



Formulation, Standardization and Microbial Viability Analysis of a Calcium-enriched, Millet-based Freeze-dried Yogurt Mix Incorporating Strawberry for Long-term Space Missions

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ABSTRACT

Background: As NASA plans to increase the number of Indian astronauts for future space missions, there is a pressing need for space-friendly food options that meet specific dietary requirements. This study addresses this need by developing a yogurt mix incorporating finger millet (*Eleusine coracana*) and strawberries (*Fragaria × ananassa*), designed to be rich in calcium and suitable for space conditions.

Methods: The yogurt was cultured with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* and various formulations were tested: T1 (50% yogurt, 50% finger millet slurry, 50% strawberry puree), T2 (50% yogurt, 50% finger millet slurry, 25% strawberry puree), T3 (50% yogurt, 25% finger millet slurry, 25% strawberry puree) and T4 as a control (standard yogurt).

Result: Nutritional analysis of the yogurt mix revealed 5 g protein, 7 g fat, 420 kcal, 82.26 g carbohydrates, 29.5 mg Vitamin C and 241.5 mg calcium per serving. Freeze-drying preserved microbial viability (above 10^7 CFU/g). Sensory evaluation with 15 panelists indicated acceptable taste and quality. PCA was also interpreted on the attributes to find the appropriate formulation. Proximate and physicochemical analyses confirmed the mix met nutritional and stability standards, offering a space-friendly, long-lasting option for astronauts' dietary needs during extended missions.

Key words: Astronaut, Finger millet, Strawberry, Space food, Yogurt, Yogurt mix.

INTRODUCTION

Astronauts face unique health challenges in space, primarily due to the effects of microgravity. Microgravity leads to bone density loss, muscle atrophy and cardiovascular health risks. The absence of mechanical load on the body weakens bones and muscles, while changes in fluid distribution and electrolyte balance affects cardiovascular health. These issues underscore the need for tailored health and nutrition strategies during space missions, particularly to combat calcium loss, muscle weakness, and other nutrient deficiencies (Pachekrepapol *et al.*, 2021). To address these challenges, one promising solution is the development of a calcium-enriched, millet-based freeze-dried yogurt mix. Yogurt is a rich source of calcium, protein and other essential nutrients, making it an ideal food to support astronauts' health. The high-quality milk proteins in yogurt provide nearly all the essential amino acids required to sustain optimal health and these proteins also aid in calcium absorption and immune function. Additionally, yogurt is naturally rich in vitamins such as A, B2, B1, B12 and others that are crucial for maintaining overall health during space missions.

Incorporating millet into the yogurt mix further enhances its nutritional profile. Finger millet, in particular, is a rich source of calcium and is more sustainable compared to other sources. It can help mitigate calcium loss experienced by astronauts in microgravity, making it a valuable addition to the diet. The inclusion of strawberry as a flavoring not only enhances palatability but also provides

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vitamin C, an important antioxidant for combating the effects of cosmic radiation. For long-term storage in space, freeze-drying the yogurt mix is an effective solution. Freeze-drying preserves the yogurt's nutritional value while significantly enhancing its shelf stability, making it suitable for long-term space missions without the need for refrigeration. This method also reduces the weight of the food, which is crucial for space travel, where payload efficiency is critical (Abdeldaiem *et al.*, 2023).

Probiotic cultures like *Lactobacillus bulgaricus* and *Streptococcus thermophilus* play a vital role in promoting astronauts' gut health. These probiotics support gut

balance, boost immunity, and inhibit pathogenic bacteria, all of which are essential for maintaining health in space. Additionally, these probiotics help improve digestion, which can be disrupted in microgravity. They also offer antioxidant and anticancer benefits, making them particularly beneficial for astronauts exposed to cosmic radiation. The inclusion of probiotics in the yogurt mix may also help mitigate stress, a common issue in space missions. Stress has been linked to gut dysbiosis, and probiotics are known to support mental health by promoting a balanced gut microbiota. Probiotics may help astronauts manage stress and anxiety, improving their overall well-being during long-duration missions (Minorette *et al.*, 2024).

