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Post-harvest physiology of microgreens

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ABSTRACT

Microgreens are seven- to ten-day-old seedlings of various vegetable crops that are packaged as young shoots, including both cotyledons and hypocotyls. To improve the marketability of this highly perishable product, shelf life must be extended by controlling respiration rates. Little information is available describing the post-harvest characteristics of this high value specialty crop, and its respiration rates have not yet been carefully quantified. The present study aims to investigate the respiration rates and shelf life of microgreens. Three crop species were evaluated in this study: arugula (*Eruca sativa*), radish (*Raphanus sativus*), and red cabbage (*Brassica oleracea* var. *rubra*). Respiration rates (estimated by CO_2 evolution) were measured at harvest and in storage for a total of three weeks using an infrared gas analyzer. Product quality was evaluated visually each week. Based on visual analysis, shelf life averaged fourteen days for arugula and red cabbage, and twenty-one days for radish when stored at 4°C. However, shelf life was reduced to seven days when stored at 10°C for red cabbage and arugula, while it was reduced to fourteen days for radish. Red cabbage and arugula had the highest respiration rate at harvest, which also corresponded to a decrease in visual quality. Beginning in week two there was a spike in respiration rates, possibly due to decay organisms, which also corresponded to a decrease in the visual quality rating of the crop. This study aims to provide a benchmark data set for future microgreens post-harvest experiments designed to lower respiration rates and increase shelf life. Increased shelf life for this specialty crop will allow for transportation to larger markets.

INTRODUCTION

Microgreens are seven-to-ten day old individual plant seedlings of various species, recently developed as specialty cut vegetables. Consumed as a garnish and in salads, microgreens consist of only the fleshy cotyledons and the hypocotyls. Microgreens are increasingly popular and have potential to attract a significant portion of a $500 million sprouts market (Brentlinger, 2007). However, their use is limited by an extremely short shelf life of approximately one week, depending on the species. This perishability impedes further market growth and export of the crop. In contrast, "sprouts" (e.g. alfalfa and bean) are typically harvested with the roots intact, which allows for a longer shelf life. This study focuses on the post-harvest characteristics of microgreens. A thorough understanding of the crops' physiological responses after harvesting will allow for the development of optimal shipping and storage protocols.

The limited shelf life of perishable goods such as cut vegetables limits their longevity in the market due to rapid post-harvest degradation (Artes *et al*., 2007). Shelf life is often dictated and controlled by the crop's respiration rate, usually quantified as carbon dioxide (CO_2) evolution per unit of time and unit of fresh weight (e.g. $\mu g\ CO_2\ g^{-1} \cdot h^{-1}$). While mature plants are photosynthetically and metabolically active, seedlings such as microgreens predominantly respire during the germination process. Cotyledon cells in the "seed leaves" metabolize stored carbohydrates and do so until carbohydrate resources are depleted and the seedling is fully matured (Chrispeels & Boulter, 1975). As carbohydrate sources are depleted, the cotyledons wither. Since microgreens are harvested at the cotyledon stage, this process can result in degradation of the primary product.

Slower respiration, which can be achieved by lowering temperatures, directly correlates with a lower rate of cellular metabolism. This modification of plant metabolic activity has been reported for most fruits and vegetables (Song *et al*., 1992; Loaiza & Cantwell, 1997), including cut greens, but not for microgreens. Slower metabolic rates induced by lowered temperatures also directly impact visual quality and can help extend shelf life. An adjunct technique used with lower temperature storage is the use of modified gas environments, both on the small scale (modified air packaging) and large scale (controlled atmosphere) storage (Song *et al*., 1992). Beyond physiological data collection, shelf life can also be quantified. However, visual quality is crucial, because it indicates the physical status of the microgreens and influences sales (Rennie *et al*., 2001).

Due to their relatively recent agricultural development, there is currently little information available specifically regarding microgreens. However, the post-harvest physiology of similar crops such as cut salad greens has previously been studied. Cantwell *et al*. (1998) measured respiration rate of various cultivars of specialty leafy greens and correlated this information to leaf size, visual quality, and temperature. Respiration rates of chopped or whole leaves were also measured. In addition to the visual quality rating the study also reported the marketable percentage, defined as the proportion of the product volume that remained saleable. These protocols provide a basis for the current microgreens study because quality was quantified on a numerical scale.

