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The impact of freeze-dried food on gut microbiota composition: a preliminary study

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ABSTRACT

Freeze-dried food is widely used during space expeditions or flights. However, evidence on how this affects the gut microbiota is limited. This study aimed to assess changes in the composition of gut microbiota in volunteers subjected to a 14-day stay in a controlled space-analogue habitat. Five adults provided stool samples at baseline and after two weeks. Meals were freeze-dried and standardized for portion size and composition. Meals were served according to a daily schedule with no additional snacks allowed. Coffee and tea were permitted. Compliance was monitored by returning and verifying the packaging. Bacterial community profiles were assessed using shallow shotgun metagenomics and analyzed using paired statistical methods, including alpha diversity indices and beta diversity ordination with permutation-based testing. Differential abundance analyses were performed to identify taxa showing trends toward change during the intervention. Overall gut bacterial diversity and community structure were essentially stable over 14 days among all participants. No statistically significant changes in alpha diversity were observed, and global beta diversity patterns did not indicate a consistent separation of the entire community between baseline and day 14. Exploratory analyses suggested small changes within individuals in the relative abundance of selected taxa; however, inter-individual variability prevailed, and the small sample size limited statistical power. It appears that a diet consisting entirely of freeze-dried foods, consumed for 14 days, did not significantly affect the overall diversity of the gut microbiota or the structure of its communities. However, these studies are preliminary in nature and provide hypotheses for use in larger, controlled studies aimed at elucidating the microbiome's response to dietary regimens based on freeze-dried products.

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1. Introduction

Freeze-drying is one of the methods of food preservation, aimed at reducing the free water content in the product. Reducing the water content in dried material prevents the growth of microorganisms and slows down or inhibits chemical reactions and physical changes, resulting in a product that is durable, safe to consume, and has a long shelf life for several years to several decades (Deng et al., 2019; Osae et al., 2020)

Fruit, vegetables, yeast, probiotics, and coffee extracts are most commonly used for freeze-drying (Bhatta et al., 2020) In this process, water or another solvent is removed from the frozen product by sublimation, mainly under reduced pressure. As a result of this process, water is removed from the product to a content of 1-4% (Kawasaki et al., 2019; Parikh, 2015; Shukla, 2011; Wang et al., 2012) The freeze-drying process has made it possible to compose nutritious, lightweight meals that take up little space for people who do not have access to traditional food, such as participants in high-altitude expeditions, space flights, underwater expeditions, polar expeditions, and military missions (Beery et al., 2023; Cooper et al., 2011; Titov et al., 2021)

This type of food is a proven way of feeding many groups of active people. However, very little is still known about how the freeze-drying process itself affects the diversity and composition of the gut microbiota of people who consume such food during short-term exclusive consumption.

The human gut microbiota (GM) is a complex ecosystem of microorganisms colonizing the human gastrointestinal tract, playing a key role in regulating biological processes (Makki et al., 2018; Moszak et al., 2020) The largest group of microorganisms inhabiting the intestines are bacteria. To date, 2776 species of bacteria have been identified in the human intestine, representing 11 different phyla. Among them, the dominant group is Bacillota (earlier Firmicutes), which accounts for as much as 65% of the intestinal microbiota. The next largest group is Bacteroidota, which accounts for 23% of the microbiota. Actinomycetota and Pseudomonadota account for 5% each. It is worth noting that the composition of the gut microbiota varies depending on diet, geographic location, age, individual factors, and the analytical method used (Zhao et al., 2026).

The diversity and composition of the GM is constantly changing, and one of the most important factors shaping the microbiome is the composition of food (Fackelmann et al., 2025). Studies show that different dietary patterns are clearly reflected in the composition of the microbiota, significantly affecting its functions. It is well known how the composition of the diet, e.g., the abundance of certain nutrients, can "reprogramme" the GM. (For example, small changes, such as adding more vegetables and fruits to the daily menu, can bring enormous benefits to intestinal health), but relatively little is known about how food preparation affects the diversity, richness, and functionality of the human GM.

The aim of this study was to elucidate how 14-day freeze-dried food ingestion affects the structure of the human gut microbiome at both the global and single-species level.

2. Materials and methods

2.1. Study design

The study was conducted at the LunAres Research Station in Piła, Poland, a space-analog habitat simulating selected conditions of manned Martian and Lunar missions (https://lunares.space) All volunteers signed up for the mission voluntarily and stayed on the mission for 14-days (winter 2021 mission).

2.2. Recruitment and qualification

Participants were recruited on a voluntary basis from among those who declared their willingness to participate in the experiment in the analog habitat. Five participants agreed to take part in the study The inclusion criteria were a declaration of good health and no symptoms of acute infection at the time of entering the experiment. No full medical qualification or in-depth screening tests were conducted prior to participation. Upon arrival at the habitat, a physician conducted a basic health assessment, including measurement of vital signs and selected basic clinical indicators (e.g., blood pressure, heart rate, body temperature). In the case of signs of acute illness or significant clinical abnormalities, the participant would not be allowed to participate.

Due to voluntary selection and reliance on self-assessment of health, the presence of undiagnosed chronic conditions cannot be completely ruled out.

The study protocol was approved by Independent Bioethics Committee for Scientific Research of Pomeranian Medical University in Szczecin (KB – **0012/105/18).**

2.3. Dietary intervention

Before the mission began, a qualified clinical dietitian prepared menus for the mission participants, which were balanced in terms of micro and macro nutrients. The calorie content of the diet was calculated taking into account the participants' anticipated physical activity (including warm-up, daily exercise on a cyclometer, strength training, and individual exercises, e.g., yoga) and amounted to 2880.5 kcal/d ± 313.1 kcal/d; 15-20% protein, 35-40% fat, 45-50% carbohydrates. The other constituents were as follows: Dietary fiber 50.7 ± 9.8 g (due to heat treatment, we estimate that the amount in the finished meal has decreased by approximately 10%). Digestible carbohydrates 261.2 ± 75 g Total carbohydrates 432.7 ± 41 g.