The main objective of the study here is to formulate and standardize the yogurt mix as a space food, and to assess the proximate qualities including calcium, vitamin C, protein, fat, energy, and carbohydrates and physicochemical parameters like moisture and pH. Additionally, the microbial characteristics that is total bacterial count, total yeast mold count and total coliform count were also assessed to examine the shelf life of the yogurt samples. Finally, the samples' sensory attributes and consumer acceptability were studied using nine-point hedonic scale with the help of 15 semi-trained panel members.

MATERIALS AND METHODS

Sample preparation

The experiment was conducted in June 2024 at the research laboratory of Hindustan Institute of Technology and Science, Padur. Yogurt was prepared using milk, bacterial cultures, stevia, maltodextrin, strawberry pulp, and finger millet slurry in various treatments (T1, T2, T3, T4 - control). Strawberry puree and millet slurry were mixed with other ingredients as shown in Table 1. Lyophilization began by sterilizing the Lyophilizer and molds with ethanol. Semi-liquid yogurt was poured into silicone molds, sealed with foil, frozen for 12 hours, and freeze-dried at -80°C. Once dried, samples were unmolded, pulverized, and stored in Mylar bags for further use.

Physicochemical analysis

Control (T4) and samples (T1, T2, T3) were taken. Infra digi pH meter was used to determine the pH of the control and yogurt samples. The moisture characterization analysis was measured according to the method reported by (Dimitrellou *et al.*, 2020).

Antioxidant activities

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The scavenging effect of the yogurt mix was assessed using the DPPH method. A DPPH stock solution (24 mg/100 mL methanol) was prepared and filtered for ~0.973 absorbance at 517 nm. Samples (100 µL) mixed with 3 mL DPPH solution were incubated in darkness for 30 minutes, and absorbance was recorded at 517 nm.

Total phenolic compounds

The total phenolic content was measured using (Rodrigues *et al.*, 2024) method. Extract (100 µL) was mixed with 2 mL 2% sodium carbonate, incubated for 2 minutes, followed by 100 µL Folin-Ciocalteu's reagent for 30 minutes in darkness. Absorbance at 720 nm was recorded, and phenol concentration was calculated using a Gallic acid calibration curve.

Proximate composition

The proximate composition of yogurt samples (T1, T2, T3) and control (T4) was analyzed for energy, protein, fat, carbohydrates, calcium, phosphorus, iron, and Vitamin C using the titration method.

Microbial analysis

Yogurt samples (T1, T2, T3, T4) were tested for total bacterial, mold, and yeast counts. Serial dilutions (up to 10⁻⁶) were plated on Plate Count Agar (bacteria) and Rose Bengal Agar (yeast/mold), incubated at 37°C for 24-72 hours. Colonies were expressed in CFU/g (Medina *et al.*, 2023).

Sensory analysis

The yogurt samples (T1, T2, T3, and T4) were assessed for acceptability through sensory evaluation on the basis of appearance, taste, flavor, texture, and overall acceptability using a nine-point hedonic scale with 15 semi-trained panelists from Hindustan Institute of Technology and Science. (Jayaweera *et al.*, 2018). Rehydrated samples (45°C) were served in labeled ceramic cups for assessment of color, flavor, taste, texture, appearance, and overall acceptability (0 = "extremely dislike", 9 = "extremely like").

Principal component analysis was also conducted to analyze the relationships between sensory attributes and compositional parameters (energy, protein, fat, etc.) of yogurt samples (T1, T2, T3, T4) are interpreted in Fig 1.

Table 1: Composition of yogurt samples (T1, T2, T3 and Control T4).

Sample ID	Yogurt	Strawberry puree	Finger millet slurry	Maltodextrin	Stevia	Total weight
T1	100 ml (25%)	70 g (17.5%)	50 g (12.5%)	20 g (5%)	20 g (5%)	260 g (100%)
T2	100 ml (25%)	60 g (15%)	50 g (12.5%)	20 g (5%)	20 g (5%)	250 g (100%)
T3	100 ml (25%)	50 g (12.5%)	20 g (5%)	20 g (5%)	20 g (5%)	210 g (100%)
T4 (Control)	100ml (25%)	40 g (10%)	20 g (5%)	20 g (5%)	20 g (5%)	200 g (100%)

The first two principal components were selected based on the eigenvalues and cumulative variance explained.

