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Can Bulgarian Yogurt enhance astronauts' performance during the Mars Missions?

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Abstract

Probiotics (*pro-for and bio- health*) from yogurt are one of the most effective means to stimulate and strengthen the immune system. They help balance and regulate the digestive system, as well as preserve and enrich the gut microbiome. Maintaining a healthy gut microbiome is crucial for human health and well-being, especially for astronauts living in confined and stressful environments, such as those on a mission to Mars. One way to promote gut microbiome diversity is through diet, and Bulgarian yogurt (kiselo mlyako in Bulgarian: кисело мляко) made with *Lactobacillus delbrueckii subsp* bulgaricus and Streptococcus thermophilus has shown positive effects on gut health. This paper explores the potential of regular production and consumption of gut-beneficial foods, such as yogurt, during space travel. It analyses whether the dietary limitations and challenges in providing varied and fresh food for astronauts could be addressed through the addition and daily consumption of Bulgarian yogurt. To investigate this, we conducted an experiment with a team of analog astronauts participating in a two-week analog mission in a closed, Mars-like environment at the Mars Desert Research Station in the Utah desert, the USA. In compliment to all recognized health effects of yogurt, the analog astronauts reported that it can be easily prepared and had a positive effect on their overall well-being and gut health. Our study demonstrated the feasibility of incorporating freshly made yogurt into the astronauts' diet and its potential to significantly contribute to achieving good health and well-being, which is an important goal in the colonization of other planets, such as Mars.

Keywords Bulgarian yogurt, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp*, Gut microbiome, Mars Desert Research Station, Analog astronaut

Introduction

Yogurt and its health benefits

For millennia, humans have consumed microorganisms through fermented foods. Fermented dairy products, such as yogurt and cheese, are highly valued as functional foods and widely consumed due to their positive health effect. In 2014, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." [1]. Probiotic (*pro-for and bio- health*) microorganisms are primarily derived from the *Lactobacillus*, *Lactococcus*, *Bifidobacteria*, *Pediococcus*, or *Bacillus* genus, with Lactobacilli being the most commonly utilized as probiotics (Georgieva et al., 2015). There are various probiotics including *Lactobacillus delbrueckii bulgaricus and its yogurt starter partner Streptococcus salivarius subsp. thermophilus*, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, *and Lactobacillus acidophilus* and three species of *Bifidobacteria*. Fermented foods offer health

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benefits through direct probiotic effects, involving live microorganisms, and indirect biogenic effects, resulting from microbial metabolites. Specific effects vary based on the species and fermentation processes. *Lactobacillus helveticus* can affect human health by inhibiting pathogens, modifying gut microbiota, modulating the immune system and improve cognitive function in the elderly. *L.casei* strain Shirota, consumed orally the necessary dose as live cells, may enhance intestinal function and metabolic activity, benefiting human health. The main benefit of probiotics is their ability to help restore balance in the intestinal microbiota [2]. Probiotics have long been reported to aid in the treatment of various gastrointestinal dysfunctions. They are also described as useful in combating oxidative stress, improving mucosal immunity [3] and enhancing general immunity [4]. Thus, they may contribute to involve new functional products, to enrich daily menu. In addition such probiotic LAB species can contribute to the production of functional foods with positive health effects in addition to its nutritional qualities. This would be very valuable especially in limiting conditions such as a long space mission or expeditions in unfavourable natural places.

The benefits of *Lactobacillus bulgaricus* in challenging environments

The yogurt Microbiota contain at least 10^6 CFU (Colony forming units)/gr live lactic acid bacteria (LAB), which may present a daily dose of probiotics for consumers. *Lactobacillus bulgaricus* named initially the Bulgarian bacillus, represents humanity's historic contribution to modern science, marking the inception of the world's first health-promoting foods. Constituted by two specific and well-defined live and active bacteria strains, namely *Lactobacillus delbrueckii* subsp. b*ulgaricus* and *Streptococcus thermophilus,* the primary benefit of Bulgarian yogurt is its high probiotic content, which supports digestion and a healthy gut [5]. The probiotic strains in Bulgarian yogurt have the ability to suppress the growth of harmful bacteria [6].

L. Bulgaricus has demonstrated remarkable and diverse health benefits, supported by global experimental and clinical research [2, 7]. During the milk fermentation the synbiotic partners—*L. bulgaricus* and *S. thermophilus* produce variety of metabolites, called postbiotics. They are main factors to regulate healthy gut homeostasis, to overcome dysbiosis and to communicate via gut-brain axis, helping also several physiological functions and systems. Unique to *L. bulgaricus* is secretion of both L (+) and D (-) lactic acid, conferring potent antioxidant and anticancer effects. This differs it from other probiotic microorganisms, which secrete only L (+) lactic acid [2]. Through the production of postbiotics with various beneficial effects, cell lysates from the lactic acid bacteria of yogurt have been shown to stimulate the immune system regulates gastrointestinal functions and maintains microbial flora. They reduce cancer risk, offers protection during radiotherapy, regulates cholesterol, and inhibits pathogenic infections. Based on unique beta galactosidase activity, *L. bulgaricus* may help to overcome the problem of lactose intolerance. Additionally, *L. bulgaricus* produces bulgaricin, a heat-resistant antimicrobial substance effective against virulent strains [8, 9]. Its superior adherence to colonic mucosa and rapid reproduction enhances its treatment and detoxification capabilities compared to other lactic acid bacteria probiotics. Specifically beneficial for women, it resembles the vaginal lactic acid microbiota, underscoring its significance in promoting health [10]. Such scientific data create a new concept about potential of yogurt and other fermented milk products as an indispensable component of nutrition in extreme or unfavorable conditions.

Yogurt and life in extremal conditions

Through the years, the continuous investigations into the advantages of Bulgarian yogurt highlight its significance as a robust and nutritious food source, especially in challenging environments. This persistence in research underscores the resilience of *Lactobacillus bulgaricus* and its adaptability, reinforcing its reputation as a valuable probiotic in various conditions. In the 1990s, *L. bulgaricus* was used in the production of probiotic functional food for astronauts, which was tested during the flight of the second Bulgarian astronaut in Space [2, 11, 12].

Another experiment was conducted to test the positive effects of L. *bulgaricus* DWT1, sourced from water, on human health and well-being in extreme temperatures and conditions during a 150-day voyage to Antarctica, as part of the Bulgarian Antarctic Expedition 2022–2023. The study included 20 sailor participants who were exposed to challenging environments and adverse working conditions [13].

