

Article

Foodomics of Rice Grains in Astrobiology: Spaceflight-Induced Modulation of Aroma, Texture, and Protein Digestibility in Thai Landrace Rice (466HM) Aboard the Shijian-19 (SJ-19) Low Earth Orbit Mission

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Abstract

Ensuring a sustainable source of nutritious food is critical for long-duration space missions. Thai landrace rice 466HM exhibits high nutritional value and stress resilience, making it a promising candidate for space cultivation, yet its response to low Earth orbit (LEO) conditions remains poorly understood. This study compared rice grains maintained under terrestrial conditions with grains stored aboard the Shijian-19 (SJ-19) reusable satellite, orbiting at ~336 km for 13.5 days under microgravity ($2^{-7} \times 10^{-7}$ g) and an absorbed radiation dose of ~0.153 rad (Si). Volatile compound profiling, texture analysis of cooked grains, and simulated gastrointestinal digestion followed by peptide mass fingerprinting were performed. LEO-exposed rice grains exhibited a 1.67-fold increase in adhesiveness compared to Earth-based rice ($p < 0.01$), while hardness remained unchanged between the two groups ($p > 0.05$), alongside distinct alterations in flavor-related volatile compounds and peptide profiles. Principal component analysis revealed clear separation between Earth and LEO-exposed samples, indicating microgravity-associated shifts in digestible peptide composition. Cytotoxicity assessment using MTT assays in HT-29 and HepG2 cells confirmed the safety of both rice types. These findings demonstrate that orbital conditions influence the compositional, functional, and sensory attributes of rice, providing insights relevant to space agriculture and astronaut nutrition.



Academic Editor: Fathi Karouia

Received: 16 January 2026

Revised: 2 February 2026

Accepted: 5 February 2026

Published: 10 February 2026

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Keywords: recoverable satellite; microgravity; jasmine rice; peptide; digestion; space agriculture

1. Introduction

Ensuring a sustainable and nutrient-rich food supply for long-duration space missions is a critical challenge as humanity transitions from short orbital visits toward extended exploration and eventual extraterrestrial colonization [1]. The impracticality of resupplying food over months or years, coupled with the degradation of nutritional value in stored provisions, necessitates exploring alternative strategies, including the use of raw food materials cultivated or maintained in space. To meet this need, initial studies have begun focusing on fundamental staple crops such as rice within low Earth orbit (LEO), aiming to understand how conditions in orbit influence their nutritional and culinary properties. In this context, integrating knowledge from plant biology, food science, and space engineering is essential for developing a closed-loop, on-board food system capable of supporting human health and well-being. Examining rice grains under LEO conditions is especially relevant because radiation and microgravity together represent a potent combination of stressors unlike those experienced by the Earth control. Galactic cosmic rays (GCRs), solar particle events (SPEs), and particles originating from the Van Allen belts create a complex radiation environment, posing health risks to astronauts and potentially altering cellular and molecular processes in plants [2,3]. Additionally, the outer edges of the Van Allen belts contain high-energy particles that can impact satellites and spacecraft in upper LEO. This radiation environment requires careful monitoring and shielding, especially for long-term missions like those on the International Space Station (ISS) [4]. Providing a raw food supply that meets the nutritional, physiological, and psychosocial needs of astronauts is critically important for space exploration. This environment not only challenges plant growth, physiology, and metabolism but may also modify the palatability and nutritional adequacy of staple foods intended for astronaut consumption [5]. Despite rice's global importance as a carbohydrate source, how radiation and related space factors influence its eating quality and nutritional composition remains largely unknown, presenting a pressing knowledge gap in space food research.

Addressing this gap becomes possible through the use of specialized orbital platforms like the Shijian-19 (SJ-19) recoverable satellite, which enables controlled exposure of biological samples to the full range of space conditions for a defined period. Unlike simulations on Earth, the SJ-19 platform circumvents atmospheric shielding and constant gravitational fields, subjecting rice grains to genuine microgravity, high-energy radiation, and extreme temperature fluctuations. These combined stressors can induce alterations to plant tissues at molecular and metabolic levels that cannot be fully replicated in ground-based experiments, thereby providing unique insights into how staple crops adapt, survive, and potentially thrive under extraterrestrial conditions [6–8]. Additionally, GCR introduces high-energy particles that penetrate cells, causing damage to genetic materials and other critical biomolecules such as lipids, proteins, and carbohydrates [9]. This radiation can induce mutations affecting protein structure and activate stress-response pathways [10]. Potentially altering the nutritional and sensory qualities of the rice grains. Identifying these adaptations is vital for designing cultivation systems that ensure adequate nutrition for astronauts on deep space missions.

Rice grains are primarily carbohydrate food for Asia. Cooked rice texture attributes, such as hardness and stickiness, are considered important factors in its palatability [11]. Within the broader diversity of rice, landrace varieties such as Hawm Mali (466HM) and

RD22 are of particular interest due to their genetic resilience to environmental stress [12,13]. Unlike modern high-yielding strains, landraces have been shaped by centuries of selection under challenging field conditions, leading to stable traits such as drought tolerance, robust aromatic profiles, and distinctive textural attributes [14]. By identifying rice varieties that can withstand the challenges of space cultivation while retaining their nutritional value and suitability for cooking, we take a crucial step toward sustainable food production for long-duration missions. This research contributes to our understanding of extraterrestrial environments, informing future efforts to grow and prepare food beyond Earth. Beneficial for various landrace rice traits, they are critical genetic resources for advancing rice breeding programs, especially in the context of environmental conditions like those found in LEO [12]. Cultivated and naturally selected over centuries within specific ecological and cultural settings, these traditional varieties have developed unique adaptations to local environments. These adaptations include resistance to pests, diseases, and abiotic stresses such as drought, salinity, and temperature extremes [15]. Their genetic diversity serves as a reservoir of traits that can be utilized to address challenges posed by fluctuating temperatures, low atmospheric pressure, and harmful radiation. The use of landrace rice enables the incorporation of traits that enhance yield stability and nutritional value [15]. Investigating how 466HM rice grains respond to LEO conditions offers an opportunity to leverage their inherent genetic strengths, adapt them for space agriculture, and ultimately guide crop selection and breeding strategies. Understanding these responses also enhances knowledge of how environmental extremes influence food quality on a molecular level, reinforcing the importance of biodiversity and traditional genetic resources in future-oriented agriculture.

Foodomics approaches, including advanced chromatographic and spectrometric techniques, provide the necessary analytical power to dissect molecular changes in rice grains exposed to orbital conditions [16]. Additionally, gas chromatography-ion mobility spectrometry (GC-IMS) enables detailed sensory analyses, helping us understand the full impact of space conditions on raw food materials from molecular changes to alterations in taste and aroma that affect consumer acceptability. Moreover, proteins and carbohydrates are essential macronutrients vital for human health. Proteins provide amino acids necessary for tissue repair, enzyme function, and hormone synthesis, while carbohydrates serve as the primary energy source for metabolic processes. Changes in the protein and carbohydrate content of rice grains due to LEO conditions could have significant implications for the nutritional adequacy of astronauts' diets. Similarly, alterations in sensory attributes such as taste, texture, and aroma could affect food intake and psychological well-being [17]. We hypothesized that LEO radiation and microgravity may alter volatile metabolism, starch physiochemistry, and protein digestibility through molecular-level stress effects. Through such integrative analyses, this work aims to inform the development of space-based agricultural systems capable of sustaining human life far beyond Earth's boundaries, paving the way for future interplanetary missions where crop cultivation will be central to mission success.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Seeds of the Thai aromatic landrace rice variety Hawm Mali-466 (466HM), identified under genetic stock number GS: 18415, were provided by the Ubon Ratchathani Rice Research Center, Thailand. This variety was selected based on its distinct aromatic profile and environmental resilience. A total of 15 g of dry seeds were used for the study. The experimental design consisted of two groups: an Earth control group maintained under terrestrial conditions and a treatment group subjected to low Earth orbit (LEO) exposure. Both groups were derived from the same seed batch to ensure genetic uniformity. The LEO

group was included in a spaceflight mission for a defined duration, while the Earth control seeds were stored under 15–20 °C with 55–60% relative humidity on Earth. After exposure, both seed groups underwent downstream analyses to compare biochemical, sensory, and digestibility parameters. This experimental setup enabled the evaluation of how short-term microgravity influences the foodomic properties of traditional 466HM rice grains.

