Veggie ISS Validation Test Results and Produce Consumption

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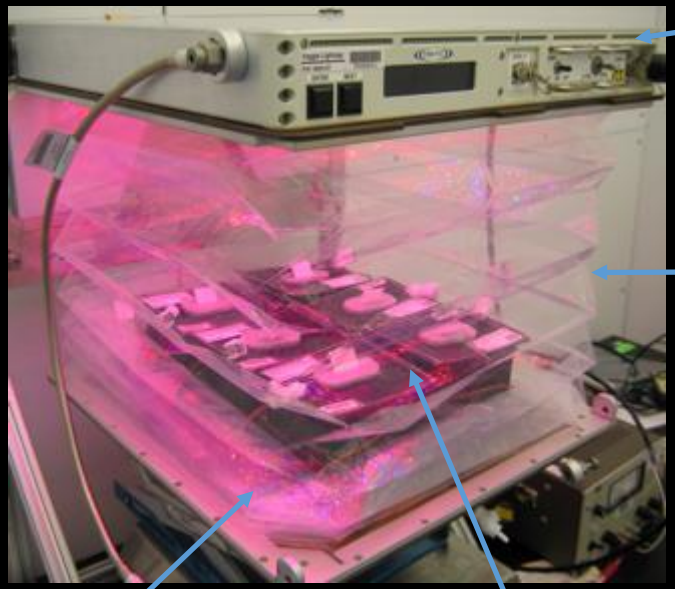
Veggie Overview



VEGGIE was designed and built by Orbital Technologies Corporation (ORBITEC)

- Small Vegetable Production System – 0.15 m^2 growing area
- Flew to ISS on SpaceX-3 and was installed in Columbus module in May, 2014
- Initial experiments validated capabilities using ‘Outredgeous’ red romaine lettuce
- Samples returned Oct., 2014

Veg-01 First Crop Harvest

VEG-01 Analysis

- Fresh Mass
- Anthocyanin/Antioxidant/Phenolic Analysis
- Elemental analysis of plants and water
- Culturable microbial assessment:
  - Plants
  - Water
  - Pillow components
  - Identification of cultured microbes
- RNA sequencing/id of total microbial population
- Crew Questionnaire
- X ray tomography of pillows

Fresh Mass

	Flight	Ground
Number	3	5
Average FM	20.61 g	15.29 g
SD FM	11.66 g	9.60 g
Max	31.51 g	26.11 g
Min	8.31 g	2.81 g

