrel	21.8 ± 1.2	7.7 ± 0.5
Sorrel	9.3 ± 1.5	3.1 ± 0.5
Wasabi	18.7 ± 2.9	7.6 ± 1.0
Coefficient of Variation	20%	16%

[a] Values are expressed as mean ± standard error (n=3).

2.5 Conclusions

In summary, the phytonutrient contents of 25 commercially available microgreens varieties have been determined. In general, microgreens contain considerably higher concentration of phytonutrients than their mature plant counterparts, although large variations were found among these 25 species tested. Maximum values of vitamin C, vitamin K1, and vitamin E were found in red cabbage, garnet amaranth and green daikon radish microgreens, respectively. In terms of carotenoids, cilantro microgreens

showed the highest concentration of lutein/zeaxanthin and violaxanthin and ranked second in β-carotene concentration. In contrast, popcorn shoots and golden pea tendrils were relatively low in phytonutrients, although they were still comparable nutritionally to some commonly consumed mature vegetables. It is also noted that golden pea tendrils, which is grown in the absence of light, processed much lower phytonutrient concentrations than pea tendrils grown under light, suggesting that light plays an important role on nutritional values during the growth of microgreens. The data generated by this research likely provides a scientific basis for evaluating the phytonutrient concentration of microgreens cotyledon leaves. It can also be used as a possible reference in estimating the dietary intake and adequacies of vitamins from microgreens. However, since growing, harvesting, and postharvest handling conditions may have a considerable impact on the synthesis and degradation of phytonutrients, additional studies maybe needed to evaluate the effect of these agricultural practices on phytonutrient retention.

Chapter 3: Evaluation and Correlation of Sensory, Chemical and Nutritional Quality Characteristics of Microgreens

3.1 Abstract

Sensory attributes, chemical compositions and nutritional values of six varieties of microgreens representing one or more of the sensory categories: mustard, herbal, veggie, mild or peppery/radish were selected from 25 varieties of microgreens by a preliminary in-house panel. Consumer acceptance of the 6 microgreen varieties was carried out by 80 consumer panelists. Representing the 'veggie' category, Bull's blood beet (*Beta vulgaris* L.) was rated highest in acceptability of appearance, texture, flavor and overall eating quality. In contrast, peppercress (*Lepidium bonariense* L.) representing the peppery/radish category, was scored lowest in acceptability of flavor and overall eating quality. Chemical compositions and nutritional values differed among six varieties. China rose radish (*Raphanus sativus* L.) had the highest titratable acidity and total sugars, while red amaranth (*Amaranthus tricolor* L.) had the highest pH value and lowest total sugars. The highest amounts of total ascorbic acid, phylloquinone, carotenoids, tocopherols and total phenolics were found in China rose radish, opal basil (*Ocimum basilicum* L.), red amaranth, China rose radish and opal basil, respectively. The relationships between sensory-sensory and sensory-chemical attributes were further studied. Overall eating quality of microgreens was best correlated with flavor scores. The pH values and total phenolic contents were strongly correlated with flavor-related sensory attributes (such as sourness, astringency, bitterness, etc.) and overall eating quality. Overall, all the

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microgreens evaluated in this study demonstrated "good" to "excellent" consumer acceptance and nutritional profile.

3.2 Introduction

Microgreens is a new specialty food product, which has garnered more attention in US. They are tiny version of regular plants produced from the seeds of vegetables, herbs and grains. They have two fully developed cotyledonary leaves with the first pair of true leaves emerging or partially expanded. Microgreens are usually 2.5−7.6 cm (1−3 in.) in height and harvested at 7−14 days after germination, depending on the species, and sold with the stem and attached cotyledons (seed leaves). Although small in size, microgreens can provide surprisingly intense flavors, vivid colors, and crisp textures and can be served as an edible garnish or a new salad ingredient. In recent years, microgreens have become a new culinary trend, currently being served in upscale restaurants and showing up in some grocery stores such as Whole Foods and Mom's (Brentlinger, 2005). Our previous study has found that microgreens were generally packed with more phytonutrients (such as α-tocopherol, β-carotene and ascorbic acid) than their mature plants (Xiao et al., 2012), which made them even more popular.

As known, sensory evaluation is very important for food quality control and product development because it could provide direct information of product related to future salability. In general, the overall eating quality of fresh produce is related to several sensory attributes, including appearance, texture, flavor, sound and feel aspects. Among all the quality attributes, appearance is the initial quality attribute that attracts consumers to a fruit or vegetable product, and affects their choice in the first

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phase of purchase; however, other organoleptic characteristics (e.g., flavor and texture) play a crucial role in consumer satisfaction and future purchases (Barrett et al., 2010; Francis et al., 2012). As consumers desire more and more nutritious and healthy foods, nutritional values of food are often intwined in consumers' purchasing decision.

To date, no comprehensive study has been performed on the emerging food product: microgreens. Therefore, the objective of this study is 1) to assess sensory properties and consumer acceptance of microgreens; 2) to investigate the relationships between chemical compositions and sensory attributes of microgreens; 3) to identify chemical measurements that may predict overall eating quality and consumer acceptability of microgreens; and 4) to evaluate the nutritional quality of microgreens.

3.3 Materials and Methods

3.3.1 Sample Materials

All the microgreens evaluated in this study were generously donated by Fresh Origins Farm (San Diego, CA, USA). They were grown in peat moss in unheated greenhouses under ambient light and harvested without roots. Samples were then immediately packed in clamshell containers (113.4 g of each × 3 containers) and shipped overnight in a cardboard box with foam and ice packs inside. All microgreens were received as bare-root seedlings at ARS Beltsville Agricultural Research Center, Beltsville, MD, where the samples were inspected to remove any defective microgreens before being used for sensory analysis. Sub-samples of each container used for chemical analysis were immediately weighed and freeze-dried for HPLC

analysis or juiced for titratable acidity and pH analysis. The remainder of the samples was stored in 1°C for 1 d prior to sensory analyses.

3.3.2 Sensory Evaluation

3.3.2.1 Descriptive analysis

So far, there is no lexicon for sensory properties of microgreens; therefore, an appropriate sensory ballot specifically for microgreens was developed for the following sensory tests. In this study, descriptive sensory analysis was first profiled. A qualitative sensory profile of microgreens was developed by an 8-member panel in which everyone had much experience on sensory evaluation of produce. The sensory components included appearance, texture, aroma, taste and flavor of microgreens. The sensory terminology and language was generated, developed and justified by evaluating 25 commercially available microgreens in five different sessions on May 4, May 10, May 11, May 17 and June 20, respectively. The 25 varieties of microgreens were then classified into five categories by the panel, mainly based on their flavor characteristics.

3.3.2.2 In-house Preliminary Test

After the sensory ballot (**Table 3.1**) was developed and flavor categories were established, an in-house preliminary test was conducted. Based on the perceived sensory characteristics, the 25 varieties of microgreens were sorted into five categories, namely: mustard group, herbal group, veggie group, mild group and peppery/radish group, respectively. The category/group information on their commercial names and scientific names were listed in **Table 3.2**. To select the most liked microgreens in each category, an in-house preliminary sensory test using 8 staff

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members (4 males and 4 females) who like eating vegetables, but were not involved

in the microgreens sensory study.

Table 3.1 On-screen ballot for sensory attributes scored from 0 to 100 in line scale for microgreens sensory evaluation.

Sensory Attributes	Left end (Score = 0)	Right end (Score = 100)
Intensity of Aroma	None	Very strong
Intensity of Astringency (drying, roughing and puckering mouth feel)	None	Very strong
Intensity of Bitterness	None	Very strong
Intensity of Grassy (Earthy, herbal or having a flavor of grass)	None	Very strong
Intensity of Heat (peppery, spicy or pungent)	None	Very strong
Intensity of Sourness	None	Very strong
Intensity of Sweetness	None	Very strong
Intensity of Texture	Tender	Tough
Acceptability of Appearance	Bad	Excellent
Acceptability of Texture	Bad	Excellent
Acceptability of Flavor	Bad	Excellent
Acceptability of Overall eating quality	Bad	Excellent

Microgreens representing the mustard and herbal, veggie, mild, and

peppery/radish categories were received in the mornings of October 25, November 1

and November 27, 2012, respectively. The sensory evaluations were conducted

immediately in the afternoons of receiving dates. All the samples were washed using

tap water thoroughly and spun to remove excess water on the surface. The

microgreens representing each of the five categories were evaluated consecutively

and there was a 10-minute break between two sessions. All sensory evaluations were

carried out on computers by using the previously designed ballot (**Table 3.2**) derived

from Compusense ® 5.0 sensory software (Guelph, Ontario, Canada). Previous tests

had indicated that five grams of microgreens were sufficient for rating all the sensory

quality attributes; therefore, five grams of microgreens were placed into a sample

container, which was labeled by a unique 3-digit random number and served in a random order.

3.3.2.3 Consumer Acceptance Test

Selected microgreens from each the five categories were evaluated in the consumer acceptance test, which was conducted in Beltsville, MD on February 13 to February 15, 2013. They are beet bull's blood, China rose radish, Dijon mustard, opal basil, peppercress and red amaranth, respectively. Pictures of selected microgreens evaluated in this study are presented in **Fig. 3.1**.The 80 consumer panelists were comprised of volunteer Beltsville Agricultural Research Center staff and University of Maryland students, who like vegetables, eat them frequently and had no knowledge of this experiment. Acceptability of appearance, texture, flavor and overall eating quality and intensity of aroma, texture, astringency, bitterness, grassy, heat, sourness and sweetness were evaluated from 5 g of sample. In the end, the demographic (age, gender, and ethnicity) information of each panelist was asked. There were eight sessions in total and 10 panelists per session. Between sessions, all the samples were kept in refrigerator (4 °C) and maintained at room temperature for 15 minutes before serving. The sensory evaluation was conducted in the same way mentioned in **3.3.2.2.**

3.3.3 Chemical Analysis

The titratable acidity (TA) was measured by titrating 10 mL aliquot of the microgreens juice with 0.1 N NaOH to the end point of pH 8.1, monitored with a pH meter (S20 SevenEasy™, Mettler-Toledo International Inc., Columbia, MD, USA) at 21 °C. The results were expressed as percentage of citric acid. The pH measurements were performed using a digital pH meter.

Table 3.2 Commercial names and scientific names of 25 commercially available microgreens in five groups evaluated in the in-house preliminary sensory test.

Category	Commercial name	Scientific name
Mustard	Arugula	*Eruca sativa* Mill.
	Dijon mustard	*Brassica juncea* (L.) Czern.
	Mizuna	*Brassica rapa* L. ssp. *nipposinica*
	Red mustard	*Brassica juncea* (L.) Czern.
	Wasabi	*Wasabia japonica* Matsum.
Herbal	Cilantro	*Coriandrum sativum* L.
	Italian basil	*Ocimum basilicum* L.
	Opal basil	*Ocimum basilicum* L.
	Red sorrel	*Rumex acetosa* L.
	Sorrel	*Rumex acetosa* L.
Veggie	Brussel sprout	*Brassica oleracea* L. var. *gemmifera*
	Bull's blood beet	*Beta vulgaris* L.
	Celery	*Apium graveolens* L.
	Merlin beet	*Beta vulgaris* L.
	Red cabbage	*Brassica oleracea* L. var. *capitata*
Mild	Magenta orach	*Atriplex hortensis* L.
	Pak choy	*Brassica rapa* L. var. *chinensis*
	Popcorn shoots	*Zea mays* L.
	Red amaranth	*Amaranthus tricolor* L.
	Tuscan kale	*Brassica oleracea* L.
Peppery/Radish	China rose radish	*Raphanus sativus* L.
	Daikon radish	*Raphanus sativus* L.
	Peppercress	*Lepidium bonariense* L.
	Purple kohlrabi	*Brassica oleracea* L. var. *Gongylodes*
	Ruby radish	*Raphanus sativus* L.

Sugar content of microgreens was analyzed following the procedure of Stommel (Stommel, 1992) with some modifications. Sugars were extracted using ethanol: Milli-Q water (80: 20, v/v). The mixture was eluted through a C18 Sep-Pak cartridge (Water Corp., Milford, MA, USA) prior to filtering through a 0.45 μm membrane filter. Sugars were determined on HPLC using a carbohydrate analysis column (Waters Corp., Milford, MA, USA) with an isocratic mobile phase of acetonitrile: water (75:25, v/v) at the flow rate of 1 mL/min. Individual sugar was detected on a

refractometer (model 410, Waters Corp., Milford, MA, USA). Total sugar content was expressed as the sum of fructose, glucose and sucrose contents.

3.3.4 Phytonutrient Analysis

3.3.4.1 Ascorbic Acid

Total ascorbic acid (TAA) and free ascorbic acid (AA) were determined using a reverse phase high performance liquid chromatography (RP-HPLC) according to the method of Bartoli et al. (2006) with modifications. In this assay, dehydroascorbic acid (DHA), the oxidized form of AA, was reduced to AA for TAA determination. Fresh tissue (3 g) was ground in 10 mL of ice-cold 5% (w/v) meta-phosphoric acid at the speed of 15,000 rpm for 1 min in ice-water bath using a polytron homogenizer (Brinkman Instruments, Westbury, NY, USA). The mixture was centrifuged at 7000 g (Beckman J2-MI, Beckman Coulter, Inc., Irving, TX, USA) for 15 min at 4 °C, and the supernatant was filtered through Whatman #4 filter paper (Millipore Corp. Bedford, MA, USA). Ascorbic acid was detected with a photodiode array detector (DAD) (Model: G1315C) on an Agilent 1200 series HPLC system (Agilent, Santa Clara, CA, USA) at 243 nm. The extract was filtered through a 0.22 μm nylon syringe filter (Millipore, Bedford, MA, USA) and then directly injected (vol. = 10 μL) into the HPLC and run through a C18 column (Luna, 5 μm, 250 × 2.0 mm, Phenomenex, Torrance, CA, USA) with an isocratic mobile phase (100 mM phosphate buffer, pH = 3.0) flowing at the rate of 0.6 mL/min. Total AA was determined by HPLC after reducing DHA by mixing the same volume of the sample filtrate with 5 mM dithiothreitol (DTT) in 150 mM phosphate buffer (pH = 7.4 with 5 mM EDTA) for 15 min in darkness. Concentrations of TAA and AA were quantified based on peak

areas using a reduced ascorbic acid standard curve ($R^2 \geq 0.99$), and their difference was equal to the concentration of DHA.

3.3.4.2 Phylloquinone

Phylloquinone was extracted under dim light and determined by RP-HPLC, as described in **2.3.3.3**.

3.3.4.3 Carotenoids and tocopherols

Carotenoids and tocopherols were simultaneously determined using an isocratic RP-HPLC according to the procedure previously established in our laboratory, as described in **2.3.3.2**.

3.3.4.4 Total phenolic content

Total phenolic concentration (TPC) was measured using Fast Blue BB (FBBB) assay developed by Medina (2011) and modified for chlorophyll-containing tissue by Lester et al. (2013). Briefly, lyophilized microgreens sample (100 mg) was extracted with 10 mL of 80% MeOH by sonicating for 30 s. Hexane (4 mL) was then added into the extraction mixture to remove chlorophyll. After sonication for 30 s and centrifugation at 6650 x g for 5 min at 4 °C, the hexane layer was removed and discarded. The hexane wash procedure was repeated two more times. The washed methanolic extract was filtered through Whatman Grade No. 4 filter paper (Millipore Corp., Bedford, MA, USA) and then diluted with DI H_2O to the appropriate concentrations. One milliliter of diluted sample, gallic acid standard or DI H_2O blank control was added to borosilicate tubes, followed by 0.1 mL of 0.1% FBBB [4-benzoylamino-2, 5-dimethoxybenzenediazonium chloride hemi (zinc chloride) salt].

The solution was mixed for 30 s, followed by adding 0.1 mL of 5% (w/v) NaOH, and mixed and incubated for 90 min under light at room temperature. Absorbance was measured at 420 nm. The total phenolic concentration of samples was measured against the gallic acid (GA) calibration standard (concentrations of 0, 10, 50, 100, 200 250, 500 mg/L) and the results were expressed as milligram of gallic acid equivalents (GAE) per gram of dried weight sample.

3.3.5 Statistical Analysis

The sensory data of the in-house preliminary test was analyzed by PROC MIXED and overall acceptability was ranked by PROC RANK (SAS 9.2, SAS Institute, Inc., Cary, NC, USA). The distribution of sensory data in consumer test was examined using PROC UNIVARIATE and analyzed using PROC MIXED. Sources of variation were varieties (6) considered fixed and the panel sessions (8) and panelists (80) considered random. Relationships of variety preference relative to gender and age were examined using analysis of covariance (ANCOVA). Chemical composition and nutrient data were analyzed using PROC GLM for one-way analysis of variance (ANOVA). Mean comparisons were evaluated using Tukey's honestly significant difference (HSD) test with P value of 0.05. Data were also analyzed using PROC CORR to determine whether there were any correlations between different sensory quality attributes and between chemical composition and sensory quality attributes, using Pearson correlation: (*), (**), and (***) are used in the text to indicate 0.05, 0.01 and 0.001 levels of significance, respectively. Sensory data was additionally examined by Factor Analysis using PROC FACTOR to extract factors which could describe variability shared in common among sensory attributes.

3.4 Results and Discussion

3.4.1 Descriptive Analysis and Preliminary Test

A sensory ballot was developed as to describe the perceived sensory attributes of microgreens by an 8-member panel (**Table 3.1**). Twelve sensory attributes were defined and developed, including the intensity of aroma, texture, astringency, bitterness, grassy, heat, sourness and sweetness and the acceptability of appearance, texture, flavor and overall eating quality. Among them, some attributes were specifically defined: astringency was defined as drying, roughing and puckering mouth feel, grassy was defined as earthy, herbal flavor or having a flavor of grass and heat was defined as peppery, spicy or pungent. In the preliminary screening test, one or two microgreens were selected for each category/group. It is worthy note the screening test was a preliminary test and not a consumer test; therefore, it was only utilized to provide reference information to select several varieties of microgreens used for the 80-panelist consumer test. All the data was not shown in this paper.

In the mustard category, **Dijon mustard** was selected as it showed highest scores of heat flavor intensity and acceptability of flavor and second highest overall eating quality, which could best represent the mustard group. In the herbal category, **opal basil** was outstanding as it has highest intensity score of sweetness and lowest intensity scores of bitterness, astringency, heat and sourness, which contributed to the highest acceptability of flavor and overall acceptability. In the veggie category, **bull's blood beet** was highlighted due to the attractive red-greenish color. Besides, the acceptability of flavor and overall eating quality of bull's blood beet was scored

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Fig. 3.1 Pictures of six varieties of microgreens evaluated in consumer acceptance test.
A = Bull's blood beet; B = Red amaranth; C = Dijon mustard;
D = China rose radish; E = Peppercress; F = Opal basil.

highest. In the mild category, **red amaranth** stood out from the rest with highest scores of acceptability of appearance, flavor and overeall eating quality. In the peppery/radish category, **China rose radish** was selected since it presented the strongest heat/peppery flavor, which was the typical flavor of radish and chili pepper. The good visual display of green leaves and red stems of China rose radish also contribute to its good overall acceptability. Meanwhile, there was another one drawing our attention: **peppercress**, which showed the highest intensity of aroma, bitterness, astringency, sourness, sweetness and grassy and the second highest of heat. Although the resultant flavor and overall acceptability of peppercress was rated lowest, it was of great interest for us to further investigate its complex sensory quality; therefore, peppercress was also included in the consumer test. Overall, six varieties of microgreens were selected for the following consumer acceptance test, which are Dijon mustard, opal basil, bull's blood beet, red amaranth, China rose radish and peppercress, respectively.

3.4.2 Consumer Acceptance Test

The make-up of 80-member consumer panel and demographic information was shown in **Table 3.3**. For all six microgreens evaluated, sensory scores of acceptability of appearance/visual quality were excellent (scores > 70), with the exception of opal basil, which was scored as good (scores of 40 to 70) (Saftner et al., 2008), as shown in **Table 3.4**. Among all microgreens, scores were excellent for acceptability of texture during chewing, with relatively high scores of 71.3 to 85.5. Scores of acceptability of flavor were generally in the good range except peppercress,

which was also scored as the lowest in overall eating quality. Since scores of overall eating quality were good to excellent (in the range of ~40/39.7 to 76.5), all the six microgreens were considered to be of at least acceptable/good eating quality, of which bull's blood beet was scored highest and considered excellent with a score of 76.5.

Among all the six microgreens, they varied in all sensory quality scores. Bull's blood beet was scored highest and peppercress was scored lowest in overall eating quality while no significantly difference was found in other four varieties. Bull's blood beet and red amaranth, each of which are partially/entirely red/garnet in color, were scored highest and second highest in overall eating quality. Both bull's blood beet and red amaranth were scored high for acceptability of appearance, texture and flavor and for intensity of sweetness, meanwhile scored low for intensity of bitterness, astringency, heat, sourness and textural toughness. Except for the high intensity of heat, Dijon mustard has similar sensory quality characteristics to those of bull's blood beet and red amaranth, and consequently it was also scored high in overall eating quality (No. 3).