2.2. Interface Payload Support, Dried Seed Installation and SJ-19 Satellite Conditions

The SJ-19 satellite was designed as a reusable platform for space experiments, capable of supporting recoverable payloads. The SJ-19 satellite provided a controlled microgravity environment for the rice seed experiments. The satellite maintained a quasi-steady acceleration level around $10^{-6} g_0$ and a vibration and jitter acceleration around $10^{-4} g$. The flight duration lasted 13.5 days, during which the satellite orbited at an altitude of 336 km with an inclination of 41.5°. The samples were housed in the reentry capsule. The propulsion and power capsule, housing the satellite's operational components, sustained a temperature range of 5–20 °C. The samples were contained in sealed plastic tubes, ensuring a low-pressure environment throughout the flight. During launch, the acceleration load on the samples ranged between 6 and 8 g_0 . The satellite's environment was controlled during pre-launch procedures in the Beijing and launch site AIT workshops, with temperatures ranging from 15 to 25 °C and relative humidity maintained at 15–60%. The dry seeds used in the experiment were packaged using a three-layer system designed specifically for the SJ-19 mission. The outer and medial layers of the package were provided by the satellite's payload system, while the interlayer, made from white cotton cloth, was prepared by the researchers. Each interlayer package contained a maximum of 200 g of seeds and had dimensions of less than 100 mm × 140 mm. The packaged seeds were placed in sealed plastic tubes to maintain low pressure during the flight. The researchers labeled each package for identification, following a predefined coding system based on the sample type and origin. The propulsion and power capsule, housing the satellite's operational components, sustained a temperature range of 6–20 °C. The temperature was recorded in the TMHR220 region. The prepared seed samples were delivered to the China National Space Administration (CNSA) for installation into the satellite's payload compartment up to 96 h before launch on 27 September 2024 around 5 p.m. UTC and back to the Earth on 11 October 2024 around 5 p.m. UTC.

2.3. GC-IMS-Based Aroma and Volatile Profiling of 466HM Rice Grains

Aroma profiling of 466HM rice grains was analyzed using GC-IMS (FlavourSpec[®], G.A.S., Dortmund, Germany). The raw rice grains from two experimental groups (Earth control and LEO-exposed SJ-19), each with three biological replicates ($n = 3$), were prepared by transferring 0.5 g to a 20 mL airtight headspace vial with a magnetic cap. Then, each sample was heated to 80 °C and agitated for 10 min prior to sampling. Once completed, the headspace from the sample was injected into the GC-IMS equipment for analysis. The volatile compound features (VOC) were separated by using FS-SE-54-CB-1 (ID 0.53 mm, 15 m) at a constant flow rate of 2 mL/min. The column temperature was 60 °C, and the carrier gas was nitrogen. The resulting ions were driven to a drift tube (9.8 cm in length), which was operated at a constant temperature (45 °C). The flow rate of the drift gas (nitrogen gas) was set at 150 mL/min. The total chromatographic run time was 25 min. Ionization was achieved by a tritium β -emitter at 5 KeV, which generates reactant ions via proton transfer in positive ion mode. This allows for the VOC to be separated by their mass-to-charge ratio, with larger molecules drifting slower than small molecules, resulting in variable drift times. The VOC is characterized by the GC-IMS retention time measured in seconds, by the IMS drift time in milliseconds, and by the intensity of the ion current signal.

2.4. GC-IMS Data Preprocessing and Multivariate Analysis

Raw intensity data from GC-IMS was collected from two experimental groups (Earth control and LEO-exposed SJ-19), each with three biological replicates ($n = 3$). The acquired dataset consisted of 6 samples (rows) and 142 aligned volatile compound features (columns), resulting in a data matrix of 6×142 (samples $\times$ compounds). Data preprocessing, including baseline correction, peak alignment, and deconvolution of overlapping signals, was performed using the instrument's software. Because the scope of this work was focused on profiling and pattern comparison, no alkane standards or retention index calibration were employed, and compound identification was not pursued. The aligned feature matrix was exported to MetaboAnalyst 5.0 for chemometric analysis [18]. Missing values were imputed by half of the minimum detected positive value, and data were standardized using z-scores. This standardization minimizes the impact of differing scales among features on the principal component analysis (PCA) results. The dataset was organized so that each sample's chemical compound intensity measurements were presented across multiple columns (areas). PCA was performed on the standardized dataset shown in score plots. A hierarchical clustering algorithm, using Euclidean distance as the metric and average linkage as the clustering method, was applied to the standardized data. This clustering approach grouped samples and compounds based on similarity, allowing the identification of patterns across the Earth control and LEO-exposed groups. The clustering results were visualized as a heatmap, using a red-to-green color scheme to denote high and low compound intensities. Biochemical pathway interpretation is constrained by the absence of compound annotation.

2.5. Cooked Rice Preparation and Texture Profile Analysis (TPA)

To investigate texture profiles, HM466 rice grains in the Earth control and LEO-exposed samples were cooked by modification methods [19,20]. Unbroken-milled rice grains in each group were cooked at a ratio of 1:1.3 (rice grain: deionized water, w/w) and were washed two times with deionized water. The rice samples in the test tube were covered by aluminum foil, then cooked in a boiling water bath (95 °C) for 20 min. The cooked rice was then placed in a water bath at 50 °C for further texture analysis. Cooked-rice grain in each group were carefully placed as per the kernel sampling method [21]. Texture Profile Analysis (TPA) properties were characterized by TA.XTplusC Texture Analyser. The setting parameters of TPA were modified as follows [19]: pre-test speed, 2 mm/s; test speed, 1 mm/s; post-test speed, 2.00 mm/s; strain, 75%; trigger force, 5 gf. The analysis was conducted in two independent experiments, with five individual rice grains measured per group (Earth and LEO) in each experiment. Data are presented as mean $\pm$ standard deviation across the replicates and experiments.

2.6. In Vitro Human Gastrointestinal Tract (GIT) Digestion

The digestion of HM466 rice grains under Earth control and LEO-exposed conditions was carried out following the method described in [22] with a slight modification. These protocols are broadly consistent with commonly applied INFOGEST-type models in terms of the sequential oral, gastric, and intestinal phases and the order of enzyme application [23]. To minimize sample bias, two rice grains were used for each experimental group under each condition. A total of 12 experimental replicates were performed for each condition. The rice grains were cooked as mentioned earlier (Section 2.5). To mimic oral digestion, the cooked rice grains were combined with 2 mL of a buffer solution (120 mM NaCl, 5 mM KCl, 5 mM CaCl₂, pH 7.0) containing 75 U/mL of α -amylase. After digestion with amylase for 3 min, 8 mL of the buffer solution was added, and the pH was adjusted to 1.5 using 6 M HCl. Pepsin was added at a final concentration of 2000 U/mL to replicate gastric

digestion, which was carried out for 45 min. After that, the pH was adjusted to 6.0 with 4 M NaHCO₃ to neutralize pepsin activity. Pancreatin (100 U/mL final concentration) and bile salts (10 mM final concentration) were added to simulate intestinal digestion. The pH of the reaction mixture was adjusted to 7.4 with 4 M NaHCO₃, and the digestion process was continued for 240 min. After digestion, all samples were centrifuged at 12,000 × g for 30 min. The resulting supernatants were collected and subjected to peptide mass fingerprinting using tandem mass spectrometry (LC-MS/MS) analysis and cell cytotoxicity testing.

2.7. Peptide Mass Fingerprint (PMF) of In Vitro Digested 466HM Rice Grains by LC-MS/MS

PMF of digestible HM466 rice grains under Earth control and LEO-exposed conditions was analyzed by direct infusion in Orbitrap Exploris™ 240 LC-MS/MS. The peptide content was measured by the Pierce Quantitative Colorimetric Peptide Assay (ThermoFisher, Co., Waltham, MA, USA) using bovine serum albumin as a protein standard. A total of 10 ng/mL of total peptide was directly injected to LC-MS/MS at 20 µL with an electrospray ionization source in positive mode (3.4 kV). The full scan spectrum was collected in *m/z* 300–4000 at a resolution of 480,000 for 0.5 min. The spectra were acquired by Xcalibur 4.7 software in .raw file format. PMF data was analyzed by first extracting CSV files obtained from the average raw file spectrum from 0.1 to 0.4 min by Freestyle (ThermoFisher, Co., Waltham, MA, USA). Each file was preprocessed to extract relevant data by skipping meta-data rows and focusing on the *m/z* and intensity columns. The *m/z* range was divided into 50 bins, and the sum of intensities within each bin was calculated to create a feature vector for each file. The feature vectors were standardized using z-scores to ensure uniform scaling. PCA was applied to the standardized data by calculating eigenvectors and eigenvalues of the covariance matrix and projecting the standardized feature vectors onto the first three principal components (PC1, PC2, and PC3) to capture the maximum variance.