Similarly, in a study conducted among 56 Bulgarian Air Force pilots in the 1990s, probiotic foods, specifically those containing *L. bulgaricus*, were administered in challenging conditions. The findings demonstrated a reduction in total cholesterol, bad cholesterol, and triglycerides, accompanied by an increase in good cholesterol. Additionally, it was observed that *L. bulgaricus* played a role in regulating blood sugar levels and enhancing immune response. Another study involving a similar number of volunteers was undertaken in simulated shipwreck conditions. The results revealed that probiotic consumption helped maintain body weight, arm muscle strength, and electrolyte balance [13].

Gut microbiome and Bulgarian yogurt

Gut microbiome diversity refers to the varied and complex community of microorganisms present and living in the human gut [14]. A diverse gut microbiome is associated with many health benefits, including a stronger immune system, improved digestion, and better mental health. However, the gut microbiome can be significantly altered due to various factors negatively impacting its balance, such as stress, the use of antibiotics, and unhealthy or a limited diet [15]. Such imbalance of gut bacteria, known as dysbiosis, promotes intestinal inflammation [14]. This results in various health problems, including digestive issues and increased susceptibility to infections. Thus, gut microbiome diversity is essential for good health and needs to be balanced, especially for people on a mission to colonize any planet, including Mars. Astronauts face several challenges to their well-being that can negatively affect their gut microbiome and potentially lead to many health issues. These challenges include radiation exposure, changes in the microbiota, stress, and a limited and monotonous diet. Such a diet cannot stimulate gut homeostasis, which is crucial for traveling through space [16]. Furthermore, the primary goals of long-duration exploratory missions should revolve around the well-being of the gut microbiome of space travelers. To mitigate these effects, it is crucial for astronauts' diet to include functional foods, such as Bulgarian yogurt, that can promote gut microbiome diversity as well as positive effects on the homeostasis and overall health.

Yogurt is an irreplaceable and valuable food with a centuries-old tradition, suitable for all ages. It can be consumed by those suffering from various diseases even when no other food can be taken. Bulgarian yogurt is a traditional fermented dairy product that has been consumed for thousands of years. It is made by fermenting milk with two specific strains of lactic acid bacteria, *L. delbrueckii* subsp *bulgaricus* and *Str. thermophilus* [5] and recognized and defined by the Codex Alimentarius Food and Agricultural Organisation of the United Nation as cultured milk product [17]. Acknowledging the advantageous qualities of live bacteria, the Codex authorities limited the definitions of "fermented milks" and "yogurt" to exclusively encompass products that include live cultures. These bacteria work symbiotically, meaning they benefit each other during the fermentation process, converting lactose (the natural sugar in milk) into lactic acid, which gives yogurt its characteristic tangy flavor and thick texture [18]. With a high number of living beneficial bacteria [5], it is widely accepted as a probiotic food.[1] Probiotics are beneficial microorganisms that can improve gut health by increasing the diversity of the gut microbiome [19]. These bacteria produce lactic acid, which helps preserve the yogurt, giving it a tangy flavor. By consuming this appetizing and nutritious product, astronauts can ensure they receive an adequate supply of probiotics and maintain a healthy gut microbiome on Mars or any other planetary mission. Bulgarian yogurt is known for its long shelf life due to its pH and the high number of beneficial bacterial cultures present at the end of production, which act as a preservative. It can also tolerate high temperatures, humidity, and exposure to air without losing its quality or safety [20]. Its ability to withstand harsh conditions makes it an ideal food item to take to Mars, as it can be stored for long periods without refrigeration. Thus, astronauts will be able to enjoy a healthy and nutritious snack without concerns of spoilage.

As a good source of probiotics, Bulgarian yogurt is associated with good health and longevity [6, 7, 21]. Furthermore, it is recognized for reducing constipation symptoms and improving mood [22, 23]. Metabolic activity of yogurt microbiota promotes the growth of beneficial bacteria in the gut, which can lead to improved digestion, stronger immunity, and better mental health. Bulgarian yogurt also contains protein, calcium, and other essential nutrients, making it a wholesome and beneficial addition to the diets of astronauts on a mission to Mars. The medicinal-dietary properties of yogurt are determined by two factors: (i) the composition and changes in milk during lactic acid fermentation under the action of *L. bulgaricus* and *Str. thermophilus*, and (ii) the dynamics of its action on the digestive tract and other organs. The purpose of this study is to explore how yogurt can be used in space travel, specifically its potential to support the gut health of astronauts during long-duration expeditions, such as a Mars mission. It also aims to develop and evaluate yogurt-making recipes that can be used under conditions fully simulating those of a real planetary mission.

Previous studies on Bulgarian yogurt have somewhat overlooked its distinct overall health benefits and positive impacts on gut microbiota and general well-being. This oversight becomes particularly critical when considering its potential as part of the dietary options for astronauts. Highlighting its ease of preparation in space conditions is a notable achievement, given the persistent challenge of providing a varied and fresh diet for astronauts. Can

[1] "Probiotic" is derived from the words "pro" (for) and "bio" (life), literally meaning "for life" or "promoting life." The field of probiotics, which emerged in the early twentieth century, has seen significant advancements, particularly in the last two decades. Probiotics are often found in fermented foods like yogurt and are considered to have positive effects on gut health and overall well-being (Sanders et al. 2018).

Bulgarian yogurt enhance astronauts' performance during the Mars Missions?

Mars Desert Research Station

The testing ground for this research was the Mars Society's Mars Desert Research Station (MDRS) in the Utah desert, in the USA. This station is one of the many terrestrial analog sites, also referred to as "*space analog*," located in different parts of our planet Earth. The mission's habitat is isolated from civilization and not open to public visits [24]. It is situated in a location that shares similarities with Mars [25]. Mars analog habitats play a crucial role in training astronauts, engineers, and researchers for the challenges they will face on future crewed missions to Mars. These specialized facilities host small teams of volunteers and immerse them in environments that simulate the harsh conditions of the Red Planet. These simulations can extend from just a few days to well over a year, allowing participants to acclimate to the psychological, physical, and technical demands of living and working in space-like conditions [25]. All these analog missions share one prominent characteristic: they assume the past or present geological, environmental, or biological conditions of celestial bodies such as the Moon or Mars. The idea behind the analog stations is to address a plethora of challenges and gather data on the strengths, limitations, and feasibility of planned human and robotic exploration missions in extraterrestrial environments. A human spaceflight to Mars is scheduled for the next decade [26]. Analog stations facilitate the development of strategies to synergize human and robotic efforts, thereby enhancing scientific exploration. Test locations vary and include the Antarctic, oceans, deserts, as well as arctic and volcanic environments.