In contrast, peppercress was scored highest in the intensity of astringency, bitterness and sourness and high in the grassy, heat, sourness, and lowest in the acceptability of flavor, resulting in the lowest score of overall eating quality. Overall, higher bitterness and astringency ratings for peppercress, Dijon mustard and China rose radish microgreens (both in *Brassicaceae* family) were likely to be associated with the presence of high concentrations of glucosinolates, which are widely recognized as bitter compounds. These results suggest flavor (such as astringency,

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Table 3.3 Age, gender and ethnicity make-up of consumer panel.

	20 or less	21 to 30	31 to 40	41 to 50	51 to 60	61 or older	Total (%)	Ethnicity	(%)
			Age (years)					Black/African-American	4
Female	3	10	7	10	11	5	46 (58%)	Asian	25
Male	2	3	8	5	10	6	34 (42%)	Hispanic	4
Total	5 (6%)	13 (16%)	15 (19%)	15 (19%)	21 (26%)	11 (14%)	80 (100%)	White	66

Table 3.4 Intensity and acceptability of microgreen sensory attributes by consumer panel across age, gender and ethnicity.

Microgreens	Aroma	Astringency	Bitter	Grassy	Heat	Sour	Sweet	Texture	Texture	Flavor	Appearance	Overall Eating Quality
			Intensity of (rating 0 to 100)							Acceptability of (rating 0 to 100)		
Bull's blood beet	20.0 c	19.3 c	31.0 bc	61.0 ab	12.7 d	13.3 b	14.4 a	23.7 b	85.5 a	66.9 a	86.4 a	76.5 a
China rose radish	21.6 c	40.7 a	40.3 ab	58.2 ab	67.2 b	24.2 ab	8.6 a	40.3 a	72.2 b	52.5 bc	72.7 b	53.7 b
Dijon mustard	24.3 bc	35.5 ab	40.8 ab	50.2 b	86.2 a	24.0 ab	11.5 a	24.3 b	80.2 ab	54.9 ab	86.2 a	61.0 b
Opal basil	49.5 a	36.6 a	51.3 a	69.5 a	30.9 c	24.0 ab	8.7 a	32.6 ab	71.3 b	46.0 bc	55.9 c	49.0 bc
Peppercress	35.1 b	41.9 a	51.9 a	61.7 ab	66.0 b	29.5 a	10.4 a	29.3 ab	71.9 b	39.5 c	77.8 ab	39.7 c
Red amaranth	29.2 bc	23.1 bc	24.1 c	69.5 a	10.8 d	17.2 b	14.6 a	23.1 b	79.2 ab	59.1 ab	82.5 a	63.1 ab

bitterness, and sourness) is a very important quality attribute determining the acceptance of microgreens. The results are also consistent with previous reports that bitter taste and astringency were identified as the main reason for consumers to reject many vegetables containing phytonutrients (e.g. glucosinolates in *Brassica* vegetables), despite their known health benefits (Drewnowski & Gomez-Carneros, 2000).
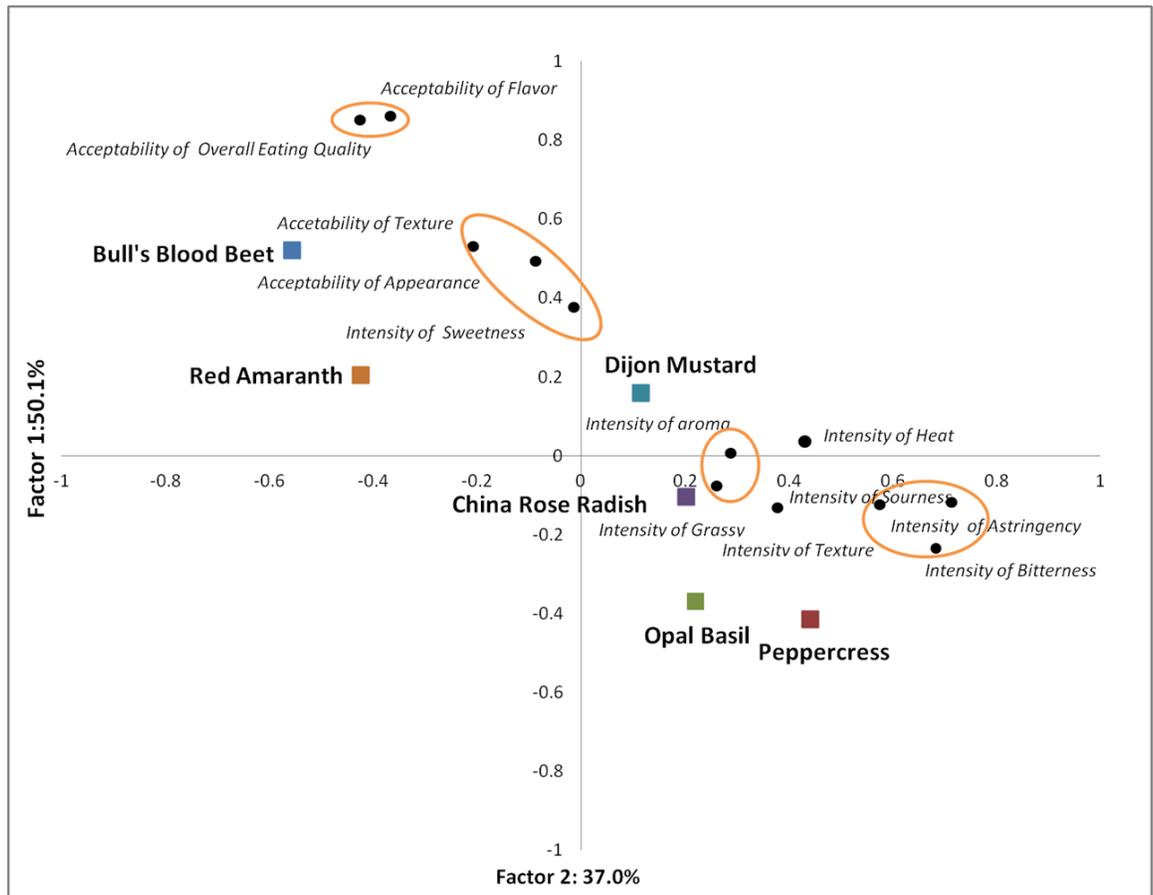
Fig. 3.2 Factor analysis of sensory data for six varieties of microgreens evaluated in consumer acceptance test.

Factor analysis was conducted on the sensory data to identify variability shared in common among the sensory attributes for all the six microgreens. Bull's blood beet and red amaranth which had generally high scores of overall eating quality, had positive scores for Factor 1 (explaining 50.1% of variation observed among the sensory descriptors), with high loading values of the acceptability of flavor, texture and appearance and the intensity of sweetness (**Fig. 3.2**). Likewise, the varieties (China rose radish, opal basil and peppercress) that had generally low scores of overall eating quality showed negative scores for Factor 1 and the variety (Dijon mustard) that had generally intermediate score of overall eating quality was scored near zero for Factor 1. Meanwhile, Factor 2 explained 37.0% of the variation observed among the sensory descriptor and the intensity of aroma, heat, grassy, sourness, astringency, bitterness and texture loaded onto this factor. Varieties (peppercress, opal basil, China rose radish and Dijon mustard) that scored generally high for the intensity of aroma, heat, grassy, sourness, astringency, bitterness and texture had positive scores for Factor 2 and varieties (red amaranth and bull's blood beet) that scored generally low in these sensory attributes had negative scores for Factor 2. To sum up, factor analysis suggested that bull's blood beet, red amaranth and Dijon mustard had higher sensory quality than China rose radish, opal basil and peppercress with bull's blood beet having the best and peppercress having the lowest overall eating quality, which was very similar to the results from other statistical analyses as described above.

In addition, we compared the intensity and acceptability sensory scores of six microgreens by gender (**Table 3.5**). Across age and ethnicity, no significant

differences were found between female and male in all the sensory attributes of six microgreens, except for the acceptability of flavor and overall eating quality of Dijon mustard. Surprisingly, female and male showed significantly different ($P = 0.001$) perception on Dijon mustard microgreens, with female demonstrating much lower acceptability scores of flavor and therefore overall eating quality.

3.4.3 Relationships between Sensory Attributes

In order to more accurately assess the impact of each sensory quality characteristic on overall eating quality, the relationships among sensory attributes were investigated. For all microgreens, overall eating quality was most strongly correlated with acceptability of flavor (r = 0.98***). Overall eating quality was also strongly correlated with scores of acceptability of texture (r = 0.82***) and intensity of sourness (r = -0.87***), bitterness (r = -0.71***), astringency (r = -0.66**), sweetness (r = 0.61**). Additionally, eating quality scores were weakly correlated with score of acceptability of appearance (r = 0.49*) and intensity of aroma (r = -0.53*). Acceptability of flavor was correlated with the intensity of some flavor-related characteristics: sourness (r = -0.84***), bitterness (r = -0.68**), astringency (r = -0.57*) and sweetness (r = 0.53*). These results suggest that flavor-related characteristics best predict consumer preferences for overall eating quality, though textural and visual quality characteristics also contribute. Among these flavor-related characteristics, there were some inherent correlations. For example, intensity of sourness was strongly correlated with intensity of astringency (r = 0.81***), bitterness (r = 0.80***), and heat (r = 0.72***) and weakly correlated with intensity of sweetness (r = -0.53*). Intensity of astringency was strongly correlated with

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intensity of bitterness (r = 0.77***) and heat (r = 0.70***). Intensity of bitterness was correlated with intensity of sweetness (r = -0.67 **), which confirmed that sweetness and bitterness are mutually suppressed in mixtures. All of these correlations showed that flavor is a gestalt perception, which is very important to overall sensory acceptance.

3.4.4 Chemical Composition

In general, TA and total sugar content are used to describe flavor of fruits and vegetables (Francis et al., 2012). TA and sugar contents of six microgreens were presented in **Table 3.6**. TA is related to the concentration of organic acids present in a food, and it is commonly used as quality parameter. The TA of fresh microgreens, expressed in grams of citric acid per 100 g of fresh microgreens, was between 0.09 and 0.019 g citric acid/100 g (Fig. 4). China rose radish and peppercress microgreens showed the highest TA values and bull's blood beet microgreen was ranked the lowest. Surprisingly, the TA data set perfectly matched with the sensory high and low scores of sourness intensity in the same order, which suggests that the difference in TA among varieties is large enough to impact sensory perception, especially for sourness. Opposite to the TA, the lowest pH value was found in China rose radish microgreen, and the highest ones were in bull's blood beet and red amaranth microgreens.

Overall, total sugar contents were low and generally presented at about 1.0 g/100g or less, with glucose (0.08–0.56 g/100g FW) and fructose (0.02–0.06 g/100g FW) being the two major sugars in all microgreens. There was no report on sugar contents of microgreens to date. Wills and coworkers reported the sugar content of bean

Table 3.5 Intensity and acceptability of microgreen sensory attributes by female and male consumer panelists across age and ethnicity [a].

Microgreens	Intensity of (rating 0 to 100)								Acceptability of (rating 0 to 100)			
	Aroma	Astringency	Bitter	Grassy	Heat	Sour	Sweet	Texture	Texture	Flavor	Appearance	Overall Eating Quality
Female												
Bull's blood beet	17.9 b	17.6 c	29.9 cd	62.5 ab	12.3 ef	10.8 c	11.0 ac	20.8 bc	86.4 a	68.4 a	87.6 a	79.2 a
China rose radish	20.1 b	44.4 a	41.4 ad	61.9 ab	68.0 bc	28.7 ab	7.1 ac	65.1 ab	71.4 bc	48.2 bd	72.3 bc	49.5 cd
Dijon mustard	25.8 b	39.8 ab	46.6 ac	55.7 bc	88.1 a	28.6 ab	8.6 ac	22.3 bc	79.2 ac	43.8 bd	88.3 a	50.0 cd
Opal basil	47.8 a	38.9 ab	51.3 ab	74.2 a	33.8 d	26.1 ab	5.2 c	30.8 ac	69.2 c	40.9 cd	56.1 c	41.8 d
Peppercress	33.6 ab	45.0 a	49.5 ab	62.9 ab	70.7 bc	31.7 a	6.5 bc	26.9 bc	71.2 ac	39.6 d	77.4 ab	38.4 d
Red amaranth	26.2 b	22.0 bc	25.1 d	63.0 ab	10.6 f	20.3 ac	15.1 a	18.6 c	78.6 ac	57.6 ac	81.7 ab	61.5 ac
Male												
Bull's blood beet	22.1 b	21.5 bc	32.9 bd	58.8 ac	13.2 ef	16.8 ac	17.8 ab	27.6 bc	84.2 ab	64.8 ab	84.8 a	72.9 ab
China rose radish	23.7 b	35.8 ab	38.9 ad	53.3 bc	66.1 bc	18.0 ac	10.2 ac	47.3 a	73.3 ac	58.3 ad	73.2 bc	59.3 ad
Dijon mustard	22.3 b	29.6 ac	32.9 bd	42.7 c	83.6 ab	17.7 ac	14.4 ac	26.9 bc	81.5 ac	68.5 a	83.4 ab	72.1 ab
Opal basil	51.9 a	33.6 ac	51.2 ab	62.9 ab	27.0 de	21.2 ac	12.2 ac	35.1 ab	74.2 ac	52.8 ad	55.6 c	56.2 bd
Peppercress	37.2 ab	37.8 ab	55.0 a	60.0 ac	59.5 c	26.5 ac	14.4 ac	32.5 ac	72.9 ac	39.2 cd	78.3 ab	41.3 d
Red amaranth	33.2 ab	24.6 bc	22.9 d	71.6 ab	11.1 ef	12.9 bc	15.2 ac	29.1 ac	79.8 ac	61.2 ab	83.6 ab	64.7 ac
Female vs. Male												
Bull's blood beet	N.S.[b]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
China rose radish	"	"	"	"	"	"	"	"	"	"	"	"
Dijon mustard	"	"	"	"	"	"	"	"	"	0.001	"	0.001
Opal basil	"	"	"	"	"	"	"	"	"	N.S	"	N.S
Peppercress	"	"	"	"	"	"	"	"	"	"	"	"
Red amaranth	"	"	"	"	"	"	"	"	"	"	"	"

[a] Values are expressed as mean ± standard error (n = 3). Values within the same column followed by the same letter are not significantly different ($P < 0.05$). [b] N.S. = Non-significant ($P < 0.05$).

Table 3.6 Analysis of titratable acidity (TA), pH, fructose, glucose, sucrose and total sugar content of six varieties of microgreens evaluated in consumer acceptance test [a].

Microgreens	TA (% citric acid)	pH	Fructose (g/100 g FW)	Glucose (g/100 g FW)	Sucrose (g/100 g FW)	Total sugar (g/100 g FW)
Bull's blood beet	0.09 ± 0.00 d	6.37 ± 0.13 a	0.33 ± 0.02 b	0.08 ± 0.01 cd	0.03 ± 0.00 abc	0.44 ± 0.03 ab
China rose radish	0.19 ± 0.00 a	5.67 ± 0.01 b	0.50 ± 0.07 ab	0.47 ± 0.08 a	0.06 ± 0.01 a	1.03 ± 0.17 a
Dijon mustard	0.16 ± 0.00 b	5.77 ± 0.07 b	0.40 ± 0.03 ab	0.35 ± 0.03 ab	0.02 ± 0.00 b	0.77 ± 0.06 ab
Opal basil	0.16 ± 0.00 b	5.82 ± 0.08 b	0.08 ± 0.01 c	0.08 ± 0.01 cd	0.04 ± 0.01 abc	0.20 ± 0.02 c
Peppercress	0.19 ± 0.00 a	5.86 ± 0.09 b	0.56 ± 0.06 a	0.26 ± 0.04 bc	0.06 ± 0.01 ab	0.88 ± 0.11 a
Red amaranth	0.13 ± 0.00 c	6.43 ± 0.03 a	0.13 ± 0.01 c	0.02 ± 0.00 d	0.02 ± 0.01 c	0.17 ± 0.00 c

[a] Values are expressed as mean ± standard error (n = 3). Values within the same column followed by the same letter are not significantly different ($P < 0.05$).

sprouts at the same level of microgreens analyzed in our study (Wills et al., 1984). In this study, high total sugar content did not result in particularly high scores of sweet taste. China rose radish microgreens had the highest contents of total sugars, glucose and sucrose, whereas it had the lowest score of sweetness intensity. Conversely, while red amaranth microgreen had the lowest contents of total sugar, fructose, glucose and sucrose, it was evaluated having the highest score of sweetness intensity. It was reported that sweetness perception can be modified by acid levels and aroma compounds (Tieman et al., 2012). The lowest score of sweetness in China rose radish microgreens could be associated with its high content of acids, which offset the perception of sweet taste. Correspondingly, red amaranth microgreens did show a relatively low TA value, probably making the sweetness standing out of other flavors.

3.4.5 Phytochemical Concentrations

The results of phytochemical analyses on ascorbic acid, phylloquinone, carotenoids, tocopherols and total phenolics of six microgreens were showed in **Table 3.7** and **Table 3.8**. The TAA concentration in six microgreens ranged from 10.6 to 68.0 mg/100g FW. Among the six samples, China rose radish microgreens had the highest TAA concentration, followed by Dijon mustard, peppercress and red amaranth. Bull's blood beet and opal basil microgreens contained the relatively low level of TAA. The phylloquinone concentration of all the six microgreens evaluated was about the same level, ranging between 2.1 and 4.0 μg/ g FW. For carotenoids, red amaranth had the highest amounts of β-carotene, lutein/zeaxanthin and violaxanthin, followed by Dijon mustard, China rose radish and peppercress. All the carotenoid data was consistent with those of our previous study (Xiao et al., 2012).Total

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Table 3.7 Analysis of water content, ascorbic acid (AA), dehydroascorbic acid (DHA), total ascorbic acid (TAA), phylloquinone (Vk1) and total phenolics (TPC) of six varieties of microgreens evaluated in consumer acceptance test [a].

Microgreens	Water content (%)	Ascorbates (mg/100g FW)			Phylloquinone (ug/g FW)	Total phenolics (mg GAE/100g FW)
		AA	DHA	TAA	Vk1	TPC
Bull's blood beet	95.1 ± 0.2 a	7.6 ± 0.2 a	5.6 ± 1.9 c	13.2 ± 1.9 c	2.1 ± 0.4 a	303.0 ± 9.8 c
China rose radish	92.1 ± 0.5 c	16.6 ± 10.2 a	51.4 ± 10.5 a	68.0 ± 3.6 a	3.2 ± 0.6 a	465.5 ± 15.9 b
Dijon mustard	94.3 ± 0.0 ab	22.6 ± 10.9 a	36.3 ± 10.8 abc	58.9 ± 0.8 a	3.2 ± 0.7 a	149.5 ± 3.9 d
Opal basil	94.3 ± 0.2 ab	2.0 ± 0.0 a	8.6 ± 0.5 bc	10.6 ± 0.5 c	4.0 ± 0.7 a	700.4 ± 9.7 a
Peppercress	93.8 ± 0.1 b	8.1 ± 2.9 a	37.9 ± 3.3 ab	46.0 ± 2.1 b	2.9 ± 0.7 a	274.7 ± 20.1 c
Red amaranth	93.5 ±0.2 b	12.6 ± 2.4 a	23.2 ± 3.4 abc	35.8 ± 2.7 b	2.3 ± 0.4 a	256.5 ± 12.0 c

[a] Values are expressed as mean ± standard error (n = 3). Values within the same column followed by the same letter are not significantly different ($P < 0.05$).

Table 3.8 Analysis of β-carotene (β-C), lutein/zeaxanthin (L/Z), violaxanthin (VX), α-tocopherol (α-T), γ-tocopherol (γ-T) of six varieties of microgreens evaluated in consumer acceptance test [a].