2.8. Cell Viability and Cytotoxicity Assessment of GIT Hydrolysates from Earth Control and LEO-Exposed Rice Grains

To evaluate the potential biosafety of rice peptides derived from gastrointestinal digestion under different gravitational conditions, cytotoxicity testing was conducted using the MTT assay. Cooked 466HM rice grains from both Earth-based and LEO-exposed groups were first subjected to the in vitro GIT process as described in Section 2.6. The resulting peptide hydrolysates, which reflected compositional and proteolytic differences observed in PMF, were used as treatment samples for cellular assays. Human intestinal epithelial cells (HT-29) and hepatic cells (HepG2) were selected as representative models for nutrient absorption and metabolic detoxification, respectively. Cells were seeded in 96-well plates at a density of 1×10^5 cells/well and incubated overnight at 37 °C in a humidified 5% CO₂ atmosphere. The cells were then treated with peptide solutions at concentrations of 25, 12.5, 6.25, and 3.125 µg/mL for 12 h. Negative controls consisted of untreated cells in culture medium. Following treatment, the medium was removed, and 0.5 mg/mL MTT reagent was added. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. All treatments were performed in two independent experiments, each with three technical replicates. The average cell viability was calculated relative to control, enabling comparison of potential cytotoxic effects associated with LEO-induced changes in peptide profiles. This assay served as a critical complement to compositional analyses, confirming the biosafety of altered peptide products resulting from orbital exposure.

2.9. Statistical Analysis

The mean and standard deviation of the normalized TGA values were computed for both conditions. An independent samples *t*-test was performed to assess whether there was a statistically significant difference between the control and treatment groups. The resulting *p*-value determined the statistical significance, with a threshold of $p < 0.05$. For multivariate PMF data, Multivariate Analysis of Variance (MANOVA) was applied to the principal component scores (PC1 and PC2) derived from PCA. In order to formally test whether the overall multivariate peptide patterns differed between the Earth control and LEO-exposed groups. Statistical significance was assessed using a two-sided test with a significance threshold of $p < 0.05$.

3. Results

3.1. Sample Installation in Payload of SJ-19 and LEO Conditions

The presence of a controlled microgravity environment aboard the SJ-19 satellite allowed 466HM rice grains to be maintained under LEO conditions throughout the 13.5-day flight, ensuring that the sealed payload design minimized environmental fluctuations. The experimental setup employed the CNSA's Sealed Plastic Tube (SPT) system, which housed cryogenic tubes containing the samples at pressures above 0.5 atm, effectively preserving sample integrity against potential outgassing or vapor loss during orbit. The workflow initiated with the insertion of rice samples into cryogenic tubes, followed by their placement into the SPT units and subsequent arrangement of SPT-loaded brackets into a passive payload container, thus providing multiple protective layers and mitigating external mechanical stresses (Figure 1A,B).

The monitored conditions indicated that the spacecraft maintained quasi-steady acceleration levels ranging from 7×10^{-7} to 2×10^{-7} g and orbited at approximately 336 km altitude with an inclination angle of 41.5° , completing each orbit in about 90 min, reflecting a typical LEO operational profile. Figure 1C presents the temperature profile recorded inside the SJ-19 reentry capsule, demonstrating stable thermal conditions critical for preserving sample integrity during spaceflight. The data show that the internal temperature was maintained near 11.3°C for the majority of the mission, with only a transient increase to a maximum of 17.05°C occurring during the high-energy phases of launch and reentry. This narrow thermal range confirms the effectiveness of the passive payload insulation and the SPT containment system in mitigating temperature fluctuations. In addition to temperature and mechanical parameters, radiation monitoring within the spacecraft revealed an absorbed dose of approximately 0.153 rad (Si), measured by a silicon-based dosimeter embedded within the payload compartment. This value reflects the internally shielded radiation exposure during the 13.5-day orbital flight, which is critical for assessing the stability of biological macromolecules and electronic systems under LEO conditions. These stable conditions support the reliability of downstream biochemical and foodomic analyses by minimizing thermally induced degradation during low Earth orbit exposure. Ground-based pre-launch procedures had carefully regulated environmental conditions, and during launch, samples experienced accelerations of 6–8 g, decreasing to less than 15 g upon reentry, confirming that the payload design successfully minimized shock loads and sustained sample stability for downstream biochemical analyses.

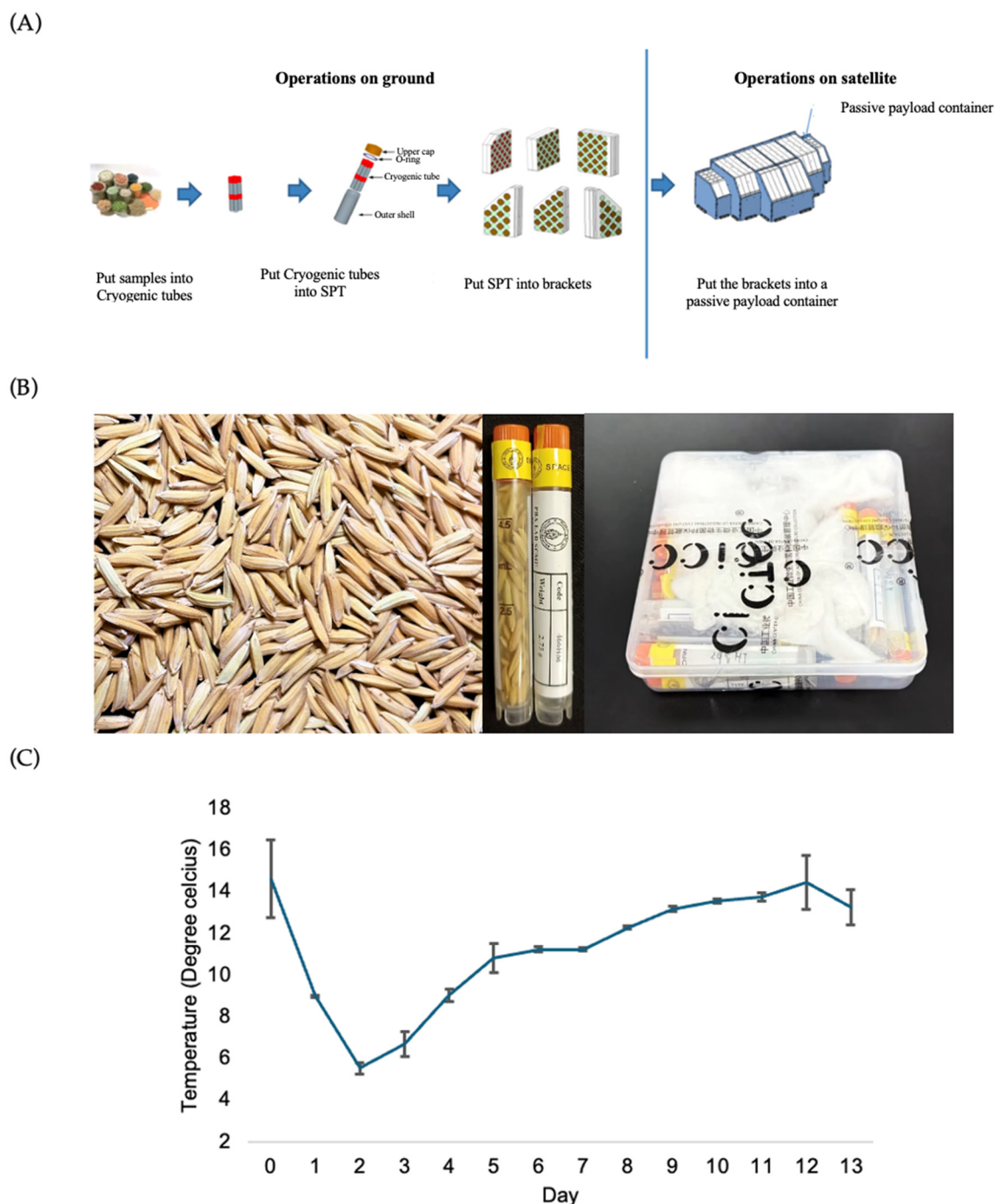


Figure 1. Overview of the SJ-19 satellite experimental setup and environmental conditions. (A) Workflow of sample installation in the payload in SJ-19. (B) Schematic showing the three-layer packaging system used to seal the rice grain samples in the SPT. The payload container was a passive system designed for microgravity with high gravity tolerance experiments. (C) Recorded temperature conditions inside the reentry capsule, with a stable temperature maintained at approximately 11.3 °C, peaking at 17.05 °C during launch and reentry phases.

