Selection of Leafy Green Vegetable Varieties for a Pick-and-Eat Diet Supplement on ISS

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Several varieties of leafy vegetables were evaluated with the goal of selecting those with the best growth, nutrition, and organoleptic acceptability for ISS. Candidate species were narrowed to commercially available cultivars with desirable growth attributes for space (e.g., short stature and rapid growth). Seeds were germinated in controlled environment chambers under conditions similar to what might be found in the Veggie plant growth chamber on ISS. Eight varieties of leafy greens were grown: 'Tyee' spinach , 'Flamingo' spinach , 'Outredgeous' Red Romaine lettuce , 'Waldmann's Dark Green' leaf lettuce, 'Bull's Blood' beet, 'Rhubarb' Swiss chard, 'Tokyo Bekana' Chinese cabbage, and Mizuna. Plants were harvested at maturity and biometric data on plant height, diameter, chlorophyll content, and fresh mass were obtained. Tissue was ground and extractions were performed to determine the tissue elemental content of Potassium (K), Magnesium (Mg), Calcium (Ca) and Iron (Fe). Following the biometric/elemental evaluation, four of the eight varieties were tested further for levels of anthocyanins, antioxidant (ORAC-fluorescein) capacity, lutein, zeaxanthin, and Vitamin K. For sensory evaluation, 'Outredgeous' lettuce, Swiss chard, Chinese cabbage, and Mizuna plants were grown, harvested when mature, packaged under refrigerated conditions, and sent to the JSC Space Food Systems Laboratory. Tasters evaluated overall acceptability, appearance, color intensity, bitterness, flavor, texture, crispness and tenderness. All varieties received acceptable scores with overall ratings greater than 6 on a 9-point hedonic scale. Chinese cabbage was the highest rated, followed by Mizuna, 'Outredgeous' lettuce, and Swiss chard. Based on our results, the selected varieties of Chinese cabbage, lettuce, Swiss chard and Mizuna seem suitable for a pick-and-eat scenario on ISS with a ranking based on all factors analyzed to help establish priority.

Nomenclature

AFT = Advanced Food Technology
Ca = Calcium

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cv	=	Cultivated variety or cultivar
DAP	=	Days after planting
Fe	=	Iron
HRP	=	Human Research Program
ISS	=	International Space Station
JSC	=	Johnson Space Center
K	=	Potassium
KSC	=	Kennedy Space Center
Mg	=	Magnesium
ORAC	=	Oxygen radical absorbance capacity
Veggie	=	Vegetable Production System

I. Introduction

THE capability to grow nutritious, palatable food for crew consumption during spaceflight will become more important as we move toward longer-duration, exploration-class missions. Critical nutrients have been shown to degrade in the current prepackaged space-food system to the point that they will not meet the shelf-life requirements of long-duration mission scenarios (Cooper, 2013). Supplementation through pharmacological capsules is not recommended due to concerns with crew compliance and possible side effects, faster degradation of nutrients in supplement form than in food form, and lack of synergistic benefits associated with the natural combination of hundreds of phytochemicals in whole-food delivery (Basu and Imrhan, 2007; Liu, 2003; Lane and Schoeller, 2000; Polivkova et al., 2010; Zwart et al., 2009). Implementation of a pick-and-eat bioregenerative produce system in spaceflight has tremendous potential to supplement nutrition over time, while supporting crew psychosocial health through the introduction of fresh foods that increase variety, texture, flavor, and color attributes of the food system (Perchonok et al., 2012). However, studies of edible produce for spaceflight have been limited, leaving a significant knowledge gap to determine the best cultivars to obtain acceptable, nutritious pick-and-eat produce for consumption in the resource constrained spaceflight environment.

Fig. 1. The Veggie plant growth hardware on the international Space Station.

Kennedy Space Center (KSC) has a long history of working on food production for bioregenerative life support (Wheeler, 2014). Recently the KSC team worked as part of the Veggie team to develop and implement plans for the hardware validation test of the Veggie hardware on ISS (Fig. 1). Veggie, a small plant growth chamber designed to produce fresh vegetables, is currently flying on ISS, and this hardware will be available to begin supplementation of astronaut diets with fresh vegetables grown in space.

The first step is to establish baseline crop growth, nutrition, and organoleptic attributes of leafy greens crops that could be grown in Veggie. KSC Food Production was tasked with delivering a list of crops to NASA's Human Research Programs (HRP) Advanced Food Technology (AFT) group with suitable nutritional, organoleptic, phytochemical and horticultural characteristics to be considered for incorporation into the diet on medium to long duration space missions (six months and longer). A draft list was submitted to HRP for feedback in December, 2013. This list was reviewed by the HRP AFT and six species were selected based on their nutritional potential to supplement the prepackaged food system and subsequently assessed under mission relevant conditions for growth, nutritional, and sensory characteristics. This report describes the baseline testing results and subsequent ranking of leafy greens varieties. It is expected that this ranking will evolve with the priority of nutrition, acceptability, and resources into a standardized metric for future crop selection.

II. Materials and Methods

A. Leafy Greens Growth Tests

Two tests were performed to identify the horticultural constraints associated with production of leafy greens salad crops suitable for pick-and-eat. Tests were essentially replicates with plant placement randomized to account for position differences. Temperature was also varied slightly between the two tests to see effects of removing the thermoperiod, and media differed slightly due to availability. Horticultural evaluation was performed on spinach, lettuce, beet, Swiss chard, Chinese cabbage, and 'Mizuna'. Seeds were either purchased or obtained from current stocks. The summary of salad crops tested and sources of seed are included in Table 1.

Table 1. Candidate pick-and-eat salad crops selected for testing, and the source of seed (web links were accessed on 23 September, 2014).

Species	Common name	Cultivar	Additional Information	Received
Spinacia oleracea	Spinach	'Tyee'	http://www.johnnyseeds.com/p-7449-tyee-f1.aspx	2/14/14
Spinacia oleracea	Spinach	'Flamingo'	http://www.johnnyseeds.com/p-8990-flamingo-f1.aspx	2/14/14
Lactuca sativa	Red Romaine lettuce	'Outredgeous'	http://www.johnnyseeds.com/p-6609-outredgeous-romaine-lettuce.aspx	1/9/09 Germ tested 1/18/13
Lactuca sativa	Leaf lettuce	'Waldmann's Dark Green'	http://www.seedsofchange.com/quickfacts.aspx?c=10103&cat=573#ad-image-ProductDetail1_aFirstImage	10/1/12
Beta vulgaris	Beet	'Bulls Blood'	http://www.johnnyseeds.com/p-5814-bulls-blood.aspx	2/14/14
Beta vulgaris subsp. cicla	Swiss chard	'Rhubarb'	http://www.johnnyseeds.com/p-7851-ruby-red-or-rhubarb-chard.aspx	2/14/14
Brassica rapa, subspecies chinensis	Chinese cabbage	'Tokyo Bekana'	http://www.johnnyseeds.com/p-5757-tokyo-bekana.aspx	2/14/14
Brassica rapa var. nipposinica	Mustard	'Mizuna'	http://www.seedsofchange.com/quickfacts.aspx?c=9536&cat=624#ad-image-ProductDetail1_aFirstImage	11/11/13