The MDRS is a privately operated space analog research facility by the Mars Society. Its primary goal is to support Earth-based research focused on developing the technology, operations, and scientific knowledge necessary for human space exploration. The facility hosts a nine-month field season, providing training opportunities for professional scientists, engineers, and college students of all levels, specifically preparing them for human operations on Mars. The facility's relative isolation enables rigorous field studies and human factors research. Most crews carry out their missions under the constraints of a simulated Mars mission within a controlled environment, lasting 2–3 weeks in duration. The advantage of MDRS is that the campus is situated in a landscape that closely resembles the geology of Mars, providing valuable opportunities for rigorous field studies simulating actual space missions. From an analytical standpoint, these analog habitats provide invaluable insights into the complexities of human adaptation to extraterrestrial environments. By subjecting volunteers to extended periods of isolation and resource constraints, researchers gain a profound understanding of the psychological resilience necessary for long-duration space travel. Moreover, these simulations enable engineers to test and refine technologies essential for sustaining life on Mars, fostering innovation and problem-solving in the face of unprecedented challenges. As humanity is coming closer to the reality of interplanetary travel, the knowledge gleaned from these analog missions becomes instrumental, paving the way for the successful execution of future crewed missions to Mars and beyond.

A significant aspect of these analog missions is food, reflecting the intricate balance between sustenance and the demands of space travel. The stringent requirements for space food which must be shelf-stable, long-lasting, compact, lightweight, and easy to prepare [27, 28] are not mere conveniences but essential factors that directly impact the physical well-being of astronauts. Additionally, in the harsh environment of space, where resources are limited and every bit of weight matters, the development of space-friendly food is a testament to human ingenuity. These foods not only meet basic nutritional needs, but also must preserve essential minerals, enzymes, and other nutrients that are prone to degradation over time. This aspect of food technology is not just about convenience. It is a meticulous science ensuring astronauts receive the necessary sustenance to maintain optimal health and prevent the debilitating effects of prolonged weightlessness. Proper nutrition is crucial to ensure astronauts maintain healthy bodies and prevent bone and muscle loss during their deployment [16].

Maintaining strong healthy gut microbiome diversity on Mars

Space exploration allows us to test and validate scientific theories developed on Earth by studying the solar system and other phenomena, such as understanding gravity, the magnetosphere, the atmosphere, fluid dynamics, and the geological evolution of other planets. Additionally, space exploration is crucial for the potential discovery of new life and the prospects of becoming a space-traveling species. It also holds importance in terms of food growing, sustainability, and promoting healthy planetary living [29] and provision of complete nutrition [30]. The success of Mars missions and any expeditions beyond Earth will heavily rely on understanding the microbiome and its profound impact on individual crew members' health. The microbiome's role is critical as it not only affects crew members' well-being, but also impacts the integrity of the spaceship and the team's cohesion, as well as the availability of a safe food supply [26]. Food's significance extends to every aspect of human physiology, as it can either contribute to excellent healthy or lead to severe health issues.

The current selection of foods available to astronauts beyond Earth predominantly consists of dehydrated products [29], supplemented occasionally by intermediate moisture products, such as dried fruits [31]. There are also rare instances of consuming frozen, fresh, thermostabilized (e.g., canned food) and irradiated foods (e.g., meat) [29, 31]. Maintaining astronauts' nutrition becomes increasingly challenging the farther they venture from Earth. For instance, a crewed mission to Mars may require provisioning food for up to five years in space. The current approach involves providing astronauts with freeze-dried and shelf-stable foods that can be easily rehydrated for preparation. Freeze-drying preserves up to 98 percent of the original foods' nutrition while reducing their weight to only 20 percent of the initial value [28]. Studies suggest that space travel can disrupt the healthy gut microbiome, which is crucial for maintaining balance and overall health during long space missions [16]. While the maintenance of a healthy microbiome is essential for extended space travel, there are various factors limiting the diversity of gut bacteria. One strategy to improve gut health is by providing space travelers with fresh food, probiotics, and prebiotics, which have the potential to enhance the balance of the gut microbiome in space travelers. However, when freeze-dried or frozen, the viability of beneficial bacteria is uncertain.

Study type, working hypothesis and aim of study

This study was experimental in nature. It aimed to build upon existing studies that have highlighted the positive impacts of yogurt, particularly Bulgarian yogurt, by exploring its potential application for astronauts during long interplanetary missions where fresh food is scarce. The unique aspect of this experiment lies not in yogurt consumption itself, but in the method employed to create yogurt: using dry milk and bypassing the need for sophisticated laboratory conditions usually associated with such processes. The study sought to demonstrate the feasibility of incorporating freshly made yogurt into the astronauts' diet, showcasing its potential benefits despite the absence of fresh produce in space. Moreover, Bulgarian yogurt was envisioned as a versatile ingredient adaptable to various culinary applications, adding a sense of novelty to the menu. What sets this experiment apart is the innovative approach to making yogurt in space conditions, employing a minimal addition of lactobacilli. Moreover, the crew underwent training to produce yogurt during the mission, highlighting the adaptability of the method to space environments despite their prior knowledge and skills, marking a significant step in space food research.

Some researchers have suggested that probiotics and prebiotics could be valuable strategies for promoting a healthy gut microbiome during long-term space missions [16]. Therefore, introducing freshly made yogurt in astronauts' diet is believed to have the potential to improve their overall well-being and health. To achieve this, we proposed a regular daily intake of yogurt containing over 1 million/g colony-forming units (CFUs) of live lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* cultures) as a strategy to promote astronauts' microbiome health. This approach is both viable and feasible as the required dried, powdered ingredients, including the lactic acid bacteria (LAB) and milk necessary for yogurt production, can be provided in accessible forms (dehydrated, freeze-dried, etc.) during interplanetary missions. Lactic acid bacteria can be stored for extended periods in lyophilized form [32], enabling their use as needed to make natural, fresh yogurt with live bacteria by using long shelf-life powdered milk stored in bulk.

Therefore, the purpose of this study was to investigate the application of yogurt in space travel, particularly its ability to enhance astronauts' self-reported gut health during extended missions like a Mars expedition. The research focused on creating and assessing yogurt-making methods suitable for the conditions of an actual planetary mission, participants' overall perceptions of the end product, as well as the level of difficulties of yogurt preparation.

