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Research Article

Candy That Cares: Investigating HETAFU's Impact on Oral Healthy Microbiota

Dr. Ranjith Kumar Kanthem[1], Sowjanya Lakkoju[2]*, Venkat bellam[3], Mounika Dandamudi[3], Swarna Deepika Yarasani[4] and Dr. Deepika Bolisetty[5]

[1]*Consultant Oral and Maxillofacial Pathologist, Department of R and D, Lasarkaali Life Sciences PVT ltd, Hyderabad, India*

[2]*M Tech Food Processing Technology, Department of R and D, Lasarkaali Life Sciences PVT ltd, Hyderabad, India*

[3]*B Tech Food Science and Technology, Department of R and D, Lasarkaali Life Sciences PVT ltd, Hyderabad, India*

[4]*B Tech Food Technology, Department of R and D, Lasarkaali Life Sciences PVT ltd, Hyderabad, India*

[5]*MDS, Department of R and D, Lasarkaali Life Sciences PVT ltd, Hyderabad, India*

***Corresponding Author:** Sowjanya Lakkoju, M Tech Food Processing Technology, Department of R and D, Lasarkaali Life Sciences PVT ltd, Hyderabad, India.

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Abstract

Background: The oral beneficiary microbiome plays a crucial role in maintaining oral health, with beneficial microorganisms such as *Streptococcus salivarius, Lactobacillus paracasei, Bifidobacterium species, Lactobacillus rhamnosus, Lactobacillus salivarius*, and *Lactobacillus reuteri* contributing to a balanced microbial environment. This study investigates the efficacy of HETAFU candy containing LLSPL-probiotics, essential oils, and DHA in enhancing the levels of beneficial oral bacteria.

Methods: A randomized controlled trial was conducted with 200 healthy children aged 5 to 14 years. Participants were randomly assigned to consume either two or five HETAFU candies daily for eight weeks. Swab samples were collected before and after the intervention to assess the prevalence and counts of the target microorganisms using appropriate culture media. Statistical analysis was performed using non-parametric tests to evaluate changes in microbial load.

Results: The study revealed no significant change in the counts of beneficial microorganisms in participants consuming HETAFU candy, with a no notable reduction in the prevalence of oral Healthy microbiome.

Conclusions: The HETAFU candy formulation demonstrates potential as an innovative, patient-friendly approach to improving oral health by not altering the beneficial oral microorganisms. Further research is warranted to explore long-term effects and practical applications in routine oral care.

Keywords: HETAFU Candy; Oral Health; Probiotics; Beneficial Microorganisms; Randomized Controlled Trial; *Streptococcus Salivarius; Lactobacillus paracasei; Bifidobacterium Species; Lactobacillus rhamnosus; Lactobacillus salivarius; Lactobacillus Reuteri*

Introduction

The oral cavity is a complex ecosystem that harbours a diverse community of microorganisms, each playing a crucial role in maintaining or disrupting oral and systemic health. This oral microbiome, which includes both beneficial and potentially harmful bacteria, exists in a delicate balance. When maintained, this balance protects against dental and periodontal diseases. However, when disrupted, it can lead to conditions such as dental caries, gingivitis, periodontitis, and even systemic effects linked to cardiovascular disease and diabetes [1].

Among the beneficial bacteria, species such as *Streptococcus salivarius, Lactobacillus paracasei, Bifidobacterium species, and Lactobacillus rhamnosus* play a pivotal role in promoting oral health by suppressing pathogenic species and contributing to a stable, protective microbial environment [2].

Streptococcus salivarius is one of the first bacteria to colonize the oral cavity and is commonly found on the tongue and mucosal surfaces. It produces bacteriocins, which are natural antibiotics that inhibit the growth of pathogenic bacteria, thus acting as a natural defence against infections. Additionally*, S. salivarius* helps regulate pH in the oral environment, limiting the growth of acid-producing bacteria that can cause tooth decay [3].

Lactobacillus paracasei has been shown to inhibit the growth of Streptococcus mutans, a primary bacterium responsible for dental caries. By creating an environment that discourages harmful species, L. paracasei helps reduce the incidence of caries and supports a balanced microbiome, potentially reducing the risk of periodontal disease [4].

Bifidobacterium species, although traditionally associated with the gut, are also present in the oral cavity. These bacteria produce lactic acid and have anti-inflammatory properties, which can benefit periodontal health. They inhibit the growth of pathogenic organisms, helping to maintain a balanced microbial community in the oral cavity and protecting against gum disease and cavities [5].

Lactobacillus rhamnosus is known for its probiotic effects on oral and gut health. In the oral cavity, *L. rhamnosus* helps reduce dental plaque and has shown potential in preventing caries by producing lactic acid, which discourages the growth of pathogenic species. This bacterium is also known for its immunomodulatory effects, supporting gum health and contributing to the prevention of oral infections [6].

Given their important roles, monitoring the counts of these beneficial microbes in the oral cavity can provide insights into overall oral health. Shifts in the levels of these bacteria may serve as early indicators of microbial imbalance, potentially signalling the onset of oral diseases or shifts due to diet, lifestyle, or hygiene practices [7].

Advanced techniques are available to accurately measure and monitor these microbial populations. While traditional culture methods have been foundational, modern techniques like quantitative polymerase chain reaction (qPCR) and next-generation sequencing (NGS) enable precise quantification and offer a comprehensive view of the oral microbiome. qPCR can be used to accurately quantify specific beneficial species, while NGS provides a broader picture of microbial diversity and community structure [8].