The proposed meals were characterized by a low and medium glycemic load. The main sources of protein used in the menu included chicken breast, low-fat dairy products, eggs, fish, and legumes, while the main sources of fat included olive oil, nuts and seeds, chia seeds, and rapeseed oil. Each meal also included vegetables and fruit. All mission participants received 5 meals/day with the following calorie distribution: 25% of the total requirement for breakfast, 10% for lunch, 35% for dinner, 10% for a snack, and 20% for supper Fig. 1. All menus were prepared in the industrial facility (Elena Kalisz, Poland) using standard recipes. After preparation, the meals were divided into portions, packaged, and freeze-dried. Meals were standardized in terms of portion size and composition for all participants and served according to set meal schedule. Snacking ad libitum was not allowed. Meals were rehydrated on site using drinking water according to standard procedure. Coffee and tea were allowed. Water intake was 35 ml/kg.

Compliance with the dietary protocol was monitored by returning and verifying meal packages during their stay in the habitats. No food recall diaries were collected.

Overview of the 14-day intervention including menu development, production od freeze-dried food sets, baseline and endpoint anthropometric measurements, stool sample collection, and microbiome and statistical analyses.

2.4. Anthropometric measurements

Anthropometric assessments were performed twice (at baseline and the end of the study). The study included measurements of height (m), body weight (kg), body composition expressed as: fat mass (percentage body fat, PBF) in %, lean mass in % and total body water (TBW) in %. All of the anthropometric parameters were measured with a multifrequency bioimpedance device, Tanita BC 601 (Tanita, Tokyo, Japan).

2.5. Stool collection and DNA extraction

Fecal samples were self-collected by crew members during the mission. Although the exact time of collection was not recorded, most

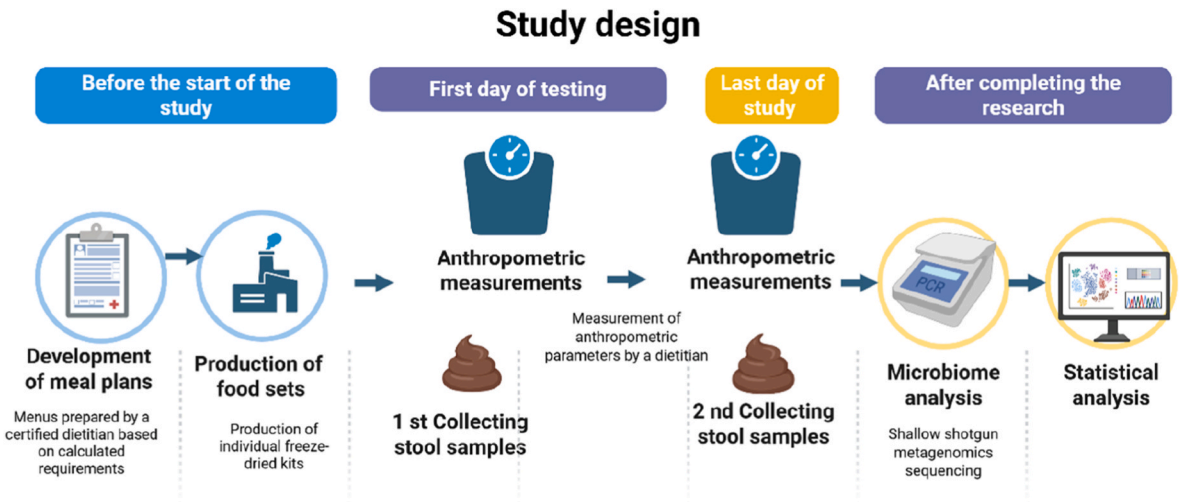

Fig. 1. Study design.

samples were obtained following the first bowel movement of the day. No stabilizing buffer was used; instead, sample preservation was achieved by immediate freezing. Following collection in the living module, stool samples were stored at −20°C for approximately two weeks. At the end of the mission, all samples were transported to the analytical laboratory in a single shipment on dry ice, with frozen conditions maintained throughout transit. Upon arrival, samples were immediately transferred to −80°C storage and kept at that temperature until analysis. Prior to DNA extraction, samples were thawed only once and processed immediately for genetic material isolation. Repeated freeze–thaw cycles were strictly avoided to minimize potential effects on DNA integrity.

Nucleic acid extraction was carried out on fecal sample using the QIAmp PowerFecal Pro DNA kit (Qiagen). In brief, material from the swabs was transferred into PowerFecal Bead tubes containing buffer C1, followed by homogenization using an Omni Bead Ruptor 12 (with 3 cycles of 30 s each, with 30-s breaks in between). Subsequent procedures were conducted following the manufacturer's instructions. Purified DNA was eluted using 70 μL of the provided elution buffer and quantified using the Quantifluor ONE dsDNA system (Promega).

2.6. Shallow shotgun metagenomics sequencing

Sequencing libraries were prepared using a reduced volume of KAPA Hyper Plus kit reagent (ROCHE, Switzerland). All steps were carried out in accordance with the manufacturer's instructions to produce libraries containing metagenomic DNA fragments of approximately 300 bp in size. Initially, metagenomic DNA samples were normalized to a concentration of 10 ng input, followed by a 10-min enzymatic digestion, indexing with KAPA Unique Dual Indexes (ROCHE), and subjected to 9 cycles of polymerase chain reaction (PCR) library amplification. Subsequently, libraries were purified and size-selected using electrophoretic techniques. The size, quantity, and quality of the selected libraries were assessed using fluorometry with Quantus (Promega) and chip electrophoresis with MultiNA (Shimadzu).

