Quality characteristics of the radish grown under reduced atmospheric pressure

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Abstract

This study addresses whether reduced atmospheric pressure (hypobaria) affects the quality traits of radish grown under such environments. Radish (*Raphanus sativus* L. cv. Cherry Bomb Hybrid II) plants were grown hydroponically in specially designed hypobaric plant growth chambers at three atmospheric pressures; 33, 66, and 96 kPa (control). Oxygen and carbon dioxide partial pressures were maintained constant at 21 and 0.12 kPa, respectively. Plants were harvested at 21 days after planting, with aerial shoots and swollen hypocotyls (edible portion of the radish referred to as the "root" hereafter) separated immediately upon removal from the chambers. Samples were subsequently evaluated for their sensory characteristics (color, taste, overall appearance, and texture), taste-determining factors (glucosinolate and soluble carbohydrate content and myrosinase activity), proximate nutrients (protein, dietary fiber, and carbohydrate) and potential health benefit attributes (antioxidant capacity). In roots of control plants, concentrations of glucosinolate, total soluble sugar, and nitrate, as well as myrosinase activity and total antioxidant capacity (measured as $ORAC_{FL}$), were 2.9, 20, 5.1, 9.4, and 1.9 times greater than the amount in leaves, respectively. There was no significant difference in total antioxidant capacity, sensory characteristics, carbohydrate composition, or proximate nutrient content among the three pressure treatments. However, glucosinolate content in the root and nitrate concentration in the leaf declined as the atmospheric pressure decreased, suggesting perturbation to some nitrogen-related metabolism.
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1. Introduction

There is no atmosphere on the Moon, and the atmospheric pressure on Mars is approximately 0.7 kPa, which is less than 1/100 that at sea level on Earth (i.e., 101 kPa). Clearly, protected, pressurized habitats are needed to support humans and associated life support systems, which might include plants. There are several reasons, however, to consider reducing the atmospheric pressure to levels less than 101 kPa for associated plant-based bioregenerative life support systems. Reduced pressures could decrease the need for more rigid structures, thereby reducing launch costs. Furthermore, low pressures would reduce gas leakage and thereby reduce resupply costs. Additionally, if the concept of using inflatable, transparent greenhouses proves feasible, new types of acceptable transparent materials will need to be developed which could lead to innovations transferable to the global

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greenhouse industry (Wheeler and Martin-Brennan, 1999; Clawson et al., 2005).

Terrestrial organisms, including higher plants, have evolved and acclimated to Earth's atmospheric pressure of 101 kPa. The effects of reduced pressure on plant growth and development is largely unknown. Studies on plant responses to reduced pressure were reviewed by Corey et al. (2002) and Richards et al. (2006). Collectively, a handful of plant species (e.g., lettuce, tomato, wheat, turnip, barley, mungbean, *Arabidopsis*) has been studied under a range of total pressures using either fixed or variable partial pressures of O_2 and CO_2. Results from these studies suggest that most plants are capable of growing vegetatively at pressures down to 25 kPa, and other species may be able to tolerate pressures as low as 10 kPa (Massimino and Andre, 1999; Goto et al., 2002). Oxygen partial pressures and pO_2/pCO_2 ratios also appear to be important when growing plants under reduced pressure, especially for processes requiring higher metabolic rates, such as seed germination, flowering, and pollination (Schwartzkopf and Mancinelli, 1991). As such, the concomitant reduction of oxygen partial pressure down to the level of 5–10 kPa with the reduction of total pressure often enhances plant growth. This typically occurs through the reduction of the energy-inefficient process of photorespiration. Further reduction of oxygen to below 5 kPa can lead to anoxia which will inhibit germination and seedling growth. Partial pressures of CO_2 can be another complicating factor in hypobaric studies with plants. There are conflicting findings as to the photosynthetic responses to hypobaria. It is well known that photosynthetic rates are dependent on the absolute supply of CO_2 substrate (Drake et al., 1997), but photosynthetic rates of wheat stands increased at reduced pressures and appeared to be dependent on the pO_2/pCO_2 ratio, even though pCO_2 was maintained at a level of 0.12 kPa, well above that considered non-limiting with regard to photosynthetic processes (Corey et al., 1997). On the other hand, no change in the photosynthetic rate of *Arabidopsis* was observed in response to a range of pO_2 from 2.1 to 21 kPa at non-limiting CO_2 of 0.07–0.10 kPa (Richards et al., 2006). Clearly more study is needed to systematically parse the effect of different essential gas components on whole plant performance and photosynthesis.