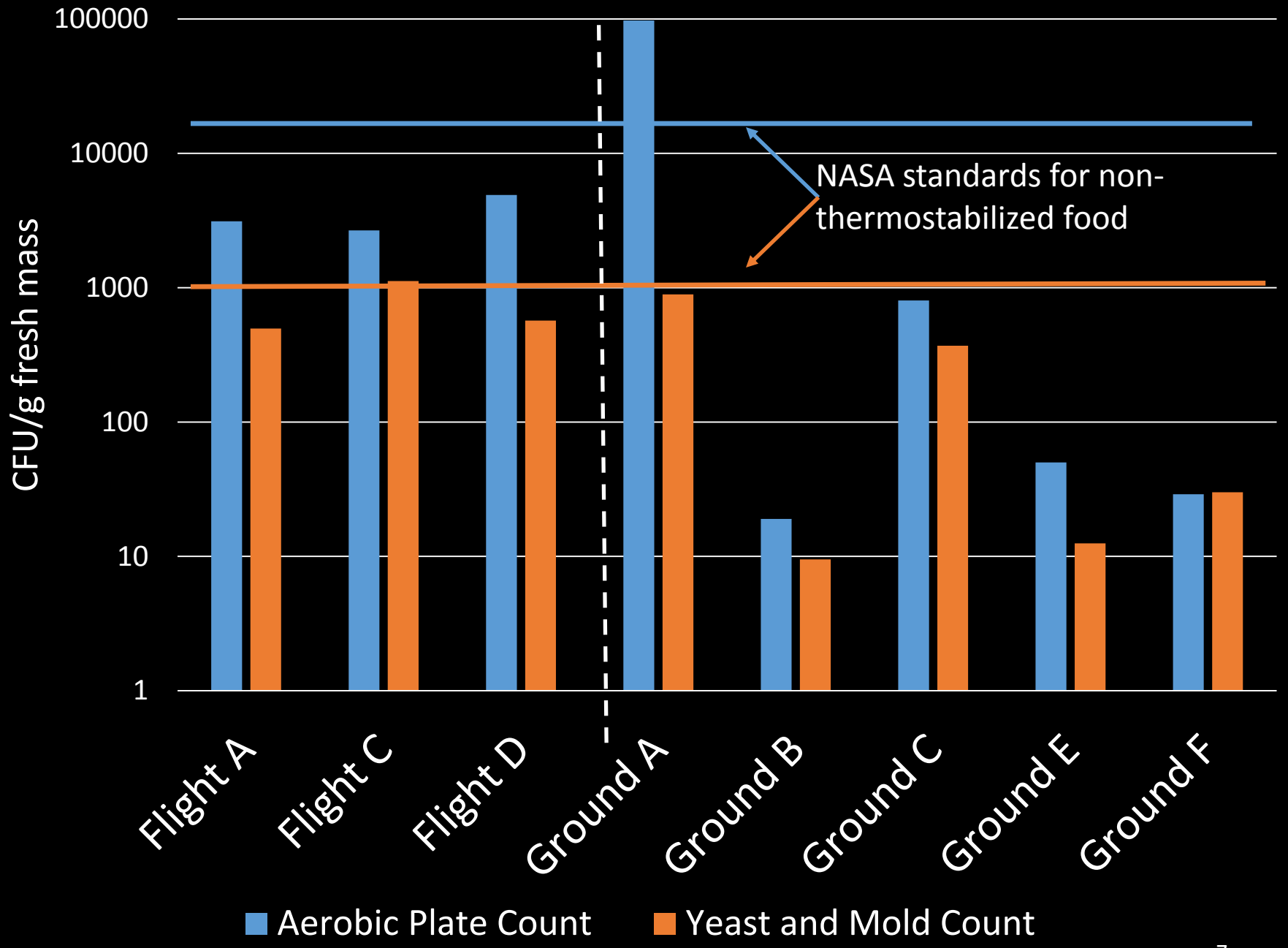
Sample priority:

1. Microbial analysis
2. Anthocyanin/Antioxidant/Phenolic Analysis
3. Elemental analysis of plants

Only samples of >19 g could be used for all three.

Microbial Assessment of ISS-grown 'Outredgeous' Red Romaine Lettuce

- Specific pathogen screens: *E. coli, S. aureus, Salmonella* sp. not found on any plants.
- Aerobic plate counts less than limit for non-thermostabilized food on all flight plants and all but one ground plant (unexplained). (Note: there are no standards for space produce so these are the closest approximate standards)
- Total yeasts and molds all below limit except on one flight plant (plant C, the largest, slightly over).
- Bacterial and fungal species isolated appear to be typical station microbes. There were some differences in the community from the ground set. (details in backup slides)


100000
10000
1000
100
10
1
CFU/g fresh mass
NASA standards for non-thermostabilized food
Flight A
Flight C
Flight D
Ground A
Ground B
Ground C
Ground E
Ground F
Aerobic Plate Count
Yeast and Mold Count

Microbial Assessment continued…

- Measured microbial levels and composition do not indicate a threat to crew health
  - Previous ground testing showed naturally low microbial levels on lettuce

- Proposed that precautionary wiping of produce with a Pro-San® sanitizing wipe would further reduce microbial levels

Pro-San® Sanitizer for Vegetables

- Commercial citric acid-based produce sanitizer
- A Pro-San® wipe was developed for cleaning the Veggie hardware: already approved for spaceflight and used on ISS
- Studies were performed to determine the efficacy of using wipes containing Pro-San® to clean and disinfect.
  - Vegetables grown in vegetable production units like Veggie (these data are in backups).
  - Veggie hardware surfaces (these data are in backups).
- Wipes reduce levels by >4 orders of magnitude.