Demographics

All analog Mars missions have a strict maximum crew limit of up to six ($n=6$) members. Similar to the selection criteria for astronauts, crew candidates needed to meet various requirements, including health status, endurance, psychological profile, professional expertise, and life experiences. In accordance with the allowed mission equipment parameters, the analog mission crew participating in this study consisted of six healthy females, aged from their early 20 s to early 50 s, from varying professional and diverse international backgrounds. The selection was based on personal experience and qualifications, matching the requirements for each of the pre-mission identified crew roles, as well as other requirements related to international travel at the time (2022) such as the compulsory full Covid-19 vaccinations. All crew members were in good health and did not report any symptoms of illness at the beginning of the mission.

As part of the Analog Mars mission's requirements, conducting experiments with control groups or implementing experimental manipulations with the study participants was prohibited. This constraint was dictated by the mission's parameters, as further elaborated in the limitation section. The sampling designs and sample size justification were developed within the scope of these restrictions.

Materials, ingredients, equipment and methods

During the analog Mars mission, the crew of six analog astronauts engaged in a daily experiment involving the production and consumption of Bulgarian yogurt. Among the participants, only one had prior experience in yogurt-making before the mission started. This individual was responsible for both making the yogurt and conducting a special training session to teach the untrained crew members how to prepare yogurt during the mission. To gather individual perspectives on yogurt quality, consumption experience, and preparation, crew members were requested to maintain daily diary entries throughout the mission. Stringent scholarly procedures were employed in developing and validating the survey guide used for data collection. Preliminary testing of the survey questions forming the basis for the daily diary entries was carried out with random participants from Curtin University, Australia, followed by testing with the Mars Society before entering the MDRS habitat. This pre-testing phase played a crucial role in determining the structure and content of the daily diary survey for its administration.

The study employed qualitative thematic analysis as its analytical method, utilizing a flexible approach to extract novel insights and concepts from the dataset. The aim of the thematic analysis was to identify and understand major themes and their interconnections within the qualitative data gathered throughout the experiment. This methodology facilitated the examination and interpretation of meaningful patterns, providing a comprehensive and overarching perspective on the dataset.

The preparation of yogurt followed a specific method to maintain the uniformity in its making. The initial yogurt preparation differed from the rest of the batches. Three ingredients were used: powdered milk, a starter Bacillus Bulgaricus (see Image 1) LAB, and water. Powdered skimmed milk (10–12% w/v) was rehydrated with boiled water tempered at 60 °C and left to cool down in a container at room temperature until it reached the optimal temperature for the lyophilized starter LAB inoculation—40 to 43 °C. Then, live cultures were introduced to the milk, stirred directly in the container, and kept in an environment that allows for reliable temperature control (usually around 40 °C degrees approximately). This is how the first batch of yogurt is created. After it is produced, the next batches are populated using live lactic acid bacteria from the yogurt made the previous day. All ingredients, equipment and methods variation used is shown in the figures.

The equipment and ingredients needed for the preparation of yogurt in the conditions of the MDRS mission are listed in Table 1.

Figure 1 visualizes the equipment, ingredients and kitchen used for the yogurt-making for the duration of the MDRS mission experiment, and Fig. 2 depicts the two yogurt processing methods.

The kitchen as we know it on Earth has little in common with the one that will most likely be on a space mission. For instance, the International Space Station (ISS) has only a small, designated area, called the "galley," which serves the purpose of allowing for food preparation and consumption" [33]. The kitchen at the MDRS mission was not so primitive like the described "galley" and instead was designed to resemble a kitchen that could be available in a closed environment during the settlement of humanity on another planet.

Results and discussion

The yogurt experiment

The yogurt experiment conducted in simulated Martian base conditions within the MDRS mission 268 station was one of the few involving foods. Another experiment focused on greens harvesting at the GreenHub. All proposed experiments for the mission were described, assessed, and approved by the Mars Society, responsible

Table 1 Yogurt preparation equipment and ingredients list

Equipment	Ingredients
Clean yogurt containers × 2	Water
Milk thermometer	Powdered milk
Stirring spoon	Lyophilized lactic acid bacteria
Electric water heater (kettle)	
A larger container (or a cavity, like a microwave or an oven)	
Towels × 2 for wrapping the yogurt containers and keeping the inoculated yogurt warm	
Freezer	

Fig. 1 Yogurt preparation equipment, ingredients and preparation place as described. Image **A** shows all the necessary equipment for yogurt-making. Image **B** depicts the type of bacteria used, and image **C** presents the available kitchen facility at the MDRS mission

for conducting the MDRS mission. The selection of the yogurt experiment was based on its scientifically identified benefits for human health [5]. The goal was to investigate whether these recognized and acclaimed benefits would manifest after yogurt consumption in a sterile, enclosed environment simulating a Martian base.

Upon its arrival at the MDRS habitat on November 13, 2022, the six-person crew, participating commenced their full simulation in the analog mission with an instruction session followed by their pre-mission determined activities. The experimental testing involving the Bulgarian yogurt-making and consumption during the whole duration of the mission, proceeded with the preparation of the first batch of yogurt. According to the pre-plan it was prepared straight after dinner to utilize the full potential of the kitchen availability, using the equipment and ingredients described earlier. The yogurt preparation began by boiling water in the kettle and mixing it with dehydrated powdered milk in a 1:1 proportion. At that point, the resulting milk's temperature was close to 75 °C and after one hour, it had dropped to 43 °C. Subsequently, the dry lyophilized culture was introduced from the original package of the Bacillus Bulgaricus Bulgarian Yogurt starter (see Fig. 1 B), following the manufacturer's instructions. The culture was stirred well until a homogeneous mix was achieved and then poured into the two empty containers (see Fig. 1 A). The two containers of the mixture were wrapped in towels to maintain a constant temperature. Subsequently, they were placed in a microwave oven (see Fig. 1 C) with the door closed to create a uniform temperature environment. The idea of using the microwave as an overnight box was made as the yogurt inoculation and preparation requires 6 to 8 h to set and the microwave itself was not used during that time of the night. The ready-set yogurt was planned to be removed in the morning before breakfast.

The microwave was chosen for its small size and insulated interior, which minimized thermal exchange with the cool environment at night. This way, the yogurt-making process could utilize the available kitchen equipment without interfering with the daily routine of the rest of the crew. To achieve better temperature control, a milk thermometer was installed inside one of the yogurt containers placed in the microwave. This allowed for monitoring the temperature changes.