There is also a lack of studies on how atmospheric pressure affects plants at the molecular level, which could lead to the better understanding of plant adaptation to hypobaria (Paul et al., 2004) and subsequently to the engineering of hypobaria or anoxia-tolerated plants. Likewise, little attention has been paid to the potential impact of hypobaria on the biochemical consequences and nutritional quality of plants. This is of great importance because plants are expected not only to fulfill bioregenerative functions (production of oxygen, removal of CO_2, and purification of water), but also provide palatable and nutritional diet supplements to the human crews. A series of experiments designed to investigate the effect of reduced total pressure at fixed O_2 and CO_2 partial pressures was initiated. The specific objectives of this study were to determine the effects of reduced total pressure alone on sensory acceptability (color, taste, and texture), taste-influencing factors, nutritional and antinutritional factors, as well as potential health benefit factors.

2. Materials and methods

2.1. Test species

Vegetables such as green onion, lettuce, radish, tomato, and pepper have been considered for both short term (e.g., ISS, CEV) and long term planetary based habitats because of their low requirements for post harvest processing and rapid production of fresh, flavorful foods to supplement the space menus (Goins et al., 2003). Many tests have been performed under the Earth atmospheric pressure; however, their performance under reduced pressures is largely unknown. For this study, radish was selected because of its rapid growth cycle, favorable harvest index, and phytonutrients content. The findings of this study will not only have direct application to space exploration, but also contribute to the understanding of source and sink physiology.

2.2. Environmental conditions

Tests were performed in the hypobaric chamber at the Controlled Environment Systems Research Facility (CES-RF) at the University of Guelph, Ontario, Canada. Three atmospheric pressures were used in these tests, i.e., 33 kPa, 66 kPa, and a control of 96 kPa. A slight negative pressure of 96 kPa served as the control in order to maintain chamber seal integrity and to minimize the leak rate. Two total pressures of 33 and 66 kPa were selected to approximate the conditions that could be anticipated for a human-rated plant growth facility since these were the pressures that human crews have worked in for sustained periods during Skylab and during pre-breath episodes prior to space walks during Shuttle missions. Regardless of the pressure treatment, O_2 and CO_2 partial pressures were controlled to 21 and 0.12 kPa, respectively. These are equivalent to 21% O_2 and 1200 ppm CO_2 at 101 kPa (1 atm) total pressure. Other environmental conditions included 22 °C air temperature, 65% relative humidity, and 300 $\mu mol\ m^{-2}\ s^{-1}$ light intensity with a 16 h photoperiod. A 3×3 (treatment × chamber) Latin Square Design was employed. All three treatments were carried out simultaneously with one day offset in three hypobaric chambers, and each pressure treatment was repeated three times in time.

2.3. Horticulture

Radish (*Raphanus sativus* L. cv Cherry Bomb Hybrid II) was grown hydroponically under three atmospheric pressures (33, 66, and 96 kPa). Radish seeds were sown in

140×40 cm stainless steel irrigation trays using Rockwool as a substrate with a final planting density of 48 plants per m². After germination and establishment for 3 days under ambient pressure, plants were thinned to one plant per hole. Chambers were subsequently closed, pressure and gas composition were brought to the set points and maintained for the rest of the experimental duration. Hydroponic nutrient solution consisted of a modified half-strength Hoagland's automatically maintained at 1200 μS cm^{-1} electrical conductivity through additions of a concentrated stock solution, and pH maintained at 5.8 with additions of 0.4 M HNO₃(Wheeler et al., 1999). At 21 days after planting (DAP), all plants were harvested 2 h into the photoperiod for all experimental replicates of three pressure treatments. Shoots and swollen hypocotyls (referred as roots hereafter) were separated and frozen in liquid nitrogen. Half of the materials were freeze-dried, ground to pass through a 40 mesh screen and stored over desiccant at -80 °C until analysis.

2.4. Sensory evaluation

At harvest, 10 plants from each treatment were collected and shipped on ice to Johnson Space Center, Houston, TX, USA. They were evaluated within 24 h of harvest for their color, taste, texture, and overall appearance by trained panelists at Johnson Space Center's Food Quality Laboratory.

2.5. Determination of glucosinolate (GS)

To determine glucosinolate content, 1 ml 70% (v/v) hot methanol and 20 μl of 3 mg ml^{-1} sinigrin monohydrate were added to 30 mg of lyophilized ground plant tissue in a 2 ml screw-cap vial. The extraction mixture was vigorously mixed and incubated at 70 °C for 30 min in an Eppendorf Thermomixer (Brinkmann Instrument, Inc., Westbury, NY) set at 1000 rpm. At the end of the incubation, the mixture was centrifuged for 10 min at 3000 rpm and the supernatant was transferred to a clean vial. The extraction was repeated twice with 1 ml solvent/extraction. The combined extract was partially purified using a strong anion exchange resin, desulphonated, and analyzed using reverse-phase HPLC coupled with APCI-tandem mass spectrometer as described by Musgrave et al. (2005). GS concentration was expressed as sinigrin equivalents.