Statistical analysis of the sensory evaluation

Statistical analysis was conducted using SPSS 16 for Windows to obtain means and standard deviations for each sample. ANOVA and the LSD-test were used to differentiate fiber responses at different levels. The level of significance was 95% ($p < 0.05$). Data for graphical treatment was imported into MS Excel's graphics program (Suwardi *et al.*, 2020).

RESULTS AND DISCUSSION

Physicochemical analysis

Control (T4) and samples (T1, T2, T3) were taken and the results are presented in Table 2. Infra digi pH meter was used to determine the pH of the control and yogurt samples (Soni *et al.*, 2021). The moisture characterization analysis was measured according to the method reported by (Dimitrellou *et al.*, 2020). Moisture content of the Freeze-Dried Yoghurt mix sample (T3) was found to be $3.04 \pm 1.03\%$ which is subsequently lower than that reported. The moisture content present in the freeze-dried control sample were also found to be $3.09 \pm 1.20\%$ is the same as discussed by Dimitrellou. The pH of the yogurt was found to be ranging from 4.0 to 4.6 as figured by (Soni *et al.*, 2020).

This shows that the moisture content is less than the store-bought yogurt and can sustain a longer shelf-life as compared with the studies made by (Zahir *et al.*, 2023).

Antioxidant activities

DPPH assay

As shown in Fig 2, the yogurt samples demonstrated an increase in DPPH-scavenging activity with the addition of strawberry puree and finger millet slurry. This increase can be attributed to the high antioxidant content of strawberries and similar berries, as reported by (Archaina *et al.*, 2019). In that study, blackcurrant yogurt candies exhibited even higher antioxidant activity, providing a valuable reference point. In a similar study, conducted by (Okur, 2022) showed increased antioxidant content with addition of red bell peppers. The yogurt samples in this study were analyzed using the DPPH radical scavenging assay. The optical density (OD) values of samples T3 and T4 indicated their antioxidant capacities, with higher OD values reflecting greater activity. Specifically, treatment T3 showed a significantly higher OD of 0.432 compared to T4, indicating superior antioxidant activity ($P < 0.05$). This finding aligns with the observations of Lee *et al.* (2020), where DPPH values increased following the addition of blueberry powder, as also noted by (Salehi *et al.*, 2021). The experiments

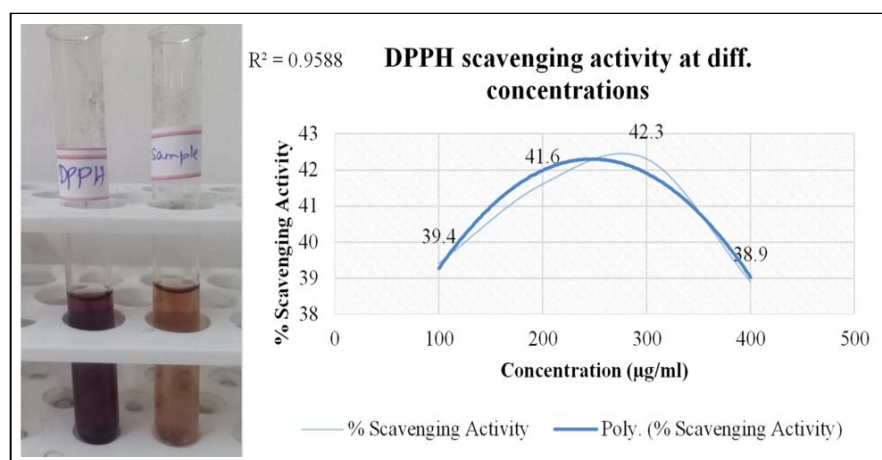


Fig 1: A line graph depicting the percentage values of moisture characterisation.

Table 2: The proximate composition of various treatments (T1, T2, T3, and T4 Control).

