

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.