2.6. Myrosinase activity

Myrosinase activity was determined by following the general procedure described by Al-Turki and Dick (2003). Frozen tissue was blended with deionized water using a 1:9 (w/v) ratio in a Warring blender for 1 min, and then centrifuged for 10 min at 3000 rpm. Two reactions were established for each sample (with or without sinigrin). Radish extract supernatant (100 μl) was transferred to a clean 1.5 ml tube containing 50 μl of 1 M potassium

phosphate buffer, pH 6.0. The negative control also contained 450 μl of deionized water, while the positive reaction contained 100 μl of 45 mM sinigrin and 350 μl of deionized water. Each reaction was incubated for 1 h at 37 °C. The amount of glucose released from sinigrin through myrosinase activity was measured with a glucose (HK) assay kit (Sigma-Aldrich, St. Louis, MO, USA).

2.7. Soluble carbohydrate

Lyophilized and ground tissue (15–20 mg) was extracted three times with 3 ml of 80% ethanol in an 80 °C water bath. All three extracts were combined and made up to a total volume of 10 ml with 80% ethanol. One milliliter of the extract was transferred to a vortex-evaporation tube, from which the solvent was evaporated to dryness under vacuum at 40 °C. The dried extract was reconstituted in 5 ml of deionized water. The solution was filtered through a 0.45 μm pore size filter into an HPLC auto-sampler vial and analyzed using a DX-500 chromatography system (Dionex Corp., Sunnyvale, CA). Soluble carbohydrates were separated on a CarboPac (PA-10) column using 52 mM carbonate free sodium hydroxide at 1.3 ml min^{-1} and selectively detected by a pulsed amperometric detector (Dionex Technical Note 20, 1989).

2.8. Tissue nitrate level

Lyophilized and ground plant material (ca. 20 mg) was quantitatively extracted with deionized water (two replicates per sample). The extract was filtered through a 0.45 μm pore size membrane filter, diluted to an appropriate concentration range, and analyzed using ion chromatography (Dionex Corp., Sunnyvale, CA, USA) coupled with suppressed conductivity detection.

2.9. Proximate analysis

Edible tissues ("roots") harvested for biometric data were dried in a convection oven set at 70 °C and ground to pass through a 40 mesh screen. Ground root tissue from each replicate ($\sim$100 g) was sent to a commercial laboratory (Eurofins Scientific Inc., Des Moines, IA, USA) for proximate analysis. Standard AOAC procedures were followed for protein by nitrogen combustion method (AOAC 992.23), crude fiber by digestion technique (AOAC method 962.09), and carbohydrates by calculation.

2.10. Total antioxidant capacity

Total antioxidant capacity of both radish root and leaf was measured by an Oxygen Radical Absorbance Capacity (ORAC$_{FL}$) assay based on changes in decay rate of a fluorescence probe when exposed to a proxyl radical in the presence of sample extracts or antioxidant (Ou et al., 2001). Sample (0.5 g) was weighed into a 50 ml conical tube and 10 ml of extraction solvent (70% acetone, 5% acetic

acid in distilled water) was added to the tube. The sample was vortexed then centrifuged for 10 min at 3000 rpm. The supernatant was transferred to a 25 ml volumetric flask. The extraction was repeated with an additional 10 ml of extraction solvent; after centrifugation the supernatant was added to the volumetric flask. The volumetric flask was filled to volume with extraction solvent and stored at 4 °C until analysis. Immediately before ORAC$_{FL}$ assay, the sample was brought to room temperature and diluted 200-fold with 75 mM phosphate buffer, pH 7.4.

3. Results and discussion

3.1. Effect of hypobaria on radish growth

Fig. 1 shows the radish plants (21 DAP) at harvest grown under 33, 66, and 96 kPa. Plants were equally healthy and had similar development across all treatments.

Fig. 1. Representative pictures of plants grown under three atmospheric pressures at the time of harvest (21 days after planting).

Decreased pressures caused a slight increase of shoot biomass (Fig. 1), but there was no significant effect of atmospheric pressure on total edible yield (storage root). A respective 32% and 8% increase in biomass of lettuce and wheat at 50 kPa compared to ambient pressure was also observed by He et al. (2003). However, the large increase was probably attributed to the concomitantly reduced oxygen level. As expected from related testing, evapotranspiration increased as the pressure decreased (Daunicht and Brinkjans, 1996). Canopy photosynthesis (CO_2 uptake) also increased slightly at both reduced pressures, and dark period respiration rate increased slightly for 33-pKa grown plants (data not shown). Previous studies have reported either increases (Corey et al., 1997) or no change in net photosynthesis in at reduced pressures (Iwabuchi and Kurata, 2003), which is likely related to the range of test conditions used (e.g., different combinations of pCO_2 and pO_2). Further details of whole plant growth and canopy gas exchange rates are beyond the scope of this paper and will be reported in a separate manuscript.