The yogurt-making setup described above was well thought out, and seemingly all the preliminary measures were taken to achieve optimal results with the final product. However, unexpected development with the constant temperature maintenance in the microwave were noticed before the crew was going to bed. When checked the temperature has shown to drop below 40 °C, indicating that the microwave was unable to sustain the optimal temperature for the next 6 h needed for the yogurt preparation. The most probable reason for this was the position of the kitchen on the outer wall of the confined MDRS habitat (see Fig. 3), which gets affected by external temperature changes. In the Utah desert, USA, the temperatures fluctuate significantly, experiencing relatively strong heat during the day and rapid cooling at night,

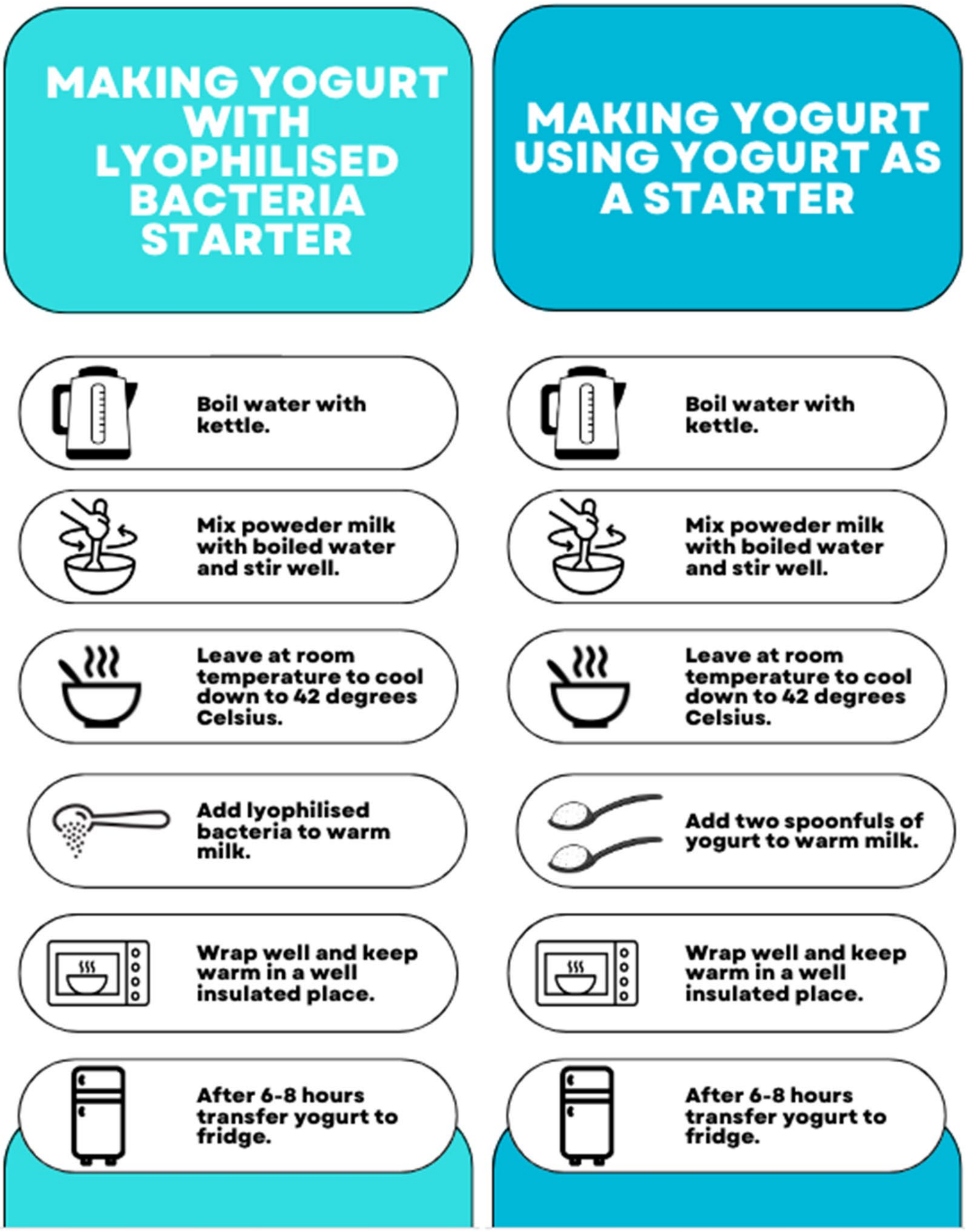

Fig. 2 Yogurt-making techniques applied during the Mars mission simulation project in Mars Analog Mission 268 station in Utah desert, USA. Only the first yogurt batch started with the lyophilized bacteria starter on the left and for the rest of the batches was followed the yogurt-making using the already made yogurt as a starter, a process described on the right

resulting in wide daily temperature ranges of over 40 degrees [34]. This finding demonstrated that a few adjustments are needed.

One solution to the varying temperatures problem was to find a spot in the inner section of the MDRS habitat that would not experience the sudden cooling effect of the lower external temperatures at night and where the heating system was more effective in maintaining a more constant temperature. Bedrooms were identified as one such area. They were positioned in the middle of the habitat floor plan, confined, insulated, and individually heated. This configuration presented a promising option for finding an appropriate area where the yogurt mixture could be left to set. The detailed

Fig. 3 A photograph of the crew's daily life illustrates that the microwave is mounted on the exterior wall of the common room of the habitat, which create opportunity for constant temperature fluctuation

A: Typical MDRS mission bedroom setting B: Cardboard box placement allocation

Fig. 4 This photograph illustrates the decision made about the new position of the cardboard box with the yogurt mixture under the bed platform where the heating pipe is built in. This way the necessary constant temperature for the yogurt-making process is assured

observation found out that the hot air outlet in the bedrooms was located under the beds, providing an ideal spot for placing the yogurt containers, still wrapped in the tea towels, inside a cardboard box on top of the heater pipe. This new experiment setting discovery ensured an even distribution of the right temperature around 40–43 °C for the entire duration of the yogurt setting process (see Fig. 4). This was reassured at 7 am the next morning when the thermometer was checked and still showed 40 °C, indicating that the allocation of the yogurt was chosen correctly and the yogurt itself had optimal conditions for inoculation more than 6 h. As initially planned the two yogurt containers were transferred to the fridge.