1. Test 1 Growth

Six plants of each cultivar were planted on 2/24/14 in 2-inch (5 cm) pots with custom potting media in Controlled Environment Chambers in the Space Life Science Laboratory, Exploration Park, Florida (Fig. 2). The media consisted of 7:3 Fafard #2 (Conrad Fafard, Agawam, MA): un-sieved turface (arcillite)(Profile Products, LLC, Buffalo Grove, IL) with Nutricote controlled release fertilizer (18-6-8 type 180) (Florikan, Sarasota, IL) mixed at a ratio of 7.5 grams fertilizer per liter of dry media. Media was premixed with water until damp, and 150 mL added to 2-inch pots. Two seeds were planted per pot with plants thinned to one per pot after germination. Pots were placed in trays equipped with a bottom watering system and lined with green capillary matting. Trays were placed in a controlled environment chamber under controlled environmental conditions similar to what could be found on ISS (Table 2).

Plants were monitored and tended daily. There were operational issues with the water reservoirs, which resulted in occasional leaking, leading to overwatering on a few occassions. This led to the development of fungi on the soil surface of some pots. Plant growth did not seem to be significantly impacted, though spinach and beet plants grew more slowly than expected, and were thus harvested at a later date than other species. It seems likely that the current growth conditions including the media and the environment were not optimized for these species.

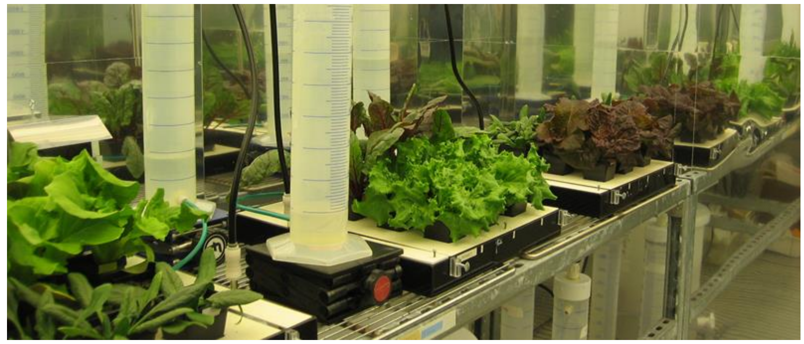

Fig. 2. Leafy greens varieties growing in a controlled environment chamber.

Table 2. Plant test 1 environmental set points

Condition	**Day**	**Night**
Photoperiod	16 hours	8 hours
Temperature	23°C	18°C
Relative Humidity	70%	70%
CO_2	400 ppm (2/14-3/14) 1000 ppm (3/14-3/31	1000 ppm
Light	~400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ CWF*	-

* CWF= cool white fluorescent with dimming ballasts.

Light was measured at three times on two week intervals to determine light changes as plants developed. The average light reading was 417 $\mu mol\ m^{-2}\ s^{-1}$ (24 moles $m^{-2}\ d^{-1}$ PAR). Environmental data (Temperature, [CO2], and % RH) were collected at 5 minute intervals. Plants were grown in the following configuration of 6 plants / cultivar / tray (Fig. 3).

Plants were grown under the above conditions, and harvested when they were horticulturally mature (≥5 fully expanded leaves). 'Mizuna' and Chinese cabbage were harvested at 23 days after planting (DAP), lettuces and Swiss chard at 28 DAP and beet and spinach varieties at 35 DAP.

2. Test 2 Growth

Following harvest of experiment one, the pots and irrigation trays were washed and all eight types of plants were replanted within each chamber on 1 April 2014. In an attempt to account for potential positional effects in the chamber the planting layout was varied. The planting map is shown in Figure 4. The conditions were similar to those in the first experiment, except

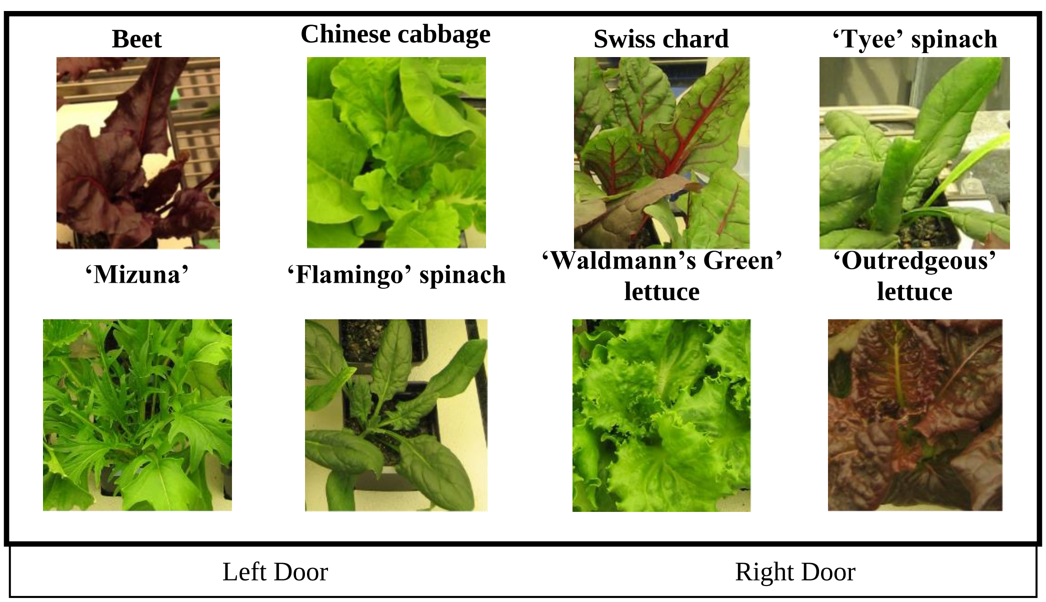

Fig. 3. Planting map of pick-and-eat salad species in controlled environment chamber (EGC M36) planted 24 February 2014. There were six plants per cultivar, each in 2 inch pots filled with potting media.

that night temperature was increased from 18°C to 23°C to maintain constant temperature to assess the impact of removing a thermoperiod on crop growth. Although a thermoperiod tends to occur in Earth-based agriculture, examining data from ISS and Veggie ground testing indicated that a thermoperiod was unlikely in this environment. The media blend used was modified slightly due to a lack of availability of previously used soilless mixes; a mix of Fafard #2 and #2B in 4:1 ratio was utilized. This resulted in a 7:3 peat/arcillite (56.9% Fafard #2: 13.1% Fafard #2B: 30% arcillite). In addition, the trays were lined with a lighter grade (thinner) gray CapMat II material in order to reduce total amount of water remaining in the tray. This CapMat II has been selected for use as a wick in the Veggie system in the future. The environmental set points are summarized in Table 3.