In addition to storage conditions, product cooling can also have an impact on post-harvest storage. Rennie *et al*. (2001)

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investigated the feasibility of vacuum coolers by applying different pressure reduction rates for air evacuation, and quantified their effect on lettuce quality. The authors measured quality with an established index. There was no statistical difference between the fast, medium, and slow-cooled lettuce heads. The quality index is a rapid and informational analysis technique for evaluating marketability and visual appeal. In addition, it can be correlated with respiration rates, which are more time consuming to quantify.

Sprouts have similar characteristics to microgreens, but are generally younger and harvested with the roots intact. DeEll *et al*. (2000) performed a study on stored mung bean sprouts (*Vigna radiata*). They examined the qualitative effects of vacuum cooling and storage temperature, and found that vacuum-cooled greens had a higher freshness rating than non-vacuum-cooled greens. Although cotyledon color was the same, the hypocotyl color rating was significantly higher in both vacuum cooled greens. Similar to Rennie *et al*. (2001), the authors found that vacuum-cooling could be a rapid and low impact post-harvest treatment for extending the shelf life of sprouts and cut greens, when treatment is followed by appropriate storage temperatures.

Lee *et al*. (2005) conducted a study that detected changes in the respiration, growth, and vitamin C content of soybean sprouts (*Glycine max*) in response to different molecular weights (low, medium, and high kDa) of the food preservative chitosan. Results showed that higher chitosan molecular weights caused increased respiration rates as measured by increased oxygen consumption. However, chitosan aided in maintaining the Vitamin C and chlorophyll content in the sprouts, both desirable characteristics for this crop. The soybean sprouts used in this study are different from microgreens, because microgreens do not have a radicle and are a cut product. Moreover, the growth improvement characteristics of chitosan, in addition to its anti-microbial properties, may aid in delaying post-harvest decay organisms in cut microgreens.

Couture *et al*. (1993) quantified physiological and quality attributes and storage life of minimally processed lettuce heads. Eight lettuce cultivated varieties (cultivars) were evaluated after storage at 0^{o}C for 24 hours. A subjective visual quality evaluation was performed as well as a quantitative evaluation of browning intensity based on spectrophotometric analysis. Additionally, different storage conditions were investigated: ambient air (0.2% CO_2) or ethylene (5 µL*L). The visual quality of different plants varied widely after six days under ethylene exposure. Browning intensity also differed among cultivars. This study is important because it shows that cultivars within a species can have different physiological responses.

Loaiza and Cantwell (1997) studied the post-harvest physiology of cilantro (*Coriandrum sativum*) in traditional cold storage as well as controlled atmosphere (CA) storage. The study quantified visual quality including color, odor pungency, respiration rates, and ethylene production. Higher temperatures correlated with increased respiration rates and decreased ethylene production. In addition, the visual quality decreased at higher temperatures, while the pungency level increased. The present study employs both a qualitative visual and odor evaluation scale, and quantitative evaluation of respiration rates to fully characterize the responses of the crop.

The present study reports the post-harvest respiration rates of microgreens at optimal storage temperature (4^{o}C) and non-optimal storage conditions (10^{o}C) over a three week period in conjunction with a visual quality evaluation system developed specifically for these crops.

MATERIALS AND METHODS

Crops and cultural protocols

Three species commercially used as microgreens, radish (*Raphanus sativa*), arugula (*Eruca sativa*), and red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), were selected. Post-harvest storage problems in these three species include premature degradation of the crop, leading to shorter than average shelf life (less than seven days), discoloration (not green), foul odor and rotting (Cross, 2008; Table 1).

Score	Description	Visual Quality
5	Essentially free from defects, freshly harvested - No profound visible defects	Excellent
4	Minor defects, not objectionable - Some (<10%) physical damage (i.e. creased cotyledons) -Product is turgid (not wilted)	Good
3	Moderately objectionable defects, marketability threshold -Slight chlorosis (yellowing) -Areas of dry and wilted microgreens (<25%)	Fair
2	Excessive defects, not saleable -Discolored hypocotyls (blue, black) -Cotyledon chlorosis (>25%) -Dry and wilted (>50%)	Poor
1	Unusable, degraded product -100% chlorotic -Mold present, foul odor -Extensive rooting -Physical degradation apparent (liquid present)	Very poor

Table 1. Microgreens qualitative evaluation scale based on overall visual analysis (modified from Rennie *et al.*, 2001).