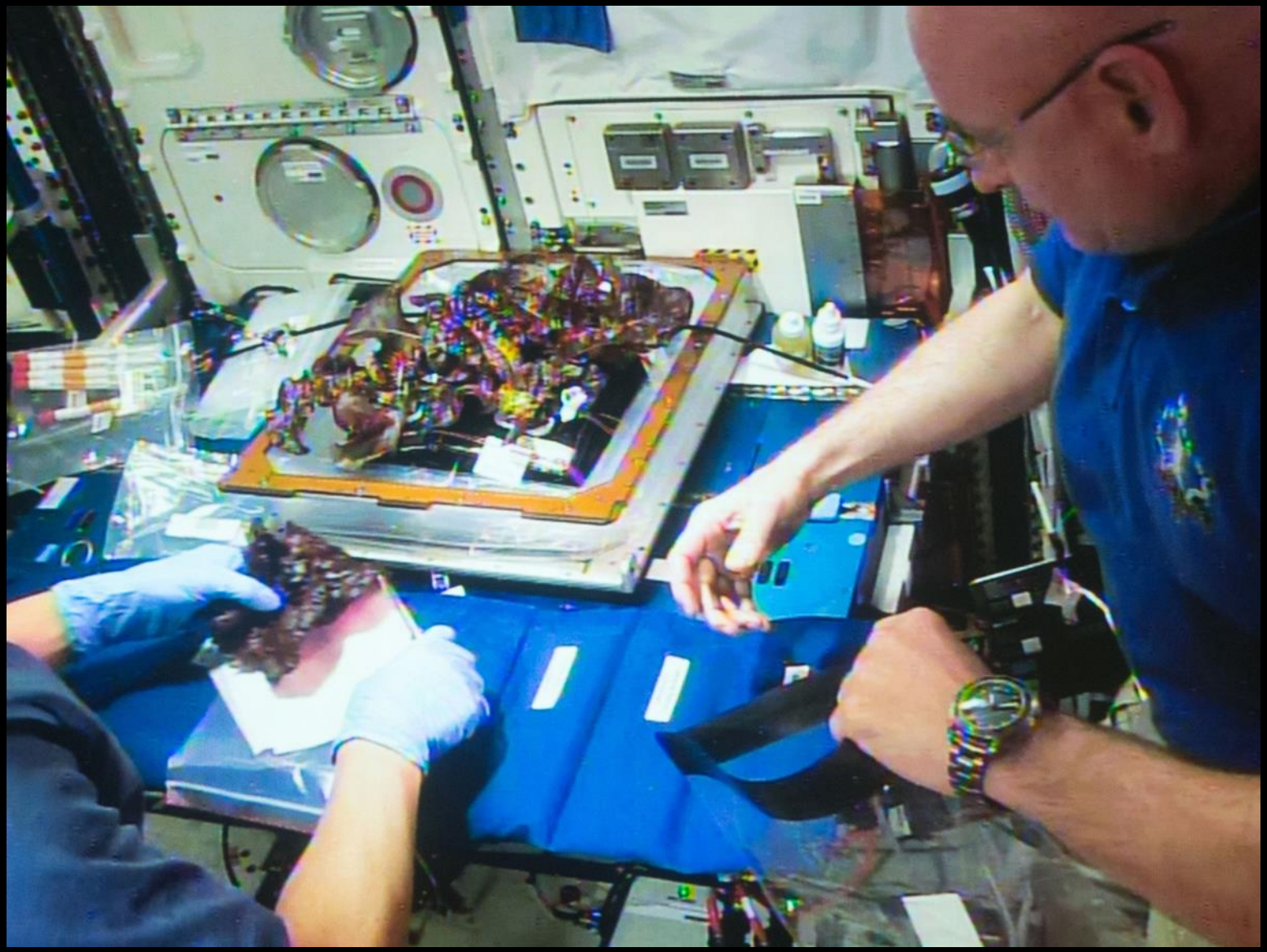
Produce Consumption Approval

- A splinter session was held at the HRP meeting to discuss Veggie results and a plan for consumption.
- Using on-board produce sanitizing wipes was tested
- Presentations were made to the Flight Medicine Board and the Tri-board (Space Medicine, Biomedical Research & Environmental Sciences, and Human Systems Engineering & Development) at JSC and received approval.
- The Payload Safety Review panel approved.
- Crew was approved to consume produce with precautionary wiping.