Composition	Treatment (T1)	Treatment (T2)	Treatment (T3)	Treatment (T4) (Control)
Energy	359.34 kcal	403.05 kcal	419.34 kcal	130.05 kcal
Protein	6.8±0.21 g	5.4±0.44 g	8.8±0.21 g	6.4±0.44 g
Fat	4.50±0.02 g	6.15±0.34 g	7.9±0.02 g	1.55±0.34 g
Carbohydrates	78.16 g	60.4 g	82.26 g	120.48 g
Vitamin C	29.5 mg	29.5 mg	29.5 mg	0
Calcium	221.5 mg	211.5 mg	241.5 mg	152 mg
Moisture content	3.24±1.33%	3.19±1.10%	3.04±1.03%	3.09±1.20%
pH	4.1	4.4	4.6	4.0

performed to assess the antioxidant properties of the yogurt samples. The findings suggest the yogurt formulations help reduce oxidative stress. This is even more so when considered in relation to environments that present higher oxidative challenges such as space where increased radiation requirements mean that there is a need for more antioxidant intake.

Total phenolic compounds

The yogurt control had TPC values of 63.24 mg/100 g discussed in Fig 3, aligning with reports that phenolic compounds (PCs) in cow's milk, derived from diet or amino acid breakdown, contribute naturally. Another study reported TPC values at 38.9 mg/100 g (C.W. Wang et al., 2024). Treatment T3 had an initial OD of 0.942, which then reduced to 0.543, yielding a scavenging percentage of 42.3%. In relation to this, Treatment T4 also began with an OD of 0.893 and ended with an OD of 0.457, yielding a scavenging percentage of 38.9%. These results have implied that both treatments have strong antioxidant properties while T3 is a little higher than T4. These findings align with that of the study by (Kulaitien, 2021) where yogurt bites

enriched with rosehip fruit powder showed the highest phenolic content at 49.05 mg·100 gH₁, followed by nettle and mulberry leaves powders at 40.51 mg·100 gH₁ and 38.81 mg·100 gH₁, respectively (Samakradhamrongthai et al., 2021).

Proximate analysis

The proximate analysis of the samples (T3) and (T4) were shown in the Table 2. The energy content present in the freshly prepared yogurt mix were found to be 419.34 kcal estimated from the analysis, while the content of energy in T4 was found to be 130.05 kcal which is significant increase as depicted in the results found by (Wang et al., 2020). The protein content estimated from the proximate analysis have showed the presence of 8.8 ± 0.21 g in the mix while the content is a little low that is 6.4 ± 0.44 g. This shows that the treatment T3 when consumed by astronauts on longer missions to meet a part of their protein requirement which will be helpful for the growth and repair of muscles. The prepared samples (T3 and T4) have reliable values of fat content 7.9 ± 0.02 g and 1.55 ± 0.34 g respectively (Wang et al., 2020). This implies that the fat content in the yogurt

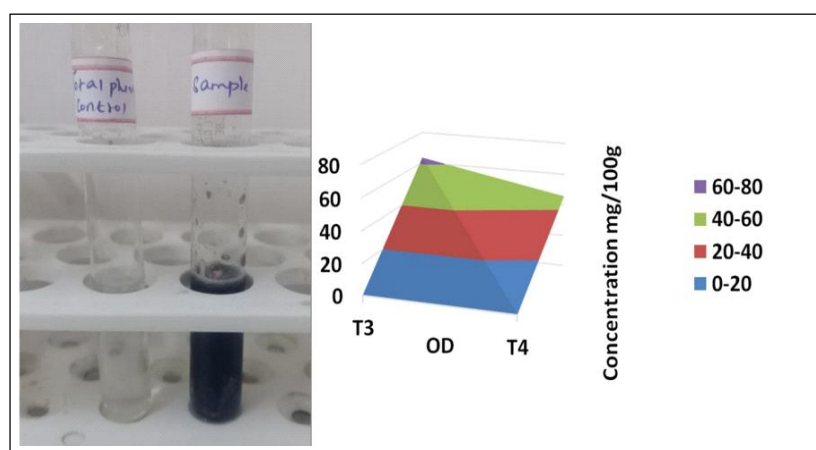


Fig 2: 3D plot showing concentration levels (mg/100 g) of total phenolic compounds across treatments (T3, T4).