Seeds were obtained from a commercial, organic microgreens seed source (Seeds of Change; Spicer, MN). The greens were produced according to commercial protocols in the industry (Cross, 2008; Franks & Richards, 2009), and also according to organic production standards (NMOCC, 2011). All crops were grown in slotted trays (20, 2.5cmx25cm slots in a 28cm x 54.5cm tray) filled with organic soil-less potting mix (Sun Gro Sunshine Organic Sphagnum Blend, SunGro

Horticulture Canada Ltd.). Seed of each species was weighed before planting: 1.4g of radish, 1.2 g of red cabbage, and 0.053g of arugula seed was used per slot. Seeds were coated with 4.9 mg of a competitive bacterial inoculant (Micro 108®, Natural Industries Inc., Houston, TX) in 950 ml of water at the first irrigation to reduce soil borne pest pressure. Pure tap water with no inoculant was used thereafter. Seeds were sown in each slot by evenly distributing them on the soil surface. Immediately after sowing, trays were watered and covered with an inverted slotted tray for 48 hours to keep humidity levels high. To ensure sufficient illumination, trays were placed 38 cm under fluorescent lighting units (two, 122 cm long 40 watt cool white fluorescent bulbs) that were illuminated 24 hours a day until harvest. The crop was kept moist until harvest, and no fertilizer was applied during crop growth.

A marketable product for all species requires a hypocotyl length of 2.5 to 5 cm (Cross, 2008). Hence, the crops were harvested by cutting at the media surface after seven days of growth for radish, nine days for arugula, and eleven days for red cabbage. After harvest, greens were fan-dried to remove surface moisture without wilting (approximately 8-10 min). Radish was fan-dried at room temperature (22°C). Arugula and red cabbage were dried in a refrigerator at 4°C, also with the aid of a fan to speed drying. Thirty six grams of surface-dried microgreens were packed into standard clam-shell packages (454g shallow hinged deli containers, AD16S, Genpak®, Greens Falls, NY) that were modified with the addition of four 3mm holes in the lid for ventilation. Packaged microgreens were immediately placed in a walk-in cooler at either 4°C or 10°C in the dark.

Respiration rate

Microgreens were placed in two temperature conditions, 4°C and 10°C, provided by a walk-in cooler. Each temperature condition contained all three species replicated four times in a completely randomized design. Randomization was simple and samples were re-randomized at each sampling date. For testing, sub-samples of 3.6g of whole microgreens were removed from each package. Respiration rate was measured using a modified conifer chamber and a portable gas exchange system equipped with an infrared gas analyzer (IRGA)(LI-6400XT, LiCor Inc., Lincoln, NE). An additional package of microgreens was used to calibrate the LiCor system. During readings, a large chamber was used (6400-05 Conifer Chamber, LiCor Inc.) and was modified by wrapping the IRGA head with a layer of aluminum foil to completely exclude light. Measurements were made at harvest (week 0) and every seven days for at least three weeks (weeks 1, 2, and 3), or until microgreens were physically degraded. Physical degradation was defined as moldy and/or discolored greens with a foul odor.

LiCor system parameters

For each temperature treatment and reading date, the LiCor unit and user were moved into the walk-in cooler and allowed to acclimate to the environment. The flow rate was set to 300 $\mu mol/mol^{-1}$, leaf area was set to 36 cm^2, CO_2 reference was maintained at 400 $\mu mol/mol^{-1}$ using a CO_2 injector and scrubber, and relative humidity was set to 80%.

Visual quality evaluation

Visual quality evaluations were made at the same time as respiration rate measurements each week. The evaluation system was modified from Rennie *et al*. (2001) and was based on the physical condition of the microgreens. The rating scale ranged from 1 (poor) to 5 (excellent). A marketability threshold was set at 3 on the visual quality scale. A rating of less than three designated an unmarketable product (Table 1).

Statistical analysis

Statistical analysis was performed using SAS (Statistical Analysis Software version 9.2, 2009, Cary, NC). Treatment means were separated using the least significant difference (LSD) with a P value of <0.05.

RESULTS

Respiration rates

Mean respiration rates were significantly lower at 4°C than at 10°C for all three crops (Figures 1-3). At harvest (week 0), radish respiration rates averaged 110µg CO_2 per gram fresh weight each hour (µg CO_2 $g^{-1}•h^{-1}$) at 4°C and 255 at 10°C; one week later mean respiration rates were 84µg and 141µg at 4°C and 10°C, respectively (Figure 1). At harvest, arugula respiration rates averaged 99µg CO_2 $g^{-1}•h^{-1}$ at 4°C and 201µg at 10°C; one week later mean respiration rates were 84µg and 158µg at 4°C and 10°C, respectively (Figure 2). At harvest, red cabbage respiration rates averaged 111µg CO_2 $g^{-1}•h^{-1}$ at 4°C and 186µg at 10°C at harvest; one week later mean respiration rates were 38µg and 143µg at 4°C and 10°C, respectively. Beginning in week two and three there was a spike in respiration rates at 10°C, particularly in the red cabbage (Figure 3).