3.2. Effect of hypobaria on radish sensory characteristics

Sensory characteristics such as color, overall appearance, taste, and texture greatly affect consumer's perception of vegetable quality, and are strongly influenced by temperature and irradiation (Schreiner et al., 2002). Radish produced from this experiment was evaluated for these attributes by trained panelists. Results showed no difference in any of the four attributes examined among the radishes grown under hypobaria compared to the control (Table 1). Consistent color may also suggest no changes in the photosynthesis of color-rendering anthocyanins in the radish periderm. However, one must bear in mind that the values in Table 1 are somewhat subjective. Thus, parameters influencing the taste were further quantified.

3.3. Effect of hypobaria on taste determining characteristics

Glucosinolate (GS) is thought to be the key determinants to radish's characteristic taste and aftertaste attributes often regarded as pungent and burning sensation (Widell et al., 1998). Moreover, they have both positive and negative implications in human health. Monosaccharides such as glucose and fructose also influence the aftertaste attributes. Therefore, these two classes of compounds were analyzed to provide more objective assessment to the taste of the radishes produced under the hypobaric conditions of this study.

GS is nitrogen- and sulfur-containing plant secondary metabolites composed of a β-thioglucose residue, a sulfonated oxime, and a side chain typically derived from an amino acid. These compounds are mainly found in plants of the order *Capparales* and coexist with myrosinase (thioglucosidase, EC 3.2.3.1), a glucosinolate-hydrolyzing enzyme. Cruciferous vegetables fall within this order, and disruption of crucifer tissues brings myrosinase and GS

Table 1
Sensory analysis of radish grown under 33, 66, or 96 kPa atmospheric pressures (NS: not significant)

Atmospheric pressure (kPa)	Appearance	Color	Taste	Texture
33	5.7 ± 1.8	3.1 ± 0.7	5.6 ± 1.8	2.5 ± 0.7
66	5.3 ± 2.0	3.0 ± 0.8	5.4 ± 1.8	2.6 ± 0.8
96	6.0 ± 1.4	2.8 ± 0.7	5.4 ± 1.8	2.7 ± 0.8
	NS[a]	NS	NS	NS

[a] NS stands for not statistically significant at $p \leqslant 0.05$, and apply to other tables.

into contact, liberating a glucose molecule and an unstable intermediate that can degrade into a variety of compounds (e.g., isothiocyanate, oxazolidine-2-thiones, nitrile, thiocyanate). It is the GS hydrolysis products that give crucifers their characteristic smell and flavor. Meanwhile, the hydrolysis products from some GS (e.g., 2-hydroxyl-3-butenyl and 3-indolylmethyl GS) have goitrogenic action in animal and humans, and hence are considered anti-nutritive compounds, while the isothiocyanate derivatives from other GS have anticarcinogenic potential (Selmar, 1999; Mithen, 2001), and are considered as phytonutrients. Sulforaphane, the isothiocyanate derivative of 4-methylsulfinylbutyl glucosinolate, is a potent anticarcinogen by functioning as a phase 2 enzyme inducer. In addition to sulforaphane, the isothiocyanate derivatives of 4-methylsulfinyl propyl and 4-methylthiobutyl GS are also phase 2 enzyme inducers, but only 10% as effective as sulforaphane (Zhang et al., 1992). An investigation of 109 radish cultivars (Carlson et al., 1985) found that intact radish roots primarily contain 4-methylthio-3-butenyl GS (accounting for 60–90% of the total GS) with small amounts of 4-methylsulfinylbutyl, 4-methylsulfinyl-3-butenyl, 3-indolymethyl GS.

Since radish contains both beneficial and potentially toxic GS in small quantities, it is of concern that altering the environmental conditions under which the plant was grown (i.e., reduced atmospheric pressure) may alter GS composition and alter the balance between beneficial and toxic GS. Previous studies have shown that growth conditions (e.g., temperature, irradiance, and deposition) can modify the proportions of the principal GS. Broccoli seedlings contained significantly greater levels of GS when grown under a 30 °C day/15 °C night temperature regime as compared to either a 22/15 °C or an 18/12 °C regime (Pereira et al., 2002). Concentration of the total and aliphatic GS in leaves of a rapid-cycling *Brassica oleracea* (Charron and Sams, 2004) were dramatically elevated in the high and low temperature treatments, but decreased in roots. Water stress during seed development in *B. napus* var. *oleifera* resulted in increases of up to 60% in overall seed GS concentration (Champolivier and Merrien, 1996). Ciska et al. (2000) also found that GS levels were significantly increased in 11 field-grown crucifers in a warmer, drier season than in the cooler, wetter one.

To our knowledge, this is the first study to examine the effect of growth under reduced atmospheric pressure on GS composition in radish plants. Analysis of both the shoot and root of the radish grown under 33, 66, and 95 kPa total

pressures indicated that 4-methylthio-3-butenyl GS remains to be the predominant GS regardless of the pressure treatment (Fig. 2). GS concentration in the root tissue was greater than in the leaf tissue, and appeared to decline as atmospheric pressure decreases (Table 2). Apparent differences within the leaf tissue were not statistically significant. In roots, the difference was statistically significant, but only when the values from third experimental replicate were eliminated because they were obviously outliers. All other minor glucosinolates were below the quantification limits, and therefore are not reported here. The activity of myrosinase paralleled glucosinolate, being much higher in root than in leaf, and not altered by reduced pressure.