3.2. GC-IMS-Based Aroma and Volatile Profiles of Earth Control and LEO-Exposed 466HM Rice Grains

Aroma and flavor profiles of cooked 466HM rice were analyzed using gas chromatography-ion mobility spectrometry (GC-IMS), detecting 177 volatile features based on signal alignment and intensity patterns. In the PCA (Figure 2A), the volatile compound profiles of LEO-exposed and Earth control samples were distinctly separated into two non-overlapping

clusters, indicating clear differences between the groups. PC1 explained 94.44% of the variance, while PC2 accounted for 2.45%, resulting in a cumulative variance of 96.89%. Heat map analysis using Euclidean clustering further confirmed these differences (Figure 2B). Earth control rice samples formed a distinct blue cluster, while LEO-exposed rice samples formed a red cluster, highlighting the divergence in volatile compound profiles. This separation highlights the significant impact of LEO exposure on the aroma and flavor profiles.

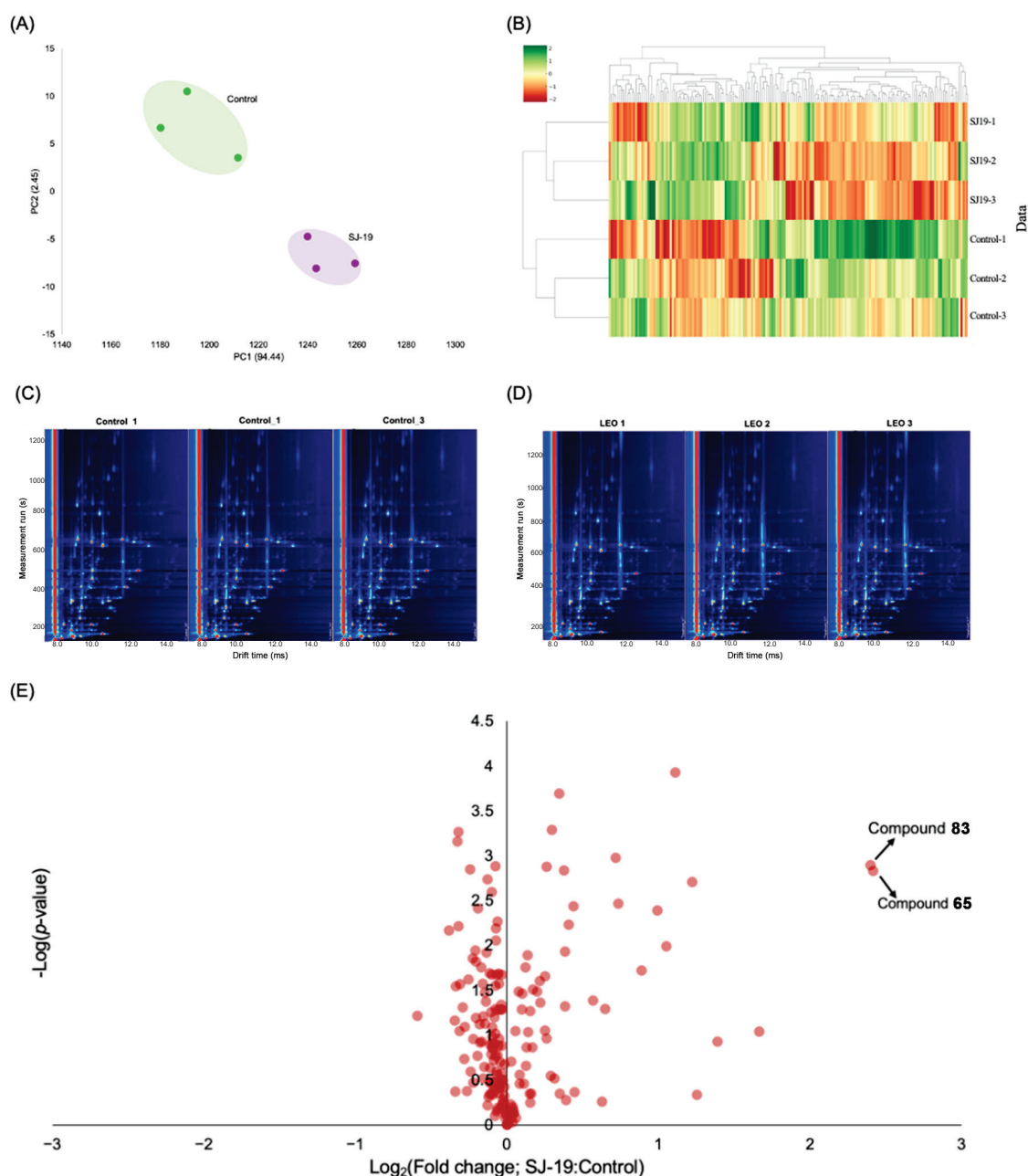


Figure 2. Aroma and flavor profiling of cooked 466HM rice grains. (A) PCA plot showing distinct separation between cooked Earth rice grains (green) and LEO-exposed rice grains (purple). (B) Heat map analysis with Euclidean clustering confirming distinct volatile compound profiles. GC-IMS biological fingerprints of volatile features in cooked 466HM rice grains under Earth control conditions (C) and LEO exposure aboard the SJ-19 satellite (D). The x-axis represents drift time (ms) and the y-axis represents measurement run (s). Each spot corresponds to a volatile feature, with signal intensity indicated by the color scale (blue = low and red = high). (E) Volcanic plot displaying the differential abundance of key volatile compounds, highlighting significant enrichment in specific volatile features under LEO-exposed conditions.

To compare the different samples, the GC-IMS spectrum of rice grains of the Earth control was used as a reference, as illustrated in Figure 2C. This reference spectrum was compared with that of rice grains exposed to LEO conditions aboard the SJ-19 satellite, as shown in Figure 2D. Among the 177 detected volatile compounds, 59 exhibited significant differences between the two conditions (Appendix A). Differential analysis indicated that 27 VOC were elevated and 32 were reduced in LEO-exposed rice compared to Earth control rice grains. The most notable changes included volatile compound feature 65, with a 5.33-fold increase (p -value = 0.0014), and compound 83, with a 5.28-fold increase (p -value = 0.0013). The findings demonstrated that LEO exposure alters the synthesis or release of specific aroma-related volatile compounds. These results emphasize the significant impact of LEO conditions on the volatile compound profiles, thereby affecting the aroma and flavor characteristics of cooked rice.

3.3. Texture Profile Analysis (TPA) of Cooked 466HM Rice Grains Under Earth Control and LEO Conditions

TPA of cooked 466HM rice was conducted in two independent experiments to ensure the reproducibility of the observed textural differences between rice samples exposed to LEO conditions and those grown and cooked under Earth conditions. The analysis focused on two key texture parameters: hardness and adhesiveness, which are critical for evaluating the mechanical and sensory properties of cooked rice.

Across both experimental replicates, the mean hardness of cooked rice exposed to the LEO environment aboard the SJ-19 satellite was 2859 ± 429 g, compared to 2434 ± 210 g in the Earth control rice (Figure 3A). Although there was a slight numerical increase in the LEO group, the difference was not statistically significant ($p > 0.05$). In contrast, adhesiveness showed a consistent and statistically significant increase under LEO conditions. Specifically, the LEO-exposed rice exhibited a significant increase in adhesiveness across both independent experiments ($p < 0.01$), corresponding to an approximate 1.5-fold increase in stickiness under LEO conditions. The mean adhesiveness of cooked rice exposed to the LEO environment aboard the SJ-19 was 149 ± 31.1 gf·s, compared to 90.07 ± 22 gf·s in the Earth control rice (Figure 3B). While hardness remained unaffected by LEO exposure, adhesiveness significantly increased, indicating that microgravity selectively alters specific textural characteristics of cooked rice grains. The consistency across two independent experiments enhances the reliability of these findings. Because only two independent experiments with a limited number of individual grains were available, the absence of a significant difference in hardness should be interpreted cautiously, and the magnitude of the observed change relative to within-group variability is more informative than statistical values alone. In this context, the approximately 1.67-fold increase in adhesiveness represents a comparatively large and reproducible effect, whereas the numerical difference in hardness corresponds to a small effect relative to the experimental variability.

3.4. PMF of In Vitro Digested 466HM Rice Grains Under Earth Control and LEO Conditions

PMF profiling of cooked HM466 rice grains under Earth control and LEO conditions revealed distinct differences in peptide distribution patterns between the two groups. The PMF for the Earth control condition is shown in Figure 4A, while that for the LEO condition is depicted in Figure 4B. The Earth control group spectra exhibited dominant peaks in the m/z range of 300–500, indicating a more uniform distribution of peptides in this region. In contrast, the LEO condition displayed higher density peaks in the m/z range of 600–750, indicating a difference in the distribution of peptide signals. These spectral differences highlight the variation in PMF under the two conditions.