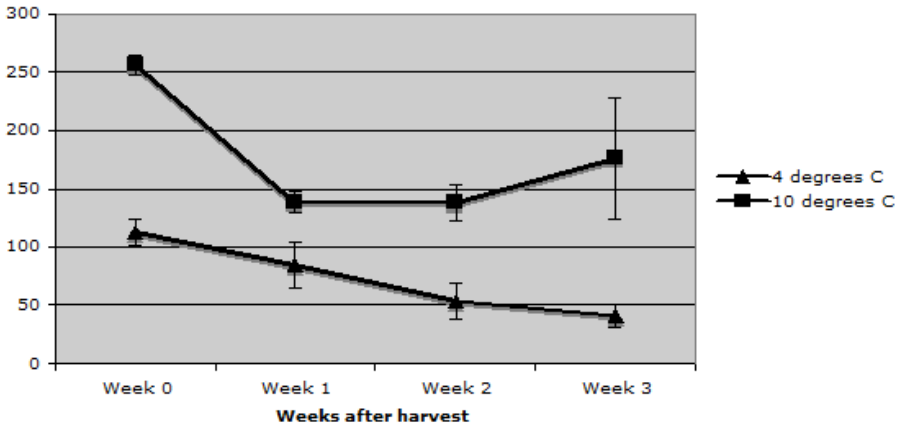

Figure 1: Radish microgreens respiration rates at two storage temperatures from harvest (week 0) until product degradation (week 3).

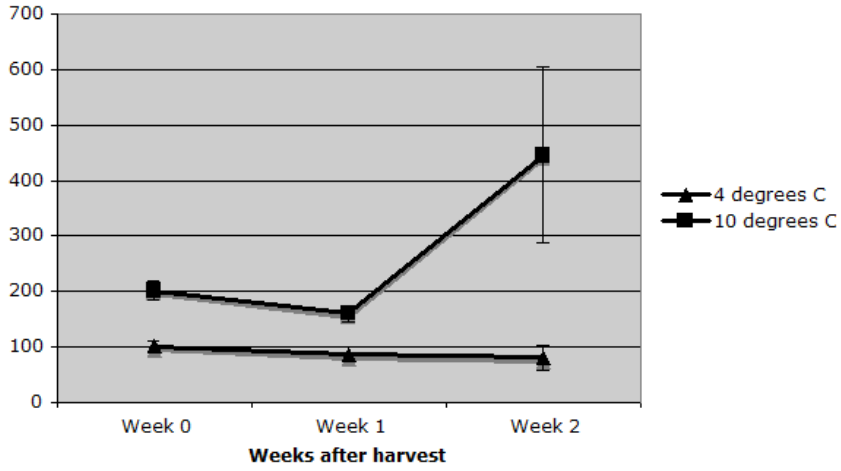

Figure 2: Arugula microgreens respiration rates at two storage temperatures from harvest (week 0) until product degradation (week 2).

Visual quality rating

Based on visual analysis, shelf life averaged two weeks for arugula and red cabbage, and three weeks for radish when stored at the control temperature of 4°C (Figures 4-6). However, shelf life was reduced to only one week when stored at 10°C for arugula and red cabbage. Shelf life was reduced to two weeks for radish stored at 10°C. There was an inverse relationship between the visual analysis and respiration rate. This was more pronounced at 10°C versus 4°C. One week after harvest, the respiration rate of arugula increased dramatically and visual quality dropped below a rating of three. This drop in visual quality manifested as yellowing cotyledons and development of decay odors. Red cabbage stored at 10°C also passed below the visual quality threshold one week after harvest, and was therefore unmarketable. Physical degradation characteristics for red cabbage included a slight decay odor and a bluish coloration to the hypocotyls. Radish, on the other hand, generally had a slower respiration rate and therefore had a longer shelf life, up to three weeks when stored at 4°C.