Soluble carbohydrate content in both leaf and root is shown in Fig. 3. Glucose, fructose, and sucrose were the major components as expected, and their concentrations in roots were 27, 32, and 1.6 times that found in the leaf tissue. The ratio of glucose, fructose, and sucrose in the leaf was approximately 1:1:1, while that in root was 20:20:1. There was no significant difference in any of the soluble sugars among pressure treatments. The proportions of these sugars could influence vegetable quality to a great
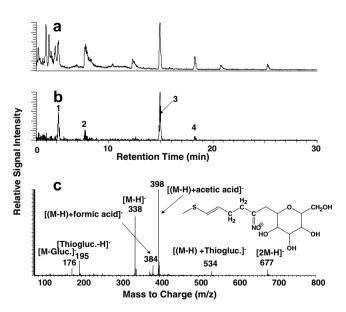

Fig. 2. Glucosinolate profile of radish extracts. (a) HPLC-APCI(-) total ion chromatogram; (b) chromatogram of an extracted ion (*m/z* 195) characteristic of glucosinolates indicating presence of four glucosinolates where peak #1 is the internal standard, sinigrin; (c) normal mass spectrum of component #3 confirming its identity as 4-methylthio-3-butenyl glucosionlate. Its desulfonated structure is shown in the inset.

Table 2
Effect of reduced atmospheric pressure on the concentration of 4-methylthio-3-butenyl GS (sinigrin equivalent µmol g^{-1} dry tissue) and myrosinase activity

Atmospheric pressure (kPa)	GS (µmol g^{-1} dry tissue)		Myrosinase activity (mg glucose g^{-1} fresh tissue)	
	Leaf	Root	Leaf	Root
33	0.8 ± 0.1	2.4 ± 0.2 (2.5 ± 0.2)[a]	0.8 ± 0.2	5.4 ± 2.1
66	0.8 ± 0.2	2.9 ± 0.6 (3.2 ± 0.2)[a]	0.6 ± 0.3	5.3 ± 2.2
96	1.1 ± 0.5	3.1 ± 0.8 (3.6 ± 0.3)[a]	0.6 ± 0.2	5.8 ± 1.2
	NS	NS	NS	NS

Values represent the mean of six independent analyses (two analytical replicates × three experimental replicates) ± the standard deviation.
[a] Average of two experimental replicates (i.e., the third experimental replicate was eliminated from the data set), resulting in statistically significant difference.
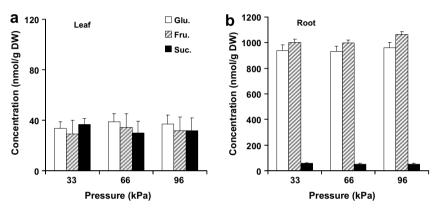

Fig. 3. Effect of hypobaria on the carbohydrate content of radish leaves and roots. Error bars represent the standard deviation of six independent analyses (two analytical replicates × three experimental replicates).

extent since fructose is 1.8 times sweeter than sucrose (Doty, 1976), and sucrose is about 1.7 times sweeter than glucose (Yamaguchi et al., 1970). Radish is not normally perceived as sweet compared to typical fruits due to its relatively low soluble sugar content. For example, radish has only one thousandth of the glucose and fructose content of the field grown strawberry (*Fragaria × ananassa* Duch) (Wang and Bunce, 2004). Nevertheless, no difference in the proportions of these sugars among the pressure treatments confirmed that there was no detectable impact of reduced pressure on the sensory characteristics of the radishes grown in this study or carbohydrate metabolism.

3.4. Effect of hypobaria on antioxidation capacity

It is well established that a diet high in fruits and vegetables is associated with a reduced risk of oxidative stress mediated diseases such as cancer, cardiovascular, and neurodegenerative diseases (Fang et al., 2002). The health beneficial effects of fruits and vegetables are attributed to their high levels of a wide range of phytochemicals serving as antioxidants to combat reactive oxygen species (ROS). Radish is rich in the antioxidants vitamin C and folate, and its red skin contains anthocyanin, a powerful antioxidant. To assess whether reduced pressure environments affect these phytonutrients, antioxidant capacity of the hydrophilic extracts of radish was determined using the modified oxygen radical absorbance

capacity (ORAC$_{FL}$) assay. This assay measures the scavenging capacity of antioxidants in samples against the peroxyl radical (ROO$^•$), one of the most common reactive oxygen species (ROS) found in the body. The assay mimics reactions of antioxidants with lipids in a physiological system and has been adopted by the USDA as a standardize test to measure antioxidant potency of foods and nutritional supplements.