These libraries were further normalized to 2 nM, pooled, denatured with NaOH, and diluted with HT1 buffer (Illumina). These prepared libraries were supplemented with 1% (v/v) PhiX control v3 (Illumina) and then sequenced on an Illumina MiSeq System using a 2x150-cycles paired-end sequencing strategy, although only the forward reads were used in the subsequent analysis. Our investigation revealed that paired-end analysis led to a significant reduction in the number of reads, coupled with a decline in overall quality. The Illumina bcl2fastq2 Conversion Software (version 2.20) was employed for demultiplexing sequencing data and converting base call files into FASTQ files using default parameters. On average, 1,000,000 reads per sample were obtained.

2.7. Metagenomics data processing

Sequences retrieved from shallow shotgun metagenomics sequencing were processed with the NGless pipeline (v1.3.0). Reads were trimmed with a Phred-score <25 and discarded if the sequence length was below 45 bp. Contamination filtering was performed by mapping the reads against GRCh38.p14. Taxonomic profiling was conducted using the NGless mOTUs module (v2.6). Here, mOTUs denotes metagenomic operational taxonomic units, i.e., species-level taxonomic units inferred using universal single-copy marker genes for shotgun metagenomic profiling. The taxon labels shown in Figs. 2, 5, and 6 were retained as generated by the NGless/mOTUs output, including species-level names and reference/meta-mOTU identifiers, to preserve traceability of the taxonomic assignments. Species level richness (Observed, Chao1), alpha diversity (Shannon, Simpson), and beta diversity were calculated using vegan (v2.6-4) package and the stats (v4.2.2) package. The beta diversity was calculated on a species-level abundance table using the Bray–Curtis dissimilarity metrics and visualized as principal coordinate analysis (PCoA). Exploratory taxon-level differential-abundance analyses were also performed using species-level mOTU assignments, while heat-tree visualizations displayed the corresponding taxonomic hierarchy. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate method. All microbiome analysis was carried out in the R statistical programming language (v4.4.0) and figures were generated using ggplot2 (v3.5.1).

2.8. Statistical analysis

Prior to statistical analysis, the raw count data were subjected to quality control and preprocessing steps. Taxa present in less than 10% of the samples were excluded to minimize the impact of rare taxa on the analysis. To address the compositional nature of microbiome data, the count data were rarefied. Because baseline and endpoint measurements were obtained from the same participants, paired baseline-endpoint comparisons were performed using the two-sided Wilcoxon signed-rank test. For exploratory taxon-level analyses, paired Wilcoxon signed-rank tests were applied where sufficient non-zero paired observations were available, followed by Benjamini-Hochberg false discovery rate correction. For beta diversity, PERMANOVA was performed on Bray-Curtis distances using permutations constrained within participant ID to account for the paired study design. To explore potential interactions among microbial taxa, we conducted pairwise correlation analyses using the Pearson correlation coefficient, which measures the