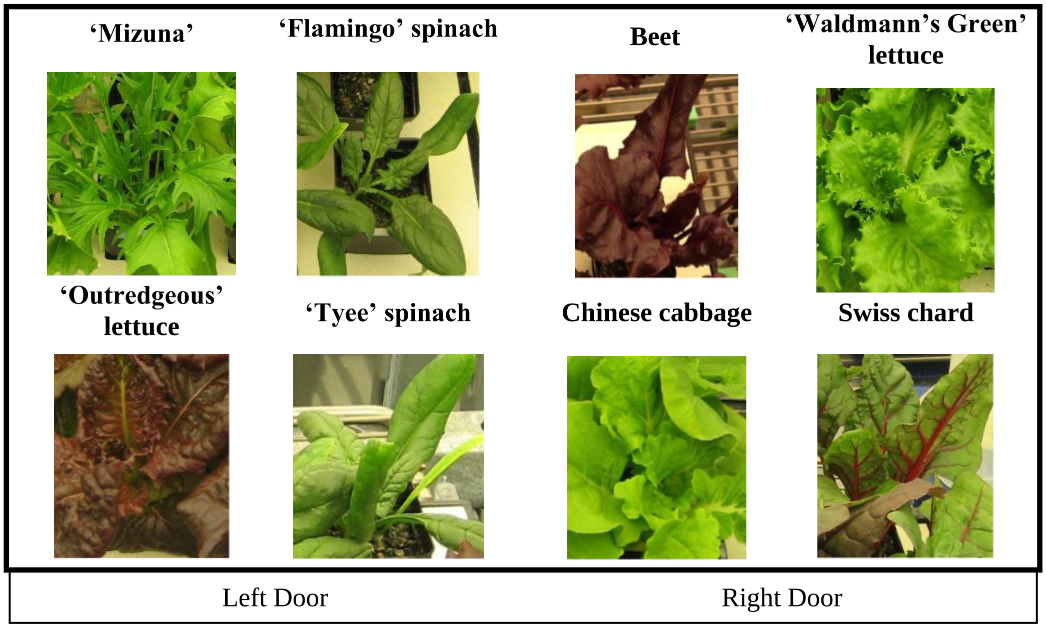

Fig. 4. Planting map of pick-and-eat salad species in controlled environment chamber (EGC M36) planted 1 April 2014. There were six plants per cultivar, each in 2 inch pots filled with potting media.

Table 3. Plant test 2 environmental set points

Condition	**Day**	**Night**
Photoperiod	16 hours	8 hours
Temperature	23°C	23°C
Relative Humidity	70%	70%
CO_2	1000 ppm	1000 ppm
Light	~400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ CWF	-

Tray 1, which supported ‘Mizuna’ and ‘Outredgeous’ lettuce had two episodes of drying, one on 8 April, 2014 (7 days after planting-DAP) and again on 13 April, 2014 (12 DAP). Protocols were put into place to increase the number of checks, and no additional periods of desiccation were observed. However, fungal growth was noted on the media after 13 days. The impact of the fungi on the plant growth and associated data is unknown. Although these types of organisms would likely be in ISS, in microgravity, the use of closed rooting pillows compared to open pots with exposed media, should reduce the incidence of media-borne disorders. Air-borne microbes may still colonize leaf surfaces, potentially requiring the development of on-orbit sanitation methods. Subsequent testing has shown that thoroughly cleaning of the chambers between planting can reduce appearance of fungi on the soil surface.

Harvest times were similar to test one, with ‘Mizuna’ and Chinese cabbage being harvested at 23 DAP (23 April, 2014) and lettuce cultivars and Swiss chard at 28 DAP (29 April 2014). Unlike Test 1, the spinach cultivars and beet were harvested at the same time as the lettuces (28 DAP) on 29 April 2014 instead of 35 DAP. As in test one, the distance between each lettuce plant was increased following harvest of ‘Mizuna’ and Chinese cabbage to minimize overlap of plant leaves. A slight chlorosis was noted on older leaves of Chinese cabbage and ‘Mizuna’, and 5 mL of ½ strength Hoagland’s nutrient solution were added to each pot on 24 April 2014. Unfortunately, there were additional overwatering events during this test, but these were detected relatively early, and corrective actions were taken.

i. Harvest Procedures

At harvest, non-destructive chlorophyll measurements (Minolta SPAD meter) averaged for three leaves per plant, plant height and two diameters were measured. Plant fresh mass was measured. In addition, one fully expanded leaf was removed, weighed, oven dried, and reweighed to calculate dry mass. The remainder of the plant was wrapped in foil and frozen at -80°C until freeze dried for nutrient analysis. Harvest procedures were identical for both tests 1 and 2.

ii. Tissue preparation for chemical analysis

All frozen plant tissue was first freeze dried for 4-5 days until completely dry. Freeze dried samples were ground with a Wiley Mill (http://www.thomassci.com/wileymill) to pass thru a 20 mesh screen (0.841 mm) and samples were refrozen at -80ºC in 50 ml tubes until analysis. Analytical methods are included as Appendix 8.1.

iii. Weighting and rankings

Weighttings for different parameters were established based on initial perceptions of the importance of these factors. This is a draft weighting list only and will be open to future modifications based on new information on the importance of these parameters for a space diet.

III. Results and Discussion

B. Horticultural Parameters

Plants were harvested at times described in the methods for both tests, and the same data were collected in both tests. In general, plants had slightly reduced fresh mass in the second test lacking a thermoperiod, however the causes of the slightly lower growth are uncertain due to differences in watering. Data from plants of both experiments were pooled below. Data were collected on horticultural parameters of edible fresh mass, percent moisture of leaves, SPAD (corresponding to chlorophyll concentration), plant area and height, which inform on the volume these plants would occupy in a facility such as Veggie. Additionally data were collected on the percent of germination and the time to harvest. All of these parameters were integrated into the final ranking.

iv. Fresh mass

Fresh mass (amount that would be consumed) varied according to species (Figure 5), and reflected the general morphology and growth structure of each species/cultivar, with the thin leafed ‘Tyee’ spinach producing an average fresh mass of 11 g/plant and Chinese cabbage producing approximately 40 g / plant. The average across all the pick-and-eat candidates was 24 g / plant, and that average value may be useful in estimating the production needs to meet a crew member’s dietary requirements.