The first yogurt batch was sampled for the first time by the crew during dinner on November 14. The yogurt

A: Yogurt garnished with strawberries and mango next to the spoon

B: The crew is enjoying their desserts complimented with yogurt

Fig. 5 Yogurt-based desserts at dinner time on November 14, 2022, served with a homemade cookie and fruits. This was the first-time yogurt was tested in the MDRS enclosed habitat

was served together with rehydrated mangos, bananas, and strawberries, arranged as a fruit yogurt dessert (see Fig. 5), complemented with a homemade cookie.

After the first dinner the next batch of yogurt was started. The process involved slight modification as instead of using the dried *Bacillus Bulgaricus Bulgarian Yogurt starter* cultures (see Fig. 1), the previously made yogurt itself was used (as shown in Fig. 2). The preparation process was exactly the same with the only difference that instead of adding the Bacillus Bulgaricus Bulgarian Yogurt starter two full spoons of yogurt from the first batch were mixed and stirred well into the milk. The use of the already cold yogurt from the first batch brought the temperature of the mixture down to 42 °C. Again, the same procedure was followed as per the initial yogurt preparation with wrapping the containers in tea towels and placing them in the cardboard box in one of the bedrooms on top of the heating pipe for the night. The next day, at 7 am, the new batch of yogurt was placed in the fridge. This became a daily routine for the rest of the MDRS mission, ensuring we always had one or two full containers of freshly made yogurt in the fridge for everyone in the crew to enjoy.

Experiment outcome

The lyophilized bacteria were used only once for the inoculation of the first batch of yogurt. For the rest of the experiment, two spoonsful of yogurt were used as a starter for each new batch. The quality of the yogurt remained consistent without noticeable variations in taste, color, texture, and consistency between batches (see Fig. 6). This was reported after observing the results of every batch and was supported by the crew's experience with yogurt consumption, briefly described in their diary entries.

Yogurt applications

Freshly made yogurt was available for every crew member throughout the entire mission. The crew embraced the opportunity to add yogurt to their daily meals, using it in a variety of forms, but predominantly as part of deserts or in sweet versions. Among the most preferred options were smoothies with blueberries and yogurt, fruit yogurt with a diversity of dried fruits available for the mission, including bananas, strawberries, blueberries,

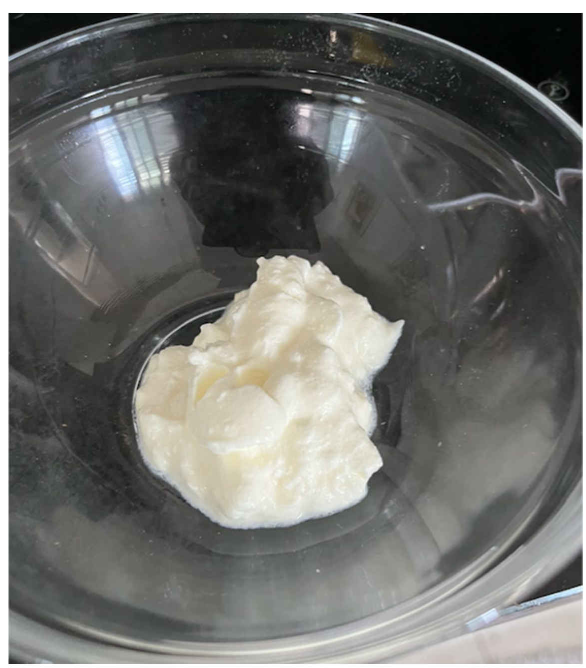

Fig. 6 The image below shows an example of the typical color and thick and creamy consistency of the yogurt made during the expedition at the MDRS

Fig. 7 Pizza base made with yogurt as part of the ingredients and the salad with yogurt-based dressing

mango, and apples. Yogurt was also used for making pancakes and consumed as a side with the pancakes. On some occasions, yogurt was served as a welcomed side for risotto, pizza, and baked potatoes. Additionally, on two occasions, yogurt was added to the salad dressing when the crew biologist harvested the greens in the GreenHub. These occasions occurred both in the middle and at the end of the MDRS mission.

Another example of yogurt use was its addition to various main meal-based dishes, offering a solution to diversify the crew's meals menu. It was incorporated as part of a yogurt-based salad dressing and used for making a pizza base with the addition of one spoonful of yogurt (see Fig. 7).

Educational sessions

Several educational sessions were held with the crew during the two-week mission. Prior to the start of the mission, the crew was briefed on the nature of all experiments proposed to be conducted during the analog mission. At the beginning of the mission, the team was introduced to the process of making yogurt by one of the crew members with prior knowledge and training of yogurt-making.

In the second week of the mission, there was a hands-on training about the benefits of yogurt for the microbiome and general health, including a demonstration of the yogurt-making technique, followed by a questions and answers (Q&A) session at the end. As a result of the educational session combined with the practical yogurt-making experience, the majority of the crew members expressed desire and visible enthusiasm toward yogurt-making. They shared their plans to start making their own yogurt after the MDRS mission is over and they are back home.

Prior experience with yogurt

All the crew members were asked to state their previous experience with consumption of yogurt. Five out of the seven crew members confirmed having some sort of previous experience with yogurt. The main reasons stated were diverse (see Table 2).

There were two people who had not consumed yogurt previously. The reasons they stated were related to different concerns, including "sensitivity" to diary and due to concerns about high sugar content: *"The store-bought yogurt doesn't have the health benefits and often contains too much sugar."* The reported previous experience with yogurt consumption was seen as a significant factor in promoting the inclusion of the yogurt in the MDRS mission daily menu.

The questionnaire and the experiment results

During the mission, all analog astronauts' part of the mission crew filled out a daily questionnaire reflecting their experience with the homemade yogurt they consumed on a regular daily basis. Overall, the addition of freshly made yogurt to the crew's menu was met with keen interest and enthusiasm, and even passion. Some of the crew members shared that despite not being accustomed to eating yogurt in their everyday lives prior to attending the mission, they thoroughly enjoyed eating yogurt during the mission. They described the experience as "*wonderful*," "*good*," "*pleasant*," "*delicious*," "*nice*," "*enjoyable*," "*fun*," "*outstanding*," and "*great*." One crew member even mentioned, "Better than yesterday," indicating their growing appreciation for yogurt consumption and embracing the opportunity.