Microgreens	Carotenoids (mg/100g FW)			Tocopherols (mg/100g FW)	
	β-C	L/Z	VX	α-T	γ-T
Bull's blood beet	3.8 ± 0.3 d	3.5 ± 0.2 d	2.0 ± 0.0 bc	0.0 ± 0.0 c	0.1 ± 0.0 d
China rose radish	5.8 ± 0.3 b	5.1 ± 0.3 b	1.7 ± 0.2 bc	2.0 ± 0.2 a	0.5 ± 0.0 a
Dijon mustard	6.1 ± 0.1 b	4.7 ± 0.2 bc	2.3 ± 0.1 b	1.1 ± 0.0 b	0.3 ± 0.0 b
Opal basil	4.3 ± 0.2 cd	3.9 ± 0.2 cd	1.2 ± 0.1 c	0.0 ± 0.0 c	0.2 ± 0.0 cd
Peppercress	5.5 ± 0.1 bc	4.6 ± 0.1 bc	1.9 ± 0.0 bc	0.0 ± 0.0 c	0.3 ± 0.0 b
Red amaranth	8.1 ± 0.5 a	6.7 ± 0.3 a	4.2 ± 0.4 a	0.0 ± 0.0 c	0.2 ± 0.0 c

[a] Values are expressed as mean ± standard error (n = 3). Values within the same column followed by the same letter are not significantly different ($P < 0.05$).

phenolic content (TPC) varied significantly among varieties from 149.5 to 700.4 mg GAE/100 g FW in the six microgreens. The highest TPC was found in opal basil, which was almost 5-fold higher than Dijon mustard, which surprisingly still contained slightly higher TPC than some commonly consumed vegetables, e.g. broccoli (35-100 mg GAE/100 g) (Turkmen et al., 2005; Zhang & Hamauzu, 2004). The α-tocopherol was not present in all the six microgreens. Only China rose radish and Dijon mustard microgreens contained certain amounts of α-tocopherol, with the number of 2.0 mg/100 g FW and 1.1 mg/100 g FW, respectively. The γ-tocopherol concentrations were ranging from 0.1 to 0.5 mg/100 g FW. These results on tocopherols were not in accordance with our previous report, in which all the 25 microgreens were rich in tocopherols. Nutritional profile of produce could be affected by many preharvest and postharvest factors, such as seed source, growth location, growth environments, storage time and duration. With respect to these phytonutrients, all the six microgreens analyzed in our study could be considered to be good sources of phytonutrients and antioxidants.

3.4.6 Relationships between Sensory and Chemical Attributes

The sensory evaluation provides information about the attributes of a product from consumers' perspectives. However, it is usually difficult to recruit enough consumers or trained panelists to get involved in sensory evaluations before a new food product is marketed. Therefore, it is significant to develop a relationship between the chemical composition of a product and its sensory attributes, as well as between sensory perceptions and acceptability for consumers (Escribano et al., 2010).

In our study, the TA values of all the six microgreens were not significantly correlated with the intensity of sourness or any other sensory quality characteristics. Not as expected, a negative correlation between TA and pH was not found in this study, nevertheless, the tendency of TA and pH were almost in the opposite way. Whilst, the pH values have correlations with many sensory attributes. It has a significant correlation with acceptability of overall eating quality ($r = -0.70***$) and flavor ($r = -0.67**$), intensity of bitterness ($r = 0.77***$), sourness ($r = 0.60**$) and astringency ($r = 0.52*$), and negative correlation with sweetness ($r = -0.50*$), suggesting that the pH value may be a good indicator for flavor and overall eating quality. Many other researchers previously reported that astringency elicited by acids was a function of pH and not concentration or anion species while sourness was influenced by concentration, pH and anion species (Goldman et al., 1999; Lawless et al., 1996; Sowalsky & Noble, 1988). This could explain why astringency was correlated with pH ($r = 0.52*$), not TA, while sourness was correlated with pH ($r = 0.60**$) and not TA ($r = 0.42$, not significant).

Total sugar content was not correlated with the sensory scores of intensity of sweetness but weakly and negatively correlated with intensity of bitterness ($r = -0.45$, not significant). Sucrose content was negatively correlated with intensity of bitterness ($r = -0.62**$) and sourness ($r = -0.48*$). Similarly, glucose was also negatively correlated with bitterness ($r = -0.62**$). Sugars were not alone in accounting for variation in the sweetness of fruits and vegetables, especially when the amount of sugar was low. The perception of sweetness may be affected by other acidic or bitter compounds present in fruits and vegetables (Keast & Breslin, 2003). Taking China

rose radish as an example, the total sugar content of China rose radish was highest; however, its intensity of sweetness score was the lowest. The difference between sensory perception and chemical components could lie in the acid contents (Abbott et al., 2004; Baldwin et al., 1998). The high acidity, shown as highest TA value and lowest pH value, could make China rose radish taste less sweet. Similarly, red amaranth that had the lowest total sugar content was scored highest for intensity of sweetness, which was probably due to the low acidity, making red amaranth perceived as much sweeter.

The TPC value was strongly correlated with overall eating quality (r = 0.66***) and sensory scores of flavor-related attributes: intensity of astringency (r = -0.73***), heat (r = -0.84***), sourness (r = -0.80***), bitterness (r = -0.60**) and sweetness (r = 0.58**) and acceptability of flavor (r = 0.58*). It is well known that food astringency is due to the presence of phenolic compounds, like procyanidins in many fruits. In addition, phenolic compounds also contribute to the bitter taste (e.g. flavanon neohesperidosides in citrus fruits) and pungent taste (e.g. capsaicins in chili peppers) (Tomás-Barberán & Espin, 2001). Based on the results obtained in current study, the TPC value may be considered as an informative parameter in evaluating the overall eating quality and flavor-related sensory attributes of microgreens. Therefore, it is of significance to measure TPC value and appropriately interpret it into sensory information.

In addition, some phytonutrient data also had relationship with the sensory attributes. Violaxanthin has strong correlations with intensity of sourness (r = 0.77***) and bitterness (r = 0.60**), acceptability of flavor (r = -0.72***) and overall

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eating quality (r = -0.7***). Both α-tocopherol and γ-tocopherol had correlations with

bitterness (r = -0.64** and r = -0.61**, respectively). However, future work is

needed to further specify the inherent mechanism for those relationships.

3.5 Conclusions

In conclusion, six selected microgreens evaluated in our study varied in sensory

quality characteristics with consumer panel ranking bull's blood beet with the highest

preference and peppercress with the lowest acceptance. Among all the sensory quality

attributes tested, flavor quality attribute best predicted overall eating quality of

microgreens. Visual and textural quality attributes also affected consumer acceptance.

The pH and TPC values could be used as indicators to provide sensory information

and predict consumer acceptability. Overall, microgreens with good consumer

acceptability have a good nutritional profile and can provide health benefits to

consumers.

Chapter 4: Postharvest Quality and Shelf Life of Radish Microgreens as Impacted by Storage Temperature, Packaging Film, and Chlorine Wash Treatment

4.1 Abstract

Microgreens are new and emerging products, which are young seedlings of vegetables and herbs. Our previous study showed that microgreens contain higher nutrients compared to their mature counterparts. However, they typically have a short shelf life (1-2 days) at ambient temperature. The objective of this study was to optimize postharvest handling conditions to reduce the quality loss and extend the shelf life of daikon radish microgreens. Storage temperature, packaging film, and wash treatment were investigated. Changes in headspace composition, quality index, chlorophyll concentration, tissue electrolyte leakage, and aerobic mesophilic bacteria (AMB) and yeast & mold (Y&M) counts were monitored periodically during storage. Results indicated that 1) storage temperature significantly ($P < 0.05$) affected package atmosphere, product quality and shelf life. One degree Celsius was the optimal temperature for storage of radish microgreens with no chilling injury observed; 2) film oxygen transmission rate (OTR) significantly ($P < 0.05$) affected O_2 and CO_2 composition, but OTR did not significantly affect quality attributes during 28 days of storage at 1°C; 3) Chlorine wash treatment (100 mg/L) significantly reduced initial

microbial populations by 0.5 log cfu/g, including AMB and Y& M. However, microbial populations rebounded after day 7.

4.2 Introduction

Microgreens have gained popularity as a new culinary trend appearing in upscale markets and restaurants over the past few years. They are tender cotyledonary-leaf plants having vivid colors, intense flavors and tender textures; therefore, they are usually served fresh as ingredients in salad, soups and sandwiches or used as an edible garnish (Treadwell et al., 2010). In a recent study, we found that microgreens generally contain higher concentrations of phytonutrients (such as α-tocopherol, β-carotene and ascorbic acid) than their mature-leaf counterparts (Xiao et al., 2012). However, microgreens are delicate and have a very short shelf life (1-2 days) at ambient temperature; and as such are categorized to be highly perishable products (Chandra et al., 2012).

Storage temperature is one of the most important factors affecting the postharvest physiology and storage behavior of produce. In general, low temperature storage can reduce quality loss and extend shelf life by depressing rates of respiration, senescence, and growth of spoilage microorganisms (Manolopoulou et al., 2010; Spinardi & Ferrante, 2012). Optimum storage temperature varies depending on the fruit or vegetable. For some chilling sensitive fruits and vegetables, the use of low temperature storage adversely affects quality attributes and causes deterioration more rapidly (Galvez et al., 2010; Paull, 1999). Thus, the selection of optimum storage temperature is crucial.

Modified atmosphere packaging (MAP) is an effective technology for maintaining freshness and prolonging shelf life of produce, which has been successfully applied in fresh and minimally processed produce, such as lettuce (*Lactuca sativa L.*), broccoli (*Brassica oleracea L. cv. Acadi*), spinach (*Spinacia oleracea L.*) and mushrooms (*Agaricus bisporus cv. U3 Sylvan 381*) (Sandhya, 2010). There are many factors influencing package atmosphere of products, including product respiration rate, packaging film oxygen transmission rate (OTR), product weight, package surface area, storage temperature and relative humidity (Sandhya, 2010). In food supply chains, package size and product weight are often pre-determined. Selecting a packaging film with suitable OTR to match the product respiration rate is the best way to maintain quality and extend shelf life of produce.

Consumer demand for fresh, convenient and nutritional foods have spurred a recent rapid growth of the minimally processed fruit and vegetable (Kobori et al., 2011). In the fresh-cut processing chain, chlorine-based solutions are very potent and efficient sanitizers and have been widely used in the fresh-cut industry in the USA. However, the use of chlorinated sanitizers is banned in some European countries due to the potential risk of undesirable disinfection by-products (DBPs) upon reaction with organic matters, such as chloroform ($CHCl_3$), haloacetic acids or other trihalomethanes (THMs) (Artes et al., 2009). In recent years, some alternatives have been proposed, e.g. irradiation, ozone, electrolyzed water, essential oils, and organic acids. However, none of them have gained widespread acceptance by the industry (Rico et al., 2007).

Currently, there is no ready-to-eat microgreens are commercially available in the food supply chains due to their perishability and high price. Green daikon radish (*Raphanus sativus L.var. longipinnatus*) is one of the most commonly-grown commercial microgreens. It has an extraordinarily high concentration of α-tocopherol (87.4 mg/100 g FW) (Xiao et al., 2012), which is an important lipid-soluble antioxidant and can protect cell membranes from oxidative stress. Moreover, the potent spicy flavor, bright green color and tender texture of daikon radish microgreens are also favorable. However, little information is available on optimal storage temperature, packaging film and wash treatment configuration of green daikon radish microgreens. Therefore, the objectives of this study were 1) to optimize storage temperature; 2) to evaluate the effect of packaging film OTR under optimum storage temperature; and 3) to investigate the effect of chlorine wash treatment under optimal storage temperature and packaging film OTR on maintaining quality and prolonging shelf life of green daikon radish microgreens.

4.3 Materials and Methods

4.3.1 Sample Preparation

Green daikon radish (*Raphanus sativus var. longipinnatus*) seeds were purchased from Living Whole Foods, Inc. (Springville, UT, USA). Seeds were sown in 28 cm W × 54 cm L × 6 cm D culture trays (Vacuum-Formed Standard 1020 Open Flats without holes, Growers Supply, Dyersville, IA, USA). The media was Fafard 3B potting soil consisting of 45% peat moss, 15% vermiculite, 15% perlite and 25% bark (Griffin Greenhouse & Nursery Supplies, Bridgeton, NJ, USA). Seeds were grown in a temperature-controlled (25°C) growth chamber. During the first three days, the

trays were covered and seeds were germinated in the dark. For the next 4 days, the

seedlings were exposed to light irradiance (42 µmol s^{-1} m^{-2}, determined by LI-1000

datalogger, LI-COR, Lincoln, NB, USA) for a 12-hr photoperiod. Seven-day-old

radish microgreens were harvested by cutting stem ends with scissors sterilized with

75 mL/100 mL alcohol. After harvest, radish microgreens were inspected prior to any

treatment and plants with defects were discarded.

4.3.1.1. Temperature Treatments

Fifteen grams of radish microgreens were packaged in polyethylene bags (15 cm

× 15 cm, Pacific Southwest Container Inc., Modesto, CA, USA) with film oxygen

transmission rate (OTR) of 16.6 pmol s^{-1} m^{-2} Pa^{-1}. All the bags were sealed and stored

at 1, 5, or 10°C cold rooms under dark for 14 days. Evaluations were performed on

day 0, 3, 7, 10 and 14. All treatments were conducted in four replicates.

4.3.1.2. Packaging Treatments

Radish microgreens (15 g) were packaged in 15 cm × 15 cm bags prepared from

polyethylene films with OTRs of 8.0, 11.6, 16.6, 21.4, or 29.5 pmol s^{-1} m^{-2} Pa^{-1}. The

permeability of the films was tested by the manufacturer (Pacific Southwest

Container Inc., Modesto, CA, USA) under conditions of 23°C and 101.3 kPa using a

MOCON apparatus according to ASTM F2714-08 and ASTM F2622-08 standards.

Four replicates of each treatment were prepared for each evaluation day (day 0, 7, 14,

21 and 28). All samples were stored at 1°C in a dark room for subsequent evaluation.

4.3.1.3. Wash Treatments

The sodium hypochlorite (NaOCl) wash solutions (50, or 100 mg/L free chlorine,

pH 6.5) were prepared using Clorox$^®$ (6 mL/100 mL sodium hypochlorite, Clorox

Co., Oakland, CA, USA) and the pH was adjusted with citric acid solution. All the free chlorine levels before treatments were measured with a chlorine photometer (CP-15, HF Scientific Inc., Fort Myers, FL, USA). Radish microgreen samples (350-400 g) were washed in pre-disinfected mesh bags with gentle agitation in 40 L wash solutions at 20°C for 1 min, followed by rinsing with 20°C tap water for 1 min. Washed samples were then centrifuged at 300 rpm for 3 min with a commercial T-304 salad centrifugal dryer (Garroute Spin Dryer, Meyer Machine Co., San Antonio, TX, USA) to remove excess surface water. Unwashed samples were used as controls. Portions (15 g) of washed and unwashed radish microgreens were placed into polyethylene bags (15 cm × 15 cm) with OTR of 29.5 pmol s^{-1} m^{-2} Pa^{-1} and stored at 1°C for 28 days in the dark. Four bags were randomly selected on each sampling day (day 0, 7, 14, 21 and 28) for quality evaluations.

4.3.2 Headspace Gas Composition

The O_2 and CO_2 contents in the headspace of packages were analyzed using an O_2/CO_2 gas analyzer (CheckMate II, PBI-Dansensor A/S, Ringsted, Denmark) by inserting the needle of the measuring assembly through a septum adhered to the packaging film.

4.3.3 Quality Index

4.3.3.1 Chlorophyll Analysis

Total chlorophyll content was determined spectrophotometrically using the method of Auderset et al. (1986) with minor modifications. Excised radish cotyledonary leaves (1.0 g) were transferred into 50-mL centrifuge tubes. After homogenization in 10 mL 80 mL/100 mL acetone (HPLC-UV grade, Pharmco-Aaper,

Brookfield, CT, USA) solution at the speed of 17, 500 rpm for 30 s (Adaptable homogenizer, VDI 25, VWR International, West Chester, PA, USA), the mixture was filtered (Grade 413 Filter Paper, Qualitative, VWR International, West Chester, PA, USA) into a 25 mL amber volumetric flask and rinsed with 80 mL/100 mL acetone solution until filter cake became colorless. The filtrate was diluted with 80 mL/100 mL acetone solution to 25 mL and stored at -20 °C until ready to measure. Absorbance was read at 646, 663, and 710 nm (UV-1700 Spectrophotometer, Shimadzu, Kyoto, Japan) and total chlorophyll was calculated by the following formula:

Total chlorophyll (µg/g FW) =

$[(A_{646} - A_{710}) * 0.01732 + (A_{663} - A_{710}) * 0.00718] *$ dilution volume (mL) * 1000/ fresh weight (g)

4.3.3.2 Electrolyte Leakage Analysis

Tissue electrolyte leakage was measured following a modified procedure from Allende et al. (Allende et al., 2004). Radish microgreens (5 g) were submerged in 150 mL deionized water at 20 °C and shaken for 30 min. The electrolyte of the solution was measured using a Model 135A Thermo Orion conductivity meter (Beverly City, MA, USA). Total electrolytes were obtained after freezing the samples at -20 °C for 24 h and subsequent thawing. Tissue electrolyte leakage was expressed as a percentage of the total electrolyte.

4.3.3.3 Overall Quality and Off-odor Evaluation

Overall visual quality and off-odor were evaluated following the procedure of Luo et al. (2004) and Meilgaard et al. (1991). Briefly, the visual quality was evaluated

on a 9-point hedonic scale, where 9, 8, 7 and 6 = like extremely, strongly, moderately and slightly, respectively, 5 = neither like nor dislike and 1, 2, 3 and 4 = dislike extremely, strongly, moderately and slightly, respectively. A score of 6 was considered the limit of salability (Kim et al., 2004). Off-odor score was based on a 0 to 4 scale where 0 = no off-odor, 1 = slight off-odor, 2 = moderate off-odor, 3 = strong off-odor, and 4 = extremely strong off-odor. All visual quality and off-odor evaluation were carried out by three trained evaluators (1 male and 2 female, aged 28 and 43 years old). The evaluators have had over five-year of research experience with fresh produce, especially performing sensory evaluation of leafy greens. Prior to the start of this experiment, additional trainings specific to the organoleptic properties of radish microgreens were provided to the evaluators.

4.3.3.4 Microbial Enumeration

Microbial growth on radish microgreens was assayed following a procedure from Luo et al. (2004) and Allende et al. (2004b) with some modifications. Samples of 3 g radish microgreens were macerated with 27 mL sterile phosphate buffered saline (PBS, 10 × solution, Fisher Scientific, Pittsburgh, PA, USA) with a stomacher blender (Model 80, Seward Medical, London, UK) for 2 min at high speed. A 50 μL sample of each filtrate or its appropriate dilution was logarithmically spread on agar plates with a Whitley automatic spiral plater (Wasp II, Don Whitley Scientific Ltd., West Yorkshire, UK). The aerobic mesophilic bacteria (AMB) population was determined by plating samples on tryptic soy agar (TSA, Difco, Detroit, MI, USA) and incubating at 28 °C for 24 h. Yeast and mold (Y&M) enumeration was performed by culturing with potato dextrose agar (PDA, Difco, Detroit, MI, USA) supplemented

with 200 mg L^{-1} chlorophenicol (Sigma-Aldrich, St Louis, MO, USA) and incubated at room temperature (22 °C) for 44-48 h. Microbial colony counting was conducted with a ProtoCOL automated colony counter (Synbiosis, Cambridge, UK) and reported as log CFU/g of tissue.

4.3.4. Statistical Analysis

Four replicates from each treatment were evaluated on each sampling day. Data was analyzed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance (ANOVA). To determine the statistical significance of the data, Tukey's honestly significant difference (HSD) test was conducted for post-hoc multiple comparisons at a significance level of 0.05. All the data was reported as the mean of 4 replicates ± standard error (SE).

4.4 Results and Discussion

4.4.1 Effect of Temperatures on Quality and Shelf Life

The changes in headspace atmospheres of packaged radish microgreens were significantly ($P < 0.05$) affected by storage temperature (**Fig. 4.1A** and **4.1B**). Packages stored at 10 °C experienced a rapid depletion of O_2 and accumulation of CO_2, with the low O_2 (9.1 kPa) and high CO_2 (2.5 kPa) levels within the packages at the end of 14-day storage. In contrast, all the packages stored at 1 and 5 °C maintained a higher level of O_2 (15.5 and 13.1 kPa, respectively) and a lower concentration of CO_2 (1.3 and 1.6 kPa, respectively) than the packages stored at higher temperature 10°C. This is likely due to the lower respiration rate of the samples stored at lower temperatures.

Decrease in chlorophyll content is associated with cellular degradation and/or senescence, which is often used to estimate quality loss of green vegetables (Hodges et al., 2000). No information has been found specifically on total chlorophyll content of microgreens. In this study, the initial chlorophyll content of radish microgreen leaves was around 754 µg/g fresh weight (FW). As shown in **Fig. 4.1C**, total chlorophyll content decreased in all samples through 14-day storage except for those held at 1 °C, at which temperature samples maintained the highest chlorophyll content (691.1 µg/g FW), and no apparent yellowing phenomenon was observed at the end of the storage period. In contrast, samples stored at 10 °C were first to show signs of yellowing on day 7, with a rapid decline in chlorophyll content with a final value of 171.8 µg/g fresh weight. Decrease in chlorophyll content is clearly temperature-dependant with lower temperature resulting in greater chlorophyll retention. It is probably due to the reduction of metabolic activity on chlorophyll degradation under low temperature (Pogson & Morris, 1997).