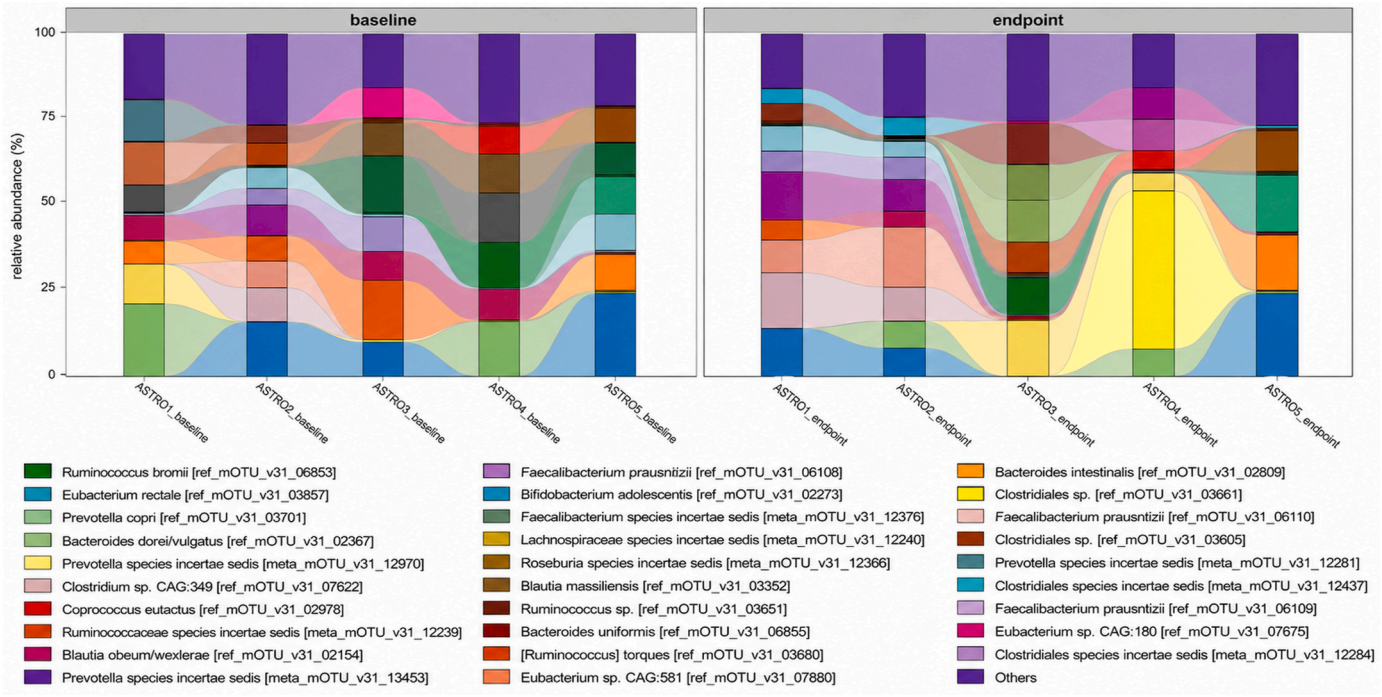

Fig. 2. Species-level taxonomic composition of fecal metagenomes at baseline and endpoint. Stacked relative-abundance profiles are shown for the top 30 species-level mOTUs, with the remaining taxa grouped as "Others." Each color corresponds to a species-level mOTU listed in the legend Samples are shown for each participant at baseline and endpoint. ASTRO1–ASTRO5 denote anonymized participant identifiers. The stacked bar plot is descriptive. Where exploratory baseline-endpoint taxon-level comparisons were performed, paired Wilcoxon signed-rank tests with Benjamini-Hochberg correction were used. mOTU, metagenomic operational taxonomic unit.

linear relationship between two continuous variables. Given the large number of pairwise comparisons, controlling for false positives was essential. The Benjamini-Hochberg procedure was applied to adjust p-values for multiple testing using the p.adjust function in the base stats package. Adjusted p-values (q-values) less than 0.05 were considered statistically significant.

Heat tree analysis was utilized to visualize the differential abundance of taxa across hierarchical taxonomic classifications between the baseline and endpoints. Differences in taxon abundance between baseline and endpoint samples were assessed using paired Wilcoxon signed-rank tests where sufficient paired observations were available. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate correction. In the heat tree visualization, each node represents a taxon, and edges denote taxonomic relationships. Node sizes correspond to the mean relative abundance of taxa, while node colors reflect the log2-fold change between groups.

The core microbiome was defined using prevalence and relative abundance thresholds. Prevalence was calculated as the proportion of samples in which a given taxon was detected above a specified abundance threshold. We set a prevalence threshold of 50%, meaning that taxa present in at least half of the samples were considered for inclusion in the core microbiome. An abundance threshold was set to filter out taxa with negligible contributions; only taxa with a relative abundance above 0.1% were included.

Microbial abundance data were processed using the microbiome package and managed as phyloseq objects using the phyloseq package v.1.22.3 (McMurdie and Holmes, 2013). Data normalization and transformation steps were performed to account for compositionality and sequencing depth variations. The prevalence of each taxon across samples was computed using the prevalence function from the microbiome package. This function calculates the fraction of samples where the abundance of a taxon exceeds the set detection threshold. Taxa were filtered based on relative abundance using the transform function to convert raw counts to relative abundances. Only taxa meeting the abundance criteria were retained for core analysis. Core taxa were identified using the core_members function, which selects taxa that meet or exceed the defined prevalence and abundance thresholds. Heatmap was generated using the plot_core () function to visualize the prevalence and abundance of core taxa across samples.

All statistical analyses were conducted using R v.4.3.2 and Python v.3.12 for Fedora Linux (v.41). Graphics were generated with ggplot2 v3.5.1 (Wickham, 2016), plotly v5.24.1 (Sievert, 2020) and seaborn v.0.13.2 (Waskom, 2021).

3. Results

Althogether 5 (ASTRO1-5) participants were included. In the text and figures, ASTRO1–ASTRO5 denote anonymized participant identifiers. Baseline and endpoint parameters are presented in Table 1. No significant differences in these outcomes were noted with regard to time point (Table 1).

Table 1
Baseline participant characteristics and anthropometric/body-composition changes during the 14-day intervention.

Variable	Baseline (n = 5)			Endpoint (n = 5)			p-value
Sex, F/M	2/3				-		-
Age, year	26	±	3.7		-		-
Height, cm	171.7	±	4.9		-		-
Weight, kg	67.5	±	9.5	65.4	±	8.5	0.063
BMI	22.9	±	2.8	22.3	±	2.5	0.125
WHR	0.82	±	0.08	0.82	±	0.08	1.0
FAT, %	24.7	±	5.6	23.6	±	5.3	0.063
TBW, %	54.8	±	4.1	55.7	±	4.1	0.063

Data are presented as mean ± SD. Baseline-endpoint comparisons were performed using the two-sided Wilcoxon signed-rank test for paired samples. F/M, female/male; BMI, body mass index; WHR, waist-to-hip ratio; TBW, total body water.

3.1. Analyzing the taxonomic characteristics

Across the 10 fecal metagenomes (five participants sampled twice), species-level mOTU profiling yielded a total of 425 species-level bacteria were identified. In the stacked relative-abundance profiles (Fig. 2 top 30 taxa shown), the top 30 taxa captured 75.0–86.2% of each sample's community (median 80.4%), with the remainder grouped as "Others" (13.8–25.0%, median 19.6%).

At baseline, community composition was strongly participant-specific, with different taxa dominating different individuals rather than a single shared signature. Nonetheless, several taxa repeatedly appeared among the most abundant across samples, including *Ruminococcus bromii* (mean 9.2%, range 0–21.4%) and *Eubacterium rectale* (mean 8.5%, range 0.03–26.0%), alongside variable contributions from *Blautia obeum wexlerae* (mean 5.2%, range 0.3–9.0%), *Roseburia* species (mean 4.4%, range 0–14.0%), *Lachnospiraceae* species (mean 4.7%, range 0.1–14.2%), and *Bacteroides dorei vulgatus* (mean 3.0%, range 0.08–14.0%). This baseline heterogeneity is consistent with individualized species-level microbiome configurations.