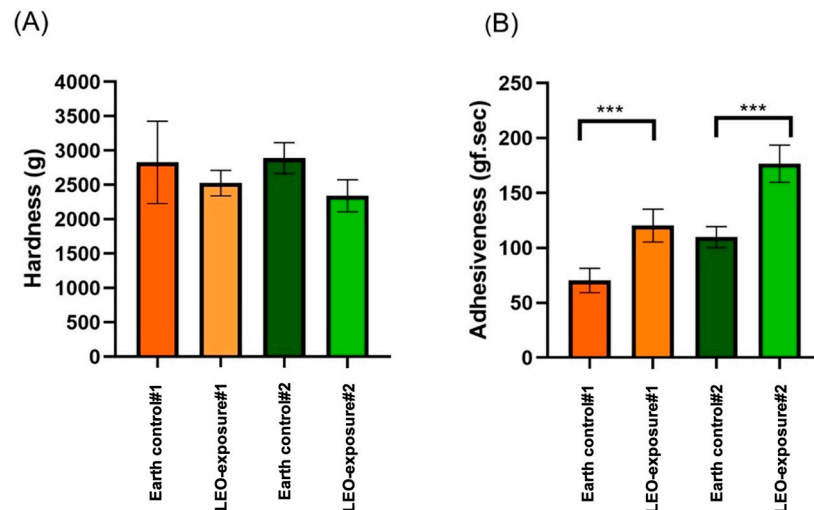


Figure 3. TPA of cooked 466HM rice comparing rice exposed to LEO conditions aboard the SJ-19 and Earth control samples. (A) Hardness values show no significant difference between the two conditions. (B) Adhesiveness values are significantly higher in LEO-exposed rice grains compared to the Earth rice grains, demonstrating an increase in stickiness. Data are presented as mean $\pm$ standard deviation from two independent experiments (independent experiments #1 and #2 are shown in orange and green, respectively), with significant differences indicated as *** $p < 0.01$.

To systematically analyze these differences and account for variations across replicates, PCA was applied. PCA was conducted using averaged spectra derived from 12 individual experiments per sample group, ensuring robust representation of the peptide profiles. The selection of MANOVA on PCA scores was selected because the PCA scores summarize the dominant sources of variance in the high-dimensional spectral data while reducing collinearity among variables. The PCA identified that the three principal components (PC1, PC2, and PC3) explained 47.36%, 8.56%, and 7.66% of the variance, respectively, collectively accounting for 63.58% of the total variance. The PCA scatter plots, shown in Figure 4C (PC1 vs. PC2) and Figure 4D (PC1 vs. PC3), illustrate the separation between the Earth control and LEO-exposed groups, reflecting distinct peptide profiles under the two experimental conditions. To confirm the statistical significance of the observed separation, multivariate analysis of variance was performed using PC1 and PC2 as dependent variables and group membership (Earth control vs. LEO-exposed sample) as the independent variable. The analysis revealed a highly significant difference between the groups (p -value < 0.001). These findings demonstrate the effectiveness of PMF combined with PCA in distinguishing the peptide fingerprints of rice grains under differing conditions.

3.5. Cell Viability and Cytotoxicity of GIT Peptide Hydrolysates Derived from Earth Control and LEO-Exposed 466HM Rice Grains

To verify the biosafety of cooked 466HM rice grains under Earth control and LEO-exposed conditions, MTT assays were performed on human intestinal (HT-29) and hepatic (HepG2) cell lines. The experimental design included two independent trials with three technical replicates each. The viability results demonstrated no statistically significant cytotoxicity in either cell line exposed to LEO or Earth rice hydrolysates. In HT-29 cells, cell viability remained consistently high across both groups (Figure 5A), while HepG2 cells similarly exhibited no viability reduction following exposure to any sample condition (Figure 5B). These findings indicate that short-term orbital cultivation did not induce any detectable toxicity in the rice, supporting its safety as a space food crop.

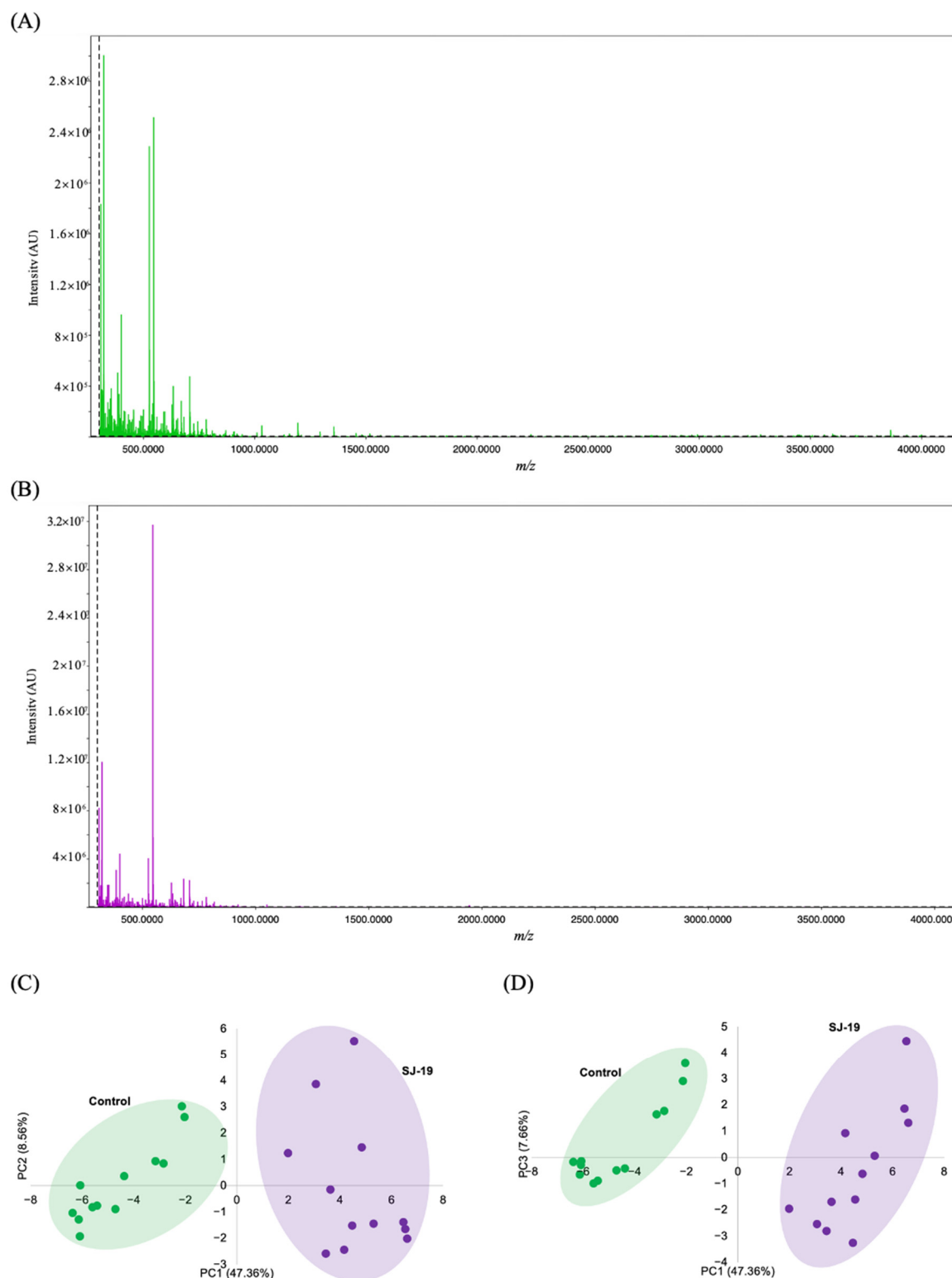


Figure 4. The PMF and PCA analyses revealed distinct peptide profiles of cooked 466HM rice grains under Earth control and LEO-exposed conditions. PMF spectra of cooked 466HM rice grains under control (A) and LEO-exposed (B) conditions. PCA scatter plots illustrating the separation of Earth control and LEO-exposed groups. (C) PCA based on PC1 and PC2 and (D) PCA based on PC1 and PC3. Each point represents the averaged spectrum from 12 individual experiments per group.