Approved Safe-Handling Procedures

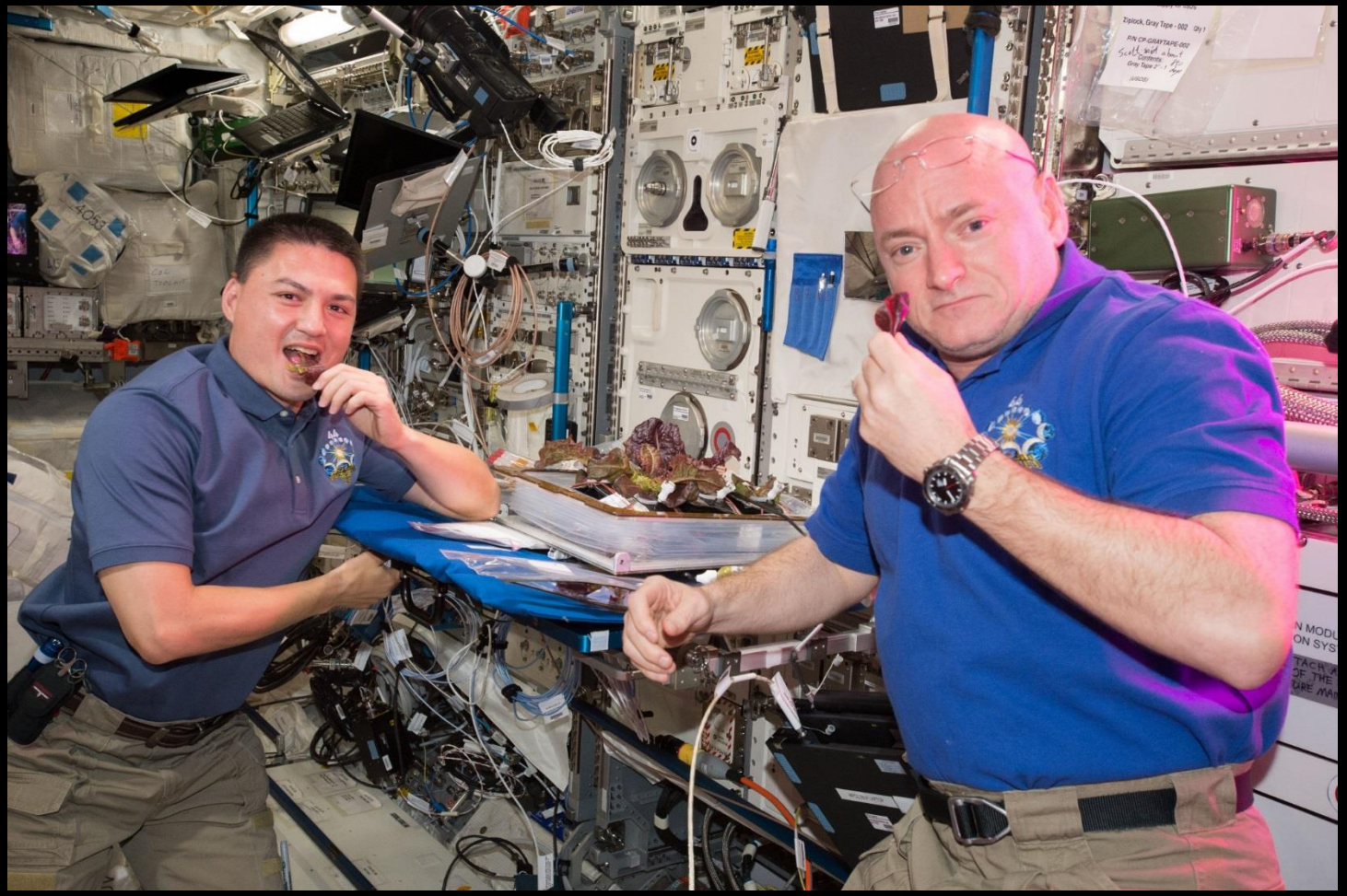
- Veggie seeds, media, wicks and pillows shall be clean and free of contaminants.
- Crew shall wear gloves when handling pillows and plants.
- Veggie hardware (bellows, etc.) shall be wiped with Pro-San wipes prior to installation of plant pillows and following growth.
- Tools shall be wiped before and after use.
- Produce shall be wiped at harvest according to produce-specific procedure.