Baseline community structures differed substantially across individuals. At baseline, ASTRO1 was dominated by *Eubacterium rectale* (26.0%) and *Bacteroides dorei vulgatus* (14.0%), whereas ASTRO2 was characterized by *Ruminococcus bromii* (15.1%) together with two *Prevotella* species meta-mOTUs (meta_mOTU_v31_12970, 8.7%; meta_mOTU_v31_13453, 7.8%). ASTRO3 displayed a distinct profile enriched for *Ruminococcaceae* species (15.7%) and *Roseburia* species (14.0%) with *Faecalibacterium prausnitzii* (9.5%). In contrast, ASTRO4 baseline was dominated by *Eubacterium rectale* (15.7%), *Lachnospiraceae* species (14.2%), and *Anaerostipes hadrus* (13.8%), while ASTRO5 baseline was driven by *Ruminococcus bromii* (21.4%) alongside *Bifidobacterium adolescentis* (9.8%), and *Coprococcus eutactus* (9.4%).

At endpoint, the cohort again showed marked inter-individual variability, and changes relative to baseline were not directionally consistent across the dominant taxa: none of the top 30 taxa increased or decreased uniformly in all five participants. Instead, endpoint profiles reflected idiosyncratic remodeling, including prominent expansions of specific taxa in individual participants. The most striking example was *Prevotella copri*, whose relative abundance was negligible at baseline ($\leq$0.25% across all participants) but increased to 44.2% in a single participant by the end of the study. This outlying value resulted in a wide end-of-study range (0–44.2%) and had a substantial impact on the group mean, increasing it to 8.8%. Endpoint samples also showed increased contributions (at least in some participants) from *Clostridium* sp (endpoint max 15.7%) and two *Prevotella*-associated species meta-mOTUs (meta_mOTU_v31_12970, endpoint max 15.0%; meta_mOTU_v31_13453, endpoint max 12.6%). In contrast, several taxa that were prominent at baseline in specific individuals, such as *Roseburia* species and *Lachnospiraceae* species, contributed <1% on average at endpoint (means 0.21% and 0.35%, respectively), reflecting pronounced reductions in the participants where they were initially abundant.

Changes from baseline to endpoint were highly individual-specific in both direction and magnitude. ASTRO1 showed a major compositional reorganization, with *Eubacterium rectale* decreasing from 26.0% to 0.06% and *Bacteroides dorei vulgatus* from 14.0% to 0.2%, accompanied by enrichment of *Prevotella*-associated taxa (meta_mOTU_v31_12970, 15.0%; meta_mOTU_v31_13453, 12.6%) and *Ruminococcus bromii* (14.1%). ASTRO2 exhibited an endpoint increase in *Clostridium* species (from 7.0% to 15.7%) together with higher *Eubacterium rectale* (from 0.03% to 6.9%), while maintaining substantial Prevotella-associated contributions. ASTRO3 shifted from a *Ruminococcaceae/Roseburia*-enriched baseline to an endpoint dominated by *Bacteroides dorei vulgatus* (16.3%) and *Bacteroides intestinalis* (10.9%) with increased *Clostridiales* mOTUs (ref_mOTU_v31_03605, 10.3%; ref_mOTU_v31_03661, 9.4%) and marked reductions in *Ruminococcaceae* species (from 15.7% to 0.4%) and *Roseburia* species (from 14.0% to 0.4%). ASTRO4 displayed the most conspicuous single-taxon expansion, with *Prevotella copri* increasing to 44.2% at endpoint (baseline 0.25%), alongside concurrent depletion of baseline-dominant taxa such as *Lachnospiraceae* species (14.2% to 0.03%) and *Anaerostipes hadrus* (13.8% to 0.08%). In contrast, ASTRO5 showed comparatively modest restructuring, with enrichment of *Coprococcus eutactus* (9.4% to 14.3%) and *Faecalibacterium* species (9.8% to 14.2%), while *Bifidobacterium adolescentis* declined sharply (9.8% to 0.06%).

Consistent with these observations, within-participant compositional change (Bray–Curtis dissimilarity between paired baseline and endpoint profiles) spanned a wide range (0.24–0.86), indicating that temporal dynamics were dominated by participant-specific shifts rather than a uniform cohort-wide transition over time.

3.2. The alpha-diversity analysis

Alpha diversity was evaluated from the species-level mOTU profiles using richness (Observed, Chao1) and diversity/evenness-sensitive indices (Shannon, Simpson) across the paired baseline and endpoint samples (n = 5; Fig. 3). At the cohort level, no statistically significant differences were detected between baseline and endpoint for any of the four indices (paired comparison; Wilcoxon signed-rank Test, Observed: p = 0.75, Chao1: p = 0.92, Shannon: p = 0.69, Simpson: p = 1.0). Changes were heterogeneous across individuals rather than directional.

Overall, alpha diversity metrics indicated stable cohort-level richness and diversity between baseline and endpoint, but substantial inter-individual variability in the direction and magnitude of change, most notably a pronounced drop in evenness-sensitive indices in individual participants – Fig. 4.

To interpret the results of the core microbiome study from metagenomic data, Fig. 5 shows the context provided by the heat map observed at two different time points. This visualization shows the hierarchical relationships between different taxonomic groups and their relative changes in abundance under different conditions (time point). Results at two time points indicate differences in microbial taxa before and after a specific intervention. In this study, we present taxa that show significant changes in abundance because these are potential indicators of how the intervention is affecting the microbial community (Table 2).

Fig. 6 illustrates taxonomic differences between endpoint and baseline samples. The color gradient and the size of nodes, edges, and labels are based on the $\log_2$ ratio of median abundance at endpoint relative to baseline. In this visualization, blue indicates lower median abundance and red indicates higher median abundance at endpoint compared with baseline. *Streptococcus thermophilus* showed lower median abundance in endpoint samples than in baseline samples, whereas *Clostridiales* sp. showed higher median abundance at endpoint.