Table 2 Reasons for yogurt consumption

I like the taste and it's healthy
Taste, gut health, ingredient in other dishes
It's part of traditional Punjabi food and it's tasty. And healthy
Good source of calcium and protein. I don't have digestion or stomach issues, but I know it helps my friends with IBS (Irritable Bowel Syndrome) and other issues
Good food

The sensory and textural aspects of yogurt have significant influence on its overall quality and appeal. The textural characteristics of the yogurt was described by some as "smooth and creamy," while others recounted the taste as "*simple, a bit sour, but yummy*" or "*sweet and yummy*" due to the "*fruit flavour mix*." Another crew member categorized it as: "*smooth, a little runny*." Others depicted the flavor as "*slightly sour but absolutely delicious*," "*thick*," "*creamy*," "*sweet and yummy*," and "*thick and creamy.*" In a similar vein in another study thickness or viscosity, smoothness opposite to lumpiness, and sliminess were pointed among the most common sensory attributes relating to yogurt [35].

One astronaut crew member characterized the texture as "*smooth but slightly thin*," while another described it as: "*very tasty, not very sour*." Another crew member shared the view that the texture was improving, stating, "*Thicker than yesterday. Definitely an improvement*." Another interesting point noted in the crew members' answers was that some of them found yogurt consumption more appropriate for the morning. One member mentioned, "*Mornings—enjoyable. Evenings—less enjoyable*." Another described it as: "*Morning sour but good. Mixed well with dried fruits*." Without explicitly stating something positive or negative about consuming yogurt during dinner, one crew member expressed the view that "*Evening taste was overpowered by orange flavouring.*" Another participating crew members wrote that yogurt "*In the morning was a bit sour, but in the afternoon, it has an orange flavour added to it*," which was considered as a massive improvement in the overall taste.

When asked whether they noticed any benefits from consuming yogurt, one of the crew members expressed an opinion that they felt "*not as thirsty*" and found yogurt consumption "*hydrating*." Another crew member noticed that regular consumption of yogurt seemed to promote digestion, stating, "*Ensured, but may have promoted digestion*." One of the participating astronauts illustrated the experience with yogurt consumption as something that helped with stomach bloating, saying, "*Yes, the bloated feeling was relieved*." Another crew member expressed the view that yogurt helped to "*keep my bowel movement regular*." One more member shared a similar experience after regular yogurt consumption:

> *"I didn't feel constipated even though I was often dehydrated in a very dry desert environment." (MDRS mission analog astronaut)*

Additionally, one of the crew members pointed out that yogurt was a "fresh" food, stating, "*I enjoyed having fresh food here in the habitat*," contributing positively to their overall diet during the mission.

Overall experience

When asked to express their overall experience with yogurt consumption during the MDRS mission, the analog astronauts were very positive and satisfied, highlighting its taste, freshness, and nutritional benefits (see Table 3). Some of them emphasized the benefits of live cultures, while others mentioned that are feeling tired of preparing it toward the end of the mission. Similar to other food presented as part of the mission's daily menu, there was a common sentiment that the novelty faded over time, indicating the importance of variety in the astronaut's diet to maintain interest and satisfaction throughout the mission. These various responses underline the potential of yogurt as a versatile and nutritious

Table 3 Experience of consuming yogurt

Great! The yogurt was tasty and full of healthy life cultures
It was good. Nice and fresh, which made a change to the other food that we had
Positive
It was delicious! It was very cool to learn how to make yogurt
Good! I enjoyed having it on the table during meals. Like all of the food in the hab. I was tired of it by Day 14

Table 4 Perceived benefits of yogurt consumption

Added good texture to certain dishes, presumably helped with digestion
Benefits—tasty, good for your gut, proteins. Negatives—none
It added variety to our menu during the mission, maintained our gut health, allowing us to consume more fruits in the form of smoothies
My body didn't agree with the dairy, but I enjoyed eating it
Good source of calcium and proteins. I either eat it unsweetened or add some splenda

addition to space mission diets, catering to diverse taste preferences and dietary needs.

Pros and cons of yogurt

The overall personal benefits and drawbacks of yogurt consumption during the mission were also noted. These were more positive than negative. Some examples are presented in Table 4. Some of the opinions shared were centered on the potential advantages for human digestion and gut health. Participants reported several positive aspects of consuming homemade yogurt, emphasizing its benefits, such as adding texture to dishes, aiding digestion, and providing essential nutrients like proteins, calcium, and gut-friendly elements. The yogurt was appreciated for its taste and versatility, contributing to a diverse menu during the mission. Others highlighted yogurt's usefulness as a good source of calcium and proteins.

Even some of the crew members who were not previously in favor of yogurt consumption due to health-related issues with dairy were regularly consuming and enjoying yogurt. Overall, the feedback was overwhelmingly positive, emphasizing the yogurt's value in enhancing both the nutritional content and culinary experience during their mission.

Organoleptic qualities of yogurt

During the MDRS mission, crew members were requested to evaluate the overall organoleptic characteristics of the consumed yogurt. The specifications of the Mars analog mission precluded the inclusion of detailed organoleptic tests within the research. As a result, these tests were not performed or discussed in the study due to these constraints. Instead, the study focused on gathering general information regarding organoleptic qualities as reported by the participants. These encompassed aspects such as taste preference, texture, and flavor, which are summarized in Table 5 to illustrate their experiences.

Opinions were diverse, indicating personal preferences. Participants noted varied observations about the taste and texture of the yogurt. Some found the initial batch slightly thick and less sour than expected, while others had mixed reactions, with the first batch being questionable, described as "iffy," but subsequent batches being excellent. Interestingly, participants appreciated the homemade texture, even those who did not enjoy store-bought yogurt.

The statement "I could go even thicker" suggests an openness to customization based on individual preferences. This feedback loop, where participants could express their preferences and see adjustments made in subsequent batches, demonstrates a participant-centered approach to the study. This adaptability likely contributed to a more positive overall experience for the participants.

Participants' comments reflect the dynamic nature of the yogurt-making process. While some slight variations were noted, the overall feedback was positive, emphasizing the value of having fresh, homemade yogurt in their meals. These responses accentuated the potential of homemade yogurt to provide a desirable food option, potentially addressing concerns related to texture and taste preferences, especially in environments with limited food variety.