Sanitizing Produce



Veg-01 Second Crop

- ½ the produce harvested for consumption, ½ for science
- Plants harvested Aug. 10, 2015 and eaten live on NASA TV



Thank you!

- Veggie and VEG-01 teams at KSC and ORBITEC
- Astronauts Steve Swanson, Rick Mastracchio, Scott Kelly, Kjell Lindgren
- Payload Operations and Integration Center
- Grace Douglas and Mark Ott

Veggie
Send more seeds!
We are hungry.
-Rick Mastracchio

- NASA Space Life and Physical Sciences, Human Research Program, and ISS Program

Backup Information

Microbial counts on Veggie lettuce samples

	Flight Plants			Ground Control Plants				
Plant Pillow	A	C	D	A	B	C	E	F
Plant weight (g)	8.6	7.6	5.8	8.0	7.9	6.9	6.0	2.6
Aerobic Plate Count (cfu gfw^{-1})	3120	2670	4900	975000	19	804	50	<29
Yeast and Mold Count (cfu gfw^{-1})	497	1120	569	891	<9.5	370	<12.5	<30
E. coli, S. aureus, Salmonella sp.	< DL**	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Microscopic Cell Count gfw^{-1}	8.45 x 10^7	8.79 x 10^7	1.38 x 10^8	3.45 x 10^7	3.98 x 10^7	4.46 x 10^7	6.73 x 10^7	1.35 x 10^8

**Below detection limit.

Identification of isolates on Veggie lettuce

	Flight Plants			Ground Control Plants				
Pillow	A	C	D	A	B	C	E	F
Bacteria isolated*	1, 4, 8	4, 5, 6, 8	1, 6, 5, 9,	1, 2, 9	Isolates not Identified	Isolates not Identified	1	none
Fungi isolated*	1, 3, 4	4	5	4	none	2	none	none

*Numbers indicate isolate identification from list below

Bacteria

1. *Burkholderia cepacia*
2. *Burkholderia fungorum*
3. *Ralstonia insidiosa*
4. *Curtobacterium flaccumfaciens*
5. *Acinetobacter genospecies 3*
6. *Ralstonia picketti*
7. *Arthrobacter ilicis*
8. *Sphingomonas parapaucimobilis.*
9. *Flavobacterium columnare (water)*

Yeast and filamentous fungi.

1. *Spordiobolus pararoseus*
2. *Cryptococcus albidus var diffluent*
3. *Rhodotorula aurantiaca B*
4. *Rhodotorula glutinis*
5. *Rhodotorula achemiour*
6. *Penicillium sp (presumptively identified by microscopy)*
7. *Aspergillus sp. (presumptively identified by microscopy)*

Produce Sanitation Validation: Ground

Vegetables:

- Two types of produce with very different surface topographies were tested: 'Outredgeous' red romaine lettuce and radish.