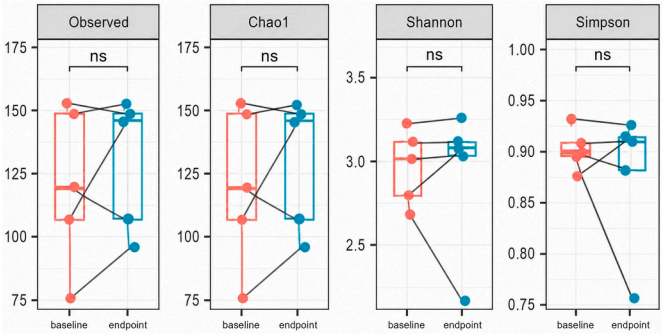

Fig. 3. Alpha-diversity indices of the fecal microbiome at baseline and endpoint. Observed richness, Chao1 richness estimator, Shannon diversity, and Simpson diversity are shown for each participant at baseline and endpoint. Paired Wilcoxon signed-rank test (with Benjamini–Hochberg correction) for baseline vs. endpoint comparisons (two-sided), performed separately for each index.

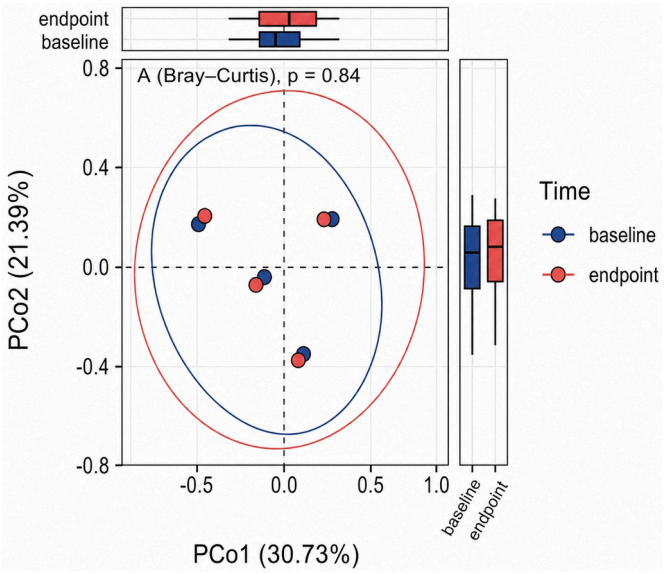

Fig. 4. Bray–Curtis beta-diversity of fecal metagenomes at baseline and endpoint visualized by principal coordinates analysis (PcoA). Points represent individual samples colored by time point; ellipses indicate group dispersion in ordination space. PERMANOVA using adonis2 on Bray-Curtis distances with 9999 permutations constrained within participant ID was used to test the association between community composition and time point.

4. Discussion

Freeze-drying is a process of dehydration at low temperatures. It is widely used in the production of meals with a long shelf life, intended, for example, for exploration expeditions or space flight trials. Thanks to low-temperature water removal technology, such food can retain the integrity of its structural components and reduce the degradation of food matrices coused by heat compared to traditional drying. Theoretically, such processing may affect the composition of gut microorganisms by altering the physicochemical properties and availability of food substrates, such as fiber-rich plant components.

It is therefore important to verify whether a fully freeze-dried, standardized meal plan disrupts the gut microbiome during short-term confinement. Studies indicate that freeze-drying effectively preserves the viability of probiotics, with survival rates ranging from 75% to 98.6% depending on the method used (Makki et al., 2018; Strasser et al., 2021)Incorporating probiotics into freeze-dried foods not only provides a convenient means of delivery but also supports the growth of beneficial gut bacteria, potentially improving overall health outcomes (Mardinoglu et al., 2018). Overall, the combination of freeze-drying technology and probiotics offers a promising approach to enhancing dietary interventions aimed at optimizing the GM (Singh et al., 2017; Strasser et al., 2021). The gut microbiome changes throughout life, and the composition of food can have a significant impact on its composition (Alasalvar et al., 2023)

However, little is known about whether the type of food preparation can alter the microbiome and whether switching to freeze-dried food can be a destabilizing factor for GM composition. Previous studies have focused on the freeze-drying process in the context of probiotic production—as shown, freeze-drying effectively preserves the viability of probiotics (Makki et al., 2018; Mardinoglu et al., 2018; Strasser et al., 2021). An important question therefore remains whether freeze-dried food can safely serve as the sole source od nutrition without adversely affecting the GM. These are very important questions in the context of using this food in defense or military missions, as well as during expeditions, e.g., high-altitude mountaineering. An important question for us was whether consuming freeze-dried food changes the GM. This microbiota is a set of microbial taxa found in the vast majority of people in a given population. This collection mainly includes bacteria, but also archaea, viruses, and fungi, which are constantly present, regardless of differences in diet, geography, and lifestyle of individuals. The core microbiome plays a key role in maintaining gut health by supporting essential functions such as digestion, nutrient absorption, and immune system modulation. Its composition and stability are considered indicators of host health, reflecting a balanced microbial ecosystem that can influence metabolic function and overall well-being. Studying the core microbiome helps us understand how microbial communities adapt to the host environment and contribute to health and disease states. Our results indicate that switching to a diet consisting exclusively of freeze-dried food has a noticeable effect on the bacterial composition of the gut microbiota. *Ruminococcus bromii* - known for its key role in the degradation of resistant starch, its stability indicates sustained functionality in carbohydrate metabolism, which is crucial for overall gut health and fermentation processes.

A positive development was the increase in the abundance of *Faecalibacterium prausnitzii,* a bacterium known for its anti-inflammatory properties and significant role in butyrate production. Its growth suggests improved gut health and possibly better immune modulation.