ORAC$_{FL}$ values were reported here as Trolox Equivalents (TE) in Fig. 4. Trolox is a water-soluble analog of vitamin E. Root extracts had 220 µmol TE g^{-1} dry weight (or 11 µmol TE g^{-1} fresh weight), which is much higher than that in leaf extract (120 µmol TE g^{-1} dry weight). No differences were found in ORAC$_{FL}$ values in roots or shoots among the three pressure treatments. The ORAC$_{FL}$ value in roots in this study was slightly higher than that of commercial radish (9.28 µmol g^{-1} FW), greater than tomato (3.13 µmol) or iceberg lettuce (4.18 µmol), and much lower than strawberry (35.41 µmol), blueberry (61.84 µmol) or raspberry (47.65 µmol) as reported by Wu et al. (2004). Although only the hydrophilic extracts were analyzed, it was previously reported that the ORAC$_{FL}$ value for the lipophilic extracts of radish is insignificant and represents only 2.8% of that in the hydrophilic extract (Wu et al., 2004). The results indicate that reduced pressure down to 1/3 of the Earth sea level atmospheric pressure during radish production does not influence its antioxidant capacity as a whole.
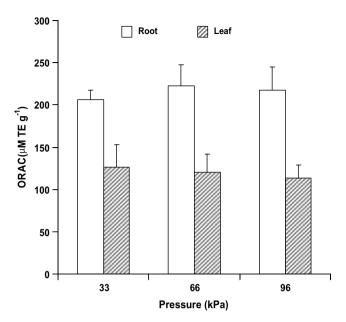
Fig. 4. Effect of hypobaria on $ORAC_{FL}$ as an antioxidant capacity indicator in both root and leaf tissue. Error bars represent the standard deviation of six independent analyses.

Fig. 5. Effect of hypobaria on nitrate level in root and leaf.

3.5. Effect of hypobaria on proximate nutrients and antinutritive factors

One of the nutritional benefits of radish is its high concentration of complex carbohydrates and dietary fiber. Protein, crude fiber, total dietary fiber, and carbohydrate contents of oven dried radish root sample are presented in Table 3. None of these parameters was affected by the reduced atmospheric pressures under which plants were grown.

Nitrate content was determined because of its significant roles in plant physiology and human health. Consistent with findings in other vegetables (Maynard et al., 1976; Levine et al., 2005), nitrate levels in radish leaf were greater than that in the root regardless of the treatment (Fig. 5). Leaf nitrate concentration was 3.9, 4.5, and 5.1 times the amount in the root for 33, 66, and 96-kPa grown plants, respectively. Although there was no difference in root nitrate levels between the pressure treatments, the leaf tissues derived from 33 and 66 kPa plants had significantly lower nitrate content than in the control 96 kPa plants ($p = 0.05$). Even though radish leaves are not typically eaten, this result is interesting because it may be consistent with other leafy vegetable crops and would be important
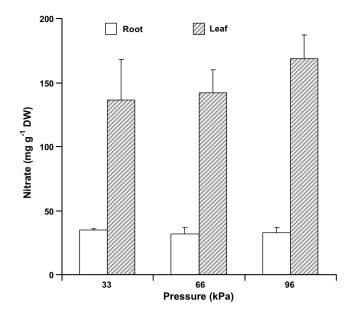
from a food quality standpoint. This is because the ingestion of vegetables with high nitrate levels presents a potential risk to human health due to the formation of nitrite (Wright and Davison, 1964; Craddock, 1983). In this experiment, plants grown under reduced atmospheric pressure had higher transpiration rates (Stutte et al., 2006), therefore the nitrate uptake via transpiration stream would be likely increased. Based on this premise, lower nitrate content in hypobaria-grown plant shoots may suggest altered activity of either nitrate transporters or nitrate reductase or both. Interestingly, reduced nitrogen content in leaves but not roots was also found in a study of turnip (*Brassica rapa* L.) under 51 kPa total pressure and 21 kPa oxygen partial pressure (Mansell et al., 1968).

4. Concluding remarks

The results indicate that reduced atmospheric pressures down to one third of the Earth's sea level pressure while maintaining oxygen partial pressure at 21 kPa had little effect on sensory perception of radish quality, or on the quantitative quality traits as well as potential health benefits. A slight difference in nitrate levels in leaves and glucosinolates in roots suggest altered metabolism in adaptation to reduced pressure or the consequence of the adaptation in reaching new homeostasis. Altered metabolism has also

Table 3
General nutritive content in the radish root following growth under 33, 66, or 96 kPa total pressure

Pressure (kPa)	% Protein	% Crude fiber	% Total dietary fiber	% Carbohydrate
33	23.4 ± 1.4	11.3 ± 1.5	28.7 ± 3.6	48.9 ± 2.8
66	23.8 ± 4.0	11.0 ± 1.8	29.6 ± 3.7	47.4 ± 4.8
96	24.2 ± 3.2	11.0 ± 2.0	29.5 ± 4.0	48.6 ± 5.9
	NS	NS	NS	NS

Values represent gram per 100 g dry tissue.

been suggested by He et al. (2003) who found that the rate of ethylene production by both lettuce and wheat was reduced under hypobaria conditions and that the hypobaric effect on reduced ethylene production was greater than that of just hypoxia (low oxygen).