Monitoring these bacteria over time allows researchers and clinicians to assess the effects of probiotic supplements, evaluate the impact of diet and hygiene on microbial balance, and tailor treatments aimed at restoring or maintaining a healthy microbiome. By understanding and supporting the levels of these beneficial organisms, healthcare providers can enhance preventive care and possibly prevent or mitigate conditions that arise from oral dysbiosis, benefiting both oral and systemic health [9].

This study aims to assess the microbial counts of beneficial bacteria *Streptococcus salivarius, Lactobacillus paracasei, Bifidobacterium species, and Lactobacillus rhamnosus* in the oral cavity, given their crucial role in maintaining oral and systemic health. These bacteria help regulate oral pH, produce antimicrobial compounds, and inhibit pathogenic species, thereby reducing the risk of dental caries, periodontal disease, and other oral conditions. Despite the known benefits of these microbes, there is limited data on their specific impact on oral health or how lifestyle factors affect their presence. Shifts in their levels may disrupt the oral microbiome's balance, leading to the dominance of harmful species. Using advanced microbial assessment techniques like quantitative PCR and next-generation sequencing, this study seeks to establish baseline data on these bacteria, correlate their levels with oral health outcomes, and identify microbial markers that may help guide preventive and therapeutic strategies for a healthier oral microbiome.

The rationale for this study lies in the urgent need for innovative, accessible, and user-friendly solutions to combat the rising prevalence of dental caries worldwide. Despite advancements in oral healthcare, many individuals struggle with maintaining traditional oral hygiene routines due to physical limitations, lack of access to care, or lifestyle challenges. High-risk groups, including children, elderly individuals, and those with special needs, are particularly vulnerable to dental decay, which is often exacerbated by limited access to regular dental care. This study aims to investigate a novel candy formulation combining probiotics, essential oils, and DHA to provide a practical alternative to traditional methods of oral care. Each component has shown individual benefits in promoting oral health by inhibiting pathogenic bacteria, reducing plaque accumulation, and supporting gum health. By combining these bioactive agents, we aim to explore their potential synergistic effects in a single, convenient delivery form that could improve oral health outcomes and accessibility.

By evaluating the effectiveness of this candy formulation, the study seeks to add to the growing body of evidence supporting the use of natural and bioactive compounds in daily oral care. Probiotics have shown promise in restoring microbial balance, essential oils possess antimicrobial properties against common oral pathogens, and DHA has been linked to anti-inflammatory benefits, which collectively could enhance oral health without the need for complex interventions. If proven effective, this approach could revolutionize preventive dental care by providing a non-invasive, enjoyable, and easy-to-integrate option for individuals at high risk of caries or those facing difficulties with traditional oral hygiene. The findings could pave the way for more accessible, holistic approaches to oral health, addressing the challenges of compliance and accessibility that conventional oral care often presents.

Methodology

This study was conducted as a randomized controlled trial to evaluate the efficacy of a HETAFU candy formulation containing LLSPL-probiotics (*Bacillus coagulans*), essential oils, and DHA on the prevalence and counts of beneficial oral microorganisms, specifically *Streptococcus salivarius*, *Lactobacillus paracasei*, *Bifidobacterium* species, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, and *Lactobacillus reuteri* in the oral cavity of participants. The study design aimed to assess the effectiveness of this innovative candy formulation in enhancing the levels of beneficial bacteria while simultaneously reducing pathogenic microorganisms associated with dental caries and oral diseases.

Participants

A total of 200 healthy participants aged 5 to 14 years were recruited from Amithabha Aadrana Vidyalaya. Inclusion criteria included children with no history of systemic diseases affecting oral health, no prior use of antibiotics or antifungal medications in the last month, and no ongoing oral diseases that would require treatment. Exclusion criteria included known allergies to any components of the candy, refusal to provide consent by parents, and children with compromised immune systems.

Randomization and group assignment

Participants were randomly assigned to one of two groups using a computer-generated random number sequence to ensure unbiased assignment:

- Group A: 100 participants who consumed two candies daily for 8 weeks.
- Group B: 100 participants who consumed five candies daily for 8 weeks.

Candy Formulation

The HETAFU candy formulation was designed to include a standardized quantity of LLSPL-Bacillus coagulants, selected essential oils (such as cinnamon oil, clove oil and peppermint oil), and DHA, ensuring that each candy contained a sufficient concentration of these active ingredients to target oral pathogens effectively.

Follow-Up Schedule

Participants were followed up at three designated intervals: 2 weeks, 4 weeks, and 8 weeks post-initiation of candy consumption. At each follow-up visit, the following procedures were conducted

Swab Collection and Analysis Procedure

Swabs were collected from participants before and after the candy consumption period to evaluate the counts of beneficial bacteria mentioned above. The swab collection procedure included

Requirements

Sterile Swabs: Pre-filled with an appropriate diluent (e.g., Peptone salt solution, Buffered peptone water, or saline).

Personal Protective Equipment: Apron, head cap, mask, and sterile gloves.

Chilled Icebox: For storing swabs at 2–8°C immediately after collection.

Swab collection procedure

Preparation: The research team donned protective gear to maintain aseptic conditions throughout the procedure.

Oral Swab Collection: A sterile swab was moistened with sterile diluent, and used to swab the entire oral cavity, including all teeth surfaces, gums, and the buccal mucosa, ensuring comprehensive sample collection.

Labeling and Storage: Each swab tube was clearly labeled with relevant participant details, including name, date of collection, and whether the swab was obtained before or after candy consumption. Labeled swabs were immediately placed in a chilled icebox to maintain a temperature of 2–8°C until analysis.

Microbial Analysis

- **Preparation of Serial Dilutions:** The collected swabs were vortexed, and a 1 mL aliquot was mixed with 9 mL of sterile diluent to prepare a 1:10 dilution, followed by serial dilutions up to 10^{-6}.
- **Inoculation:** Each dilution was inoculated onto sterile petri plates containing specific selective media for each microorganism: *Streptococcus salivarius*: Mitis Salivarius Agar at 37°C for 48 hours.