Another bacterium whose numbers decreased was *Bifidobacterium adolescentis,* which is involved in the digestion of oligosaccharides and the production of lactic acid, which affects the pH in the intestines and inhibits the growth of pathogenic bacteria. Other types of bacteria that appeared after the diet were *Bacteroides dorei* vulgatus, a bacterium associated with the control of the intestinal barrier and immune system, and a large group of different Clostridiales species, known for their high fermentation activity.

At the same time, a reduction in the abundance of *Eubacterium rectale, Blautia obeum wexerae*, Ruminococcaceae sp., Roseburia sp., Lachnospiraceae sp., Coprococcus eutoctus, Blautia massiliensis – known producers of butyrate. It therefore appears that freeze-dried food with a good composition and adequate fiber content does not adversely affect the composition of the microbiota. In another interesting study (van der Merwe et al., 2021)women were supplemented with dried powdered fruit and vegetable juices for 16 weeks. Analysis of the fecal microbiome collected from the subjects at the end of the study showed no significant differences in composition compared to the placebo (which received microcrystalline cellulose). Interestingly, these changes were not observed after extending the study by 4 weeks and additional supplementation with a cocktail containing a significant amount of dietary fiber (no change in α and β diversity was observed) (van der Merwe et al., 2021).The impact of a dried fruit and vegetable supplement and fiber rich shake on gut and health parameters in female healthcare workers: a placebo-controlled, double-blind, randomized clinical trial (van der Merwe et al., 2021). (Other studies in which dried products were used for 14 days (a diet enriched with dried raisins administered in three portions of 28.3 g each) did not affect the overall composition of the microbiota of healthy volunteers, but did affect the fecal GM by increasing the content of *Faecalibacterium prausnitzii*, *Bacteroidetes* sp. and *Ruminococcus* sp. and reducing *Klebsiella* sp., *Prevotella* sp. and *Bifidobacterium* spp (Wijayabahu et al., 2019)

In subsequent studies (Hiel et al., 2019) for 14 days, researchers fed healthy volunteers a diet modified so that meals contained significant amounts of inulin (15 g of inulin per day). The results indicated changes in the composition of the fecal GM (similar to our research), in which the proportion of *Bifidobacterium* and *Actinobacterium*, among others, increased.

When analyzing the impact of a diet rich in animal products as a control and supplemented with 30 g of freeze-dried cranberries for 5 days, the results showed that both dietary models—the control diet and the cranberry diet—did not change the α and β diversity of the microbiome. However, the diet rich in animal products (control) affected the abundance of fecal bacteria, causing a decrease in the number of Bacteroidota with a simultaneous increase in the number of Bacillota

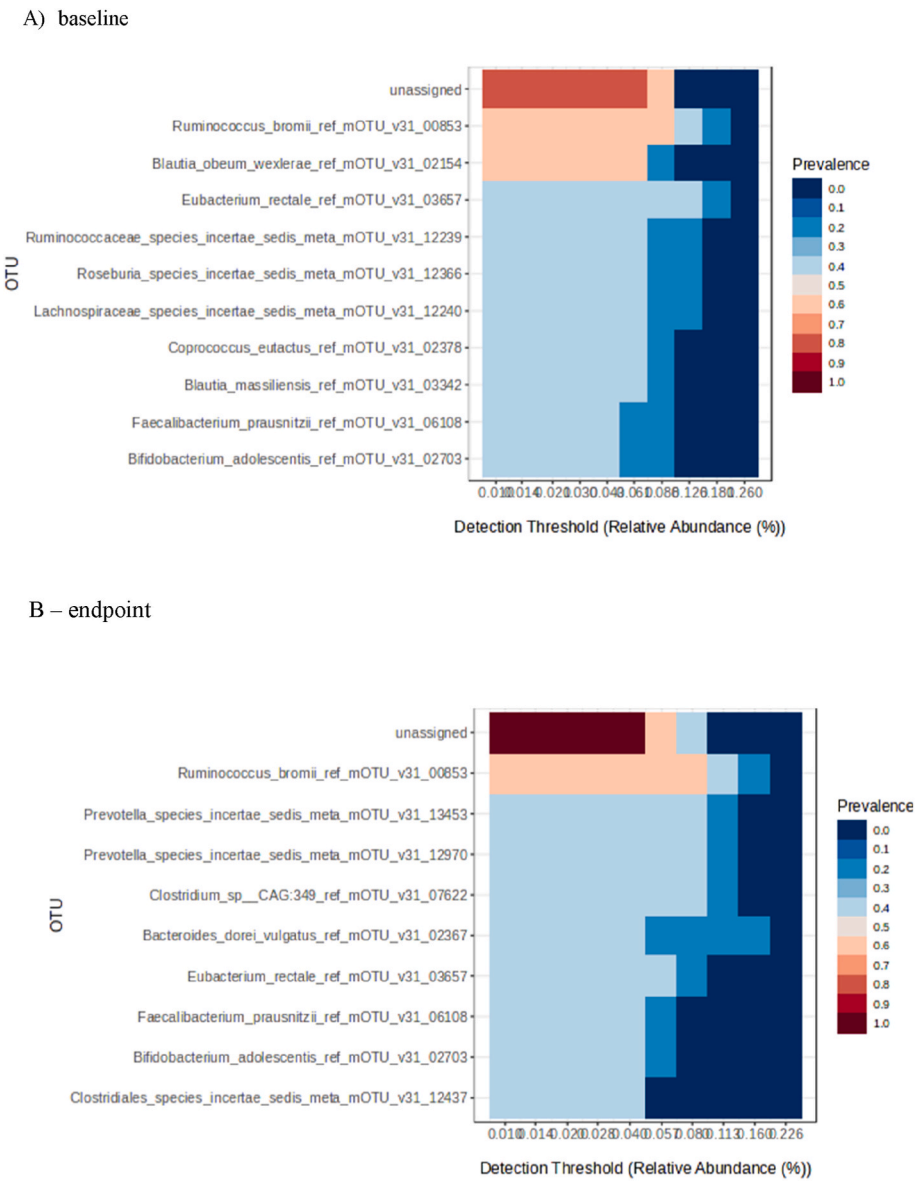

Fig. 5. The heatmap of hierarchical relationships between different taxonomic groups and their relative changes in abundance under different conditions (core microbiome analysis). (A) baseline, (B) endpoint. Warmer colors denote higher prevalence (fraction of samples above threshold). mOTU, metagenomic operational taxonomic unit.

bacteria.

