

1 Leafy Greens Grown on the International Space Station May Provide a Nutritious Supplement to
2 Astronauts' Diet

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6 Supplemental safe food production has been an essential goal of NASA to meet the nutritional
7 needs of astronauts on the International Space Station (ISS) as well as for future long duration
8 missions to the moon and beyond. Food crops grown in space experience different environmental
9 conditions than plants grown on Earth (i.e. microgravity and spaceflight physical sciences

10 impacts). To test the growth methods and effects of the space environment, red romaine lettuce
11 *Lactuca sativa cv. 'Outredgeous'*, was grown in Veggie plant growth chambers on the ISS.

12 Microbiological food safety of the plants grown on the ISS was determined by heterotrophic
13 plate counts to assess total microbial load for bacteria and fungi as well as screening for specific
14 pathogens and isolate identification. Molecular characterization was completed using Next
15 Generation Sequencing (NGS) to provide valuable information on the taxonomic composition
16 and community structure of the plant microbiome. Chemical analyses of plant tissue were
17 conducted to understand spaceflight-induced changes in key elements in the space diet,

18 phenolics, anthocyanin levels, and Oxygen radical absorbance capacity (ORAC), a measure of
19 antioxidant capacity. Three growth tests of red romaine lettuce were completed on ISS, VEG-
20 01A, VEG-01B, and VEG-03A. Plants were harvested using two harvest methods, either a
21 single terminal harvest (after 33 days) or cut-and-come-again repetitive harvesting (64 days total

22 growth). Ground controls were grown simultaneously with a delay to accommodate condition
23 monitoring and replication. A comparison of the plant tissue returned to Earth showed leaves

24 from the second grow-out had significantly higher bacterial counts than the preceding or
25 subsequent growth test or any of the ground controls. Fungal counts were significantly higher on
26 the final cut-and-come-again harvest of the third grow out. None of the potential foodborne
27 pathogens that were screened for were detected. Bacterial and fungal isolate identification and
28 community characterization indicated similar diversity between VEG-01A and VEG-01B growth
29 tests, however, there appeared to be subtle differences in diversity and distribution among the
30 three growth tests. Chemical analysis of plant tissue revealed significant variation in a few
31 elemental data, but variation in levels of phenolics, anthocyanins, and ORAC was not
32 significantly different. This study indicated that leafy vegetable crops could safely provide an
33 edible supplement to astronauts' diet, and our analysis provided baseline data for continual
34 operation of the Veggie plant growth units on ISS. This research was funded by NASA's space
35 biology program.