Lactobacillus paracasei, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Lactobacillus reuteri*: MRS Agar at 30°C for 72 hours.

Bifidobacterium species: *Bifidobacterium* Agar at 37°C for 48 hours under anaerobic conditions.

Counting and reporting

After incubation, colony-forming units (CFUs) for each microorganism were counted. The CFUs for *Streptococcus salivarius*, *Lactobacillus paracasei*, *Bifidobacterium* species, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, and *Lactobacillus reuteri* were recorded for both pre- and post-candy samples.

Data collection

In addition to microbial analysis, participants completed a structured questionnaire at each follow-up visit to assess adherence to candy consumption, any adverse effects experienced, and changes in oral hygiene practices.

Data analysis

The primary outcome measured was the change in counts of beneficial microorganisms from baseline to each follow-up time point. Data analysis was performed using non-parametric tests following normality assessments, with appropriate statistical methods i.e Kruskal-Wallis test, Mann-Whitney U test and chi square were applied to evaluate differences in microbial counts among groups at different time points. A p-value of <0.05 was considered statistically significant.

Ethical considerations

Ethical approval for the study was obtained from the Institutional Review Board (IRB). Informed consent was secured from the parents of all participants, ensuring that they understood the study's purpose, procedures, and any potential risks involved.

Results

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
Baseline Streptococcus salivarius 10^5 CFU/ml	.166	200	.000	.921	200	.000
Baseline Lactobacillus paracasei 10^5 CFU/ml	.075	200	.008	.954	200	.000
Baseline Bifidobacterium species 10^5 CFU/ml	.119	200	.000	.952	200	.000
Baseline Lactobacillus rhamnosus 10^5 CFU/ml	.181	200	.000	.844	200	.000
Baseline Lactobacillus salivarius	.382	200	.000	.627	200	.000
Baseline Lactobacillus reuteri	.392	200	.000	.622	200	.000
a. Lilliefors Significance Correction						

Table 1: Evaluation of Normality for Baseline Microbial Levels in Participants.

The table 1 summarizes the results of normality tests for various bacterial counts using the Kolmogorov-Smirnov (K-S) and Shapiro-Wilk (S-W) tests. Both tests assess whether the sample distributions follow a normal distribution, which is essential for many statistical analyses. Each test yielded a statistic indicating the degree of deviation from normality, with significance levels (p-values) provided for each bacterial type. In all cases, the p-values were less than 0.05, leading to the rejection of the null hypothesis of normality for all samples, including Baseline Streptococcus salivarius, *Lactobacillus paracasei*, *Bifidobacterium* species, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, and Lactobacillus reuteri. This consistent finding suggests that none of these bacterial counts are normally distributed, indicating that alternative analytical methods may be necessary. The mention of the Lilliefors Significance Correction implies that adjustments were made for the K-S test to account for sample size or non-normality, further supporting the conclusion that transformations or non-parametric approaches may be needed for subsequent analyses (Table 2).

Group		Gender		Total
		Male	Female	
2 Candies group	Count	45	55	100
	% within Group	45.0%	55.0%	100.0%
5 Candies group	Count	45	55	100
	% within Group	45.0%	55.0%	100.0%
Total	Count	90	110	200
	% within Group	45.0%	55.0%	100.0%

Table 2: Gender Distribution Across Candy Groups.

The table 2 summarizes the gender distribution among participants in two candy groups: the 2 Candies group and the 5 Candies group. In both groups, there are 45 males and 55 females, resulting in a total of 100 participants per group, with females making up 55.0% and males 45.0% within each group. Overall, across both groups, the total count of participants is 90 males (45.0%) and 110 females (55.0%), indicating a consistent gender distribution across the candy groups.

	Group	N	Mean	Std. Deviation	Std. Error Mean	P value
Age	2 Candies group	100	9.08	2.880	.288	0.9
	5 Candies group	100	9.13	2.762	.276	

Table 3: Age Distribution Between Candy Groups.

The table 3 presents the age distribution of participants in two candy groups: the 2 Candies group and the 5 Candies group. Both groups consist of 100 participants each, with the 2 Candies group having a mean age of 9.08 years (standard deviation = 2.880, standard error = 0.288) and the 5 Candies group showing a slightly higher mean age of 9.13 years (standard deviation = 2.762, standard error = 0.276). The P value of 0.9 indicates no statistically significant difference in mean age between the two groups, suggesting that the age distributions are comparable.

Streptococcus	Group	N	Mean Rank	Sum of Ranks	Z	P value
Baseline Streptococcus salivarius 10^5 CFU/ml	2 Candies group	100	100.17	10016.50	-0.082	0.24
	5 Candies group	100	100.84	10083.50		
2 Weeks Streptococcus salivarius 10^5 CFU/ml	2 Candies group	100	96.10	9610.00	-1.078	0.281
	5 Candies group	100	104.90	10490.00		
4 Weeks Streptococcus salivarius 10^5 CFU/ml	2 Candies group	100	98.89	9889.00	-0.394	0.693
	5 Candies group	100	102.11	10211.00		
8 Weeks Streptococcus salivarius 10^5 CFU/ml	2 Candies group	100	97.27	9727.00	-12.097	0.429
	5 Candies group	100	103.73	10373.00		

Table 4: Streptococcus Levels Across Candy Groups at Different Time Points.