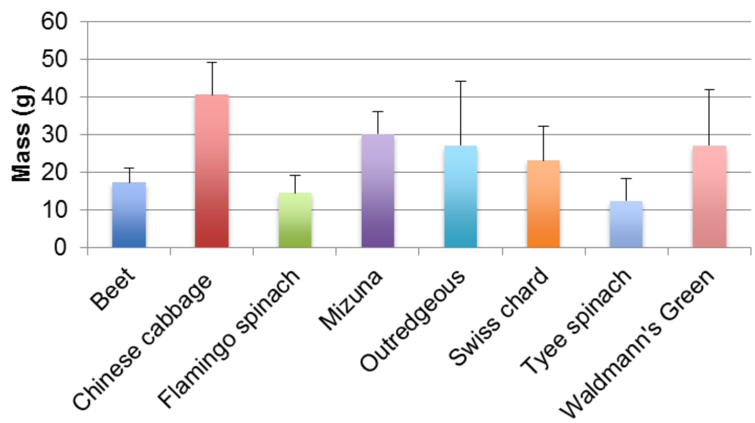

Fig. 5. Fresh mass (g/plant) of salad crops at final harvest. Error bars are standard deviations.

v. Percent moisture

The percent moisture was consistent across all salad crop species, ranging from 78% in spinach (‘Flamingo’) to 85% in ‘Outredgeous’ lettuce (Figure 6). These values were slightly lower for hydroponic and controlled environment grown salad crops (e.g., Wheeler et al., 1994).

vi. Chlorophyll and growth characteristics

Upon harvest, two measurements of plant shoot (canopy) diameter were made, which were averaged, and then converted to an approximate canopy area (cm^2) in order to determine the area required per plant. The height of each plant was also recorded as an indicator of growth, and can be used, in conjunction with area data, to calculate a minimum volume requirement for each plant (Figure 7).

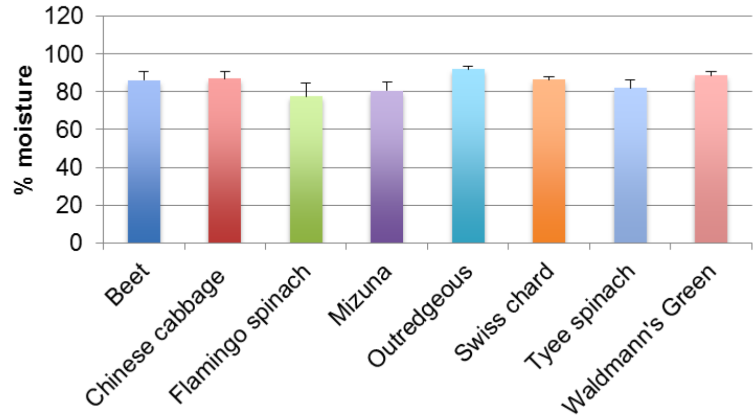

Fig. 6. Percent moisture of candidate salad crops. Error bars are standard deviations.

In addition to height, canopy area reading, non-destructive measurements of chlorophyll content were made using a Minolta SPAD chlorophyll meter (Figure 7). This measurement functions as 'proxy' for plant health, and stress. Chlorophyll content was greater than 30 SPAD units for all salad crops except 'Outredgeous' lettuce (28.7 SPAD units), which is indicative of low stress, and sufficient chlorophyll to support photosynthetic functions. 'Mizuna', Swiss chard, and both spinach varieties had very high chlorophyll readings (>50 SPAD Units), while beet, Chinese cabbage, and 'Waldmann's Green' lettuce were intermediate, with values between 30 and 50 SPAD units.

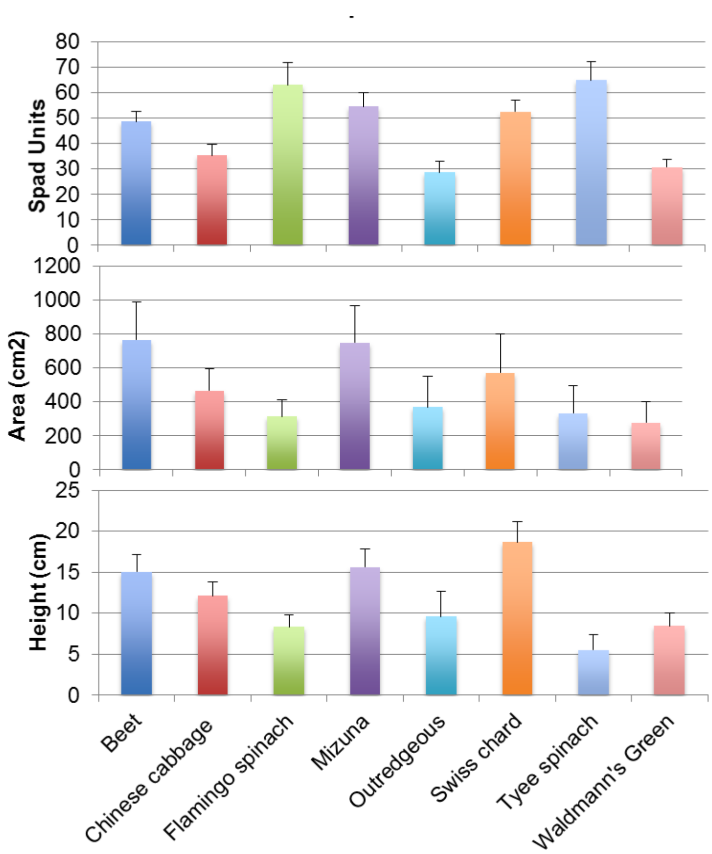

Fig. 7. SPAD, canopy area, and plant height of eight pick-and-eat salad species. Error bars are standard deviations.

There were differences in the growth area need to support the different salad species, depending upon their growth habit. Beet and 'Mizuna' have a somewhat spreading growth habit, and had canopy areas of ~700 cm^2/ plant. In contrast, both spinach and both lettuce varieties required less than 400 cm^2/plant at horticultural maturity. Chinese cabbage and Swiss chard had intermediate values.

All the species selected were relatively compact and suitable for long duration space missions, with both lettuce and spinach cultivars being less than 10 cm in height. Chinese cabbage, beet and 'Mizuna' slight taller (12, 15, 15.5 cm respectively), and Swiss chard at 18-20 cm in height. The Veggie hardware has an adjustable height up to 47 cm when empty with a growth height of approximately 42 cm when the reservoir is installed. The footprint is 29.2 cm x 36.8 cm, and we have successfully grown six 'Outredgeous' lettuce plants in the interior with area to spare. Testing would need to be performed to demonstrate that six plants of the larger area varieties (beet and 'Mizuna') could be accommodated, but it is likely that their open loose structure would allow growth in this area.