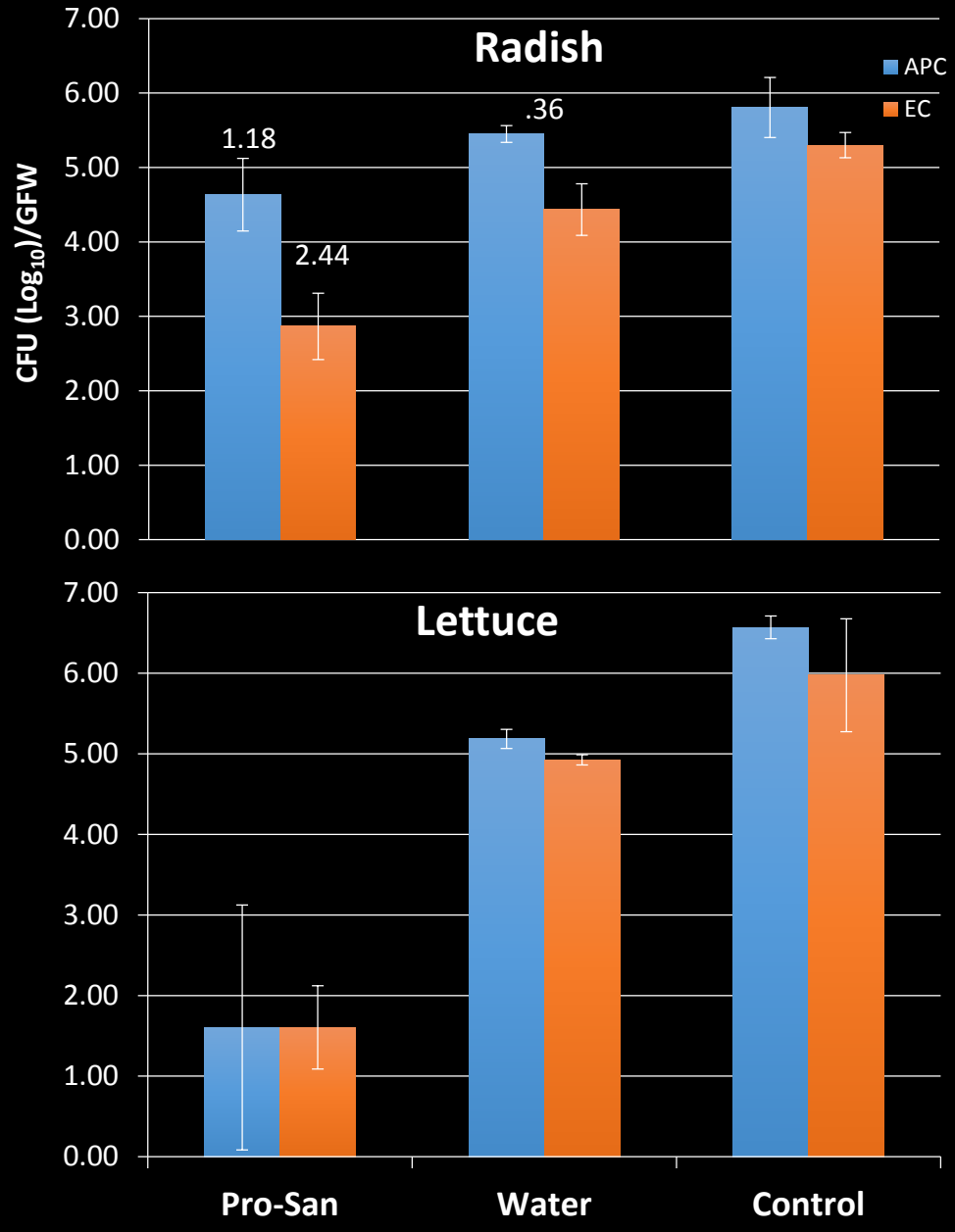
Challenge inoculation:

- Produce was inoculated with known levels of *Staphylococcus aureus (*ATCC *25923), Escherichia coli (*ATCC 4495562), and *Pseudomonas aeruginosa (*ATCC 3513563*).*

Sanitizing wipes:

- Polypropylene crew wipes (Kimberly Clark) were used to coincide with materials currently on ISS. Wipes were saturated with a 1% solution of Microcide® Pro-San sanitizer. Controls included water saturated wipes and no cleansing treatment.