The table 4 presents Streptococcus salivarius levels measured across groups consuming different quantities of candies (2 and 5 candies) at baseline, 2 weeks, 4 weeks, and 8 weeks, with each group comprising 100 participants. At baseline, the mean rank of Streptococcus levels was similar between the 2-candies group (100.17) and the 5-candies group (100.84), with no significant difference (Z = -0.082, P = 0.24). At the 2-week mark, the 2-candies group showed a slightly lower mean rank (96.10) compared to the 5-candies group (104.90), yet this difference was not statistically significant (Z = -1.078, P = 0.281). At 4 weeks, the mean ranks were again close (98.89 for the 2-candies group and 102.11 for the 5-candies group), with no significant difference (Z = -0.394, P = 0.693). At 8 weeks, Streptococcus levels also showed comparable mean ranks between groups (97.27 for the 2-candies group and 103.73 for the 5-candies group), with a non-significant result (Z = -12.097, P = 0.429). Overall, there were no statistically significant differences in Streptococcus levels across time points between the two candy groups.

Lactobacillus paracasei	Group	N	Mean Rank	Sum of Ranks	Z	P value
Baseline Lactobacillus paracasei 10^5 CFU/ml	2 Candies group	100	105.54	10554.00	-0.082	0.24
	5 Candies group	100	95.46	9546.00		
2 Weeks Lactobacillus paracasei 10^5 CFU/ml	2 Candies group	100	107.11	10711.00	-1.078	0.181
	5 Candies group	100	93.89	9389.00		
4 Weeks Lactobacillus paracasei 10^5 CFU/ml	2 Candies group	100	104.59	10459.00	-0.394	0.393
	5 Candies group	100	96.41	9641.00		
8 Weeks Lactobacillus paracasei 10^5 CFU/ml	2 Candies group	100	104.67	10467.00	-12.097	0.329
	5 Candies group	100	96.33	9633.00		

Table 5: Lactobacillus paracasei Levels Across Candy Groups at Different Time Points.

The table 5 summarizes *Lactobacillus paracasei* levels across two groups consuming either 2 or 5 candies, measured at baseline, 2 weeks, 4 weeks, and 8 weeks, with each group consisting of 100 participants. At baseline, the mean rank of Lactobacillus levels was higher in the 2-candies group (105.54) compared to the 5-candies group (95.46), but this difference was not statistically significant (Z = -0.082, P = 0.24). At the 2-week interval, the 2-candies group showed a slightly higher mean rank (107.11) compared to the 5-candies group (93.89), yet the difference remained non-significant (Z = -1.078, P = 0.181). Similarly, at 4 weeks, mean ranks were close (104.59 for the 2-candies group and 96.41 for the 5-candies group), with no statistically significant difference (Z = -0.394, P = 0.393). At 8 weeks, the mean ranks were nearly the same (104.67 for the 2-candies group and 96.33 for the 5-candies group), with a non-significant result (Z = -12.097, P = 0.329). Overall, there were no statistically significant differences in *Lactobacillus paracasei* levels between the two candy groups across all time points (Table 6).

The table 6 reports *Bifidobacterium* levels across two candy groups (2 and 5 candies) over baseline, 2 weeks, 4 weeks, and 8 weeks, with each group comprising 100 participants. At baseline, the 2-candies group had a mean rank of 98.70, slightly lower than the 5-candies group (102.30), with no statistically significant difference (Z = -0.482, P = 0.624). At the 2-week mark, mean ranks were again close (99.05 for the 2-candies group and 101.96 for the 5-candies group), and this difference was not significant (Z = -0.378, P = 0.81). At 4 weeks, the mean ranks were 99.36 and 101.64 for the 2 and 5-candies groups, respectively, also with no significant difference (Z = -0.274, P = 0.73). At 8 weeks, the mean ranks remained similar (99.92 for the 2-candies group and 101.08 for the 5-candies group), with a non-significant result (Z = -0.197, P = 0.89). Overall, *Bifidobacterium* levels did not significantly differ between the two candy groups at any time point (Table 7).

The table 7 shows levels of *Lactobacillus rhamnosus* across groups consuming either 2 or 5 candies, measured at baseline, 2 weeks, 4 weeks, and 8 weeks, with each group including 100 participants. At baseline, the mean rank of *Lactobacillus rhamnosus* levels was lower in the 2-candies group (92.70) compared to the 5-candies group (108.30), and this difference approached signifi-

Bifido bacterium	Group	N	Mean Rank	Sum of Ranks	Z	P value
Baseline Bifido bacterium species 10^5 CFU/ml	2 Candies group	100	98.70	9870.00	-0.482	0.624
	5 Candies group	100	102.30	10230.00		
2 Weeks Bifido bacterium species 10^5 CFU/ml	2 Candies group	100	99.05	9904.50	-0.378	0.81
	5 Candies group	100	101.96	10195.50		
4 Weeks Bifido bacterium species 10^5 CFU/ml	2 Candies group	100	99.36	9936.00	-0.274	0.73
	5 Candies group	100	101.64	10164.00		
8 Weeks Bifido bacterium species 10^5 CFU/ml	2 Candies group	100	99.92	9992.00	-0.197	0.89
	5 Candies group	100	101.08	10108.00		

Table 6: Bifido bacterium Levels Across Candy Groups at Different Time Points.