C. Chemical Analysis

vii. Chemical analysis (Ca, Fe, K, Mg, ORAC, Vitamin K, Lutein, Zeaxanthin)

Dried tissue samples of all eight crops were analyzed for elemental content using ICP-OES elemental analysis ESC-PS2-PLP006, and the four crops selected for subsequent sensory analysis also underwent analysis for phytonutrient content (analytical methods included as appendices). The results from elemental analysis are shown in Table 4.

Table 4. Mean values (dry mass, DM) of elemental content of crops (*italics indicate crops sent to NASA JSC for organoleptic testing*). Text in bold shows highest value for Ca, K and Mg, and the lowest level for Fe, a desirable condition.

	Ca	Fe	K	Mg
		(µg/g DM)		
Beet	14787.05[a,b]	286.51[a]	30416.27[a]	**25843.99[a]**
Chinese cabbage	**17068.20[b]**	**6.79[b]**	20036.45[b]	7993.75[b]
Spinach ('Flamingo')	8493.08[a,c,d]	47.52[b]	12854.27[b]	9966.16[b]
'Mizuna'	14140.41[a,b]	16.73[b]	16964.88[b]	6761.41[b]
***Lettuce* ('Outredgeous')**	8168.93[c,d]	14.02[b]	**36169.85[a,c]**	6541.36[b]
Swiss chard	14332.48[a,b]	65.02[b]	26887.21[a,d]	17808.77[c]
Spinach ('Tyee')	8601.98[d]	36.60[b]	22427.80[a,b]	10990.26[b]
Lettuce ('Waldman's Green')	5916.18[d]	20.85[b]	23656.95[a,b]	4467.57[b]

Different letters indicate significant differences among column data (p<.05).

All the salad crops were good sources of Ca, K and Mg. Not surprisingly, there were differences between the samples, with no particular species being the standout: Chinese cabbage had the highest concentration of Ca, beet of Mg, 'Outredgeous' lettuce of K. Chinese cabbage had the lowest Fe levels. All nutrient contents in Table 5 and discussed below are on a per gram of edible dry mass basis. Final consideration should be given to edible mass expected from each plant, as a plant with a moderate amount of a nutrient may provide the greatest amount due to high edible mass. The factor of percent moisture coupled with the measured fresh mass, serves as a conversion factor between edible fresh mass and dry mass.

Calcium (Ca): Calcium is an essential dietary element that is critical to regulation of heart and muscle action, and is necessary to develop and maintain bone structure (Power et al., 1999). All the leafy greens tested had relatively high Ca content, with Chinese cabbage being the highest, with 17 mg/g dry mass (DM), following closely by beet, Swiss chard and 'Mizuna' with 14.8, 14.3, and 14.1 mg/ g DM respectively.

Iron (Fe): Iron is a necessary co-factor for many critical enzymatic systems, most notably as a cofactor for hemoglobin content in blood. However, high iron levels in astronauts after prolonged exposure to microgravity have been implicated in accelerating bone loss (Zwart et al., 2013). Beet had the highest concentration of Fe, with 286 µg/g DM, over 4X higher than Swiss chard with 65 µg/g DM. The four lowest (most advantageous for astronaut diet) were Swiss chard, 'Outredgeous' lettuce, 'Mizuna', and 'Waldmann's Green' lettuce.

Potassium (K): Potassium is an essential electrolyte to maintain charge balance and energy transfer in cells. The crew diets are somewhat limited in potassium (Lane and Schoeller, 2000) and it was indicated as a high priority nutrient. All species tested had relatively high concentrations of K (>12 mg/ g DM), although there was a three-fold range of concentrations with a high of 36.2 mg/g DM in 'Outredgeous' lettuce to a low of 12.8 mg/g DM in 'Flamingo' spinach.

Magnesium (Mg): Magnesium, along with Ca, is necessary for the development and maintenance of bone structure (Vernikos and Schneider, 2010). Magnesium level was highest in beet (25.8 mg/g DM) and Swiss chard (17.8 mg/g DM), and lowest in 'Waldmann's Green' lettuce (4.5 mg/g DM).

Four of the species were selected for sensory analysis and additional nutrient analysis. The weighting and prioritization is described fully in section III.E. Selected crops were 'Tokyo Bekana' Chinese cabbage, 'Mizuna', 'Outredgeous' lettuce, and 'Rhubarb' Swiss chard.

Once a down-selection to four crops for sensory evaluation was completed based on germination, plant growth, and elemental characteristics (see Table 10), additional testing on phytonutrient and vitamin content was conducted to establish phytonutrient content. These included overall anti-oxidant potential, using the oxygen radical absorbance capacity (ORAC) assay as a general indicator of bioactive components, anthocyanins as countermeasure against oxidative stresses, lutein and zeaxanthin as compounds supporting vision health (Demmig-Adams and Adams, 2013), and Vitamin K as a potential biological countermeasure for bone loss associated with long duration space flight. The results of those analyses are shown in Table 5.

Table 5. Mean values (g DM basis) of phytochemical analysis of crops (*italics indicate crops sent to NASA JSC for organoleptic testing*). Text in bold shows highest value for the 4 crops selected for testing at JSC.

	ORAC	Anthocyanin	Lutein	Zeaxanthin	Vit. K
	µmol TE/g	mg/g	mg/g	mg/g	mg/100g
Beet	ND*	ND	ND	ND	ND
Chinese cabbage	156.41[a]	0	2.28[a]	0.02[a]	0.65[a]
Spinach ('Flamingo')	ND	ND	ND	ND	ND
'Mizuna'	121.84[a]	0	3.87[b]	0.02[a]	**2.32[b]**
Lettuce ('Outredgeous')	**1119.59[b]**	**12.47**	3.16[b]	0.02[a]	0.16[c]
Swiss chard	1107.80[b]	2.45	**5.94[c]**	**0.04[a]**	0.79[d]
Spinach ('Tyee')	ND	ND	ND	ND	ND
Lettuce ('Waldman's Green')	ND	ND	ND	ND	ND

*ND-no data. Different letters indicate significant differences among column data ($p<0.05$) using Tukey mean separation test.

As observed with elemental analysis, there were clear differences in phytochemicals between the four species, with each having particularly high values in a different nutrient.