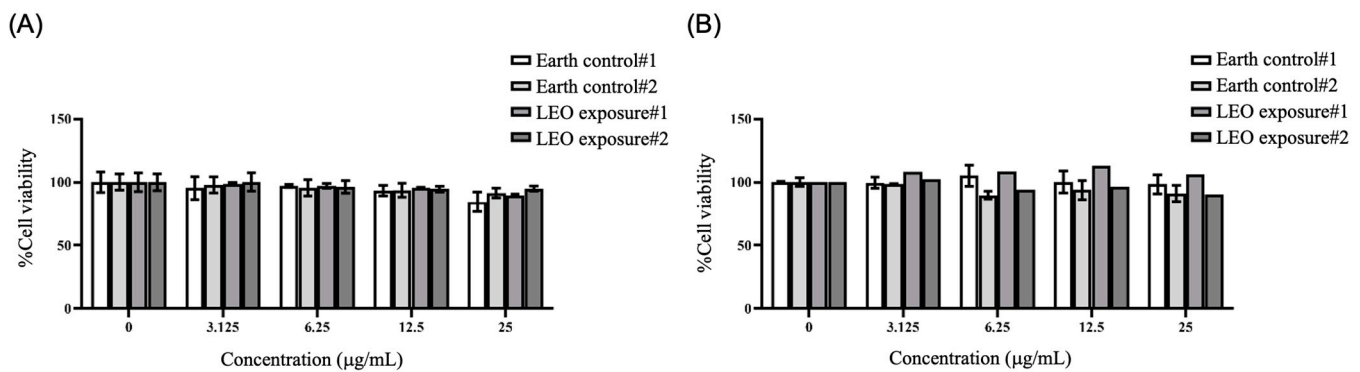


Figure 5. Cytotoxicity assessment of cooked 466HM rice grains under Earth control and LEO conditions aboard SJ-19. **(A)** Cell viability of HT-29 cells treated with rice hydrolysates from Earth control and LEO-exposed samples. **(B)** Cell viability of HepG2 cells treated with rice hydrolysates from Earth control and SJ-19-exposed samples. Cell viability was evaluated using the MTT assay in two independent experiments, each with three technical replicates.

4. Discussion

The findings demonstrate that short-term exposure of rice grains to LEO conditions can induce significant yet subtle biochemical and structural modifications, providing a new perspective on how staple crops adapt in extraterrestrial environments. Through the examination of volatile compounds, protein digestibility patterns, and textural attributes in Hawm Mali-466 (466HM) rice, it becomes evident that even brief orbital exposure can alter metabolic fluxes and structural integrity within plant tissues, laying the foundation for future research in space-based agricultural systems [24]. These discoveries underscore the importance of understanding plant adaptation to microgravity and radiation stressors and thus connect directly to the global imperative of developing robust food systems for extended missions beyond Earth orbit. These outcomes highlight that enhancing the resilience and culinary quality of staple crops, including rice, will be central to ensuring the nutritional needs of astronauts during long-duration space travel [25,26]. Rice serves as a primary carbohydrate source worldwide, and the ancient landrace HM446, from which the renowned KDML105 variety was developed, offers a valuable genetic background for understanding stress tolerance and aroma traits under space conditions [27]. The primitive genetics of HM446 provide a reference point for evaluating how LEO exposure may shape the sensory and nutritional profiles of staple grains, thus bridging traditional breeding knowledge with the emerging demands of extraterrestrial agriculture. The critical role of HM446 lies in its foundational traits, including resilience to environmental stress and a distinctive aromatic profile that contributes to the flavor of derived aromatic rice varieties [27]. Such attributes are essential for breeding strategies aimed at developing rice lines adapted to off-Earth cultivation, where microgravity, radiation, and limited resources prevail. By leveraging the genetic heritage of HM446, it becomes possible to refine the selection of suitable rice varieties for space missions, thereby linking landrace genetics to the optimization of flavor, texture, and nutritional stability under extraterrestrial conditions.

The examination of the volatile feature fingerprints in 466HM rice reveals that LEO conditions can shift the abundance and distribution of key aroma components, modifying both sensory quality and metabolic pathways. These changes may be explained by the effects of cosmic rays and microgravity on volatile compound metabolism. For example, ionizing radiation can penetrate pea protein isolates, demonstrating its potential to limit sensory damage caused by thermal treatments, while showing that the process significantly increased volatile features, particularly aldehydes and sulfurous notes, linked to protein and lipid oxidation [28]. These may correlate with our finding that volatile features 63 and

83 were significantly abundant in LEO-exposed conditions. Additionally, gravity alters enzymatic pathways involved in VOC ester biosynthesis, such as alcohol dehydrogenase [29]. It is critical in aroma compound generation and may exhibit altered activity in reduced gravity, influencing the diversity and abundance of volatile features. The distinct patterns of volatile feature distribution under the two conditions are visually evident in the GC-IMS spectra (Figure 6), where Figure 6A represents Earth-rice grains and Figure 6B represents SJ-19-exposed rice grains. To confirm the VOC fingerprint differences, the relative abundances of key compounds, including 65, 83, 67, 157, 106, 74, 176, 76, 98, and 720, were highlighted.

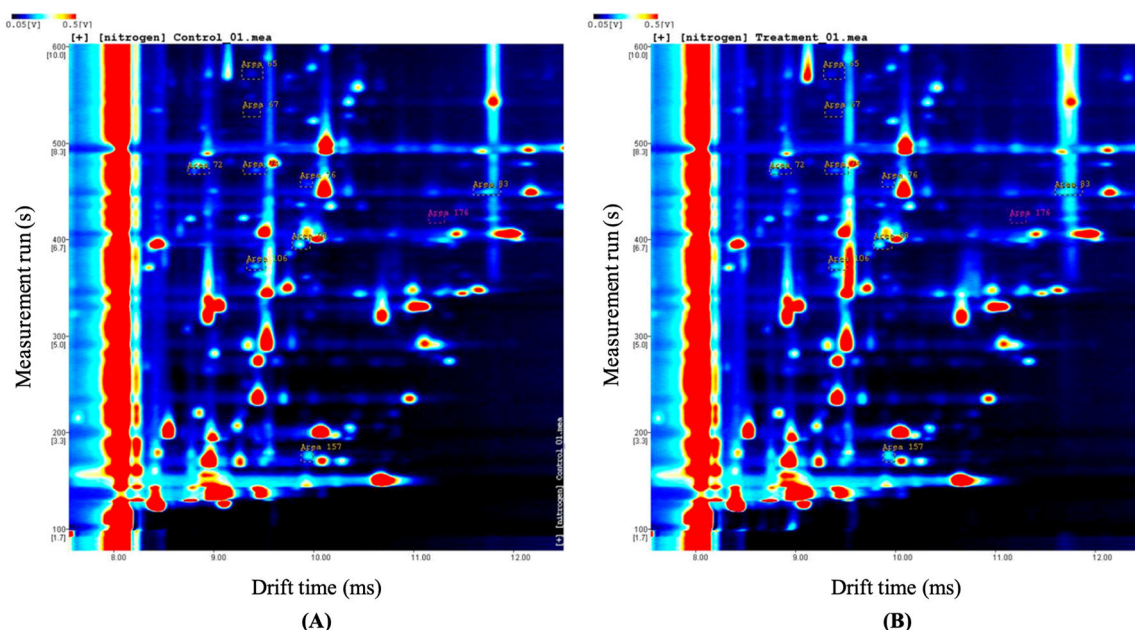


Figure 6. GC-IMS spectra illustrating the VOC fingerprints of cooked 466HM rice grains under different conditions. (A) VOC fingerprint of rice grains under Earth conditions, showing distinct spatial distribution and intensity patterns. (B) VOC fingerprint of rice grains exposed to low Earth orbit (LEO) conditions aboard the SJ-19, highlighting unique alterations in volatile compound profiles. Significant difference compounds (65, 83, 67, 157, 106, 74, 176, 76, 98, and 720) are labeled between the two conditions.

In the Earth-rice grains condition (Figure 6A), the spatial distribution and intensity of certain compounds, such as 65 and 83, appear relatively lower than in the LEO-exposed rice (Figure 6B). This observation indicates that exposure to LEO conditions aboard the SJ-19 influenced the synthesis or accumulation of these compounds. Specifically, compound features 65 and 83, which demonstrated the highest differential abundance in the previous analysis, exhibit increased signal intensity under LEO conditions, correlating with their significant fold changes. Additionally, compound features 106, 67, and 176 also show unique patterns under LEO-exposed conditions, further supporting the impact of microgravity and the space environment on volatile compound profiles. Such changes suggest that cosmic rays, microgravity, and associated oxidative stressors may affect enzymatic pathways critical to aroma generation, emphasizing the importance of understanding biochemical responses at the molecular level to guide the cultivation and processing of crops in orbit.