Lactobacillus rhamnosus	Group	N	Mean Rank	Sum of Ranks	Z	P value
Baseline Lactobacillus rhamnosus species 10^5 CFU/ml	2 Candies group	100	92.70	9270.00	-.4182	0.0574
	5 Candies group	100	108.30	10830.00		
2 Weeks Lactobacillus rhamnosus species 10^5 CFU/ml	2 Candies group	100	92.39	9238.50	-1.378	0.094
	5 Candies group	100	108.62	10861.50		
4 Weeks Lactobacillus rhamnosus species 10^5 CFU/ml	2 Candies group	100	93.63	9362.50	-1.274	0.49
	5 Candies group	100	107.38	10737.50		
8 Weeks Lactobacillus rhamnosus species 10^5 CFU/ml	2 Candies group	100	94.18	9417.50	-1.197	0.12
	5 Candies group	100	106.83	10682.50		

Table 7: Lactobacillus rhamnosus Levels Across Candy Groups at Different Time Points.

cance (Z = -0.4182, P = 0.0574). At the 2-week mark, the mean rank for the 2-candies group remained slightly lower (92.39) than the 5-candies group (108.62), though not statistically significant (Z = -1.378, P = 0.094). By the 4-week interval, mean ranks were similar (93.63 for the 2-candies group and 107.38 for the 5-candies group), with no significant difference (Z = -1.274, P = 0.49). At 8 weeks, the mean rank remained slightly lower in the 2-candies group (94.18) than in the 5-candies group (106.83), with the difference remaining non-significant (Z = -1.197, P = 0.12). Overall, there were no statistically significant differences in *Lactobacillus rhamnosus* levels between the two candy groups at any time point (Table 8).

The table 8 presents data on the presence and absence of *Lactobacillus salivarius* across two candy groups (2 candies and 5 candies) over baseline, 2 weeks, 4 weeks, and 8 weeks. At baseline, *Lactobacillus salivarius* was present in 57% of the 2-candies group and 59% of the 5-candies group, with no statistically significant difference (χ^2 = 0.082, P = 0.443). At the 2-week mark, both groups showed identical presence rates at 50%, with no significant difference (χ^2 = 0.000, P = 0.556). By 4 weeks, *Lactobacillus salivarius* was present in 53% of participants in both groups, again with no significant difference (χ^2 = 0.000, P = 0.556). At 8 weeks, presence rates were slightly higher, with 65% in the 2-candies group and 64% in the 5-candies group, but this difference was not significant (χ^2 = 0.02, P = 0.5). Overall, no significant differences in *Lactobacillus salivarius* presence were observed between the two candy groups at any time point (Table 9).

The table 9 shows the levels of *Lactobacillus reuteri* in two candy groups (2 candies and 5 candies) at baseline, 2 weeks, 4 weeks, and 8 weeks. At baseline, *Lactobacillus reuteri* was present in 60% of participants in both groups, with no significant difference (χ^2 = 0.00, P = 0.57). At 2 weeks, presence rates were 59% in the 2-candies group and 58% in the 5-candies group, which was also non-significant (χ^2 = 0.021, P = 0.58). At 4 weeks, both groups

			Group		Total	χ^2	p value
			2 Candies group	5 Candies group			
Baseline Lactobacillus salivarius	Present	Count	57	59	116	0.082[a]	0.443
		% within group	57.0%	59.0%	58.0%		
	Absent	Count	43	41	84		
		% within group	43.0%	41.0%	42.0%		
2 weeks Lactobacillus salivarius	Present	Count	50	50	100	0.000[a]	0.556
		% within group	50.0%	50.0%	50.0%		
	Absent	Count	50	50	100		
		% within group	50.0%	50.0%	50.0%		
4 weeks Lactobacillus salivarius	Present	Count	53	53	106	0.000[a]	0.556
		% within group	53.0%	53.0%	53.0%		
	Absent	Count	47	47	94		
		% within group	47.0%	47.0%	47.0%		
8 weeks Lactobacillus salivarius	Present	Count	65	64	129	0.02[a]	0.5
		% within group	65.0%	64.0%	64.5%		
	Absent	Count	35	36	71		
		% within group	35.0%	36.0%	35.5%		

Table 8: Lactobacillus salivarius Levels Across Candy Groups at Different Time Points.

			Group		Total	χ^2	p value
			2 Candies group	5 Candies group			
Baseline Lactobacillus reuteri	Present	Count	60	60	120	0.00	0.57
		% within group	60.0%	60.0%	60.0%		
	Absent	Count	40	40	80		
		% within group	40.0%	40.0%	40.0%		
2 weeks Lactobacillus reuteri	Present	Count	59	58	117	0.021[a]	0.58
		% within group	59.0%	58.0%	58.5%		
	Absent	Count	41	42	83		
		% within group	41.0%	42.0%	41.5%		
4 weeks Lactobacillus reuteri	Present	Count	65	65	130	0.000[a]	0.556
		% within group	65.0%	65.0%	65.0%		
	Absent	Count	35	35	70		
		% within group	35.0%	35.0%	35.0%		
8 weeks Lactobacillus reuteri	Present	Count	58	60	118	0.083[a]	0.44
		% within group	58.0%	60.0%	59.0%		
	Absent	Count	42	40	82		
		% within group	42.0%	40.0%	41.0%		

Table 9: Lactobacillus salivarius Levels Across Candy Groups at Different Time Points.

had equal presence rates at 65%, showing no statistical difference (χ^2 = 0.000, P = 0.556). At 8 weeks, the presence rate was 58% in the 2-candies group and 60% in the 5-candies group, again with no significant difference (χ^2 = 0.083, P = 0.44). Overall, there were no statistically significant differences in *Lactobacillus reuteri* levels between the two candy groups at any time point.