Many previous studies of hypobaric effects on plants were carried by uniformly reducing all the major constituent gases, hence O_2 partial pressures also dropped with total pressure. This study is unique in that the hypobaria chamber facility at CESRF allows the precise control of both total pressure and gas composition. It is unclear whether the traits observed in this study with a constant pO_2 would be similar at reduced pO_2 levels (e.g., 14 and 7 kPa O_2). Follow up experiments systematically employing reducing pressure alone or reducing pO_2 and total pressure simultaneously are needed to delineate between the effects of hypobaria and hypoxia, which are equally important for planning future bioregenerative life support systems for space.

Acknowledgements

References

Al-Turki, A.I., Dick, W.A. Myrosinase activity in soil. Soil Sci. Soc. Am. J. 67, 139–145, 2003.

AOAC. Official Methods of Analysis, 17th ed Association of Official Analytical Chemists, Washington DC, 2000.

Carlson, D.G., Daxenbichler, M.E., VanEtten, C.H., Hill, C.B., Williams, P.H. Glucosinolates in radish cultivars. J. Am. Soc. Hort. Sci. 110 (5), 634–638, 1985.

Champolivier, L., Merrien, A. Effects of water stress applied at different growth stages to *Brassica napus* L. var. *oleifera* on yield, yield components and seed quality. Eur. J. Agron. 5, 153–160, 1996.

Charron, C.S., Sams, C.E. Glucosinolate content and myrosinase activity in rapid-cycling *Brassica olearacea* grown in a controlled environment. J. Am. Soc. Hort. Sci. 129 (3), 321–330, 2004.

Ciska, E., Martyniak-Przybyszewska, B., Kozlowska, H. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. J. Agric. Food Chem. 48, 2862–2867, 2000.

Clawson, J.M., Hoehn, A., Wheeler, R.M. Inflatable transparent structures for Mars greenhouse applications. SAE Technical Paper 2005-01-2846, 2005.

Corey, K.A., Barta, D.J., Henninger, D.L. Photosynthesis and respiration of a wheat stand at reduced pressure atmospheric pressure and reduced oxygen. Adv. Space Res. 20 (10), 1869–1877, 1997.

Corey, K.A., Barta, D.J., Wheeler, R.M. Toward Martian agriculture: response of plants to hypobaria. Life Support Biosph. Sci. 8, 103–114, 2002.

Craddock, V.M. Nitrosamines and human cancer: proof of an association? Nature 306, 638, 1983.

Daunicht, H.J., Brinkjans, H.J. Plant responses to reduced air pressure: advanced techniques and results. Adv. Space Res. 18, 273–281, 1996.

Dionex Corp., Technical Note 20, Analysis of carbohydrate by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD), 1989.

Doty, T.E. Fructose sweetness: a new dimension. Cereal Food World 21, 62–63, 1976.

Drake, B.G., Gonzalez-Mele, M.A., Long, S.P. More efficient plants: a consequence of rising atmospheric CO_2? Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 609–639, 1997.

Fang, Y., Yang, S., Wu, G. Free radicals, antioxidants, and nutrition. Nutrition 18, 872–879, 2002.

Goins, G.D., Yorio, N.C., Stutte, G.W., Wheeler, R.M., Sager, J.C. Baseline environmental testing of candidate salad crops with horticultural approaches and constraints typical of spaceflight. SAE Technical Paper 2003-01-2481, 2003.

Goto, E., Arai, Y., Omasa, K. Growth and development of higher plants under hypobaric conditions. SAE Technical Paper 2002-01-2439, 2002.

He, C., Davies Jr., F.T., Lacey, R.E., Drew, M.C., Brown, D.L. Effect of hypobaric conditions on ethylene evolution and growth of lettuce and wheat. J. Plant Physiol. 160, 1341–1350, 2003.

Iwabuchi, K., Kurata, K. Short-term and long-term effects of low total pressure on gas exchange rates of spinach. Adv. Space Res. 31 (1), 241–244, 2003.

Levine, L.H., Bauer, J., Edney, S., Richards, J., Yorio, N., Li, K., Pare, P., Wheeler, R. Scallion (*Allium fistulosum* L.) chemistry affected by variety and environmental conditions (light and CO_2). Paper #2005-01-2770, in: Proceedings of the 35th International Conferences on Environmental Systems and 8th European Symposium on Space Environmental Control Systems, July 11–14, Rome, Italy, 2005.