Anti-Oxidant Potential: 'Outredgeous' lettuce and Swiss chard both had ORAC values which were approximately an order of magnitude higher than Chinese cabbage or 'Mizuna'. As the data show, no anthocyanins were detected in Chinese cabbage or 'Mizuna', while 'Outredgeous' lettuce has the highest concentration of the four species. While it is generally believed that the presence of anthocyanins (see below), and the resultant increase in ORAC value, provides a measure of overall bioprotection in the diet, it has been difficult to establish the correlation *in vivo* (Lobo et al., 2010).

Anthocyanin: Anthocyanins are water soluble pigments (red, blue, and purple) which have very strong anti-oxidant potential, and have been suggested to provide a biological countermeasure to radiation stress on long duration space missions (Stutte et al. 2009). Anthocyanins were detected in 'Outredgeous' lettuce (12.47 mg/g DM) and Swiss chard (2.45 mg/g DM), both species with red pigments in the leaves and stems, and not in Chinese cabbage or 'Mizuna', which have green pigmented leaves. These samples were obtained from leaf tissue only, and it would be anticipated that relatively high concentrations of anthocyanins would be present in petiole tissue, based on the intensity of pigmentation of harvested produce.

Lutein and Zeaxanthin: Lutein and zeaxanthin are carotenoids that are found in a number of green leafy vegetables (Demmig-Adams and Adams, 2013). They have identical chemical formulas and are isomers, with lutein being the predominant form. These two pigments accumulate in the retina of the eye and are believed to function by filtering harmful UV wavelengths and dissipating excess light energy. Swiss chard has the highest concentration of both lutein (5.94 mg/g DM), and zeaxanthin (0.04 mg/g DM) of the four species tested. The lutein concentration in Swiss chard was over 40% higher than values in lettuce (3.16 mg/g DM) and 'Mizuna' (3.87 mg/g DM), and over twice as high as Chinese cabbage (2.28 mg/g DM). Although the zeaxanthin value was higher in Swiss chard than the other species, there were no statistical differences between them.

Vitamin K: Vitamin K has been shown to be correlated with reduction of arterial calcification and bone loss associated with osteoporosis, and is considered a critical cofactor in the incorporation and retention of Ca in bone structures (Tang et al., 2007). The packaged space diet is deficient in vitiamin K (G. Douglas, pers. comm.). In the four species tested, 'Mizuna' was a standout source of both Ca (14.14 mg/g DM) and Vitamin K (2.32 mg/100 g DM). This Vitamin K level was approximately three-fold higher than either Swiss chard (0.79 mg/ 100g DM) or Chinese cabbage (0.65 mg/100 g DM) and fourteen-fold higher than 'Outredgeous' lettuce with 0.16 mg/ 100 g DM).

D. Sensory Analysis.

Leafy greens from down selected varieties were grown in environment chambers at KSC, harvested, fresh mass was recorded, and then plants were packaged and sent via overnight delivery to JSC Food Sensory Laboratory for organoleptic/sensory evaluations. A test run of the shipping protocols was conducted on 11 August 2014 to verify the packing protocols. Following identification and mitigation of some minor issues (leaking ice pack, moderate wilting) Chinese cabbage, 'Mizuna', 'Outredgeous' lettuce and Swiss chard were pre-cooled to 10°C in the growth chambers, harvested, and prepared for shipping. The harvested produce was packed into plastic food containers prior to packing into freezer boxes lined with 4°C ice packs, and shipped to Johnson Space Center for sensory analysis. Upon receipt, the produce was washed, photographed, and presented to a sensory panel (n=35) for evaluation (Figure 8).

Fig. 8. Produce shipped to JSC for sensory analysis. Images were taken after produce was washed in preparation for panel assessment.

viii. Sensory Analysis Results

Taste panelists (n=35) were presented with samples of the washed produce, and asked to evaluate using a 9-point Hedonic Scale (1=dislike extremely, 2= Dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=Like slightly, 7=Like moderately, 8=Like very much, 9=Like extremely) for overall acceptability, appearance, color intensity, bitterness, flavor and intensity. The results of the panel assessments, and standard deviation are shown in Table 6.

These results clearly indicate that the produce was acceptable with the panelists, with all assessments being 6 (slightly like, or greater). It is of particular interest that the assessment of Chinese cabbage was >7 for all categories except bitterness. 'Outredgeous' lettuce has lowest average rating, but this may have been due to slight wilting that was observed after shipping. This, however, did not appear to make it unacceptable for the consumers.

Table 6. Summary of Sensory Analysis attributes judged on a 9-point Hedonic Scale. Values are means (standard deviation, n=35). Values in bold indicate the highest scoring crop in each category.

Crop	Attribute	Overall Acceptability	Appearance	Color Intensity	Bitterness	Flavor	Texture
Chinese cabbage		**7.23** (1.42)	8.10 (1.05)	8.34 (0.94)	6.03 (1.52)	**7.11** (1.64)	**7.97** (1.12)
'Mizuna'		6.89 (1.18)	7.29 (1.73)	8.03 (1.18)	**6.23** (1.46)	6.83 (1.36)	7.31 (1.32)
'Outredgeous' lettuce		6.69 (1.79)	7.66 (1.53)	7.77 (1.46)	6.14 (1.54)	6.51 (2.13)	6.66 (1.97)
Swiss chard		6.51 (2.12)	**8.51** (0.89)	**8.57** (0.85)	6.09 (1.50)	6.14 (2.58)	7.31 (1.63)

Panelists were also asked to evaluate the produce for crispness and tenderness using a 5 point "Just About Right" scale (1=not nearly crisp/tender enough, 2=not crisp/tender enough, 3= just right, 4=too crisp/tender , 5= much too crisp/tender). The results are presented in Table 7.

Table 7. Summary of crispness and tenderness using a 5-point "Just About Right" scale. Values are means (standard deviation, n=35). Values in bold indicate the highest scoring crop in each category.

Crop	Attribute	Crispness	Tenderness
Chinese cabbage		**3.0** (0.0)	**3.0** (0.0)
'Mizuna'		2.94 (0.23)	2.94 (0.34)
'Outredgeous' lettuce		2.46 (0.51)	3.31 (0.47)
Swiss chard		2.97 (0.17)	2.94 (0.42)

These analyses indicate that Chinese cabbage was "just right" for both crispness and tenderness, and that 'Mizuna' and Swiss chard were within a standard deviation of the "just right" score. 'Outredgeous' lettuce, on the other hand, had greater variation, with it not being quite crisp enough, and a bit too tender. This would be consistent with the slight wilting observed following shipping, which would not be a factor for produce consumed upon harvest on ISS. However, the values are still within acceptable values for consumption.