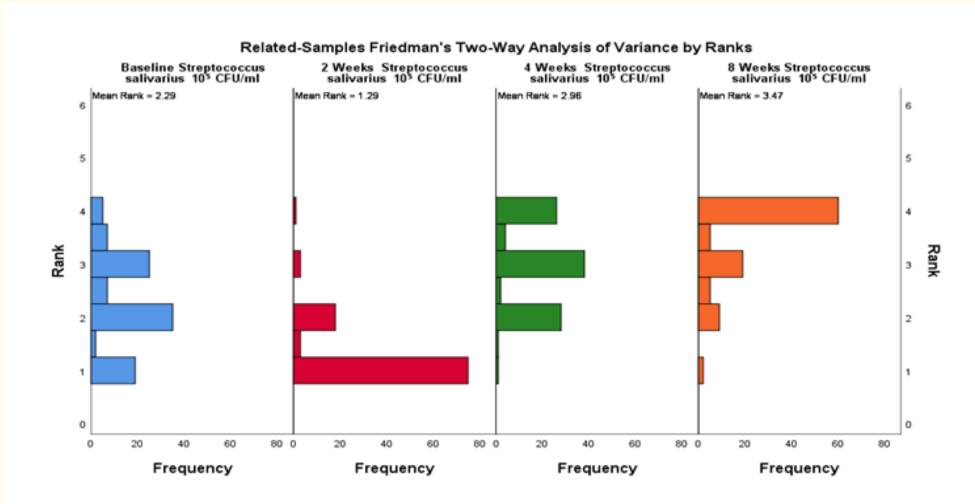
The table 10 summarizes pairwise comparisons of Streptococcus salivarius levels at different time points within the 2-candies group. When comparing the 2-week mark to baseline, there was a positive test statistic of 1.005 and a standard test statistic of 5.505, yielding a non-significant p-value of 0.25. Similarly, comparisons between 2 weeks and 4 weeks resulted in a test statistic of -1.670

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	p value
2 Weeks Streptococcus salivarius 10^5 CFU/ml-Baseline Streptococcus salivarius 10^5 CFU/ml	1.005	.183	5.505	0.25
2 Weeks Streptococcus salivarius 10^5 CFU/ml-4 Weeks Streptococcus salivarius 10^5 CFU/ml	-1.670	.183	-9.147	0.34
2 Weeks Streptococcus salivarius 10^5 CFU/ml-8 Weeks Streptococcus salivarius 10^5 CFU/ml	-2.185	.183	-11.968	0.21
Baseline Streptococcus salivarius 10^5 CFU/ml-4 Weeks Streptococcus salivarius 10^5 CFU/ml	-.665	.183	-3.642	0.94
Baseline Streptococcus salivarius 10^5 CFU/ml-8 Weeks Streptococcus salivarius 10^5 CFU/ml	-1.180	.183	-6.463	0.29
4 Weeks Streptococcus salivarius 10^5 CFU/ml-8 Weeks Streptococcus salivarius 10^5 CFU/ml	-.515	.183	-2.821	0.87

Table 10: Pair wise comparisons of 2 candy group of Streptococcus salivarius.

and a standard test statistic of -9.147 (p = 0.34), while the comparison between 2 weeks and 8 weeks showed a test statistic of -2.185 and a standard test statistic of -11.968 (p = 0.21), both indicating no significant differences. Between baseline and 4 weeks, the test statistic was -0.665 with a standard test statistic of -3.642, and the p-value was 0.94. Comparing baseline to 8 weeks, the test statistic was -1.180 and the standard test statistic -6.463 (p = 0.29). Finally, the comparison between 4 weeks and 8 weeks yielded a test statistic of -0.515 and a standard test statistic of -2.821, with a p-value of 0.87. Across all intervals, none of the pairwise comparisons showed statistically significant changes in Streptococcus salivarius levels, indicating stability in these levels within the 2-candies group over time.

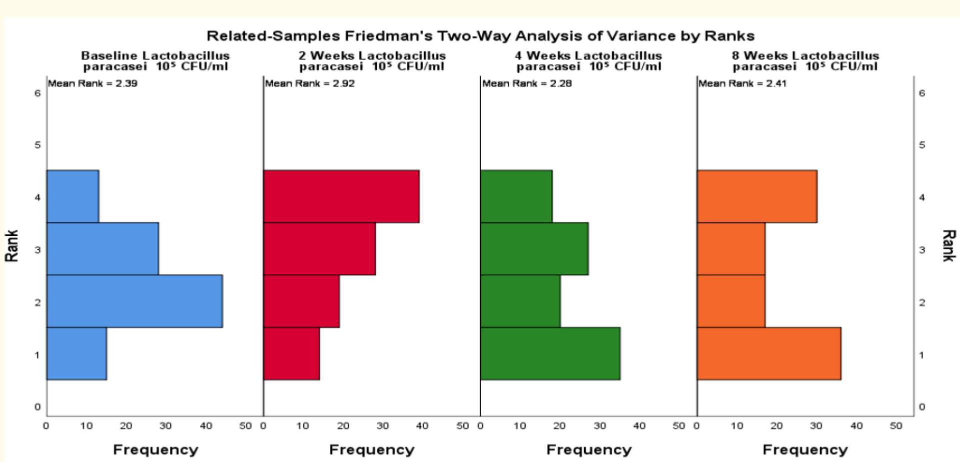
The table 11 presents pairwise comparisons of *Lactobacillus paracasei* levels at various time points within the 2-candies group. When comparing 4 weeks to baseline, the test statistic was 0.110 with a standard test statistic of 0.602, and a p-value of 1, indicating no significant difference. Similarly, comparing 4 weeks to 8 weeks, the test statistic was -0.130 with a standard test statistic of -0.712 (p = 1), again showing no significant change. The comparison between 4 weeks and 2 weeks yielded a test statistic of 0.640, with a standard test statistic of 3.505 and a p-value of 0.284, indicating no statistical significance. When comparing baseline to 8 weeks, the test statistic was -0.020 and the standard test statistic -0.110, with a p-value of 0.947. The comparison of baseline to 2 weeks showed a test statistic of -0.530 and a standard test statistic of -2.903 (p = 0.341), while comparing 8 weeks to 2 weeks resulted in a test statistic of 0.510 and a standard test statistic of 2.793, with a p-value of 1. Overall, these results indicate that there were no statistically significant changes in *Lactobacillus paracasei* levels across the various time points within the 2-candies group (Table 12).