It is safe to say that a diet rich in animal products (proteins and fats) and low in dietary fiber, the so-called Western diet, reduces the total content of intestinal bacteria and also affects its composition. This diet promotes a reduction in the content of, among others, *Bifi*dobacterium, Eubacterium, Lactobacillus and an increase in Enterobacteria Bilophila, Alistipes, Blautia, Ruminococcus and Bacterioides (Ross et al., 2024) In turn, the Mediterranean diet increases the total amount of intestinal bacteria, including in particular the content of Bifidobacterium, Eubacterium, Lactobacillus, Bacterioides, Prevotella, Faecalibacterium, Roseburia, while reducing the content of Collinsella and Ruminococcus (Lopez et al., 2014; Singh et al., 2017)

4.1. Limitations

This study has several limitations that should be considered when interpreting the results. First, the sample size was very small (n = 5), which substantially limits the statistical power and generalizability of the findings. The exploratory nature of the study means that subtle but biologically meaningful microbiome changes may have remained undetected due to insufficient power.Second, participants were recruited on a voluntary basis and were not subjected to extensive medical screening before the intervention. Although basic health assessments were performed upon arrival, the presence of undiagnosed conditions or individual lifestyle differences prior to the mission cannot be completely excluded and could have influenced baseline microbiome composition. Third, the intervention lasted only 14 days, which may not be sufficient to capture longer-term microbiome adaptations to a fully freeze-dried dietary pattern. While short-term microbial fluctuations can occur rapidly, sustained dietary effects on community structure and metabolic function often require longer observation periods. Fourth, although the diet was standardized and compliance was monitored through returned packaging, no detailed dietary records or biomarkers of dietary adherence were collected. Therefore, minor deviations from the planned dietary protocol cannot be entirely ruled out.Another limitation concerns the analytical approach. Shallow shotgun metagenomic sequencing was used, which provides reliable taxonomic profiling but may have limited resolution for detecting low-abundance taxa and functional pathways

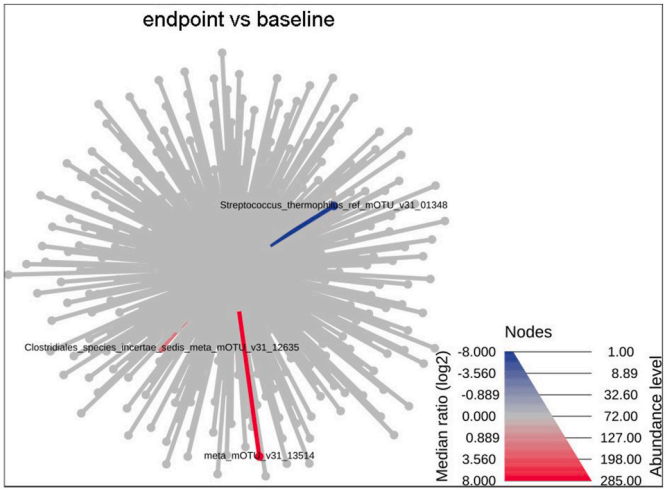

Fig. 6. Heat tree of gut microbiome taxonomic differences between baseline and endpoint. Nodes were scaled by abundance and colored by $\log_2$ median ratio (endpoint/baseline); Wilcoxon rank–sum tests with Benjamini–Hochberg adjustment. mOTU, metagenomic operational taxonomic unit.

Table 2
Taxa highlighted in exploratory core-microbiome and heat-tree analyses.

Stable core species:	*Ruminococcus bromii*
Increased abundance:	*Faecalibacterium prausnitzii,*
Decreased abundance:	*Eubacterium rectale, Bifidobacterium adolescentis*
Emerging species:	*Prevotella* sp. and *Clostridium* sp *Bacteroides dorei vulgate, Clostridiales* sp.-
Species that disappeared after the intervention:	*Blautia obeum wexerae*, *Roseburia* sp., *Lachnospiraceae* sp., *Coprococcus eutoctus, Blautia massiliensis, Ruminococcaceae* sp.,

compared with deeper sequencing approaches. Additionally, only forward reads were used in downstream analyses, which may have reduced the overall taxonomic resolution.Finally, the study was conducted in a specific analog habitat environment designed to simulate space mission conditions. While this provides a unique and controlled setting, factors related to confinement, altered daily routines, stress, or physical activity patterns may have independently influenced the gut microbiome, making it difficult to attribute observed microbial changes solely to the freeze-dried diet.

Future studies should therefore include larger cohorts, longer intervention periods, more comprehensive health screening, and deeper metagenomic or multi-omics analyses to better characterize the impact of freeze-dried diets on the human gut microbiome.

5. Conclusions

Freeze-drying is a popular method of food preservation that is becoming available to an increasingly wider group of consumers. In this preliminary study, a 14-day fully freeze-dried diet was not associated with statistically significant changes in overall gut microbiota diversity; however, larger controlled studies are needed to confirm safety of such dietary regimen.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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