E. Weightings and Prioritization

ix. Weighting

A weighting was decided based on the parameters measured and this weighting was used to modify the ranking process of the plant candidates. Table 8 highlights the parameters, assigned weightings, and rationale for this weighting. It is important to note that this is a first attempt at this weighting process, and as new information is accrued on these parameters pertaining to value to the space diet, weighting factors may shift. The weighting used was simple, with factors characterized as either of most importance (x 2 value), moderate importance (x 1.5 value), normal (x 1) or not impacting (x 0). Factors considered to have the most important contribution to the success of a particular crop included fresh mass as an indication of the amount of food produced, Potassium due to a strong need for this element in the space diet, and overall taste as a unified metric for produce appeal. Moderately important factors included the horticultural factors germination, plant volume and days to maturity. These factors have a large impact on plant growth in the Veggie hardware and on the edible amount of plant tissue. Other moderately important factors include iron and magnesium, all of the nutrient factors, and the organoleptic evaluator flavor. The roles of iron and magnesium are well defined and the levels are important for the diet. The nutrient factors are also very desirable for the diet. Flavor should be a strong indicator of crew interest in eating the produce. Percent moisture is weighted as normal with the thought that it does not vary much and will not contribute significantly. Calcium is weighted normally because while it is an important nutrient to have at adequate levels in the diet, too much could have negative impacts. Appearance, color, bitter, texture, crispness and tenderness are all weighted normally with the belief that these individually have slight bearing on whether produce will be eaten. SPAD, a metric of chlorophyll content, is interesting purely from a plant physiological perspective and has no bearing on plant growth, nutritional quality or flavor; thus it was weighted zero.

Table 8. Assigned weightings of different crop selection parameters. Inverted factors are those where smaller values are considered more desirable.

Parameter	Weighting	Rationale
		Plant Growth Factors
Germination	x 1.5	Germination indicates how easy plants are to grow.
SPAD	x 0	SPAD is a factor of plant growth (chlorophyll content) but not important for diet or a yield parameter
Volume	x 1.5 (inverted)	Volume is important because it gives information on how much space the crop will occupy and it is a constraint for spaceflight
Fresh Mass	x 2	Fresh mass indicates crop yield - a principal factor
Days to maturity	x 1.5 (inverted)	Indicates how quickly plants could be grown
% moist	x 1 (inverted)	Percent moisture indicates amount of dry mass ~calories
		Elemental Factors
Ca	x 1	Calcium is important but desired amount remains unclear
Fe	x 1.5 (inverted)	Too much Iron can cause issues so low iron is desired
K	x 2	Space diet is deficient in Potassium - a principal factor
Mg	x 1.5	More Magnesium is desirable
		Nutrient Factors
ORAC	x 1.5	Antioxidants may help protect from radiation damage
Lutein	x 1.5	Lutein is potentially important for eye health
Zeaxanthin	x 1.5	Zeaxanthin is potentially important for eye health
Vitamin K	x 1.5	The space diet is deficient in Vitamin K
		Organoleptic Factors
Overall taste	x 2	Overall taste is a principal factor
Appearance	x 1	Appearance is not important as long as it is acceptable
Color	x 1	Color is not important as long as it is acceptable
Bitter	x 1	Bitterness might be good or bad
Flavor	x 1.5	Flavor is important
Texture	x 1	Texture is not important as long as it is acceptable
Crispness	x 1	Crispness is not important as long as it is acceptable
Tenderness	x 1	Tenderness is not important as long as it is acceptable

x. Prioritization

Average values of each parameter were calculated for the plants of tests 1 and 2 (maximum of n=2 plants but some varieties had fewer plants due to poor germination). All averages were normalized so that the minimum value equates to zero and the maximum value equates to 1. For factors where lower values were considered more desirable (plant volume, days to maturity, percentage moisture and Fe levels) normalized data were inverted so that the minimum value equaled one and the maximum value equaled zero. All normalized averages were then weighted based on the parameters outlined in Table 8. Table 9 shows the weighted normalized plant growth and elemental factors for the eight crops combined from tests 1 and 2.

Table 9. Normalized, weighted rankings of crops tested. Rankings are based on the plant growth and elemental factors listed in Table 8. Values are normalized on a 0-1 scale and weightings are applied. Factor values are sums of the normalized, weighted averages for all plants in tests 1 and 2. Blue shading indicates crops selected for further testing. Data are averages based on N=12 planted crops, though in cases of poor germination N is lower.

Crop	Plant Growth Factors	Plant Growth Rank	Elemental Factors	Elemental Rank	Plant Growth + Elemental	PG+E rank
'Bull's Blood' beet	2.30	8	3.80	2	6.10	6
'Tokyo Bekana' Chinese cabbage	6.26	1	3.36	4	9.62	**1**
'Flamingo' spinach	2.52	7	1.94	8	4.46	8
'Mizuna'	5.05	2	2.68	6	7.73	**3**
'Outredgeous' lettuce	4.23	4	3.73	3	7.96	**2**
'Rhubarb' Swiss chard	3.45	5	3.94	1	7.39	**4**
'Tyee' spinach	2.94	6	2.83	5	5.77	7
'Waldmann's Green' lettuce	4.82	3	2.31	7	7.12	5

The top four crops from tests 1 and 2 were processed for additional nutrient analysis (anthocyanin, ORAC, Vitamin K, Lutein and Zeaxanthin). Anthocyanin data were excluded because only two species had measurable amounts. These same crops were then regrown for organoleptic evaluation at JSC. Data from both aspects of testing were normalized and weighted as described above and are presented in Table 10. The "just-about-right" data were normalized based on the absolute value of the percentage difference from the central value of 3.

Table 10. Normalized, weighted rankings of down-selected crops for nutrient analysis (ORAC, Vitamin K, Lutein and Zeaxanthin) and organoleptic evaluation based on those factors listed in Table 8. Overall ranking indicates the final ranking of these crops including the plant growth and elemental factors from Table 9. Nutrient data are averages based on N=12 planted crops (except 'Outredgeous' which had N=11). Organoleptic data are based on a different group of plants with N=35 evaluators.

Crop	Nutrients	Nutrient Rank	Organol. Factors	Organol. Rank	Overall	Overall Rank
'Bull's Blood' beet						
'Tokyo Bekana' Chinese cabbage	0.63	4	8.05	1	18.30	**1**
'Flamingo' spinach						
'Mizuna'	2.15	2	5.64	2	15.52	**3**
'Outredgeous' lettuce	1.96	3	1.93	4	11.85	**4**
'Rhubarb' Swiss chard	4.92	1	4.55	3	16.86	**2**
'Tyee' spinach						
'Waldmann's Green' lettuce						

IV. Conclusions

All of the four crops tested for nutrient and organoleptic evaluation seem to be good candidates for growth in the Veggie hardware on ISS. The top selection, Chinese cabbage, has excellent rapid growth, a good elemental profile, and is highly palatable to tasters. The second selection, Swiss chard, has slower growth, but it is very nutritious, both in elements and other nutrients measured, and it is also very palatable. 'Mizuna', the third choice, also has excellent growth and it is fairly nutritious and palatable. 'Outredgeous' lettuce, has good growth, good nutrition and generally good palatability, though plants did not handle storage and shipping as well as other varieties. This aspect is unlikely to be a factor in the pick-and-eat scenario envisioned for ISS. Future work on leafy greens crops will include testing these in Veggie plant pillows and in the Veggie hardware on ISS.