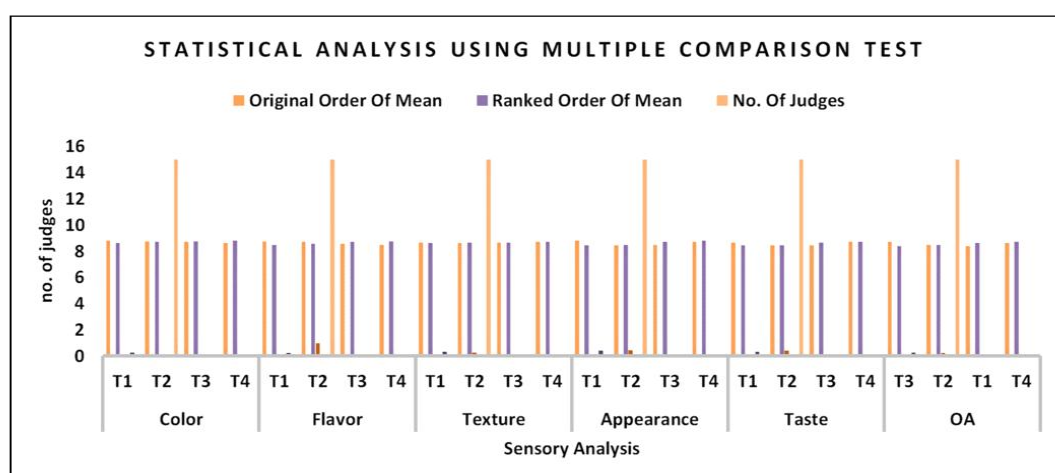


Fig 3: Statistical score (T1, T2, T3, T4).

mix might account to the mouthfeel and satiety of the astronaut in spite of the raise in total calorie amount (Vila-Real *et al.*, 2022). The carbohydrate content in treatments T3 (82.26 mg) and T4 (120.48 mg) differed, likely due to natural sugars from strawberry and finger millet. The calcium content (241.5 mg in T3 and 152 mg in T4) is crucial for combating bone density loss in astronauts. Customizing nutrient variations for astronauts ensures optimal health during missions (Thomas *et al.*, 2023).

Microbial analysis

A food product cannot assist the host therapeutically unless it contains at least 103 CFU/mL live bacterial colonies when consumed. The microbial analysis of the prepared sample of strawberry and finger millet yogurt mix were found to be 72×10^3 CFU/ml of total bacterial count and the total yeast, mold and coliform count were found to be negligible. Similar studies (Mat Sarif *et al.*, 2022) underline the need of keeping bacterial counts within permissible ranges to ensure food safety and quality. This is in alignment with observations in which yogurt formulas, such as those in T1 to T4 which is depicted in Table 3 often have zero counts of yeast, mold and coliforms, indicating hygienic production standards critical for customer safety (Plessas *et al.*, 2024). The results have showed that the decrease in colony count was visible with the addition of strawberry slurry. The treatment T4 which is the control group have found to be shown with a slightly higher colony count than the treatments T2 and T3 which showed the impact of enrichment. This shows that the microbial counts have been on negligible number accounting to the shelf stability of the space food (Grasso *et al.*, 2020).

Sensory analysis

The results of the sensory analysis done by nine-point hedonic scale that have been displayed in Table 4, have showed that the treatment 3 was discovered to be the most preferred in terms of color, flavor, taste, appearance,

texture and overall acceptability (Mariam and Kumari, 2024). The samples were given after being reconstituted in hot water (7 g/50 mL). The colour of the treatments T3 and T4 were found to be acceptable than the treatments T1 and T2. While the flavor and appearance of the yogurt treatments, T1 and T3 scored high, the taste of the treatment T3 remained highly scored. Finally, the overall acceptability of the Treatment T3 scored higher than the rest of the treatments. A similar study by (Katke and Deshpande, 2022) showed better overall acceptability with psyllium husk incorporated frozen probiotic yogurt. The selected treatment was given to further analysis (Plessas *et al.*, 2024).

Statistical analysis

One of the most used techniques for comparing treatment means is the Duncan's multiple range test (DMRT) (Shankar *et al.*, 2022). One method for testing an experimental hypothesis is the Tukey test. It is activated when there is a mutually statistically significant interaction between three or more factors, which regrettably isn't just the sum or product of the significance levels of the individual variables (Zajác *et al.*, 2020). The chart compares the sensory evaluation of different yogurt mix formulations (T1, T2, T3, T4) based on color, flavor, texture, appearance, taste, and overall acceptance (OA). T3 consistently ranks higher in sensory attributes like appearance and overall taste, indicating the formulation was more preferred by judges in comparison to T1 and T4, which scored lower in key parameters. The highest ranking in overall acceptance was observed for T3. This suggests that T3, with its balanced sensory properties, is the most favorable among the formulations tested, making it the most acceptable formulation for astronaut consumption based on taste and sensory appeal which are displayed in Fig 4 (Tavakoli and Mokhtarian, 2021).