Tissue electrolyte leakage is an indicator of cell membrane damage (Fan & Sokorai, 2005) and has been closely related to quality loss in fresh-cut produce during storage (Kim et al., 2005; Luo et al., 2004). During this study, there was no significant difference found in three temperature treatments (1, 5, and 10 °C) (**Fig. 4.1D**). All the samples showed minimal increase (0.3 - 0.9%) in electrical conductivity during the entire 14-day storage, indicating that the samples stored at low temperature did not lose cell membrane integrity. It was also suggested that daikon radish microgreen is not susceptible to chilling injury (Chandra et al., 2012).
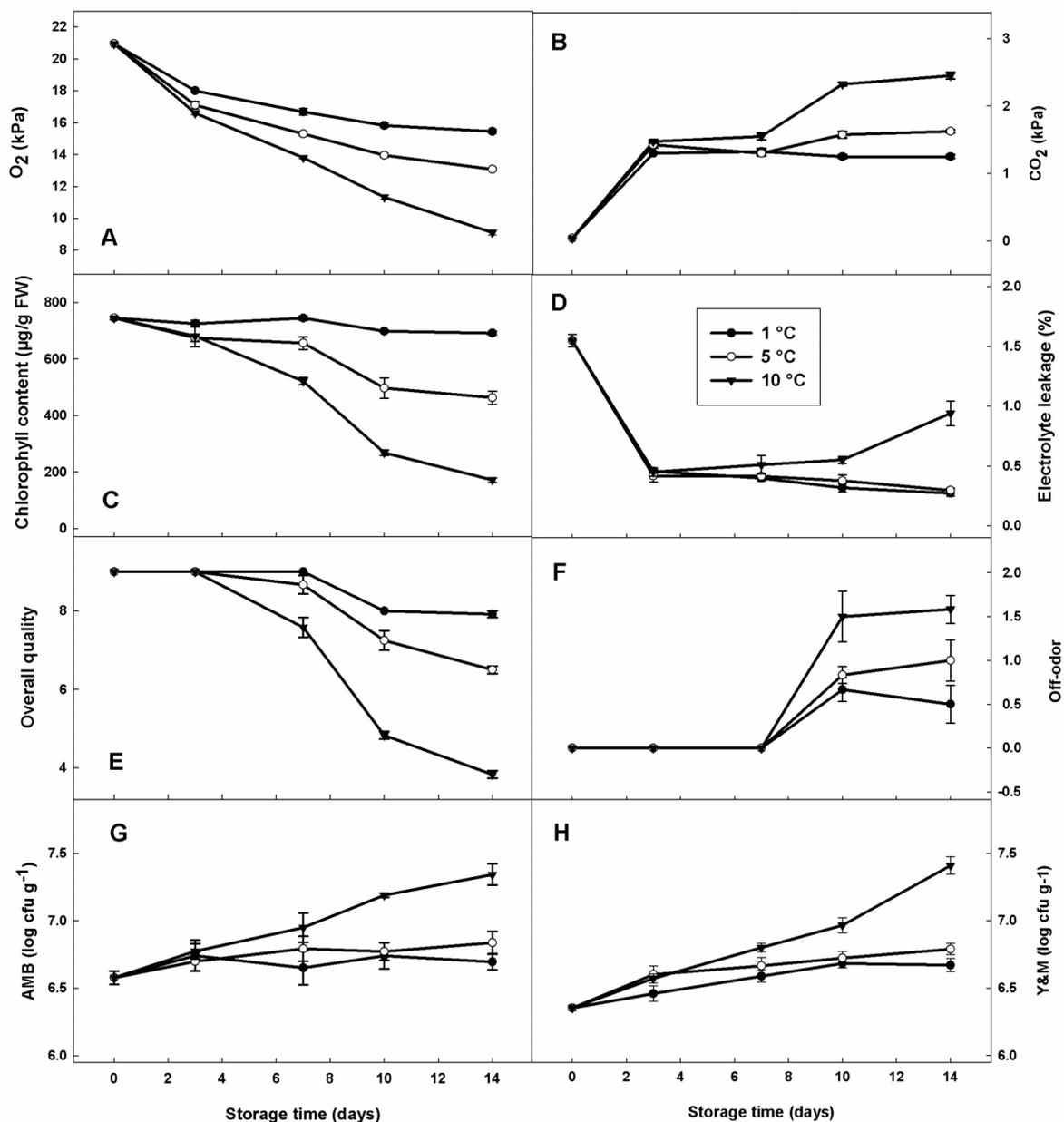
Fig. 4.1 Effect of temperature on the changes in O_2 (A) and CO_2 (B) partial pressures within packages, chlorophyll content (C), electrolyte leakage (D), overall quality (E), off-odor (F), aerobic mesophilic bacteria (AMB) (G) and yeast & mold (Y&M) (H) populations of packaged green daikon radish microgreens using 16.6 pmol s^{-1} m^{-2} Pa^{-1} OTR film during storage (n = 4).

Vertical bar represents ± standard error.

Overall visual quality and off-odor are important factors influencing the marketability of a food product. In this experiment, storage temperature significantly affected visual quality deterioration and off-odor development (**Fig. 4.1E** and **4.1F**). Throughout the whole 14-day storage period, radish microgreens stored at 1 °C were rated highest in overall quality, followed by samples at 5 °C, with the final score of 7.9 and 6.5 on day 14, respectively. Samples stored at 10 °C maintained acceptable visual quality (a score of 7.6) until day 7, however, after day 7, yellowing was observed and all these samples experienced a sharp decline in overall quality which became unacceptable (scored 4.8) within 10 days of storage, indicating that temperature abuse is severely detrimental for the delicate radish microgreens. No off-odor was detected on radish microgreens before day 7 for all treatments. On day 10, all the three treatments displayed slight to moderate off-odor and the higher the storage temperature was, the higher the intensity of off-odor was detected. At the end of 14-day storage, only slight off-odors were detected (scored 0.5 and 1.0, respectively) in the samples stored at 1 and 5 °C and moderate off-odor (scored 1.6) was detected in 10 °C treatment. The development of off-odors had a positive correlation with the decrease of O_2 and the increase of electrolyte leakage, suggesting that tissue senescence and deterioration resulted in cell membrane damage and undesirable fermentative volatiles, such as ethanol and acetaldehyde (Kim et al., 2005).

Changes in aerobic mesophilic bacteria (AMB) and yeast and mold (Y&M) populations on radish microgreens stored at different temperatures were shown in **Fig. 4.1G** and **Fig. 4.1H.** Storage temperatures significantly ($P < 0.05$) affected

microbial growth rate. During the 14-day storage, AMB populations on radish

microgreens stored at 10 °C increased more rapidly than those stored at 1and 5 °C.

AMB populations at 10 °C increased by a total of 0.8 log cfu/g, compared to 0.1 and

0.2log for 1 and 5 °C, respectively. Y&M growth followed a similar trend. Low

temperature significantly inhibited the growth of AMB and Y&M and samples stored

at 1 °C maintained a relatively lower bacterial population than samples stored at 5 °C.

Storage temperature significantly affected the quality attributes and microbial

growth of green daikon radish microgreens. Samples stored at 1 °C maintained the

best quality during the 14-day storage; therefore, 1 °C was considered to be the

optimal storage temperature for green daikon radish microgreens and was selected for

the following packaging film and chlorine wash experiments.

4.4.2 Effect of Modified Atmosphere Packaging on Quality and Shelf Life

Packaging film OTR significantly ($P < 0.05$) affected the headspace O_2 and CO_2

concentrations of radish microgreens packages at 1°C (**Fig. 4.2A** and **4.2B**).

Atmospheres in the packages prepared with higher OTR films (21.4 and 29.5 pmol s^{-1}

m^{-2} Pa^{-1}) equilibrated at higher levels of O_2 (15.0 - 16.0 kPa) and lower levels of CO_2

(1.2 - 1.3 kPa). This finding is in accordance with a previous report on fresh-cut salad

savory (Kim et al., 2004). Packages prepared with 8 pmol s^{-1} m^{-2} Pa^{-1} film OTR

exhibited a relatively more rapid depletion of O_2 and accumulation of CO_2, than those

occurred in all other treatments. However, the headspace O_2 concentrations were as

high as 8.8 kPa and CO_2 concentrations were relatively low (3.0 kPa) on day 28,

indicating that the tissues had not experienced anaerobic respiration.

After the 28-day storage at 1 °C, the content of total chlorophyll had declined slightly to a final range of 656-678 μg/g FW. Among all packaging film treatments, the total chlorophyll contents did not vary significantly over the entire storage time (**Fig. 4.2C**). Compared to 8.0 and 11.6 pmol $s^{-1} m^{-2} Pa^{-1}$ film OTR treatments, total chlorophyll loss of samples in 21.4 and 29.5 pmol $s^{-1} m^{-2} Pa^{-1}$ OTR film packages were slightly greater at the end of storage, however, the difference was not statistically significant ($P < 0.05$).

There was no significant difference on the tissue electrolyte leakage of radish microgreens among different packaging film OTRs (**Fig. 4.2D**). Interestingly, it was noted that there was a sharp decrease in tissue electrolyte leakage for all treatments from day 0 to day 7, and also a slight decrease in the following seven days (from day 7 to day 14). This phenomenon was also observed in fresh-cut cilantro leaves during the early stages of storage at 0 °C by Luo et al. (2004). This decrease in electrolyte leakage on day 7 suggested that a cell membrane damage recovery process may exist in plants/produce in the early stages of cold storage (Luo et al., 2004). During subsequent storage, increased electrolyte leakage was recorded for all packaging treatments. At the end of the 28-day storage period, samples packaged in 29.5 pmol $s^{-1} m^{-2} Pa^{-1}$ OTR film had the lowest electrolyte leakage percentage (0.9%), whereas, the highest value (1.3%) was found in the lowest (8.0 pmol $s^{-1} m^{-2} Pa^{-1}$) OTR film package.
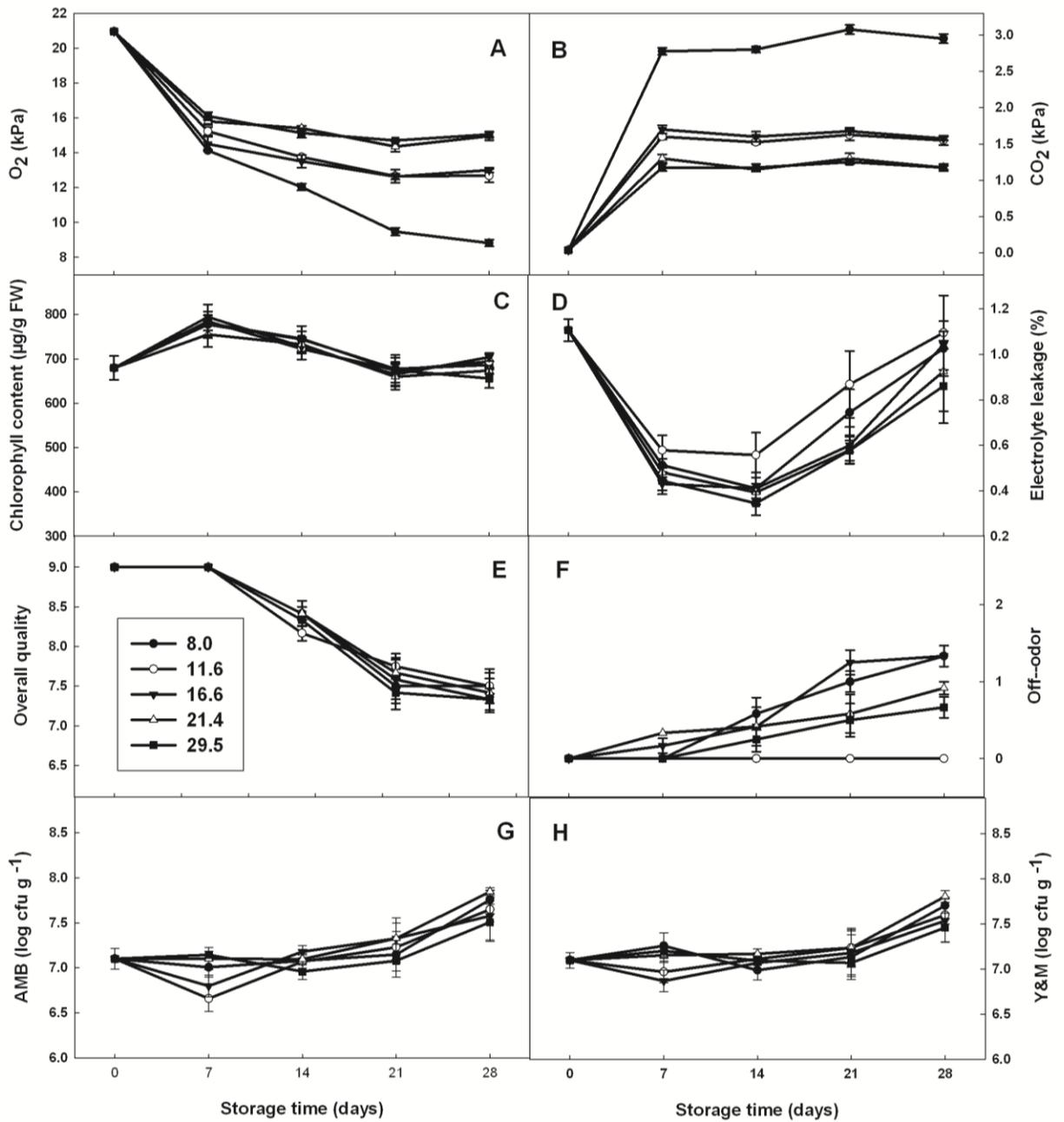
Fig. 4.2 Effect of packaging film OTR on the changes in O_2 (A) and CO_2 (B) partial pressures within packages, chlorophyll content (C), electrolyte leakage (D), overall quality (E), off-odor (F), aerobic mesophilic bacteria (AMB) (G) and yeast & mold (Y&M) (H) populations of packaged green daikon radish microgreens of green daikon radish microgreens during 1 °C storage (n = 4).

Vertical bar represents ± standard error.

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There was no noticeable quality loss among all treatments from day 0 to day 7 (**Fig. 4.2E**). Starting on day 14, tiny black spots were observed on the leaves of radish microgreens, resulting in reduced quality scores. At the end of storage, the overall quality scores of radish microgreens in all treatments had declined to 7.3-7.5, which was above the acceptable level.

Slight off-odor (a score of 0.7-1.3) developed in all samples at the end of storage (**Fig. 4.2F**). Samples from 29.5 pmol s^{-1} m^{-2} Pa^{-1} OTR film packages developed the least off-odor, followed by 21.4 pmol s^{-1} m^{-2} Pa^{-1} OTR film packages. This trend of increasing values with decrease in film OTR was in accordance with that found for electrolyte leakage, indicating that the development of off-odor was associated with loss of cell membrane integrity (Wang et al., 2005). In addition, it is noted that no off-odor (a score of 0) was detected in samples packaged in 11.6 pmol s^{-1} m^{-2} Pa^{-1} OTR film bags; instead, a pleasant but incongruent smell was present on all sampling days. The same experiments were repeated in another 1°C chamber, and the same results were obtained. No satisfactory explanation was found.

The initial microbial load on radish microgreens was relatively high (7.1 log cfu/g of AMB and Y&M), similar to that found in baby spinach leaves (Allende et al., 2004). The result is also consistent with the finding recently reported by Chandra et al. (2012) for unwashed 'Tah Tasai' Chinese cabbage microgreens. It was also hypothesized by Chandra et al. (2012) that the delicate microgreen stalks may be more vulnerable to microbial attachment and growth than mature ones. From day 7 to day 21, AMB and Y&M populations (**Fig. 4.2G** and **4.2H**) on radish microgreens remained stable at 1 °C. After day 21, the growth of AMB and Y&M increased

slowly with the final count of 7.5-7.8 log cfu/g. Although gas compositions were significantly affected by different packaging treatments, there was no significant difference in the growth of AMB and Y&M among treatments ($P < 0.05$), suggesting that gas composition did not influence the overall growth of AMB and Y&M of radish microgreens under 1 °C storage. Luo et al. (2004) found similar results for fresh-cut cilantro leaves. Therefore, it may be deduced from these results that temperature is the predominant factor influencing growth for most microorganisms (Koseki & Itoh, 2002).

Among all the OTR film treatments, no significant difference was found on maintaining the quality and prolonging the shelf life of radish microgreens. Overall, samples packaged in 29.5 pmol s^{-1} m^{-2} Pa^{-1} OTR film maintained relatively better quality during 28-day storage under 1 °C, demonstrating lowest tissue electrolyte leakage, AMB and Y&M counts, and off-odor score (except the suspicious off-odor score of 11.6 pmol s^{-1} m^{-2} Pa^{-1} OTR film treatment); thus, the film with 29.5 pmol s^{-1} m^{-2} Pa^{-1} OTR was chosen to be used in the subsequent wash study of daikon radish microgreens.

4.4.3 Effect of Wash Treatment on Quality and Shelf Life

During the entire 28-day storage period, no significant difference ($P < 0.05$) was found in the changes of O_2 and CO_2 composition in packages among all wash treatments (**Fig. 4.3A** and **4.3B**). In the first seven days, the headspace O_2 concentration of all bags dropped rapidly, nearly reaching equilibrium by day 7. All treatments maintained a constant high level of O_2 (14.0-16.0 kPa) until the end of storage. Meanwhile, the CO_2 level increased rapidly during the 1st 7 days followed by

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a slight decline. This result suggests that wash treatment had no significant ($P < 0.05$) effect on O_2 reduction and CO_2 evolution rates of radish microgreens packaged in the same permeable polyethylene bags (OTR = 29.5 pmol s^{-1} m^{-2} Pa^{-1}) and stored at low temperature (1°C).

During the 28-day storage at 1°C, the total chlorophyll content did not vary significantly ($P < 0.05$) (**Fig. 4.3C**). There was no direct relationship between wash treatment and total chlorophyll content. This lack of discernable effect due to wash treatment may be the result of large sample variation obscuring the variation attributable to wash treatment.

On day 0, unwashed samples (control) showed higher tissue electrolyte leakage than all other washed samples, probably due to tissue fluids exuded from cut ends during washing. Meanwhile, water-washed samples exhibited lowest electrolyte leakage (0.8%) of all wash treatments, which is the same result reported for a recent study on 'Tah Tasai' Chinese cabbage microgreens (Chandra et al., 2012). After day 14, tissue electrolyte leakage increased rapidly for 100 mg/L chlorine treated samples and the values were significantly ($P < 0.05$) higher than those of other treatments on day 21 and day 28. On the contrary, no significant difference was observed in the changes of electrolyte leakage during subsequent storage among other treatments (**Fig. 4.3D**).
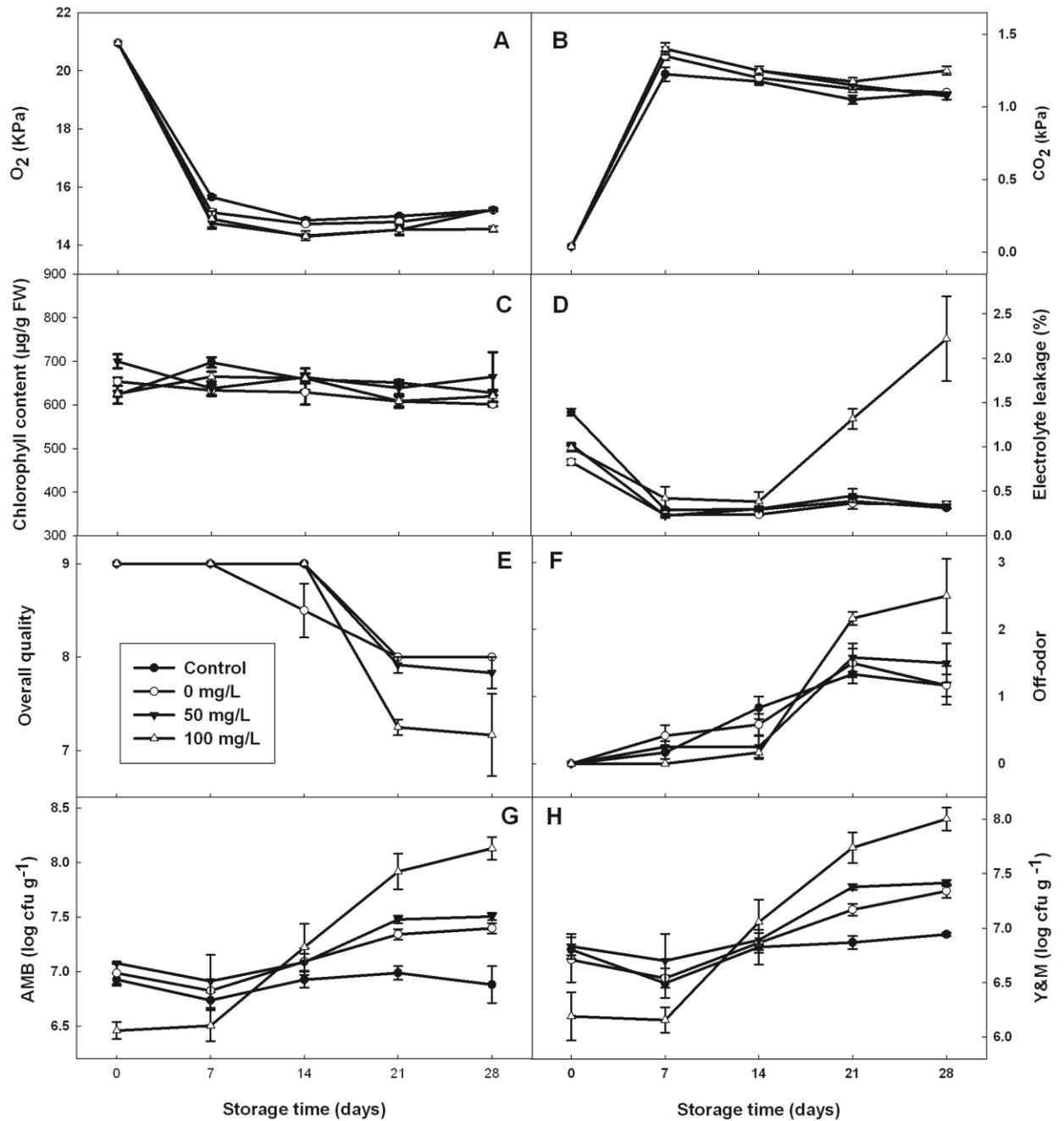
Fig. 4.3 Effect of chlorine wash treatment on the changes in O_2 (A) and CO_2 (B) partial pressures within packages, chlorophyll content (C), electrolyte leakage (D), overall quality (E), off-odor (F), aerobic mesophilic bacteria (AMB) (G) and yeast & mold (Y&M) (H) populations of green daikon radish microgreens during 1 °C storage using 29.5 pmol s^{-1} m^{-2} Pa^{-1} OTR film (n = 4). Control represents unwashed sample.