Graph 1: Related-Samples Friedman's Two-Way Analysis of Variance by Ranks.

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	p value
4 Weeks Lactobacillus paracasei 10^5 CFU/ml-Baseline Lactobacillus paracasei 10^5 CFU/ml	.110	.183	.602	1
4 Weeks Lactobacillus paracasei 10^5 CFU/ml-8 Weeks Lactobacillus paracasei 10^5 CFU/ml	-.130	.183	-.712	1
4 Weeks Lactobacillus paracasei 10^5 CFU/ml-2 Weeks Lactobacillus paracasei 10^5 CFU/ml	.640	.183	3.505	0.284
Baseline Lactobacillus paracasei 10^5 CFU/ml-8 Weeks Lactobacillus paracasei 10^5 CFU/ml	-.020	.183	-.110	0.947
Baseline Lactobacillus paracasei 10^5 CFU/ml-2 Weeks Lactobacillus paracasei 10^5 CFU/ml	-.530	.183	-2.903	0.341
8 Weeks Lactobacillus paracasei 10^5 CFU/ml-2 Weeks Lactobacillus paracasei 10^5 CFU/ml	.510	.183	2.793	1

Table 11: Pair wise comparisons of 2 candy group of Lactobacillus paracasei.

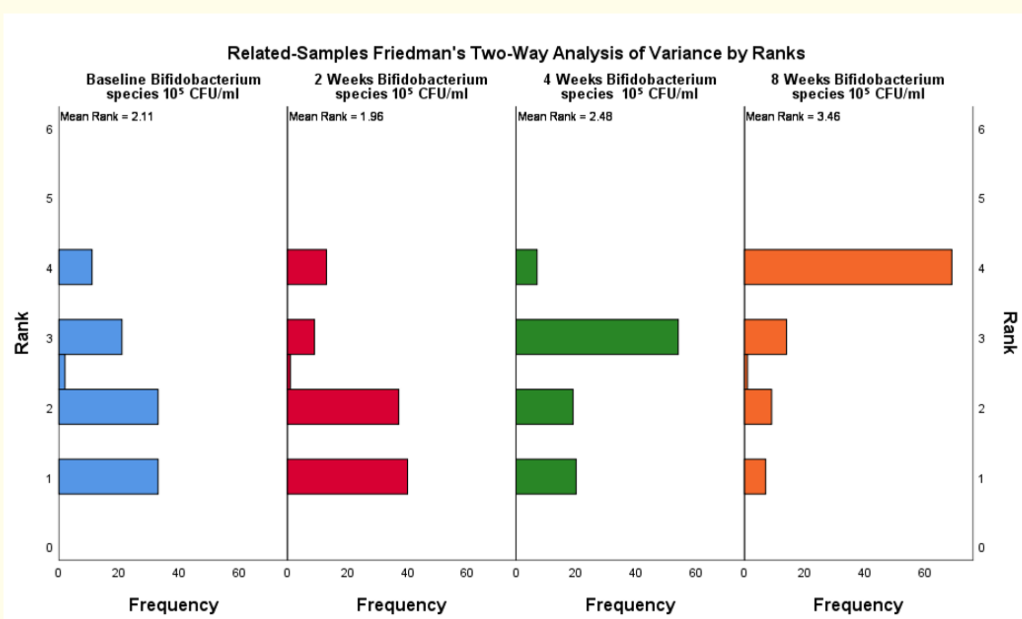


Graph 2: Related-Samples Friedman's Two-Way Analysis of Variance by Ranks.

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	p value
2 Weeks Bifidobacterium species 10^5 CFU/ml-Baseline Bifidobacterium species 10^5 CFU/ml	.155	.183	.849	0.1
2 Weeks Bifidobacterium species 10^5 CFU/ml-4 Weeks Bifidobacterium species 10^5 CFU/ml	-.525	.183	-2.876	0.281
2 Weeks Bifidobacterium species 10^5 CFU/ml-8 Weeks Bifidobacterium species 10^5 CFU/ml	-1.500	.183	-8.216	0.584
Baseline Bifidobacterium species 10^5 CFU/ml-4 Weeks Bifidobacterium species 10^5 CFU/ml	-.370	.183	-2.027	0.77
Baseline Bifidobacterium species 10^5 CFU/ml-8 Weeks Bifidobacterium species 10^5 CFU/ml	-1.345	.183	-7.367	0.481
4 Weeks Bifidobacterium species 10^5 CFU/ml-8 Weeks Bifidobacterium species 10^5 CFU/ml	-.975	.183	-5.340	0.82

Table 12: Pair wise comparisons of 2 candy group of Bifidobacterium species.

Table 12 presents the pairwise comparisons of *Bifidobacterium* species levels in the 2 candies group across various time points, measured in 10^5 CFU/ml. At 2 weeks compared to the baseline, the test statistic is 0.155 with a standard error of 0.183, yielding a standardized test statistic of 0.849 and a p-value of 0.1, indicating no statistically significant difference. Comparing 2 weeks to 4 weeks, the test statistic is -0.525 with a standardized test statistic of -2.876 and a p-value of 0.281, also showing no significant difference. Similarly, comparisons between other time points—2 weeks vs. 8 weeks, baseline vs. 4 weeks, baseline vs. 8 weeks, and 4 weeks vs. 8 weeks-all exhibit p-values greater than 0.1, indicating no statistically significant differences in *Bifidobacterium* levels between these intervals (Table 13).