Appendix

Analytical methods for plant elemental and nutrient assessments.

xi. Elemental (Ca, Fe, K, Mg,) Analysis:

0.5 g of dried and ground plant material is digested in an open vessel system using 70 mLHD polyethylene vials (CPI International, Amsterdam, Holland) using a graphite-heating block (Mod Block, PN 4370-010007, CPI International, Amsterdam, Holland). The plant material is digested at 95°C using a slight modification of the EPA (Environmental Protection Agency, USA) Method 3050B, as described below. 5 mL of 70% HNO_3 (Nitric Acid 70% Trace Metal Grade, Fisher Scientific, Suwanee, GA) is added to the samples and then boiled for approximately 25 minutes. After cooling 2.5 mL 70% HNO_3 is added and the samples are reheated. After 35 minutes, samples are cooled and 1.5 mL H_2O_2 (Fisher Science) was applied. When the peroxide reaction ceased, another 1 mL H_2O_2 is added and the samples are reheated for approximately 50 minutes. During the digestion, vials were covered by watched glasses (CPI International). Samples were cooled overnight, passed through a 25 mm 0.45 μm syringe filter (Whatman) and then diluted to 50 mL with ultra-pure DI water.
Single element standards (CPI International) were diluted to the same acid concentration as the samples (11%) and quantification was done by external calibration.

xii. Vitamin K:

The reference method is a proprietary method developed by Eurofins Nutritional Analysis Center (Des Moines, Iowa) from a Journal of Agricultural Food Chemistry compendium. Vol#42, p. 295-300, 1994, Evaluation of an HPLC Method for the determination of Phylloquinone in Various Food Matrices. "

xiii. ORAC- fluorescein Assay

Approximately 20 mg of the dried sample was added to the ASE350 (Dionex) extraction cell. Sample was extracted in Acetone: Water: Acetic Acid (AWA) at a ratio of 70%: 29.5%: 0.5% to a final volume of 11.7 mL. Aliquots of 0.2 mL of extract were tested for antioxidant capacity using the $ORAC_{FL}$ assay. Dilutions of the extracted leafy green samples were prepared in a 75 mM phosphate buffer. Aliquots of 20 μL of the diluted sample were placed in a 96 well transparent flat bottom microplate, along with appropriately diluted Trolox standards, under subdued light. A "forward-then-reverse" pattern was adopted to place samples in the 96-well plate to account for possible positional effects. Additionally, the edge wells were not used for standards or samples due to possible "plate effects" which can result in significant measurement variability as compared to other parts of the plate. All samples were analyzed in triplicate (analytical replicates). The BioTek Synergy Hybrid plate reader automatically added 200 μL of fluorescein to each well, followed by 20 μL of the peroxyl radical generator (AAPH [2'2'azobis(2-amidinopropane)]) which then reacts with the fluorescent probe, fluorescein, to quench the fluorescence signal. The assay is monitored on the reader every few minutes for 2 h, and the presence of antioxidants extends the time required for the fluorescence decay to reach an endpoint. The $ORAC_{FL}$ value is calculated from the area under the decay curve, and is reported as μmol Trolox (a Vitamin E analog) equivalents (TE) over a range of concentrations.

$ORAC_{FL}$ has been established as a useful assay for the *in vitro* investigation of antioxidant capacity because the peroxyl radical produced in this assay is one of the foremost radicals present in the human body.

xiv. Anthocyanin Analysis

Approximately 100 mg of the dried sample were added to a 15 ml tube for the anthocyanin analysis. Aliquots of 4.0 ml of 99% Methanol: 1% HCl were added to each tube and the extracts were placed in darkness at 4°C for 24 h. After 24 h the samples were centrifuged for 10 min at 3500 rpm. The supernatant containing the anthocyanins was decanted and another 1.0 ml of the methanol extraction mixture was added to the pellet. Tubes were vortexed and centrifuged once again as previously described and supernatants were combined (total volume 5.0 mL) for anthocyanin content analysis. Absorbance measurements were taken at 530 nm and quantified based on a standard curve of the anthocyanin cyanidin-3-0-glucoside.

xv. Lutein and zeaxanthin

Extraction Procedure *(Modified from Perry et al., 2009*.)

Xanthophyll (lutein and zeaxanthin) contents in fruits, vegetables, and corn and egg products. 100 mg samples of freeze dried and ground leafy green material was extracted overnight at 4ºC in 5 ml of 100% methanol. After incubation, the samples were centrifuged at 3500 rpm, 4ºC for 12 min. The methanol layer was decanted into a 50 ml tube followed by the addition 5 ml THF. Samples were vortexed for 30 sec, then centrifuged again at the prior conditions. The THF layer was decanted into the methanol extract, and this procedure was repeated two more times, combining all of the extracts into one tube. The samples were brought to a final volume of 20 mL with THF, and centrifuged to pelletize any plant material. Aliquots of 4 mL of the extract were dried under nitrogen, and resuspended in 1 ml of 2:1 (ethanol: methyl tertiary butyl ether [MTBE]). Sample were filtered through a 0.2 µm filter and subsequently analyzed via HPLC analysis.

Identification and Quantification of Lutein and Zeaxanthin via HPLC

HPLC System: Agilent 1100 or 1260 HPLC System
Column: YMC America C30 Carotenoid Analytical Column, 100 x 2.0 mm
(P/N CT99S03-1002WT)
YMC America Guard Column, 10 x 2.1 mm
(P/N CT99S03-1002WT)
Mobile Phase: Solvent A: MeOH:MTBE:Water (95:3:3)
Solvent B: MeOH:MTBE:Water (8:90:2)
Detection: Diode Array; UV 445 nm
Flow: 0.2 mL/min.
Temperature: 20°C
Injection Vol.: 3 µL
Analysis Time: 38 min.
Gradient: 100% Solvent A
12 min. linear gradient to 45% Solvent A and 55% Solvent B
1 min. hold at 45% Solvent A and 55% Solvent B
6 min. gradient to 5% Solvent A and 95% Solvent B
2 min. hold at 5% Solvent A and 95% Solvent B
1 min gradient to 100% Solvent A
16 min hold at 100% Solvent A

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