Vertical bar represents ± standard error.

All samples subjected to different wash treatments maintained the highest possible visual score of 9.0 during storage at 1°C until day 14 (**Fig. 4.3E**). After day 14, the visual quality of 100 mg/L chlorine treated samples declined rapidly, receiving the lowest overall score (a score of 7.2) at the end of the 28-day storage. However, no significant difference was found for overall quality of all other treatments (unwashed, water, 50 mg/L chlorine), which maintained good visual quality (scores of 7.8-8.0) at the end of storage. This suggests that the 100 mg/L chlorine treatment may have caused tissue damage during wash, which led to the quality loss during storage. However, the wash treatment with 50 mg/L free chlorine did not appear to have a detrimental effect on quality.

The results for off-odor development followed the same trends as those for visual quality (**Fig. 4.3F**). Only trace amount of off-odor (scored 0.2 - 0.8) was detected for each treatment on day 14. At the end of storage, a slight to moderate level of off-odor (scored 1.2 - 1.5) was detected from all samples except for 100 mg/L chlorine treated samples, which developed the strongest off-odor with a moderate to strong score of 2.5. The sensory results agreed well with those from tissue electrolyte leakage, suggesting that the loss of freshness and development of off-odor was related to tissue damage and senescence.

A wash treatment with 100 mg/L free chlorine significantly reduced microbial population on day 0, while no difference was found among all other treatments (**Fig. 4.3G** and **4.3H**). Microbial populations increased after day 7 of storage. However, samples that received the 100 mg/L chlorine treatment had a more significant increase in microbial populations than the other treatments. AMB and Y&M growth on

samples treated with 100 mg/L chlorine overtook that of all other treatments on day 14 and continued to outgrow others until the end of storage. This is in agreement with the more rapid quality loss observed in this treatment, probably due to tissue damage incurred during washing. A similar result was reported for 'Tah Tasai' Chinese cabbage microgreens treated with chlorinated water (Lee et al., 2009). Water washed samples experienced slightly less microbial growth after day 14 than did 50 mg/L chlorine washed samples. Among all treatments, unwashed samples maintained lowest microbial growth after the 14th day of storage, which is in accordance with the finding in a recent study of Kou et al. (2013). This result is probably due to the lower moisture content in these packages. Excess moisture remaining on washed leaf surfaces, and the possible damaged incurred from agitation during washing and drying may have promoted microbial growth in those packages. Radish microgreens have young and delicate leaf tissues that can be easily damaged during preparation. Since removal of excess water without tissue injury is often closely related to the maintenance of quality and shelf life of fresh or fresh-cut produce, future studies may need to optimize the washing, and drying processes.

4.5 Conclusions

The quality and shelf life of radish microgreens as impacted by three major postharvest treatment factors, i.e. storage temperature, packaging film OTR, and chlorine wash treatment, were evaluated in this study. Storage temperature had a significant impact on package atmosphere, product visual quality, microbial growth and membrane integrity. A storage temperature of 1 °C was rated as the best treatment followed by 5 °C storage. Samples stored at 1 °C maintained the highest

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overall visual quality with minimum off-odor development, lowest AMB and Y&M counts. This treatment also maintained the highest tissue integrity with minimum chlorophyll degradation, whereas those stored at 10 °C lost quality more rapidly.

Packaging film OTR significantly affected headspace gas composition during 1 °C storage; however, it did not have a significant effect ($P < 0.05$) on the quality and shelf life of the product, probably due to the presence of high level O_2 and low level CO_2 within the packages over time and low respiration rate of microgreens stored at 1 °C. In our study, microgreens packaged in all OTR film bags stored at 1 °C maintained good quality and shelf life throughout 28 days.

Among all wash treatments and control (no wash), 100 mg/L free chlorine wash treatment had a significant impact on the reduction of microbial population initially. However, microbial growth on these samples exceeded those of all other treatments after 7 days. In this study, the use of chlorine washing solutions did not achieve the goal of producing ready-to-eat radish microgreens with low microbial load and long shelf life, therefore, some alternative treatments need to be further investigated in the future study.

Chapter 5: Effect of Light Exposure on Sensorial Quality, Concentrations of Bioactive Compounds and Antioxidant Capacity of Radish Microgreens during Low Temperature Storage

Xiao, Z., Lester, G.E., Luo, Y., Xie, Z., Yu, L., Wang, Q. (2014) Food Chemistry, 151, 472-479.

5.1 Abstract

Radish microgreens constitute a good source of bioactive compounds; however, they are very delicate and have a short shelf life. In this study, we investigated the impact of light exposure and modified atmosphere packaging on sensorial quality, bioactive compound concentrations and antioxidant capacity of radish microgreens during storage. Results showed 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 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity between light and dark treatments. Radish microgreens in bags of 29.5 pmol s^{-1} m^{-2} Pa^{-1} oxygen transmission rate (OTR) maintained better quality than those within laser-microperforated bags. In conclusion, light exposure accelerated deterioration of radish microgreens, while dark storage maintained quality; and application of OTR bags was beneficial in extending shelf life.

5.2 Introduction

Microgreens are a new class of specialty vegetables that are often harvested at the cotyledonary leaf stage without roots and seed coats. Microgreens are favored by chefs and consumers in high-end restaurants for their attractive colors, tender texture, and intense flavors. A recent report on phytonutrient studies (Xiao et al., 2012) demonstrated that most microgreens contain substantially higher levels of bioactive compounds, such as ascorbic acid, phylloquinone, tocopherols and carotenoids, than their more mature true-leaf forms. As the demand for microgreens increases, and they begin to appear in farmer's markets and specialty grocery stores, the optimization of their postharvest storage conditions is therefore becoming important.

Commercially, containers used for microgreens are plastic clamshell containers, in which the gas composition is atmospheric. In order to accurately measure the headspace gas composition, laser microperforated plastic bags were used in the current study as a substitute for clamshell containers. However, our previous studies found that using optimized modified atmosphere packaging and low temperature storage considerably extended the shelf-life of radish microgreens (Xiao et al., 2013). Thus both packaging conditions will be investigated.

Fresh produce, including microgreens, are usually displayed under light in grocery stores. Recently, the effect of light exposure on quality and phytochemical concentrations of different vegetables has been studied extensively. Büchert et al. (2011) reported that continuous low intensity light delayed yellowing and postharvest senescence of broccoli florets (*Brassica oleracea* L.). Noichinda, et al. (2007) reported that low intensity fluorescent light accelerated fresh weight loss, but

prevented the loss of vitamin C of Chinese kale (*Brassica oleracea* var. *alboglabra*) during storage. Lester et al. (2010b) found that continuous light exposure prevented loss of ascorbic acid and was beneficial in enhancing carotenoids and tocopherols of baby-leaf spinach (*Spinacia oleracea* L.). Studies also showed that light exposure could cause detrimental effects on produce quality. A study by Sanz et al. (2009) demonstrated that asparagus (*Asparagus officinalis* L.) stored under light experienced accelerated deterioration and shortened shelf life than those stored under continuous dark. Martínez-Sánchez et al. (2011) also observed that light exposure could promote browning of fresh-cut Romaine lettuce.

Daikon radish microgreens (*Raphanus sativus* var. *longipinnatus*) were chosen in this study due to its abundance in bioactive compounds relevant to human health (Xiao et al., 2012) and broad usage in restaurants in the US. The objective of this study is to determine the effect of light exposure and packaging conditions on sensorial quality, concentrations of bioactive compounds, and antioxidant capacity of daikon radish microgreens during cold storage.

5.3 Materials and Methods

5.3.1 Sample Preparation

Daikon radish microgreens (*Raphanus sativus* var. *longipinnatus*) were grown by Sun Grown Organic Distributors, Inc. (San Diego, CA, USA) in an unheated greenhouse and under ambient light. Samples were harvested without roots, packed in clamshell containers and shipped overnight in insulated containers with ice packs. When received, all samples were inspected prior to packaging and defective plant tissues were discarded. Samples (20 g) were re-packaged in 12.5 cm × 12.5 cm

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plastic bags, which were made of either polyethylene film (Pacific Southwest

Container Inc., Modesto, CA, USA) with OTR of 29.5 pmol s^{-1} m^{-2} Pa^{-1} or laser-

microperforated oriented polypropylene film (LMP), provided by Dole Fresh

Vegetables, Inc. (Salinas, CA, USA), respectively. The samples in each packaging

type were further randomly divided into two groups and subjected to light and dark

treatments. The samples subjected to light were stored under continues fluorescent

light (light intensity $\approx$ 30 µmol·s^{-1}·m^{-2}) and those receiving dark treatment were stored

in two-layer brown paper bags (light intensity $\approx$ 0.1 µmol·s^{-1}·m^{-2}). The light intensity

was measured by LI-1000 data loggers (LI-COR, Lincoln, NB, USA) at the top of

packages. Three packages of radish microgreens were randomly selected from each

treatment on day 0, 4, 8, 12 and 16 for evaluations.

5.3.2 Headspace Gas Composition

Packaging headspace gas samples were withdrawn by inserting the needle through

a septum adhered to the packaging film. The gas composition (O_2 and CO_2) was

measured using an O_2/CO_2 gas analyzer (CheckMate II, PBI-Dansensor A/S,

Ringsted, Denmark).

5.3.3 Quality Attributes

5.3.3.1 Color (*L*, C*, h°*)

Color coordinates (CIE *L*, C*, h°*) were directly measured on the products using

a model CR-410 colorimeter (Konica Minolta, Ramsey, NJ, USA) with a 50 mm

diameter viewing aperture. The equipment was calibrated with a standard white plate

(Y = 94.0, x = 0.3130 and y = 0.3191). The concentrations of each package of radish

microgreens were transferred to a clear plastic tray. Color was measured at ten

locations and the mean value was taken to ensure that color readings were

representative of each sample. Three replicate packages were evaluated for each

treatment on each sampling day (day 0, 4, 8, 12 and 16). The results were expressed

as lightness (L^*), Chroma (C^*) and hue angle ($h°$) values.

5.3.3.2 Weight Loss

Weight loss was determined by weighing the bagged samples from at the

beginning of storage and during storage. Three replicates were evaluated for each

treatment on each sampling day (day 0, 4, 8, 12 and 16). Results were expressed as

percentage of weight loss relative to the initial fresh weight.

5.3.3.3 Sensory Evaluation

Sensory evaluation was conducted by a six-member trained panel using a ballot

designed with Compusense® 5.0 system (Guelph, Canada). All the samples were

evaluated under controlled yellow light in individually partitioned sensory booths. A

total of 4 samples, one from each of the four treatments were served one at a time to

each panelist. Each sample was labeled with a random 3-digit number and served to

the panel members in random orders. The visual quality was rated using a 9-point

hedonic scale, anchored by 9 = like extremely, 5 = neither like nor dislike and 1=

dislike extremely (Meilgaard et al., 1991); a score of 6 was considered the limit of

salability (Kim et al., 2004). Off-odor was scored on a 0 to 4 scale where 0 = no off-

odor, 1 = slight off-odor, 2 = moderate off-odor, 3 = strong off-odor, and 4 =

extremely strong off-odor.

5.3.4 Analysis of Bioactive Compounds

5.3.4.1 Ascorbic Acid

Total ascorbic acid (TAA) and free ascorbic acid (AA) were determined using a reverse phase high performance liquid chromatography (RP-HPLC) using the protocol described in **3.3.4.1**. In this assay, the amount of dehydroascorbic acid (DHA) was equal to the difference between TAA and AA.

5.3.4.2 Carotenoids and Tocopherols

Carotenoids and tocopherols were simultaneously determined using an isocratic RP-HPLC according to the procedure previously established in our laboratory (Xiao et al., 2012), as described in **2.3.3.2**.

5.3.5 Determination of Antioxidant Capacity

5.3.5.1 Total Phenolics

Total phenolic content (TPC) was measured using Fast Blue BB (FBBB) assay developed by Medina (2011) and modified for chlorophyll-containing tissue by Lester et al. (2013), as described in **3.3.4.4**.

5.3.5.2 Relative DPPH Radical Scavenging

The relative 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (DPPH) was evaluated according to the method of Cheng et al. (2006). The test solution (0.1 mL) of sample extracts, Trolox standards, or blank solvent control was added to 0.1 mL of freshly prepared DPPH solution to initiate the reaction. The absorbance of the reaction mixture was measured at 515 nm for 40 min of reaction in

the dark. DPPH values were calculated using areas under the curve and expressed as micromoles of Trolox equivalents (TE) per gram of dried weight sample.

5.3.5.3 Hydroxyl Radical Scavenging Capacity

Hydroxyl radical scavenging capacity (HOSC) assay was conducted according to the protocol of Moore et al. (2006) using a Victor multilabel plate reader (PerkinElmer, Turku, Finland). Aqueous sample extracts were evaporated to dryness and redissolved in 50% acetone. Reaction mixtures consisted of 170 µL of 9.28×10^{-8} M fluorescein prepared in 75 mM sodium phosphate buffer, 30 µL of standard, sample extract, or blank, 40 µL of 0.1990M H_2O_2 and 60 µL of 3.43 mM $FeCl_3$. Fluorescence was measured every minute for 3 h with an excitation wavelength of 485 nm and emission wavelength of 535 nm. HOSC values were expressed as micromoles of Trolox equivalents (TE) per gram of sample on a dry weight basis.

5.3.6 Statistical Analysis

Package atmospheres, dry weight, weight loss, antioxidant activity, color, nutrient, and quality and off odor data were analyzed as three-factor linear models using the PROC MIXED procedure (SAS Institute Inc., Version 9.2, Cary, NC, USA). Analysis factors were storage time (5 levels), package film (2 levels), and light condition (2 levels). Three replications were evaluated per treatment on each sampling day for all parameters except quality and off odor for which 6 replications were evaluated per treatment per sampling day. Quality and off-odor evaluator ratings were averaged for each sample. Different samples were analyzed on each evaluation day for all studies. Assumptions of normality and variance homogeneity of the linear

model were checked and the variance grouping technique was used to correct for variance heterogeneity. When effects were statistically significant, means were compared using Sidak adjusted p-values to maintain experiment-wise error ≤ 0.05. Data was reported as the mean of 3 replicates $\pm$ standard error (SE).

5.4 Results and Discussions

5.4.1 Effect on Headspace Gas Composition

The gas composition in all laser microperforated bags (LMP) was maintained at atmospheric and not affected by the light treatment during the storage period (**Fig. 5.1A**). This suggests that the laser microperforated films used in this study allowed sufficient gas exchange with the surrounding environment to compensate for O_2 consumption and CO_2 production that may have been produced by the microgreens. In contrast, the O_2 and CO_2 levels in OTR bags were significantly impacted by the light exposure. Within the first 4 days of storage, there was a sharp decline in O_2 levels inside the packages of both light and dark treatment. However, O_2 levels increased in light-stored packages while under dark remained unchanged from day 4 until the end of storage (day 16). Similar to O_2, under both light and dark conditions the package headspace CO_2 levels remained nearly unchanged in laser microperforated films throughout the entire storage period (**Fig. 5.1B**). In OTR bags, the CO_2 levels differed significantly under light vs. dark storage. Under light conditions, CO_2 levels remained nearly unchanged from day 0 to day 12, followed by a slight increase (up to 0.8) from day 12 to day 16. While there was a sharp increase in CO_2 from day 0 to day 4 (up to 3.8) and remained high throughout the storage under dark condition. The large difference in gas compositions observed under light

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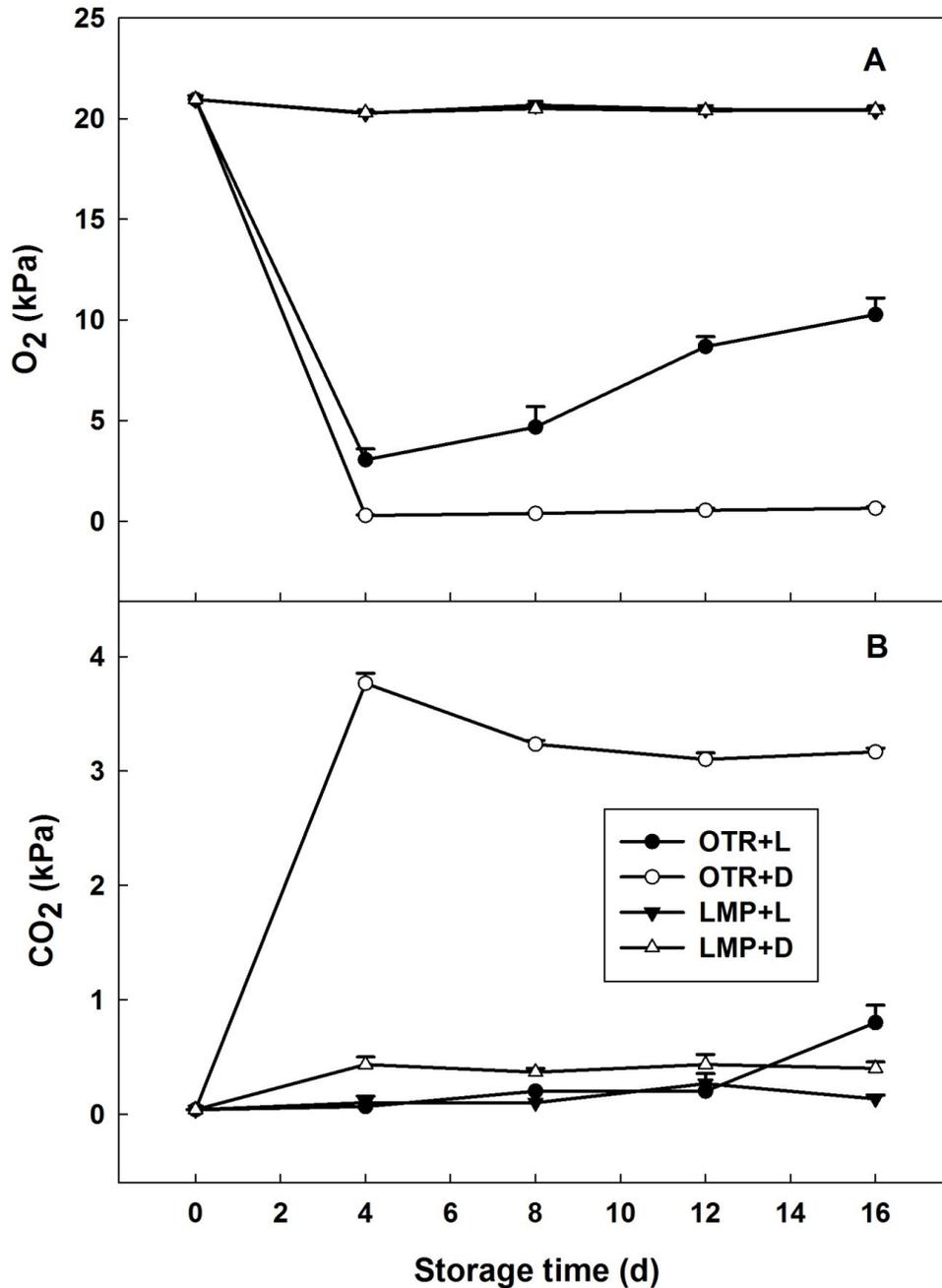
Fig. 5.1 Effect of light exposure on the headspace gas composition in oxygen transmission bags (OTR) and laser microperforated bags (LMP) at 5 °C for 16 days.

OTR+L, OTR+D, LMP+L and LMP+D represents radish microgreens in OTR bags subjected to light, in OTR bags subjected to dark, in LMP bags subjected to light, and in LMP bags subjected to dark, respectively. Values are means ± standard errors of three replicates.

versus dark conditions is likely attributable to the respiratory and photosynthetic activities of microgreens.