Table 3: The microbial composition of various treatments (T1, T2, T3 and T4 Control).

Composition	Treatment (T1)	Treatment (T2)	Treatment (T3)	Treatment (T4) (Control)
TBC	98×10^3 CFU/ml	86×10^3 CFU/ml	72×10^3 CFU/ml	88×10^3 CFU/ml
TYC	NIL	NIL	NIL	NIL
TMC	NIL	NIL	NIL	NIL
TCC	NIL	NIL	NIL	NIL

Table 4: Displaying sensory evaluation scores for treatments (T1, T2, T3 and T4 Control).

Sample ID	Color	Flavor	Texture	Appearance	Taste	OA
T1	8.8±0.41	8.75±0.47	8.65±0.49	8.8±0.41	8.65±0.49	8.4±0.52
T2	8.75±0.44	8.7±0.47	8.65±0.49	8.45±0.60	8.45±0.60	8.5±0.51
T3	8.8±0.47	8.75±0.51	8.76±0.50	8.85±0.61	8.95±0.51	8.7±0.50
T4 (Control)	8.8±0.41	8.5±0.51	8.6±0.50	8.45±0.60	8.35±0.67	8.65±0.51

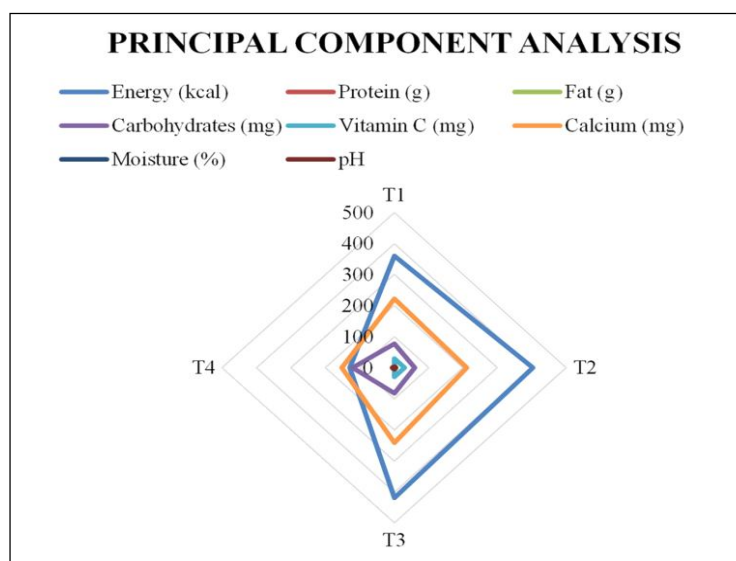


Fig 4: Principal component analysis.

CONCLUSION

The development of strawberry and finger millet incorporated yogurt mix using stevia as a sugar substitute has been successfully prepared and tested for various aspects. The results have shown that the incorporation of strawberry and finger millet has significantly increased the quality and nutritional values than that of regular yogurt. In conclusion, Treatment T3 of the strawberry and finger millet yogurt mix demonstrated superior sensory qualities, including color, flavor, texture, and overall acceptability. It exhibited favorable physicochemical properties, with lower moisture content and appropriate pH levels, ensuring a longer shelf-life. The proximate analysis revealed high protein, fat, calcium and energy content, making it a suitable option for astronauts. Additionally, T3 showed strong antioxidant activity and total phenolic content, contributing to its nutritional benefits. Microbial analysis confirmed the safety of the yogurt samples, with negligible yeast, mold and coliform counts, highlighting the hygienic production standards. The results obtained from the study have showed the qualities of the enriched yogurt mix with strawberry and finger millet, highlighting the potential to be presented as a space food for astronauts. Additionally, packaging of the space food must be evaluated to potentially keep the strawberry and finger millet yogurt mix shelf-stable and easily accessible for astronauts.

Conflict of interest

All authors declare that there is no conflict of interest.

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