Mansell, R.L., Rose, G.W., Richardson, B., Miller, R.L. Effects of prolonged reduced pressure on the growth and nitrogen content of turnip (*Brassica rapa* L.). Tech. Rep. 68-100. Brooks AFB, TX: U.S.A.F. School of Aerospace Medicine, 1968.

Massimino, D., Andre, M. Growth of wheat under one tenth of the atmospheric pressure. Adv. Space Res. 24 (3), 293–296, 1999.

Maynard, D.N., Barker, A.V., Minotti, P.L., Peck, N.H. Nitrate accumulation in vegetables. Adv. Agron. 28, 71–118, 1976.

Mithen, R. Glucosinolates – biochemistry, genetics, and biological activity. Plant Growth Regul. 34, 91–103, 2001.

Musgrave, M.E., Kuang, A., Tuominen, L.K., Levine, L.H., Morrow, B.C. Seed storage reserves and glucosinolates in *Brassica rapa* L. grown on the International Space Station. J. Am. Hort. Sci. 130 (6), 848–856, 2005.

Ou, B., Hampsch-Woodill, M., Prior, R.L. Development and validation of oxygen radical absorbance activity using fluorescein as the fluorescent probe. J. Agric. Food Chem. 49, 4619–4626, 2001.

Paul, A.-L., Schuerger, A.C., Popp, M.P., Richards, J.T., Manak, M.S., Ferl, R.J. Hypobaric biology: *Arabidopsis* gene expression at low atmospheric pressure. Plant Physiol. 134, 215–223, 2004.

Pereira, F.M., Rosa, E., Fahey, J.W., Stephenson, K.K., Carvalho, R., Aires, A. Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleraca* Var. *italica*) sprouts and their effect on the induction of mammalian phase 2 enzymes. J. Agric. Food Chem. 50, 6239–6244, 2002.

Richards, J.L., Corey, K.A., Paul, A.L., Ferl, R.J., Wheeler, R.M., Schuerger, A.C. Exposure of *Arabidopsis thaliana* to hypobaria environmentals: implications for low-pressure bioregenerative life support systems for human exploration missions and terraforming on Mars. Astrobiology 6 (6), 851–866, 2006.

Schreiner, M., Huyskens-Keil, S., Peters, P., Schonhof, I., Krumbein, A., Widell, S. Seasonal climate effects on root colour and compounds of red radish. J. Sci. Food Agric. 82, 1325–1333, 2002.

Schwartzkopf, S.H., Mancinelli, R.L. Germination and growth of wheat in simulated Martian atmospheres. Acta Astronaut. 25 (4), 245–247, 1991.

Selmar, D. Biosynthesis of cyanogenic glycosides, glucosinolates and nonprotein amino acids, in: Wink, Michael (Ed.), Annu. Plant Rev.: Biochem. Plant Secondary Metab., Vol. 2. CRC Press, Boca Raton, FL, pp. 123–124, 1999.

Stutte,G., Yorio, N.C., Richards, J.T., Edney, S.L., Sisko, M.D., Wheeler, R.M., Stasiak, M.A., Dixon, M.A. Habitation 2006 abstract # HLS 123, 2006.

Wang, S.Y., Bunce, J.A. Elevated carbon dioxide affects fruit flavor in field-grown strawberries (*Fragaria × ananassa* Duch). J. Sci. Food Agric. 84, 1464–1468, 2004.

Wheeler, R.M., Martin-Brennan. Mars greenhouses: concepts and challenges, in: Proceedings from a 1999 Workshop. NASA Tech. Mem. 208577, 2000.

Wheeler, R.M., Sager, J.C., Mackowiak, C.L., Stutte, G.W., Yorio, N.C., Berry, W.L. Nutrient, acid and water budgets of hydroponically grown crops. Acta Hort. 481, 655–661, 1999.

Widell, S., Krumbein, A., Auerswald, H. Glucosinolate in Radies und sensorische bewertung. Wissenschaftlichen Arbeistagung der Deuts-chen Gartenbauwissenschaftlichen Gesellschaft und des BDGL 35, 151, 1998.

Wright, M.G., Davison, K.L. Nitrate accumulation in crops and nitrate poisoning of animals. Adv. Agron. 16, 197–247, 1964.

Wu, X., Gu, L., Holden, J., Haytowitz, D.B., Gebhardt, S.E., Beecher, G., Prior, R.L. Development of a database for total antioxidant capacity in foods: a preliminary study. J. Food Composition Anal. 17, 407–422, 2004.

Yamaguchi, S., Yoshikawa, T., Ikeda, S., Ninomiy, T. Stuides on the taste of some sweet substances. Part I. Measurement of the relative sweetness. Agric. Biol. Chem. 34, 181–186, 1970.

Zhang, Y., Talalay, P., Cho, C.G., Posner, G.H. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc. Natl. Acad. Sci. USA 89, 2399–2403, 1992.