In general, exposure to light during storage induces stomatal opening, resulting in increased respiratory activity, which leads to the consumption of O_2 and release of CO_2 (Sanz et al., 2008). In the presence of light, chlorophyll-containing tissues could also continue photosynthetic activity, which depletes CO_2 and releases O_2 in packages (Olarte et al., 2009). In our study, the high levels of O_2 and lower levels of CO_2 in light-stored microgreens packages suggests active photosynthetic processes in microgreens during storage under light exposure.

5.4.2 Effect on Quality Attributes

5.4.2.1 Color (L^*, C^*, h°)

Color changes were presented in terms of the coordinates, lightness (L^*), chroma (C^*) and hue (h°) (**Fig. 5.2**). The color of radish microgreens was significantly affected by light exposure, packaging atmosphere and storage duration. In general, L^* values initially decreased during storage and then slowly increased returning to the original value, except for the treatment of LMP+D which increased beyond the original value (**Fig. 5.2A**). Irrespective of packaging treatments, the L^* values of light-stored samples were significantly lower than those of dark-stored samples, which indicated that light-stored samples were darker. The tendency of samples to darken when exposed to light has been reported by previous studies on green parts of chard and leek (Ayala et al., 2009; Sanz et al., 2008). The darkening of plant samples observed in these instances was probably caused by the photosynthetic activity in the

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Fig. 5.2 Effect of light exposure on lightness (L^*), chroma (C^*), hue angle ($h°$) of radish microgreens stored in oxygen transmission bags (OTR) and laser microperforated bags (LMP) at 5 °C for 16 days.

OTR+L, OTR+D, LMP+L and LMP+D represents radish microgreens in OTR bags subjected to light, in OTR bags subjected to dark, in LMP bags subjected to light, and in LMP bags subjected to dark, respectively. Values are means ± standard errors of three replicates.
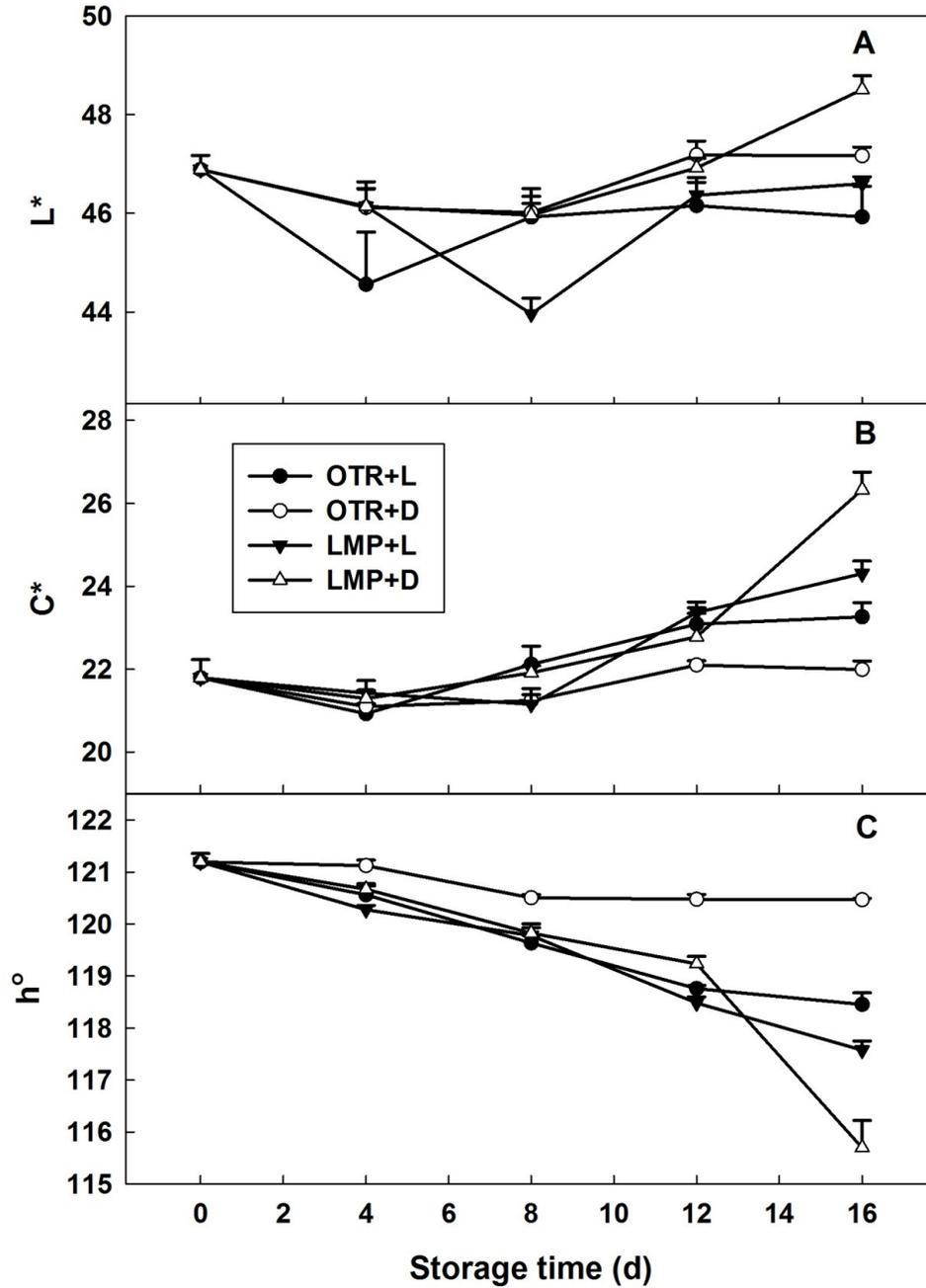
presence of light during postharvest storage, through which the green pigment chlorophyll was produced.

Chroma (C^*) represents the intensity or purity of the hue (Gómez et al., 2010). The higher the C* value, the more intense the sample hue appears. Among all four treatments, the least change in C^* was found in dark-stored OTR microgreens samples, indicating that the intensity of hue of the tissue was not significantly affected by this storage condition (**Fig. 5.2B**).

In this study, all the h° (color) values were in the quadrant of 90° (yellow) to 180° (green); therefore, the decrease of h° value means that samples tend to turn from green to yellow-green. The higher the h° value, the greener the microgreens. Our results demonstrate that light exposure clearly affected h° value of samples stored in OTR bags with a progressive decrease in h° during storage, while samples in dark-stored OTR bags were minimally affected with only a slight decrease in h° values on day 8, and no change thereafter (**Fig. 5.2C**). The yellowing observed in the light-stored samples at the end of the storage period provides additional evidence that light exposure accelerates discoloration of radish microgreens. Hue values of microgreens packaged in LMP bags decreased during storage but no significant differences were found between light and dark treatments, indicating that LMP packaging played a more important role in tissue discoloration than OTR packaging. The high oxygen concentration in LMP packages resulted in more rapid yellowing, an indication of tissue senescence and chlorophyll degradation (Heaton & Marangoni, 1996).

Contradictory reports were found in literature regarding the effect of light exposure on quality maintenance of fresh produce. Studies from Sanz et al. (2008)

and Ayala et al. (2009) showed a similar gradual decrease in hue angle values of chard and leek, respectively, during storage. However, Kasim and Kasim (2007) reported that low intensity light exposure was beneficial to the preservation of green color of Brussels sprout and broccoli florets. Based on the $L^*, C^*, h^°$ coordinate data in our study, it was concluded that the impact of packaging film is more important than that of light or dark exposure in maintaining visual quality of radish microgreens light- and dark-stored OTR bags maintained the freshest appearance compared to light- and dark-stored LMP bags following 16 days at low temperature storage.

5.4.2.2 Effect on Sensorial Quality

The effect of light exposure on the sensorial quality, irrespective of packaging, resulted in that dark-stored microgreens having significantly better visual quality than samples in lighted storage. Initially, all microgreens had a fresh appearance on day 4 with no significant difference among treatments. However, by day 8, microgreens stored under dark received significantly higher visual quality scores than those stored under light (**Fig. 5.3A**). After 8 days of storage, OTR samples stored in darkness maintained high visual quality while the visual quality of light-exposed OTR samples and all the LMP samples were below the limit of acceptability. The visual quality of dark-stored OTR microgreens was rated above 5.0 (i.e. considered likeable) throughout the entire 16 day storage while all other microgreens continued sharp decline in visual quality. Accompanying the changes in visual quality, radish microgreens exposed to light produced an off-odor more slowly than those stored under dark conditions (**Fig. 5.3B**). While light exposure initially prevented the occurrence of off-odor in OTR samples, after 8 days of storage the intensity of off-
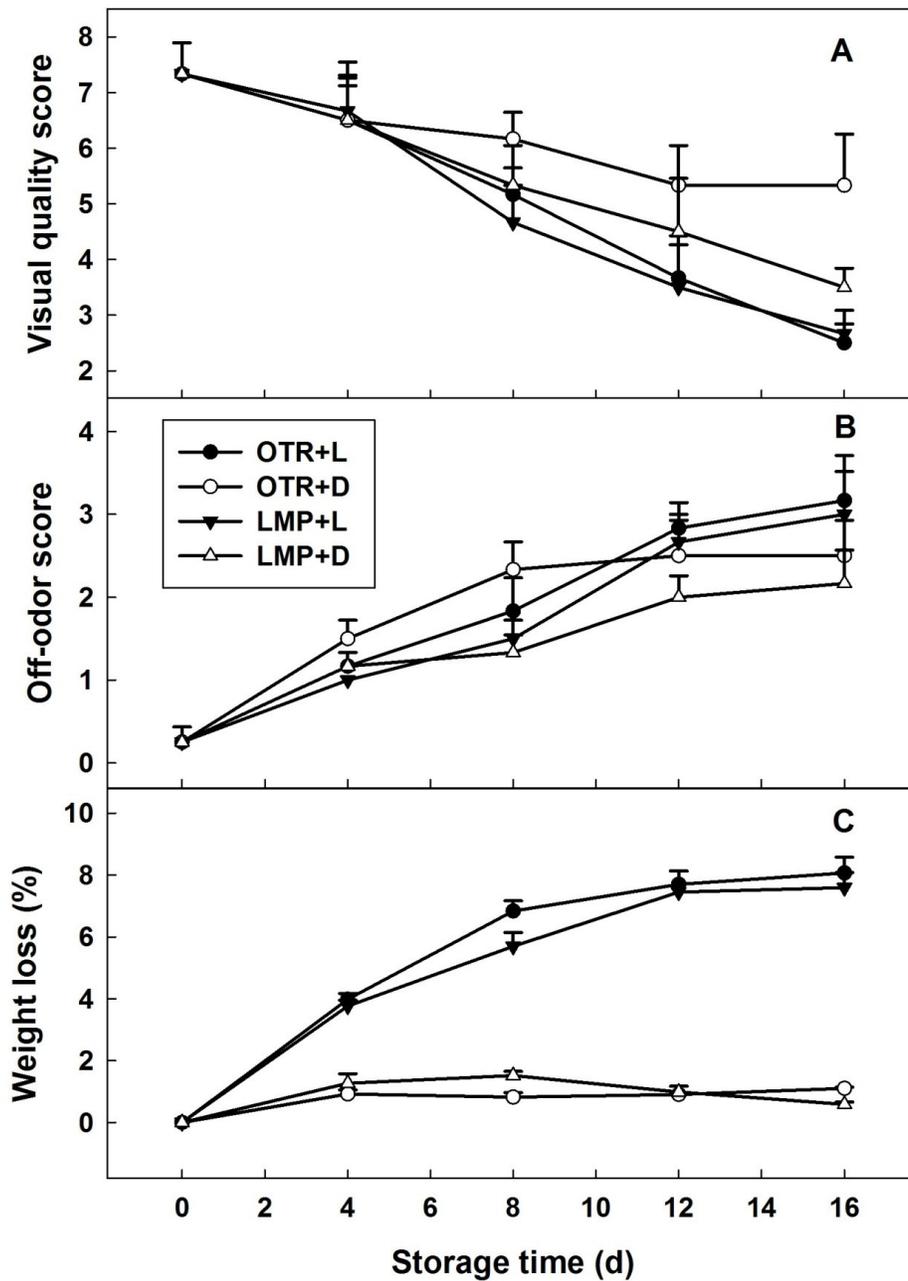
Fig. 5.3 Effect of light exposure on visual, off-odor and weight loss of radish microgreens stored in oxygen transmission bags (OTR) and laser microperforated bags (LMP) at 5 °C for 16 days.

OTR+L, OTR+D, LMP+L and LMP+D represents radish microgreens in OTR bags subjected to light, in OTR bags subjected to dark, in LMP bags subjected to light, and in LMP bags subjected to dark, respectively. Values are means ± standard errors of six replicates.

odor of light-exposed microgreens was higher than that of dark-stored samples. Samples stored in LMP packages in darkness showed less intensity of off-odors at each sampling day than those stored in OTR packages except for day 4 when there was no significant difference between packaging treatments. The lower off-odor scores for LMP bags than for OTR bags were probably due to higher permeability of LMP film. In our study, exposure to light during postharvest storage was found to have a negative effect on the sensorial quality maintenance of radish microgreens, which is in accordance with the findings reported by some previous researchers on minimally processed chard, leek, asparagus and Romaine lettuce (Ayala et al., 2009; Martínez-Sánchez et al., 2011; Sanz et al., 2008; Sanz et al., 2009).

5.4.2.3 Effect on Weight Loss

Weight loss of radish microgreens under light exposure was significantly ($P <$ 0.05) higher than that for dark-stored samples on each sampling day (**Fig. 5.3C**). The weight loss of light-stored microgreens increased throughout storage, ending up with 8.1% and 7.6% of weight loss in OTR and LMP packages, respectively. In contrast, samples stored in the dark maintained a stable fresh weight and with little weight loss (around 1.0%) during the entire 16-day storage period. Similar research findings were reported for light-exposed storage of Brussels sprout, Chinese kale, broccoli and cauliflower (Kasim & Kasim, 2007; Noichinda et al., 2007; Olarte et al., 2009), which showed that stomata were closed in darkness within 1 day of storage, but remained open during storage under light conditions. It is generally recognized that the degree of stomatal opening is directly related to both the transpiration rate and the diffusion of CO_2 for photosynthesis. Under light exposure, an increased transpiration

rate, due to more stomatal openings, consequently accelerates the loss of water vapor from tissues, which is the likely cause of the higher weight loss. It was also observed that there was substantial moisture condensation inside the packaging film in light exposed packages, which was further evidence of high leaf transpiration rate under light.

5.4.3 Effect on Bioactive Compounds

5.4.3.1 Ascorbic Acid

Dry weight percentage was shown in **Fig. 5.4A**, which was used for calculating all the nutrient concentrations. The changes in AA concentration of radish microgreens in all treatment groups followed a similar trend over the 16-day low temperature (5°C) storage (**Fig. 5.4B**). With the exception of samples stored in OTR bags and exposed to light (OTR+L), free ascorbic acid concentration increased initially (from day 0 to day 8), decreased until day 12, and then increased again until the end of storage. Compared with the samples stored in dark, radish microgreens stored under light showed significantly ($P < 0.01$) higher AA concentrations during the entire storage. Dehydroascorbic acid (DHA), the oxidized form of AA followed the opposite trend of AA, decreasing during the first 4 days of storage for all samples except samples packaged in LMP bags and stored in dark (LMP+D) and then slightly increasing until day 12, and declining thereafter (**Fig. 5.4C**). Dehydroascorbic acid for LMP+D samples increased gradually until day 8 and then increased rapidly through the end of the storage. The increase in total ascorbic acid (TAA) which occurred in all radish microgreens during the 16-day storage period regardless of the presence of light and packaging treatments (**Fig. 5.4D**) is due to the combination of
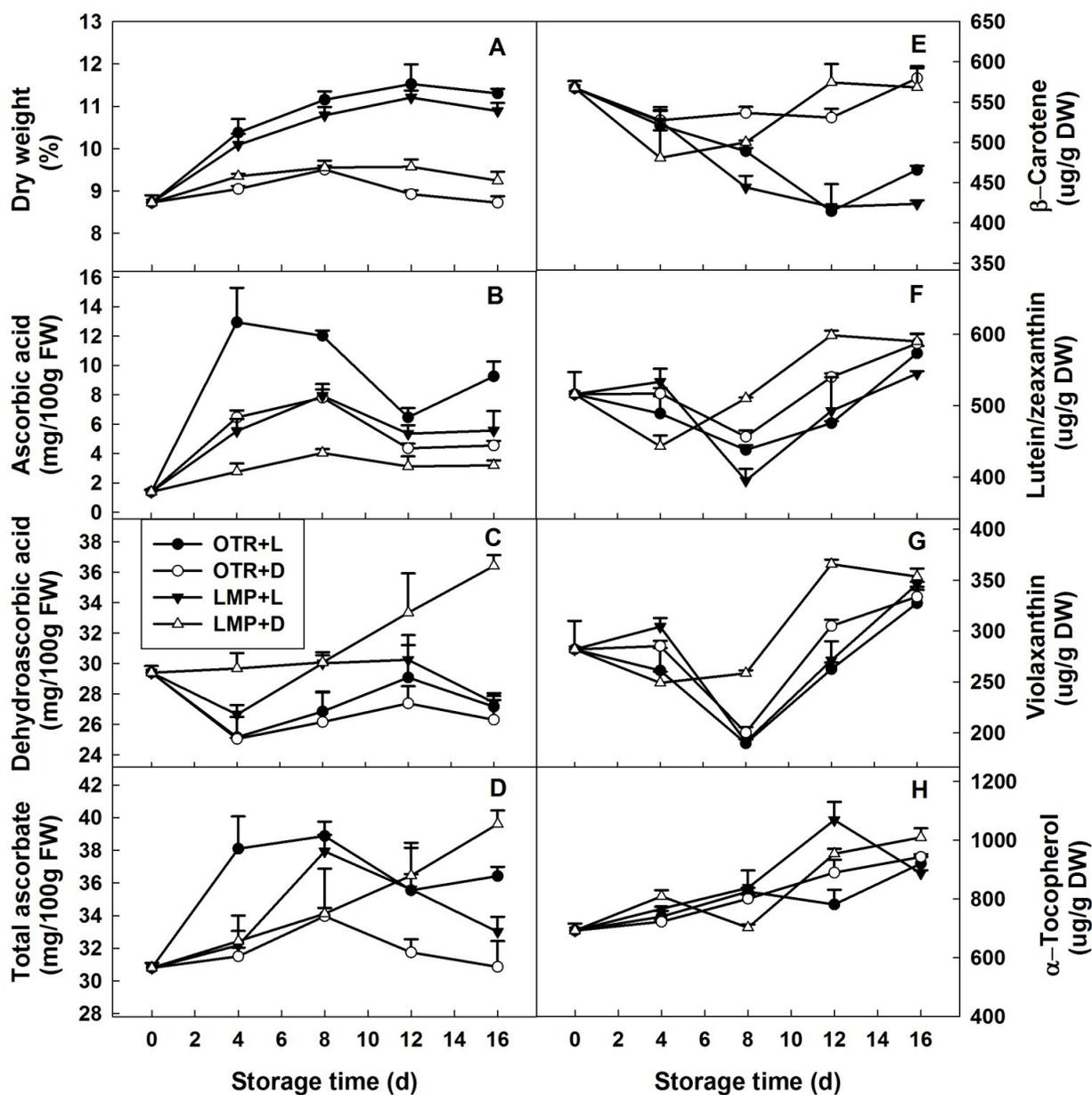
Fig. 5.4 Effect of light exposure on dry weight, ascorbic acid, dehydroascorbic acid, total ascorbic acid, β-carotene, lutein/zeaxanthin, violaxanthin, α-tocopherol of radish microgreens stored in oxygen transmission bags (OTR) and laser microperforated bags (LMP) at 5 °C for 16 days.

OTR+L, OTR+D, LMP+L and LMP+D represents radish microgreens in OTR bags subjected to light, in OTR bags subjected to dark, in LMP bags subjected to light, and in LMP bags subjected to dark, respectively. Values are means ± standard errors of three replicates.

increase in AA or DHA. In general, light-stored samples showed higher TAA concentrations than dark-stored samples over the entire storage period. The TAA concentration of radish microgreens was initially 30.8 mg/100mg FW on day 0 and increased by 18.3% in OTR samples after 16-day light exposure. In comparison, OTR samples stored in the dark retained very stable level of TAA (30.8-31.7 mg/100mg FW) during storage, with a small increase in the middle of the period (34.0 mg/100mg FW). Samples stored in OTR films preserved more AA and less DHA than those stored in LMP films during storage, regardless of light or dark storage.

In our study, the exposure to light contributed to higher levels of TAA over time. As expected, light exposure may increase the photosynthetic capacity of radish microgreens during postharvest storage, which resulted in production of D-glucose, which is the precursor of AA synthesis (Zhan et al., 2012). Interestingly, there was no TAA loss in radish microgreens packaged in OTR film bags stored at 5°C regardless of light or dark storage. Instead, light exposure positively boosted the total amount of TAA. A similar trend for total ascorbic acid in light-exposed baby spinach was found by Lester et al. (2010b).