Rice starch is predominantly composed of amylopectin, accounting for 70–80% of its weight, alongside amylose. Amylopectin is a highly branched polymer of glucose units, interconnected by $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ glycosidic bonds, forming a complex, semi-crystalline structure within starch granules. The alterations in rice texture, specifically the increase in adhesiveness, indicate that LEO conditions can also influence carbohydrate structures, potentially through radiation-induced depolymerization and modifications to

amylopectin and other polysaccharides [30,31]. The radiation can cause physical damage to the surface of starch granules, leading to the development of cracks, fissures, and altered surface roughness. This phenomenon results in damaged starch, which is known to be more hygroscopic and capable of absorbing water quickly [30]. While damaged starch absorbs more water, it may not retain it as effectively, potentially releasing it during cooking or processing, which can contribute to increased stickiness or adhesiveness. The increased water absorption and swelling power observed in some irradiated starches directly correlate with increased adhesiveness.

The observation of increased adhesiveness without a corresponding change in hardness is a pivotal finding for understanding the selective impact of LEO conditions on rice texture. Hardness is typically associated with the bulk structural integrity and resistance to deformation of the rice grain, whereas adhesiveness (or stickiness) is more influenced by the surface properties of starch granules and their interaction with water during cooking. The increased adhesiveness may reflect radiation-associated depolymerization and oxidation of amylopectin and other polysaccharides, as reported in previous irradiation studies [32,33]. This breakdown creates more exposed hydroxyl groups and smaller, more mobile polysaccharide chains. These changes may enhance the interaction of starch molecules with water, which is consistent with the increased adhesiveness observed in the present study. One of the limitations is that only two independent experiments with a limited number of individual grains were available; the interpretation of small numerical differences in hardness should be made cautiously, and effect magnitude is more informative than statistical significance alone.

These physicochemical changes align with previous findings on other starch-rich crops where radiation exposure altered swelling, solubility, and gelatinization behaviors [34]. One report on potato starch exposed to gamma radiation at increasing doses ranging from 0 to 120 kGy noted enhanced water solubility and enthalpy of gelatinization, consistent with the increased adhesiveness observed in this study [35]. The lack of significant changes in hardness alongside increased adhesiveness suggests that microgravity and radiation may primarily affect surface-layer properties of starch, without altering the overall matrix strength. These findings underscore the complex and selective impact of LEO conditions on rice grain texture and contribute to broader research on food stability in space environments. Although the hardness of rice grains remained relatively unchanged, the selective increase in adhesiveness suggests that microgravity and radiation environments may affect surface-layer properties of starch granules, thereby encouraging future studies that focus on refining the textural attributes of space staples.

The thermogravimetric analysis (TGA) results reveal significant differences in the thermal degradation behavior between starch extracted from seeds under Earth conditions (control) and seeds exposed to LEO. Both samples exhibit similar initial thermal stability, with minimal mass loss below 200 °C, suggesting that fundamental moisture content and initial decomposition mechanisms remain consistent. However, as the temperature increases, the treatment sample demonstrates a faster degradation rate, indicating a reduction in thermal stability compared to the control. The observed differences at high temperature could be attributed to modifications in starch composition, such as changes in crystallinity, amylose-to-amylopectin ratio, or the presence of additional functional groups introduced during the treatment process. These factors can influence thermal stability by affecting intra- and intermolecular hydrogen bonding, which plays a crucial role in the structural integrity of starch under heat exposure. This difference could be attributed to the unique environmental conditions experienced in space, including microgravity, cosmic radiation, and extreme temperature fluctuations. These factors may have induced molecular modifications in the starch, such as changes in polymer structure, crystallinity, or amylose-to-amylopectin

ratio. Prolonged exposure to radiation in low orbit could also cause oxidative or free radical-induced degradation, weakening the starch's structural integrity.

The distinct peptide profiles arising from *in vitro* gastrointestinal (GIT) digestion of LEO-exposed rice demonstrate that protein folding and digestive enzyme efficiency can be influenced by extraterrestrial conditions. Proteins subjected to enzymatic digestion during the oral, gastric, and intestinal phases yielded distinct peptide profiles under Earth control and LEO-exposed conditions, as shown by differences in PMF and PCA analyses. These differences may be associated with structural alterations in proteins induced under LEO conditions, such as changes in folding and stability due to gravity, radiation, and oxidative stress [36,37]. Such structural alterations in proteins can influence the specificity and efficiency of digestive enzymes, leading to unique peptide fragmentation patterns [38]. These structural alterations also can lead to altered susceptibility to enzymatic cleavage, affecting the efficiency and specificity of digestive enzymes such as amylase, pepsin, and trypsin. As a result, the peptide profiles generated during GIT digestion may differ significantly between the two conditions. The enrichment of peptide ions in higher m/z ranges may reflect altered protein substrate properties, such as carbonylation, aggregation, or conformational changes, as reported in previous spaceflight and irradiation studies. Moreover, the unique peptide composition observed in LEO-exposed samples may reflect modifications in protein folding induced by microgravity-like conditions. These changes could potentially expose or shield specific cleavage sites, thereby influencing the digestion process. Additionally, oxidative stress in LEO may result in post-translational modifications such as protein carbonylation, further altering enzymatic recognition and cleavage efficiency. However, while peptide profiling reveals clear biochemical differences in digestion products, the implications for absorption and nutritional value remain uncertain. In a realistic biological context, the small intestine selectively absorbs amino acids and dipeptides, meaning that even with variations in peptide profiles, the overall nutritional contribution of the digested protein may remain similar [39,40]. Further studies are needed to evaluate whether the observed differences in peptide profiles translate to changes in bioavailability or metabolic effects *in vivo*.

Cooked rice grains stored aboard in SJ-19 were evaluated for cytotoxicity on human intestinal (HT-29) and hepatic (HepG2) cell lines using the MTT assay. No significant cytotoxic effects were observed in either cell line compared to rice on Earth, indicating that LEO exposure did not introduce any harmful compounds that compromise cell viability. This suggests that, from a food safety perspective, LEO-exposed rice grains are as safe as their Earth-based counterparts. These findings align with reports on other space-grown crops; for example, red romaine lettuce grown aboard the International Space Station was found to be free of pathogens and as nutritious as Earth-grown lettuce, confirming the safety of produce grown in microgravity [41]. These results, when considered alongside previously observed foodomic alterations—such as changes in volatile profiles, textural adhesiveness, thermogravimetric stability, and peptide digestibility—highlight the complex physiological and biochemical responses of plants to extraterrestrial conditions. The conclusions drawn from the cytotoxicity experiment (Figure 5) must also be interpreted in light of the limited experimental scale, which primarily supports exclusion of overt acute toxicity rather than comprehensive nutritional safety. The use of two cell lines, two independent experiments, and short exposure duration provides only moderate sensitivity for detecting subtle or cumulative biological effects, particularly when sample numbers are restricted and only one viability endpoint is measured. Consequently, while the absence of reduced viability supports short-term biosafety of LEO-exposed rice hydrolysates, the current sample size and assay format are insufficient to rule out low-level functional disturbances that may be relevant under repeated dietary intake. These limitations justify the need for future studies

with a larger experimental scale and additional functional endpoints to better connect the observed foodomic alterations with nutritional safety and physiological relevance.

The differences in temperature and packaging profiles between the flight and ground controls are important and were carefully evaluated through additional thermodynamic analyses. Thermodynamic stability of representative aroma compounds was evaluated using Arrhenius-based rate modeling and Clausius–Clapeyron volatility calculations [42,43]. Hexanal was selected as a model aldehyde, and literature values for activation energies of oxidative deterioration in food lipids (50–80 kJ mol^{−1}) and enthalpy of vaporization (~41 kJ mol^{−1}) were used as inputs [42,43]. Reaction rate constants and equilibrium vapor pressures were calculated at 11.3 °C (mean capsule temperature) and 17.0 °C (upper bound of control storage) using standard Arrhenius and Clausius–Clapeyron relationships. Additional consideration was given to Maillard-derived VOCs such as 2-AP, for which published kinetic data and storage studies in fragrant rice were used to estimate plausible activation energies [44,45]. The temperature conditions recorded inside the SJ-19 capsule fluctuated within a narrow range of 11.3–17.0 °C, a difference insufficient to induce major alterations in dried rice grain chemistry. These predicted temperature-dependent effects are substantially smaller than the fold changes observed for the GC-IMS volatile features in the present study (volatile features 65 and 83), indicating that the experimentally observed modulation of volatile profiles cannot be explained by temperature differences alone. Arrhenius-based modelling using established activation energies for aldehyde oxidation (50–80 kJ mol^{−1}) predicted that this 6 °C variation would alter reaction rates by only ~1.6 to 2.0-fold, while Clausius–Clapeyron calculations for representative aldehydes with $\Delta H_{\text{vap}} \approx 41 \text{ kJ mol}^{-1}$ indicated a volatility change of just ~1.4-fold. These combined effects remain well below the 5.33-fold and 5.28-fold differences observed for key VOC features in the LEO-exposed samples (volatile features 65 and 83), suggesting that the temperature discrepancy cannot account for the magnitude of compositional changes detected in GC-IMS profiling. Moreover, all analytical procedures, including headspace extraction at 80 °C and drift-tube IMS at 45 °C standardize thermal pre-equilibration conditions across samples, effectively overriding minor prior storage-temperature differences. Importantly, the predicted temperature-driven changes in reaction rate and volatility are substantially smaller than the experimentally observed differences in GC-IMS volatile feature intensities, particularly for the most responsive features (65 and 83, showing >5-fold changes), directly linking the thermodynamic modelling to the magnitude of the experimental results. Together, these thermodynamic simulations and controlled analytical conditions support the conclusion that the biochemical modulation observed in LEO-exposed rice is primarily attributable to authentic LEO microgravity and radiation exposure rather than temperature or packaging effects.