Yogurt-making process

The study meticulously assessed the complexity of the yogurt-making process with particular attention to the challenges faced by analog astronauts. The responses from participants varied, indicating a mix of perceptions regarding the yogurt-making process. Many found it relatively easy, appreciating the quick turnaround (overnight) and the simplicity of inoculation. When asked about the level of difficulties, the majority of analog astronauts mentioned that managing the required and necessary constant temperature for a longer period of time presented a significant challenge. Participants mentioned the struggle of finding suitable locations or resources for consistent temperature control. Interestingly, some participants creatively found solutions, such as placing the yogurt under their bed.

Table 6 depicts the sentiments of the crew members dealing with yogurt-making. While the process of making yogurt was generally perceived as manageable, the critical factor of temperature control posed a notable challenge. This aspect crucial to yogurt fermentation was an important part of the experiment, aimed at establishing yogurt as an ideal candidate to play a crucial role in fulfilling the dietary needs of astronauts during interplanetary missions. These insights not only highlight the practical hurdles in space food production but also emphasize the strategic importance of mastering these processes for future space exploration endeavors.

Table 5 Organoleptic qualities of MDRS mission's yogurt

It was a bit thick and not as sour as usual
The first batch was iffy. But the rest was excellent. It wasn't too sour…It was nice and thick. I could go even thicker:)
It tasted fine, and it was nice having something fresh for meals
I actually liked the texture of the yogurt when normally I don't like the texture of store-bought yogurt

Table 6 Difficulties during yogurt-making

Relatively easy
Temperature management could be difficult depending on available resources
I thought it was so cool that it only took an overnight to make
The temperature should be perfect. Luckily it was warm in the station
I didn't make it, but I believe it was just finding the right place to put it to get the temperature right under your bed:)
Yes, as long as you have the right place, with the right temperature
It is relative to making other food in an austere environment with mostly dehydrated foods, yes
Easy inoculation but difficult to maintain temperature control

These diverse responses underscore the adaptability of individuals in austere environments but also emphasize the importance of developing precise temperature management solutions for successful yogurt production. This insight is crucial for future space missions, where efficient and reliable food production methods are essential for the well-being of astronauts.

Conclusions

Making fresh yogurt from powdered milk during space missions to provide astronauts with the health benefits of live lactic acid bacteria is not only feasible but also highly advantageous. By utilizing the available lyophilized food and utilities and adding only a small amount of yogurt starter and a milk thermometer to the payload, crews could enjoy high-quality fresh yogurt even on long space flights. The process requires minimal time and effort, and reliable temperature control is easily achievable through thermal insulation in a small container or by utilizing an existing heat-emitting device. Any crew member can make it by following simple steps; no training is required.

These minimal efforts bring substantial health benefits to the human microbiome. The psychological impact of the food variety achieved by adding yogurt to the menu, the additional taste and texture, as well as the satisfaction of being able to enjoy homemade products, are all positive aspects that significantly overweigh the investment of time and effort required for yogurt-making.

In conclusion, maintaining gut microbiome diversity is paramount for the health and well-being of astronauts on a mission to Mars. Bulgarian yogurt and its two lactic acid bacterial species—*Lactobacillus delbrueckii subsp. bulgaricus* (L. bulgaricus) and *Streptococcus thermophilus*—play a crucial role in promoting gut microbiome diversity and maintaining overall gut health by providing beneficial bacteria, probiotics, and essential nutrients. By including Bulgarian yogurt in their diet, astronauts on a mission to Mars can take a significant step toward maintaining a healthy gut microbiome and enhancing their overall health and well-being.

The astronauts' assessment of the Bulgarian yogurt's properties in this context not only expands our understanding of its perceived health benefits, but also positions it as a practical solution to the limitations and difficulties faced in space missions. Highlighting its ease of preparation in space conditions is a notable achievement, as providing varied and fresh food for astronauts has been a persistent challenge. Its adaptability as a space food option, coupled with its positive impact on astronauts' overall health and well-being, emphasizes the need for further exploration and integration of this ancient dietary practice into modern space nutrition strategies.

Incorporating Bulgarian yogurt into the diet of astronauts on Mars is an effective and practical approach to maintaining a diverse and healthy gut microbiome. With its long shelf life and probiotic properties, Bulgarian yogurt proves to be a valuable food item for colonizing the Red Planet. By prioritizing their gut health, astronauts will be better equipped to perform at their best and remain healthy throughout their mission on Mars.

Based on these findings, we strongly recommend that homemade yogurt be included as a standard menu item for space crews. The benefits of yogurt consumption during space missions go beyond nutrition; it also contributes to crew morale and well-being, making it a valuable addition to future interplanetary expeditions. This research not only contributes to the space exploration field, but also underscores the versatility of Bulgarian yogurt beyond its traditional culinary uses.

Study limitations

The study was conducted within specific conditions and a constrained experimental scope, adhering to the research parameters approved by the Martian society. The limited crew size, restricted to six members for a single analog MARS mission, made it impractical to create two distinct groups—one consuming yogurt and the other not—as all crew members expressed interest in the yogurt group. This constraint also hindered a more comprehensive analysis of gut microbe diversity, and related inquiries were omitted from the research due to mission requirements. Additionally, any human research during the missions required Institutional Review Board (IRB) or Ethics approval, imposing further constraints.

The experiment obtained mission approval under the stipulation that it adhered to the necessary constraints for exemption under the third IRB exception category. This category encompasses studies aimed at assessing public benefit or service programs' performance, evaluating food taste, quality, or consumer acceptance.

As a result of these constraints, our study did not include specific inquiries about toilet visit frequency, and we were unable to conduct organoleptic, physiological, and psychological tests. Similarly, the restrictions prevented us from collecting bacterial screening data from study participants to assess the viability of live lactic acid bacteria cells in the gastrointestinal tract pre- and post-yogurt consumption. While these assessments, including changes in flatulence frequency and gut microbiome diversity analysis, were not the primary focus, exploring these aspects in future studies with more flexibility or in different settings could offer further insights.

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Author contributions
Conceptualization was done by IS, DB, SD, and MY; methodology was done by IS, DB, SD, and MY; experiment and data collection were done by IS; data analysis was done by DB and IS; original draft preparation was done by IS and DB; project administration was done by DB; supervision was done by SD and MY; writing and review and editing were done by IS, DB, MY, and SD.

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Declarations

Ethics approval and consent to participate
Ethical approval of the research was obtained by the Curtin University Human Research Ethics Committee (in accordance with the National Statement on Ethical Conduct in Human Research, 2007; Approval no.: HRE2022-0041).

Consent for publication
Informed consent was obtained from all subjects involved in the study.

Competing interests
The authors declare no conflict of interest.

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