It is noted that AA at 1.4 mg/100g FW only accounted for 4% of TAA concentration on day 0, meaning that most of the vitamin C was present as DHA, its oxidized form. This phenomenon is thought to be associated with postharvest oxidative stresses, resulting from a variety of factors, including physical damage, temperature fluctuation, and internal senescence, all of which may contribute to the decrease in AA by oxidizing it to DHA (Hodges et al., 2004).

5.4.3.2 Carotenoids

Light exposure significantly accelerated β-carotene degradation in radish microgreens during storage in both packaging treatments, whereas the samples stored in the dark showed little change in β-carotene level throughout the storage period (**Fig. 5.4E**). Other researchers reported similarly that more β-carotene concentration was preserved in spinach leaves stored in dark condition than in light condition (Lester et al., 2010b). The changes of lutein/zeaxanthin concentrations in all treatments followed a similar trend during storage, with LMP+D samples leading the way with faster decrease and earlier increase, but all treatments increasing after day 8 (**Fig. 5.4F**). Similarly to β-carotene, lutein/zeaxanthin and violaxanthin (**Fig. 5.4G**) concentration was lower in the samples stored under light than in those stored in darkness. It is known that when the light energy is excessive to the need of plants for photosynthesis, a reversible xanthophylls conversion of violaxanthin to zeaxanthin via the intermediate antheraxanthin (violaxanthin cycle) occurs, whereby violaxanthin can be de-epoxidized into zeaxanthin (Havaux & Niyogi, 1999; Jahns et al., 2009). In our study, more violaxanthin was observed in dark condition, which indicated that the epoxidation reaction may occur in this storage condition. Zeaxanthin epoxidation to form violaxanthin is commonly induced under low light conditions (Lubián & Montero, 1998). Although the lutein/zeaxanthin concentration in radish microgreens did not decrease in the dark storage as the violaxanthin increased, this data was the sum of lutein and zeaxanthin, which may not reflect the change in zeaxanthin.

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5.4.3.3 Tocopherols

It is generally recognized that α-tocopherol is the predominant form found in leaves, while γ-tocopherol and tocotrienols accumulate to higher levels in seed of many plant species (Demurin et al., 1996; Tan, 1989). Gamma-Tocopherol is the precursor of α-tocopherol (Lester et al., 2010b). In higher plants, γ-tocopherol methyltransferase is an important enzyme in the biosynthetic pathway of α-tocopherol, which catalyzes the last step of α-tocopherol biosynthesis (Dwiyanti et al., 2011). During storage, the α-tocopherol concentration increased (**Fig. 5.4H**) over time, irrespective of light/dark treatment. Conversely, a substantial decline in γ-tocopherol occurred during the 16 day storage. However, there was no consistent difference between γ-tocopherol concentrations of light and dark treated samples (data not shown). Although there was no clear trend in γ-tocopherol concentrations, the γ-tocopherol concentration tended to decrease as the α-tocopherol increased and vice versa for most of the treatments and storage periods. In a previous study, Lester et al. (2010b) reported that continuous light exposure helped retain more α-tocopherol and γ-tocopherol in baby spinach during storage than did dark storage. In this case, photo-oxidative stress may have induced an increase in α-tocopherol (Porfirova et al., 2002).

5.4.4 Effect on Antioxidant Properties

There were no significant differences in TPC among package or light treatments over the storage period (**Fig. 5.5A**). A previous study on fresh-cut romaine lettuce showed that TPC was not significantly different among samples stored under light but dark storage tended to have higher level of TPC (Martínez-Sánchez et al., 2011). It is

well known that phenolic compounds are generally synthesized via the phenylpropanoid metabolic pathway, in which L-phenylalanine is converted into trans-cinnamic acid by the enzyme phenylalanine ammonia lyase (PAL, EC 4.3.1.5) and some other phenolic compounds are subsequently produced, such as chlorogenic acid (Martínez-Sánchez et al., 2011; Zhan et al., 2012). Phenolic compounds also act as important antioxidants and they can be oxidized to quinone during oxidative stress. Therefore, the TPC value assayed actually depends on the balance of synthesis and oxidation (Zhan et al., 2012).

DPPH radical scavenging capacity was not significantly affected by light exposure or storage duration during the 16 days they were held at 5°C (**Fig. 5.5B**). The trend was very similar to that of TPC described above, which may be due to the same antioxidant mechanism of electron transfer (ET) reaction. Differently, the HOSC assay is measuring the scavenging capacity of hydroxyl radical, in which hydrogen atom transfer (HAT) reaction is involved (Huang et al., 2005). Our data showed that the HOSC values of radish microgreens were significantly affected by different light and packaging film treatments (**Fig. 5.5C**). The HOSC of radish microgreens in all conditions underwent a substantial decline for the first 8 days of storage and a slight increase thereafter until the end of storage period. Compared to samples under darkness, those under light exposure had significantly lower HOSC values at each sampling day, irrespective of packaging film. In food system, antioxidants (such as vitamin C) may act as pro-oxidants by indirectly catalyzing the hydroxyl radical generation (Huang et al., 2005).Thus, as AA concentration increased (described above), more hydroxyl radicals may have been generated,
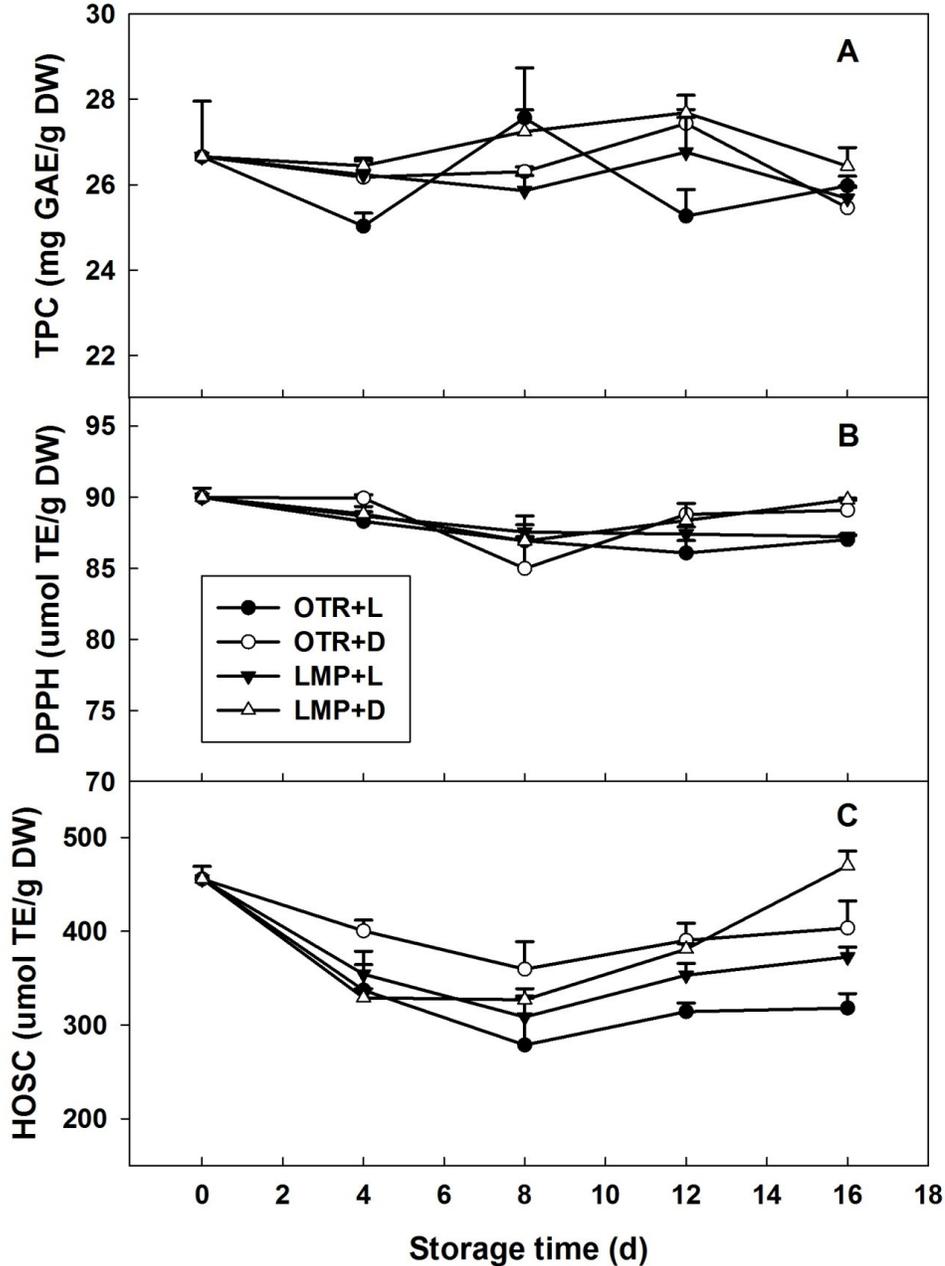
Fig. 5.5 Effect of light exposure on total phenolics, relative DPPH radical scavenging capacity (DPPH) and hydroxyl radical scavenging capacity (HOSC) of radish microgreens stored in oxygen transmission bags (OTR) and laser microperforated bags (LMP) at 5 °C for 16 days.

OTR+L, OTR+D, LMP+L and LMP+D represents radish microgreens in OTR bags subjected to light, in OTR bags subjected to dark, in LMP bags subjected to light, and in LMP bags subjected to dark, respectively. Values are means ± standard errors of three replicates.

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resulting in decreased HOSC antioxidant activity in light exposed samples. Studies

have shown that antioxidants such as phenolics may go through synthesis and

metabolism during storage (Kalt et al., 1999). The change in the bioactive compounds

profile may decrease or increase the overall antioxidant capacities of fruits and

vegetables (Kevers et al., 2007). Overall, the trend in the change of the antioxidant

capacities sheds light on the complexity of bioactive behavior in microgreens during

storage. Unfortunately this cannot be explained by the amount change of bioactive

compounds as determined in this study. A further study examining the metabolism of

individual bioactive compound in radish microgreens during storage may provide us

more understanding on these activities.

5.5 Conclusions

In conclusion, light exposure during storage contributed to the maintenance of a

higher concentration of ascorbic acid in daikon radish microgreens; but it accelerated

quality deterioration and weight loss during storage. Dark storage helped to preserve

the quality and prolong the shelf life of radish microgreens. Additionally, dark-stored

radish microgreens maintained higher levels of β-carotene, lutein/zeaxanthin and

HOSC antioxidant activity. No significant differences in α-tocopherol concentration,

total phenolics concentration and DPPH antioxidant capacity were found between

light and dark stored OTR and LMP packaged microgreens. These results showed

that postharvest environmental conditions need to be considered carefully in order to

maintain consumer acceptability, concentration of bioactive compounds and storage

life.

Chapter 6: Comparison of the Growth of *Escherichia coli* O157: H7 and O104: H4 during Sprouting and Microgreen Production from Contaminated Radish Seeds

6.1 Abstract

Radish sprouts and microgreens were produced using seeds inoculated with *Escherichia coli* O157: H7 and O104: H4. Sprouts were harvested after 5 days and microgreens harvested after 7 days, and E. coli populations on sprouts and microgreens were compared. Both *E. coli* O157:H7 and O104:H4 proliferated rapidly during sprouting, reaching contamination levels of 5.8 to 8.1 log cfu/g and 5.2 to 7.3 log cfu/g, respectively, depending on the initial inoculation of the seeds. In comparison, *E. coli* O157:H7 and O104:H4 populations on harvested microgreens ranged from 0.8 to 4.5 log cfu/g and from 0.6 to 4.0 log cfu/g, respectively, at corresponding seeds contamination levels. Although harvested microgreens carried significantly less ($P < 0.001$) *E. coli* than the corresponding sprouts, significant proliferation by *E. coli* O157:H7 and O104:H4 occurred during both sprouting and microgreen growth.

6.2 Introduction

As consumers' demand for healthy and convenient food increases, raw seed sprouts have gained popularity worldwide as they are perceived as healthier sources of carbohydrates, proteins, minerals, and vitamins (Martínez-Villaluenga et al., 2008). However, sprouts consumption has recently been implicated in several foodborne

illnesses outbreaks. An outbreak of enterohemorrhagic *Escherichia coli* (EHEC) O157: H7 which affected over 6000 people in Japan in 1996, was linked to the consumption of contaminated radish sprouts (Taormina et al., 1999). The recent Jimmy John's *Salmonella* outbreak associated with consumption of alfalfa sprouts in December 2010 sickened 88 people across 15 states in the United States. In 2011, sprouts from an organic farm in Germany were determined the sources of an outbreak of enteroaggregative E. coli (EAEC) O104:H4, which infected nearly 4000 people, and caused 53 deaths (Uphoff et al., 2013). These outbreaks have heightened consumer concerns to the safety of sprouts, and prompted many food retail/service establishments to institute policies restricting the availability of sprouts.

In recent years, consumer demands for microgreens have also grown rapidly. Microgreens are tender cotyledonary-leaf plants with cotyledonary leaves fully developed and the first pair of true leaves emerged or partially expanded. Compared to mature leafy produce, microgreens exhibit more vivid colors, intense flavors and tender textures. A recent study showed that microgreens generally contained higher concentrations of phytonutrients (such as α-tocopherol, β-carotene and ascorbic acid) than their mature-leaf counterparts (Xiao et al., 2012).

Unlike mature fresh produce, both sprouts and microgreens are grown in facilities that restrict the access of insects and wild animals, and minimize other factors of environmental contaminations. Contaminated seeds are generally the source in most sprout-related outbreaks (NACMCF, 1999), which would also likely be true for microgreens. For commercial production, sprouts typically germinate from seeds in rolling drums with high humidity and frequent watering. The conditions for sprouting

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are conducive of bacterial growth, and it has been reported that *E. coli* can exceed 7 log cfu/g during sprout production without negatively affecting the appearance of the sprouts (Taormina et al., 1999). In contrast, microgreens are grown hydroponically or in a shallow layer of soil/soil substitutes in green houses as real plants. To date, there is a lack of scientific information relative to the microbiological safety risks of microgreens. The primary objective of this work was to investigate the survival and proliferation of *E.coli* O157: H7 and O104: H4 on radish sprouts and microgreens cultured under laboratory conditions simulating commercial sprout and microgreen productions.

6.3 Materials and Methods

6.3.1 Bacterial strains and inoculum preparation

E. coli O157: H7 strain ATCC 43888 harboring a stable plasmid (pGFP) that encode for green fluorescence protein (GFP) and ampicillin-resistance (Fratamico et al., 1997), and strain ATCC 43895 were from EMFSL collections. E. coli O157:H7 strain EC415, which was isolated from spinach outbreak in 2006, was provided by Dr. M. Marmmel (FDA CFSAN, Laurel, MD). *E. coli* O104:H4 strain TW16133, which was isolated from Germany sprout outbreak in 2011, was obtained from Dr. Shannon D. Manning of Michigan State University (Al Safadi et al., 2012). Both ATCC 43895 and EC415 were transformed with pGFP extracted from ATCC 43888/pGFP. Plasmid stability of the transformed strains was evaluated by two consecutive overnight subculturings (approximately 60 generations) in the absence of selective antibiotic (Ampicillin) followed by plating on non-selective agar plates. All the colonies examined expressed GFP, indicating stable maintenance of the plasmid.

All strains were maintained at -80 °C in brain heart infusion broth (BHI, Difco, Detroit, MI, USA) with 20% glycerol.

Individual strains were re-activated on tryptic soy agar (TSA, Difco, USA) plate and single colonies were then inoculated into 20 tryptic soy broth (TSB, Difco, USA) containing 100 μg/mL Ampicillin and grown at 37 °C overnight with shaking at 200 rpm. Cells were harvested by centrifugation at $6000 \times g$ for 5 min at 4 °C and resuspended in sterile phosphate buffered saline (PBS) solution. Aliquots of cell suspensions were further diluted in sterile distilled (DI) water to obtain desired concentrations for inoculation. Cell suspensions of the three O157:H7 strains were combined as a cocktail for seed inoculation, and the O104:H4 strain was used separately for inoculation.

6.3.2 Seeds and inoculation

Daikon radish (*Raphanus sativus* var. *longipinnatus*) seeds were purchased from a commercial provider (Living Whole Foods, Springville, UT, USA) and stored at 4 °C in sealed plastic bags until use. For inoculation, radish seeds were visually inspected and those with visible defects purged. A portion of 100 g seeds were immersed in 200 mL of appropriate inoculum suspension with gentle swirling for 5 min at ambient temperature. To achieve targeted low and high levels of inoculation on seeds, the concentrations of inoculum suspensions were $10^2 \sim 10^3$ and $10^5 \sim 10^6$ cfu/mL, respectively. After draining, inoculated seeds were spread over sterile absorbent sheets and air-dried overnight under a laminar/ventilated flow biological safety hood. After drying, inoculated seeds were stored in refrigerator and used for sprout germination or microgreen planting with 48 hours. The targeted low level

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inoculation of radish seeds was 1 log cfu/g, and that of high level inoculation was 4 log cfu/g. Radish seeds with high inoculums density and un-inoculated seeds were also mixed at a ratio of 1:99 (w/w) to form sporadically inoculated seed batches. The same batches of seeds with specific inoculation levels were used for sprouting and growing microgreens.

6.3.3 Sprouting

Inoculated radish seeds (10 g) were placed in a sterile sprouting glass jar (source, location) and soaked in sterile DI water for 4 hours at ambient temperature. After draining, seeds were incubated at 25 °C with relative humidity of 70 ± 5% in the dark for 3 days. Sprouting jars were kept at an angle that ensured proper drainage during the incubation. Germinating seeds were rinsed with water twice daily. Radish sprouts were exposed to light on day 4 and harvested on day 5 as whole plants, including undeveloped leaf buds, stem and roots, for microbial enumeration.

6.3.4 Microgreen growth

Fafard Super Fine Germination Mix (Griffin Greenhouse & Nursery Supplies, Bridgeton, NJ, USA), a soil substitute commonly used by microgreen growers, was evenly spread in standard 1020 flat plastic culture trays (28 cm W x 54 cm L x 6 cm D, Growers Supply, Dyersville, IA, USA) to form a thin layer of approximately 2.5 cm in depth. The germination mix was moisturized with sterile water before seeding. Inoculated seeds (10 g) were evenly spread on top of the germination mix in each tray and incubated in a temperature-controlled growth chamber set at 25/18°C (day/night) with 12 hour photoperiods. During the first three days, trays were covered and seeds germinated in the dark. On day 4, the seedlings were exposed to white fluorescent

light (light irradiance = ~150 μmol s^{-1} m^{-2}, determined by LI-1000 datalogger, LI-

COR, Lincoln, NE, USA). Radish microgreens were daily irrigated to saturation

using an overhead sprayer. On the 7th day, microgreens were harvested by cutting

stems at 1 cm above the substratum surface with sterilized scissors. A 5-g subsample

of randomly selected microgreens from each tray (replicate) was collected for

microbial enumeration.

6.3.5 Enumeration of *E. coli*

E. coli populations on the seeds, sprouts and microgreens were enumerated

following a procedure from Luo and coworkers (Luo et al., 2004) with some

modifications. A combination of direct plating technique and most probable number

(MPN) method for enumeration was used in this study. In preliminary study, bacterial

enumeration data obtained using direct plating and MPN methods were compared and

no significant difference ($P < 0.05$) was found when identical samples were tested.

The results were also consistent with a previous report of Line and coworkers (Line et

al., 2001). Therefore, MPN method was used for complementing the direct plating

method, especially when the microbial population was lower than the detection limit

of direct plating. In this study, when the detection limit was equal or higher than 2.30

log cfu/g, direct plating method was used. When detection limit was lower than 2.30

log cfu/g, MPN method was used.

Five grams of seeds, aseptically harvested sprouts, or microgreens were

pummeled with 45 mL sterile PBS in filtered bags using a stomacher (Model 80,

Seward Medical, London, UK) for 2 min at high speed mode. Filtered solution was

used for microbial enumeration either by plating or by Most Probably Number (MPN)

procedure. For direct plating, 50 µLfiltrate or appropriate dilution was spiral plated on Sorbitol MacConkey agar (SMAC, Neogen Inc., Lansing, MI, USA) plates supplemented with 200 µg/mL ampicillin using a Whitley automatic spiral plater (Wasp II, Don Whitley Scientific Ltd., West Yorkshire, UK) and incubated at 37 °C overnight. Colonies of *E. coli* O157:H7 or O104:H4 were counted with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK) and reported as log cfu/g of sample. For the purpose of direct quantitative comparison, E. coli counts on sprouts and microgreens were also reported as log cfu per "gram seed equivalent" (gse), which represented cell counts on sprouts or microgreens germinated from 1 g of seed and was calculated by factoring in the average yields of sprouts or microgreens. MPN procedure (Luo et al., 2011) was used when the expected cell population was low (< 2.3 log cfu/g). Eight 3-mL aliquots of sample filtrate were 10-fold serially diluted using TSB supplemented with 100 µg/mL ampicillin in a deepwell microplate (5.0 mL x 48 wells) and incubated overnight at 37 °C. Subsequently, 2.5 µL of the enriched bacterial solution in each well was arrayed on SMAC plates with 200 mg/L ampicillin and incubation overnight 37 °C. Corresponding patches with green fluorescence were counted as positive for *E. coli* O157:H7, and red (sorbitol fermenting) patches counted as *E. coli* O104:H4. The *E. coli* population was calculated using an online MPN calculator (Curiale, 2004). The results were expressed as log cfu/g of sample.