By applying both biochemical and food science perspectives, the present study extends previous rice-related space biology research into a food-quality context that has received comparatively limited attention. Most prior investigations of rice under altered gravity or spaceflight conditions have primarily focused on germination performance, seedling growth, mutation frequency, or oxidative stress responses, often using clinostat-simulated microgravity or short-duration orbital exposure [13]. While these studies provide important insights into plant stress adaptation and viability, they do not address properties directly relevant to rice consumption after cooking. In contrast, this work examines dry rice grains exposed to authentic LEO conditions and evaluates post-processing attributes, including aroma profiles, textural characteristics, and in vitro protein digestibility. By shifting the analytical focus from plant development to food-quality endpoints, the present study positions rice not only as a biological payload but also as a functional food material for space nutrition. This distinction clarifies the scope and novelty of the work, linking

spaceflight exposure to sensory and digestibility traits that are directly relevant to astronaut diet design and bioregenerative life-support systems.

5. Conclusions

This study demonstrates that short-term exposure of Thai landrace rice (466HM) to low Earth orbit conditions aboard the SJ-19 is associated with changes in volatile feature profiles, increased adhesiveness, and altered peptide mass fingerprints following *in vitro* digestion. In contrast, rice hardness and cell viability in HT-29 and HepG2 cell models remained unchanged, indicating no detectable adverse effects on basic textural firmness or biosafety. Collectively, these findings highlight the influence of microgravity and cosmic radiation on the sensory-related, digestibility, and physical properties of a staple food crop. The use of traditional aromatic rice with resilient genetic traits, such as 466HM, offers strategic advantages for future space farming. This work advances the understanding of how spaceflight alters nutritional and sensory attributes and supports the inclusion of landrace rice in bioregenerative life support systems for extended space missions.

Author Contributions: Conceptualization, S.K. and T.T.; Data curation, Y.Y., K.T., C.B., J.K., P.M.-N., P.V., P.P., S.T. and T.T.; Formal analysis, Y.Y., P.M.-N. and T.T.; Funding acquisition, T.T. and S.K.; Investigation, Y.Y. and M.C.Y.; Methodology, Y.Y., C.B., P.P. and A.M.; Project administration, S.K.; Resources, Y.Y., S.C. and T.T.; Software, Y.Y., K.T., A.K., P.P. and S.K.; Supervision, S.C. and T.T.; Visualization, T.T. and Y.Y.; Writing—original draft, T.T. and Y.Y.; Writing—review and editing, S.K., Y.Y. and T.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research project was partially supported by a postdoctoral fellowship award from Mahidol University (to K.T.). Financial support was provided by King Mongkut's Institute of Technology Ladkrabang (grant number KREF186730 to P.P.). This research was supported by Mahidol University (Basic Research Fund: fiscal year 2021 (BRF1-A11/2564) for A.M. Additional funding was received from the Program Management Unit for Human Resources and Institutional Development, Research and Innovation (PMU-B) under grant numbers B11B680023 awarded to T.T., and B13F660122 awarded to S.K. Further support was provided by Thailand Science Research and Innovation (P.M.-N. and S.K.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

Acknowledgments: This research was made possible through the collaborative support of the China National Space Administration (CNSA) and the Geo-Informatics and Space Technology Development Agency (GISTDA), Thailand, for facilitating the integration, coordination, and deployment of experimental payloads aboard the Shijian-19 (SJ-19) recoverable satellite. The authors extend their sincere gratitude to Pasupha Chinvarasopak, Royal Thai Embassy, for her invaluable assistance in diplomatic communication and logistical arrangements, which ensured the smooth execution of the experimental procedures. Appreciation is also expressed to Sutinee Sibirunwong of GISTDA for her dedicated coordination and operational support throughout the project. The authors further acknowledge BUAA Sun Lab at Beihang University, with special thanks to Lian-Wen Sun, Xiao Yang, and Wu Xintong, for providing laboratory facilities and instrumentation essential for sample preparation. Finally, the authors express their sincere appreciation to Weng Jingnong of Beihang University for his key role in coordinating and facilitating institutional collaboration between Thailand and China during the experimental period.

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding this research publication. The funding bodies did not participate in the study's design, the collection of plant samples, data analysis, data interpretation, and the writing of the manuscript.

Appendix A

Table A1. Top significantly altered volatile features (VOC signals) detected by GC-IMS in 466HM rice grains exposed to low Earth orbit (LEO) aboard SJ-19 compared with the Earth control. Fold changes greater than 1 indicate higher abundance in LEO-exposed rice, while values below 1 indicate reduction. Statistical significance was determined using Student's *t*-test ($p < 0.05$).

Compounds	Fold Change	Log2 (Fold Change)	<i>p</i> -Value
Cmd 65	5.33	2.42	0.0015
Cmd 83	5.28	2.40	0.0013
Cmd 67	2.33	1.22	0.0019
Cmd 157	2.16	1.11	0.0001
Cmd 106	2.07	1.05	0.0102
Cmd 74	1.99	0.99	0.0041
Cmd 176	1.85	0.89	0.0191
Cmd 76	1.66	0.73	0.0034
Cmd 98	1.64	0.72	0.0011
Cmd 72	1.48	0.57	0.0412
Cmd 105	1.35	0.44	0.0037
Cmd 167	1.33	0.41	0.0058
Cmd 94	1.31	0.39	0.0116
Cmd 152	1.30	0.38	0.0477
Cmd 164	1.30	0.38	0.0015
Cmd 38	1.27	0.35	0.0002
Cmd 61	1.23	0.30	0.0005
Cmd 53	1.20	0.26	0.0013
Cmd 99	1.19	0.25	0.0219
Cmd 57	1.17	0.22	0.0432
Cmd 86	1.16	0.22	0.0249
Cmd 150	1.15	0.20	0.0325
Cmd 119	1.13	0.17	0.0307
Cmd 130	1.10	0.14	0.0128
Cmd 31	1.09	0.12	0.0175
Cmd 154	1.07	0.10	0.0344
Cmd 24	1.06	0.08	0.0325
Cmd 50	0.98	−0.04	0.0211
Cmd 77	0.97	−0.04	0.0457
Cmd 137	0.96	−0.05	0.0266
Cmd 48	0.96	−0.05	0.0205
Cmd 44	0.96	−0.06	0.0054
Cmd 28	0.95	−0.07	0.0213
Cmd 12	0.95	−0.07	0.0088
Cmd 27	0.95	−0.07	0.0064
Cmd 103	0.95	−0.08	0.0279
Cmd 6	0.95	−0.08	0.0013
Cmd 153	0.93	−0.10	0.0025
Cmd 88	0.93	−0.10	0.0210
Cmd 148	0.92	−0.11	0.0202
Cmd 136	0.92	−0.13	0.0018
Cmd 116	0.91	−0.13	0.0120
Cmd 75	0.91	−0.14	0.0424
Cmd 36	0.90	−0.15	0.0335
Cmd 5	0.89	−0.16	0.0266
Cmd 46	0.89	−0.17	0.0175
Cmd 139	0.88	−0.19	0.0039
Cmd 109	0.87	−0.20	0.0150

Table A1. Cont.

Compounds	Fold Change	Log2 (Fold Change)	p-Value
Cmd 91	0.87	−0.21	0.0113
Cmd 13	0.85	−0.23	0.0139
Cmd 141	0.84	−0.24	0.0014
Cmd 26	0.84	−0.25	0.0239
Cmd 69	0.82	−0.29	0.0488
Cmd 104	0.81	−0.31	0.0271
Cmd 115	0.80	−0.32	0.0005
Cmd 62	0.80	−0.32	0.0061
Cmd 59	0.80	−0.33	0.0007
Cmd 15	0.79	−0.34	0.0287
Cmd 56	0.77	−0.38	0.0068

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