Produce Sanitizing



- The numbers above each bar on the graphs indicate the $\log_{10}$ reduction of total aerobic bacteria **(APC)** and *E. coli* **(EC)** compared to controls. (e.g. 1 $\log_{10}$=90% reduction in counts)
- The Pro-San sanitization procedure reduced the average **APC** by 1.18 log per gram on the radish and **EC** was reduced by 2.44 log.
- Pro-San sanitization achieved a 5.25 log reduction in **APC** and a 4.37 log reduction in **EC** on lettuce.

(Starting *E.coli* inoculum on radish= ~6 x 10^7 , lettuce=~4 x 10^7)

Materials and Methods-Surface Sanitization

Surfaces:

- Two different Veggie surface materials were tested; Teflon Coated Kevlar (TCK) which is used on Veggie pillows and the clear bellows material.

Challenge inoculation:

- Plastics were inoculated with *Staphylococcus aureus (*ATCC *25923), Escherichia coli (ATCC 4495562),* and *Pseudomonas aeruginosa (*ATCC 3513563*).*

Sanitizing wipes:

- Two different wipe materials were tested for plastic surface sanitization. Initially a product called Mighty Wipes™ was used. These are low lint, clean room wipes.
- Subsequently, polypropylene crew wipes (Kimberly Clark) were used to coincide with materials currently approved for use on ISS. Wipes were saturated with a 1% solution of Microcide™ Pro-San sanitizer. Controls included water saturated wipes and no cleansing treatment.

Surface Sanitizing

Log reduction (from untreated surfaces) in microbial counts (CFU) after 30 sec. wipe (Mighty Wipe™) with sterile water or 1% sanitizer. (Two surfaces were tested based on materials used for the Veggie hardware.)

	Teflon Coated Kevlar		Clear Plastic	
Organisms	Water	Sanitizer	Water	Sanitizer
P.aeruginosa	1.219	5.079	3.292	6.333
S. aureus	2.592	6.767	3.920	5.698
E.coli	3.169	6.614	4.748	6.098

Log reduction (from untreated surfaces) in microbial counts (CFU) after 30 sec. wipe with sterile water or 1% Pro-San® saturated polypropylene wipes.

	Teflon Coated Kevlar		Clear Plastic	
Organisms	Water	Sanitizer	Water	Sanitizer
P.aeruginosa	0	4.182	1.792	4.509
S. aureus	3.725	3.957	4.477	4.807
E. coli	No growth detected on any treatments*			

*In this test *E. coli* apparently did not survive the desiccation on the plastic surface.

Surface Sanitizing Summary

- The sanitizer was effective in reducing the number of challenge bacteria by 4 to >6 orders of magnitude on the Teflon Coated Kevlar (TCK) and clear plastic when assayed using surface swabbing.
- Dry wipe material type had an effect on the reduction of bacteria from both surfaces.
- Cleaning with water saturated wipes reduced the numbers of bacteria on surfaces however, microorganisms could survive on the wipes after 48 hr of storage while sanitizer soaked wipes exhibited a biocidal effect with no to few bacteria surviving after use and storage (data not shown).