6.3.6 Microbiological profile of growth media and seeds

The microbiological profiles of germination mix and un-inoculated radish seeds were determined using 3M™ Petrifilms (3M Inc., St. Paul, MN, USA). Germination

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mix (5 g) or radish seeds (5 g) was mixed with 45 mL PBS and stomached for 2 min as above. Aliquots of 1 mL filtrate or its dilution was placed on appropriate biofilms and incubated as follow. Aerobic mesophilic bacteria (AMB) were determined on 3M™ Petrifilm™ Aerobic Count Plates incubated at 37 °C for 24h; yeasts and molds (Y&M) were determined on 3M™ Petrifilm™ Yeast and Mold Count Plates incubated at 25 °C for 5d; Enterobacteriaceae (EB) counts were determined on 3M™ Petrifilm™ Enterobacteriaceae Count Plates incubated at 37 °C for 24h; and total coliforms (TC) were determined on 3M™ Petrifilm™ E. coli/Coliform Count Plates incubated at 37 °C for 24h. The filtrates of germination mix and radish seeds were also spread on SMAC containing 100 μg/mL of ampicillin to screen for the presence of bacteria that might form colonies indistinguishable from that of E. coli O104:H4. No such colonies were observed.

6.3.7 Statistical analysis

All the experiments were conducted in four replications. Reported data were expressed as the mean ± standard error (SE). Microbial data were log transformed. Univariate analysis of variance (ANOVA) was performed with SPSS 13.0 for Windows (SPSS Inc, Chicago, IL, USA). The statistical significance of the data was determined by performing Tukey's honestly significant difference (HSD) tests for post hoc multiple comparisons at an experiment-wise significance level of 0.05.

6.4 Results and Discussion

6.4.1 Microbiological profile of growth medium and seeds

Fig. 6.1 showed the microbial populations of germination mix and raw radish

seeds. The main microbiological group in germination mix was yeasts and molds at

5.7 log cfu/g and followed by total aerobic mesophilic bacteria at 3.7 log cfu/g.
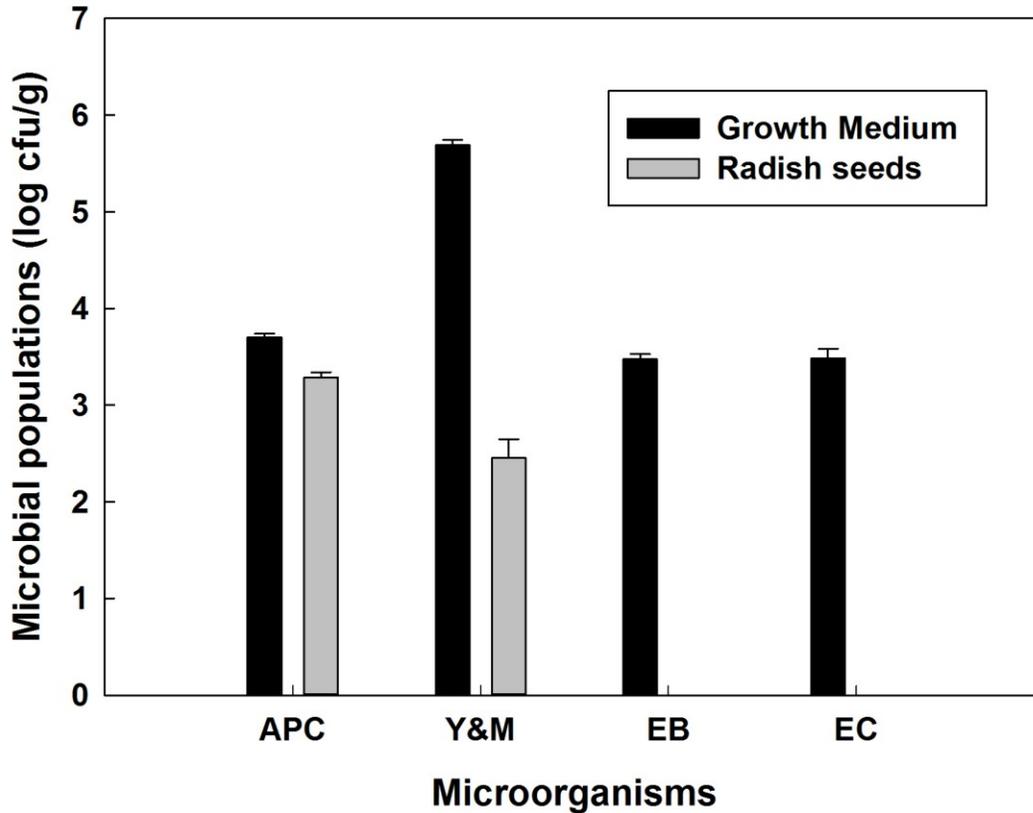
**Fig. 6.1 Populations of total aerobic plate counts (APC), yeast and mold (Y&M),
Enterobacteriaceae count (EB) and E. coli/Coliforms count (EC) on growth
medium and radish seeds.**

Vertical bars represent standard errors (n = 4).

Enterobacteriaceae counts in germination mix amounted to 3.5 log cfu/g, same as

total *E. coli*/Coliform, indicating Enterobacteriaceae in germination mix was

primarily composed of *E. coli*/Coliforms. The main component of germination mix is

Sphagnum peat moss which could inhibit bacterial growth due to the low pH, but had

a less effect on yeast and molds (Cocozza et al., 2003; Everett et al., 2013). This could explain why the yeasts and molds count was high and target pathogens were not found in the growth medium.

Indigenous microbial populations on raw radish seeds were mainly composed of aerobic mesophilic bacteria and yeast & mold, amounting to 3.3 and 2.5 log cfu/g, respectively. These results agree with previously published observations showing that seeds typically carried microbial loads ranging from 10^3 to 10^6 cfu/g (Kim et al., 2009; Robertson et al., 2002). Enterobacteriaceae or *E. coli*/Coliform bacteria were not detected on radish seeds.

6.4.2 *E. coli* O157: H7 growth on radish sprouts and microgreens

The survival and growth of *E. coli* O157: H7 on radish sprouts and microgreens produced from seeds with different inoculation levels were shown in **Fig. 6.2**. *E. coli* O157: H7 was not detected on non-inoculated radish seeds or the resultant sprouts and microgreens. By the end of 5-day sprouting, sprouts germinated from radish seeds inoculated at high level (4.6 log cfu/g) carried *E. coli* O157: H7 population of 8.1 log cfu/g (or 9.0 log cfu/gse). Those from radish seeds inoculated at low level (1.5 log cfu/g) carried a much lower *E. coli* O157:H7 population (7.6 log cfu/g or 8.5 log cfu/gse). For sporadically contaminated seeds (1% seed inoculated), *E. coli* O157: H7 populations on sprouts reached 5.8 log cfu/g (or 6.7 log cfu/gse). Although no attempt was made to quantify the growth of *E. coli* O157:H7 during sprouting, the above data indicated that *E. coli* O157:H7 was capable of growth by at least 3.2 to 5.1 logs. Previous studies showed that low levels of *Salmonella* species inoculated on alfalfa seeds increased by as much as 4 to 5 logs in the germinated sprouts (Andrews et al.,

1982) and *E. coli* O157: H7 inoculated alfalfa seeds increased by 2 to 4 logs in the

sprouts (Stewart et al., 2001). Prokopowich et al. (1991) previously reported that
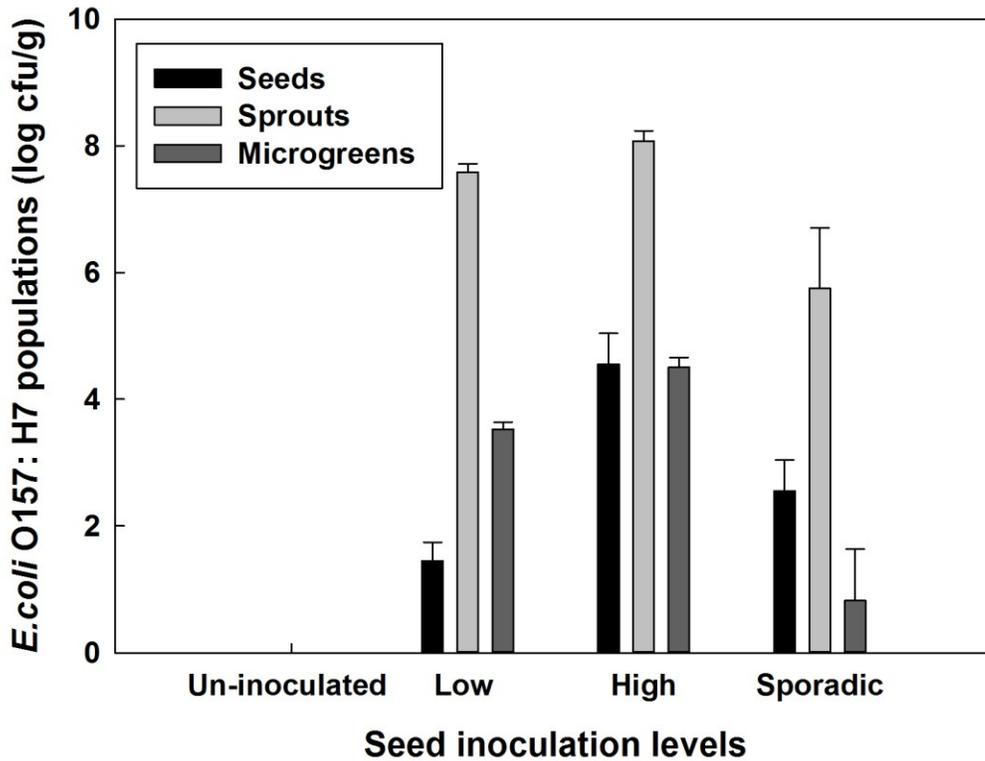
Fig. 6.2 Populations of *E. coli* O157: H7 on radish seeds, sprouts, and microgreens, produced from un-inoculated, low level, high level and sporadically inoculated seeds.

Sporadically contaminated seed treatment is a mix of 1% high level inoculated seeds with un-inoculated seeds. Vertical bars represent standard errors (n = 4).

microbial populations on sprouts obtained from retail stores reached as high as 10^9

cfu/g. In contrast, microgreens grown from similarly inoculated radish seeds carried

significantly lower ($P < 0.001$) population of *E. coli* O157: H7 cells. *E. coli* O157:H7

population reached 3.5, 4.5, and 0.8 log cfu/g on microgreens grown from low, high,

and sporadically inoculated radish seeds, respectively, which corresponded to 4.5, 5.5

and 1.8 log cfu/gse.

6.4.3 *E. coli* O104: H4 growth on radish sprouts and microgreens

The initial inoculation levels of *E. coli* O104: H4 on radish seeds were 4.3 log

cfu/g for high level, 0.8 log cfu/g for low level and 2.3 log cfu/g for sporadic

inoculation which is a mix of 1% high level inoculated seeds with un-inoculated

seeds. Un-inoculated seeds were also carried out as control, with no target bacteria

detected in either sprouts or microgreens. As with *E. coli* O157:H7, the populations of
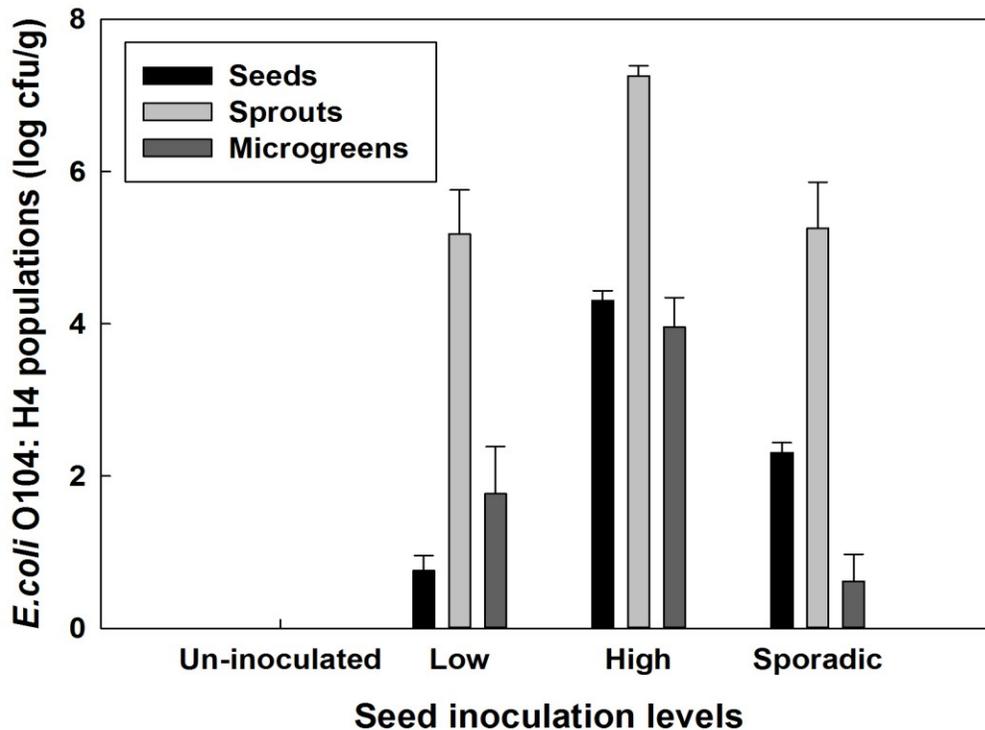
E. coli

Fig. 6.3 Populations of *E. coli* O104: H4 on radish seeds, sprouts, and microgreens, produced from un-inoculated, low level, high level and sporadically inoculated seeds.

Sporadically contaminated seed treatment is a mix of 1% high level inoculated seeds with un-inoculated seeds. Vertical bars represent standard errors (n = 4).

O104: H4 on radish sprouts were significantly ($P < 0.001$) higher (3.3 to 4.6 logs) than those on the seeds, indicating significant proliferation during seed germination and sprout growth. *E. coli* O104:H4 populations on sprouts reached 5.2, 7.3 and 5.3 log cfu/g, or 6.1, 8.2 and 6.2 log cfu/gse, respectively, for those germinated from low, high, and sporadically inoculated radish seeds. Disregarding *E. coli* O104:H4 cells removed with daily rinsing, these results represented a proliferation of *E. coli* O104:H4 during sprouting by 2.9 - 4.4 logs from seeds inoculated at different levels.

On the other hand, the proliferation of *E. coli* O104:H4 on the edible part of microgreens was less significant. *E. coli* O104:H4 populations on harvested microgreens reached 1.8, 4.0 and 0.6 log cfu/g, or 2.8, 5.0, and 1.6 log cfu/gse, respectively, for seeds inoculated at low level, high level, and those inoculated sporadically. These would represent proliferation of *E. coli* O104:H4 by -0.6, 0.0 and 1.0 logs on the microgreens. However, since it was likely that a large fraction of the proliferations by *E. coli* O104:H4 was not counted for by analyzing the harvested microgreen tissues, the actual proliferation by *E. coli* O104:H4 was likely more significant. The low proliferation of *E. coli* O104:H4 on microgreens germinated from sporadically inoculated seeds could be due to un-uniform distribution of bacteria and sampling of microgreens.

Overall, both *E. coli* O157:H7 and O104: H4 on inoculated radish seeds significantly proliferated during sprouting, and to a lesser extent, during the germination and growth of microgreens, regardless of the initial inoculation levels. At the same seed contamination level, cell counts of *E. coli* O157:H7 and O104:H4 on harvested microgreens tend to be 3-4 logs lower than those on sprouts. The higher

growth of *E. coli* on sprouts could be primarily due to the frequent rinsing and mixing, which greatly promote the dispersion and redistribution of bacterial cells on various parts of sprouts. Other factors unique to sprouting, such as high humidity and darkness, could also contribute to the higher growth of bacterial pathogens on sprouts. Another evident explanation for the lower bacterial cell counts on microgreens is the fact that only a part of the plants is harvested, leaving bacterial cells on seed coats, roots, and lower stems, and in growth substrata unaccounted.

Since neither sprouts nor microgreens generally involve a kill step for bacterial inactivation before consumption, preventing seed contamination is the key to ensuring the safety of these products. Current FDA guidelines require effective antimicrobial seed treatments for sprouting. However, most of the available seed treatments fail to completely inactivate bacteria cells attached to various seeds used for sprouting (Chyer et al., 2003; Weissinger & Beuchat, 2000). Data presented here indicated that significant proliferation by both *E. coli* O157:H7 and O104:H4 occurred during sprouting at low levels of seed contamination.

6.5 Conclusions

In conclusion, *E. coli* populations on harvested radish microgreens were 3-4 logs lower than that on sprouts produced from seeds with same contamination levels. In our laboratory study, microgreens seemed to present relatively low food safety risks in comparison to sprouts germinated from seeds with the same contamination levels; however, significant proliferation of bacterial pathogens occurs during microgreen growth. Therefore, it is of great importance to minimize bacterial contamination of seeds for sprouts and microgreens.

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Chapter 7: Conclusions and Future Work

7.1 Conclusions

This research project is the first comprehensive study the new specialty food product: microgreens. The main research findings are as follows:

- Microgreens provide attractive appearance, tender texture and intense flavor, and serve as excellent sources of healthful nutrients.

- Microgreens are generally more nutrient-dense than their mature counterparts, compared with the records in USDA National Nutrient Database.

- All microgreens evaluated in our study demonstrated "good" to "excellent" consumer acceptance and nutritional profile.

- Overall acceptability of microgreens was strongly correlated to flavor acceptability.

- The quality maintenance and shelf life extension of radish microgreens could be achieved by using low temperature and modified atmosphere packaging.

- Light exposure speeded up transpiration, thus accelerated quality deterioration and increased weight loss of radish microgreens during postharvest storage.

- During postharvest storage, transportation and retail of radish microgreens, light exposure should be avoided as it hasten senescence attributes.

- *E. coli* populations on radish microgreens were 3-4 logs lower than that on sprouts produced from seeds with same contamination levels.

- Compared to sprouts, microgreens seem to bear relatively lower safety risks.

7.2 Future Work

Several research directions arise from our research findings in this dissertation.

7.2.1 Chemical, Enzymatic and Molecular Analysis of Microgreens.

It is our intention to investigate thoroughly the underlying mechanism of the observation that microgreens are more nutrient-dense than mature plants. The starting point will be nutrient biosynthesis during growth and gene expression. Taking ascorbic acid as an example, as reported in the literatures (Dan et al., 1996), ascorbic acid is synthesized from L-galactono-1, 4-lactone by L-galactono-γ-lactone dehydrogenase (GLDH; EC 1.3.2.3); therefore, enzymatic and molecular analysis of plants at different growth stages could provide us the information on the changes in enzyme activity and gene expression occurred during seed germination and plant growth.

7.2.2 Ready-to-eat Microgreens Versus Living Microgreens

Our quality study on microgreens has shown us the optimal condition for postharvest storage, however, wash treatment did not work well on developing ready-to-eat microgreens products. To date, there is no ready-to-eat microgreens available in supermarkets, therefore, it is necessary to develop ready-to-eat microgreens with good quality, long shelf life and ensured safety. Our aim is to develop and explore appropriate wash and dry processes, well-performed packaging materials and effective sanitizers to minimize the safety risk of microgreens consumption. As seen in some restaurants and grocery stores, microgreens are sold as living forms in containers with growth medium in it. It seems like the living microgreens look fresher and have longer shelf life. Is it true? As living plants, microgreens need

adequate light exposure to maintain photosynthesis, otherwise, yellowing and wilting could happen. During transportation and storage, living microgreens may undergo a long period of dark time, which may have an impact on sensorial and nutritional quality. It will be of interest to carry out a comparative postharvest study between ready-to-eat microgreens and living microgreens.

7.2.3 Microbiological Safety Study of Microgreens

Based on our research finding on the comparison of *E. coli* growth on sprouts and microgreens, it can be seen that microgreens seem to present a lower food safety risk. Therefore, the mechanisms of bacterial distribution, attachment and interaction with the microgreens plants could be further investigated. As reported, specific virulence genes are required for bacterial attachment to plant tissues (Barak et al., 2005); therefore, molecular genetic analysis would help to explore the interaction of pathogens and microgreens. In addition, microscopy strategy, such as confocal scanning laser microscopy, can be used to observe the morphology of pathogen and plant cells and interaction between them.

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