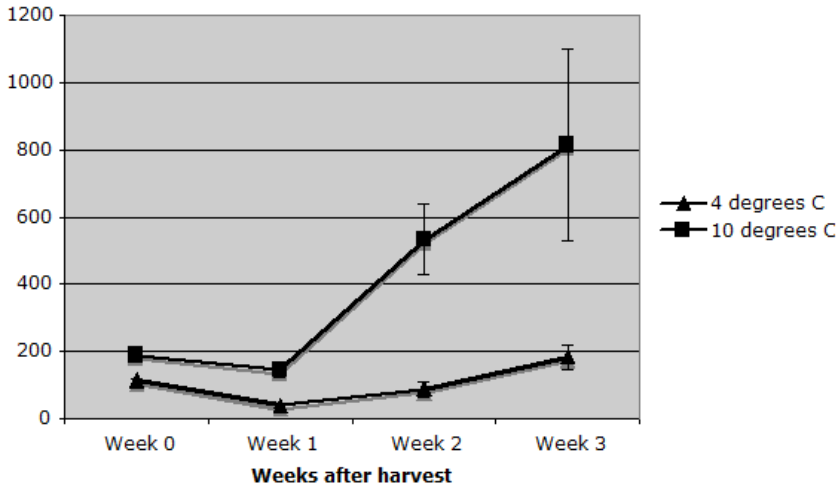

Figure 3: Red cabbage microgreens respiration rates at two storage temperatures from harvest (week 0) until product degradation (week 3).

DISCUSSION

The overall goal of this study was to observe how different temperatures affect the respiration rates and visual quality of microgreens. A comparative study was performed to evaluate the effect of temperature on post-harvest shelf life of three crop species. These three species (radish, arugula, and red cabbage) are commercially used in microgreens production, but were identified by a collaborating producer as having particularly short shelf lives relative to other common microgreens species (Cross, 2008).

Effects of temperature on respiration rates

In this study, higher storage temperatures correlated with higher respiration rates. There were also differences in shelf life and respiration rate between the three species. Radish maintained a relatively constant respiration rate while visual quality was maintained (a rating of more than three) for more than three weeks. At 10°C, arugula had a reduced shelf life of only one week. For each crop, higher temperature induced higher respiration rate. There may have been other factors influencing the respiration rate such as microbial activity and decay organisms, particularly at week two and three. Microgreens are considered a "raw agricultural commodity" and are typically not washed or surface disinfested prior to packaging (Cross, 2008). Future studies should address this as a contributor to post-harvest degradation by culturing samples for "native" populations or including a surface disinfested control.

Visual quality evaluation

A more rapid decline in visual quality corresponded to increased respiration rate, demonstrating an inverse relationship. All crops had the same initial visual rating of five out of five. For all species, visual quality was higher at each evaluation date when stored at 4°C than 10°C.

Overall, radish had lower respiration rates and higher visual quality ratings even at 10°C, resulting in a longer shelf life than the other two crops. Red cabbage, on the other hand, demonstrated the lowest visual quality and highest respiration rate resulting in a shorter shelf life (approximately seven days) at 10°C. This is surprising since red cabbage is a *Brassica* (Brassicaceae), as are the other two species in this study, and typically has a long shelf life as a horticulturally mature vegetable crop. The red pigment associated with red cabbage (anthocyanin) degraded more rapidly than the chlorophyll associated with the other two species, disproportionately and negatively affecting visual quality ratings of this crop. This characteristic may impact species selection for export markets.

CONCLUSION

This study quantifies for the first time baseline respiration rates for three species of specialty cut greens, or microgreens. A modified visual quality evaluation protocol was developed, which establishes product characteristics and marketability threshold for future studies. Additional studies could focus on the post-harvest life of plant metabolites such as vitamin C and antioxidants. Quantification of these human health-promoting components of the crop would complement the shelf life data.

In addition, this study identifies arugula and red cabbage as subjects of further study due to their high respiration rates and particularly short shelf lives. Additional consideration should be given to the control of biotic factors that can contribute to premature post-harvest degradation. Once identified, these may be controlled with additional product drying, disinfestation, or modified atmosphere packaging. More storage temperatures should be examined as well as quantification of other degradation gases such as ethylene. In addition, more frequent sampling dates (every two to three days) would allow for more precise quantification of shelf life.

In the present study, shelf life may have been influenced by the age of the seedlings at harvest. For example, radish was harvested at seven days after planting; arugula nine days; and red cabbage eleven days according to industry standards and to achieve marketable hypocotyl length. Each time a given crop was grown, it was harvested at the same interval. Future studies should investigate the role of seedling age on post-harvest shelf life.

A thorough understanding of microgreens and their storage characteristics is crucial for extending their shelf life and for expanding these products into larger markets. This data provides insight towards extending their shelf life, and will add to the knowledge base about the management and processing of this new specialty crop.

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