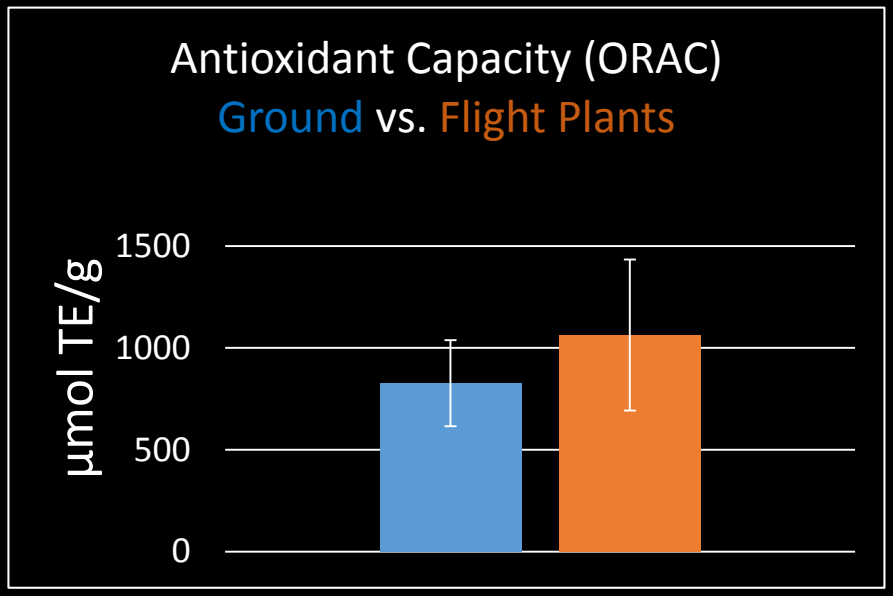
Produce Preparation for ISS

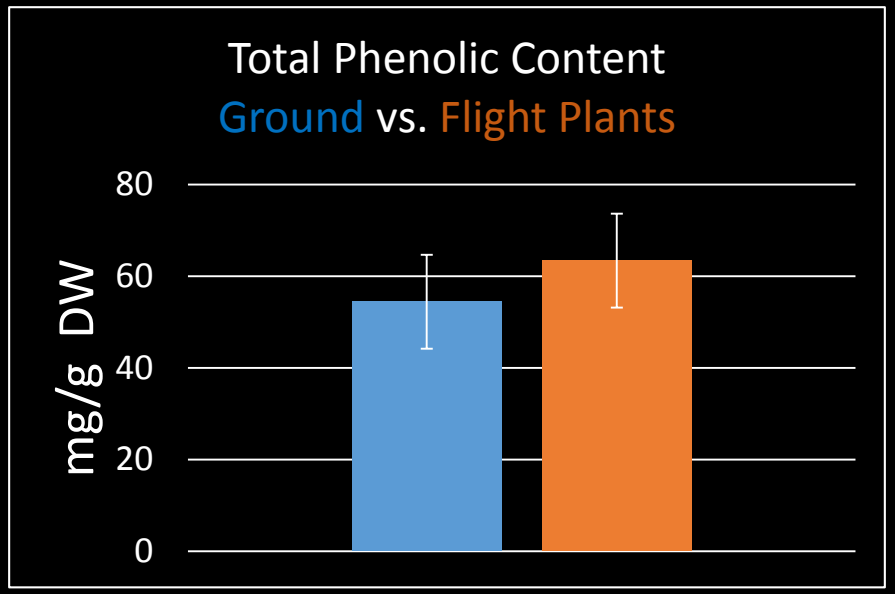
This procedure is intended to provide the basic rules & standards applicable to the handling and preparation of fresh food for stowage – Space Food Systems

- Product shall be clean and free from foreign matter, approved for food use and have typical odor, color and flavor.
- Product shall be in excellent condition at time of use and from the freshest lot available.
- Clean produce thoroughly in flowing potable water and drain.
- Dip products into 200ppm chlorine solution for a minimum of 30 seconds.
- Let products air dry on a clean dry surface.
- Bag and pack for flight.

Anthocyanins / Antioxidants / Phenolics

- Anthocyanins were the same between ground and flight
- Antioxidants and Phenolics were slightly higher in flight plants





Elemental Analysis

- Iron levels were identical and Calcium, Molybdenum and Phosphorus were similar between flight and ground.
- Boron, Copper, Magnesium, Manganese, Sodium and Sulfur were slightly higher in flight plants.
- Potassium was slightly higher in ground plants.
- Nickel and Zinc were significantly higher in flight plants.

- Flight water had low levels of Sodium, Potassium, Chlorine, Sulfate and Nitrate at the start; ground water had Fluorine, Chlorine, Sulfate and Nitrate.