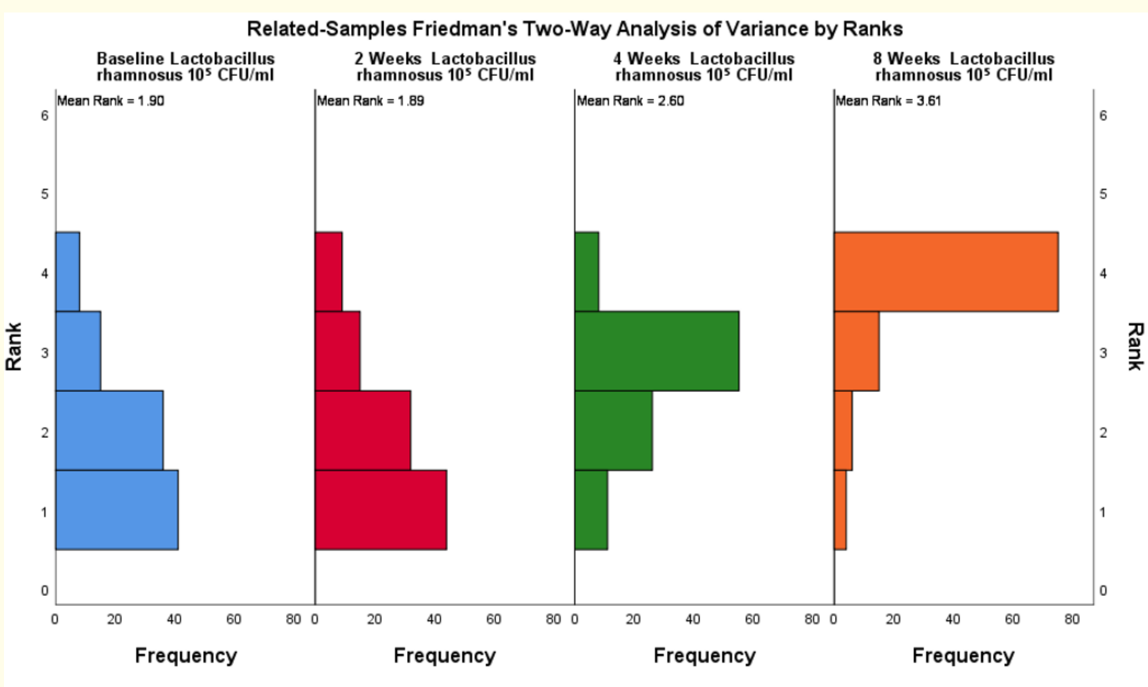


Graph 3: Related-Samples Friedman's Two-Way Analysis of Variance by Ranks.

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	p value
2 Weeks Lactobacillus rhamnosus 10^5 CFU/ml-Baseline Lactobacillus rhamnosus 10^5 CFU/ml	.010	.183	.055	1
2 Weeks Lactobacillus rhamnosus 10^5 CFU/ml-4 Weeks Lactobacillus rhamnosus 10^5 CFU/ml	-.710	.183	-3.889	0.871
2 Weeks Lactobacillus rhamnosus 10^5 CFU/ml-8 Weeks Lactobacillus rhamnosus 10^5 CFU/ml	-1.720	.183	-9.421	0.427
Baseline Lactobacillus rhamnosus 10^5 CFU/ml-4 Weeks Lactobacillus rhamnosus 10^5 CFU/ml	-.700	.183	-3.834	0.94
Baseline Lactobacillus rhamnosus 10^5 CFU/ml-8 Weeks Lactobacillus rhamnosus 10^5 CFU/ml	-1.710	.183	-9.366	0.172
4 Weeks Lactobacillus rhamnosus 10^5 CFU/ml-8 Weeks Lactobacillus rhamnosus 10^5 CFU/ml	-1.010	.183	-5.532	0.342

Table 13: Pair wise comparisons of 2 candy group of Lactobacillus rhamnosus.

Table 13 presents pairwise comparisons of *Lactobacillus rhamnosus* levels in the 2 candies group across different time points, measured in 10^5 CFU/ml. The comparison between 2 weeks and baseline shows a test statistic of 0.010, with a standard error of 0.183 and a standardized test statistic of 0.055, yielding a p-value of 1, indicating no significant difference. Comparing 2 weeks to 4 weeks, the test statistic is -0.710 with a standardized test statistic of -3.889 and a p-value of 0.871, suggesting no statistically significant difference. Likewise, other pairwise comparisons, such as 2 weeks vs. 8 weeks, baseline vs. 4 weeks, baseline vs. 8 weeks, and 4 weeks vs. 8 weeks, show p-values above 0.1, indicating no significant differences in *Lactobacillus rhamnosus* levels between these time intervals



Graph 4: Related-Samples Friedman's Two-Way Analysis of Variance by Ranks.

Discussion

Dental caries remains a prevalent and challenging public health issue, affecting diverse populations and carrying implications for overall health and quality of life. Traditional methods of caries prevention and management, such as fluoride treatments, oral hygiene practices, and diet modifications, though effective, often rely heavily on patient compliance and access to dental care. Given the limitations of conventional approaches, alternative strategies that are both accessible and user-friendly hold substantial promise in enhancing preventive care, particularly among high-risk or underserved populations.

In this context, our study investigates a novel approach involving the use of Hetafu, a candy formulation incorporating probiotics, essential oils, and DHA. This formulation is designed not only to deliver bioactive compounds directly to the oral cavity in a convenient format but also to leverage their synergistic effects to reduce the risk of dental caries. By focusing on natural ingredients with antimicrobial and anti-inflammatory properties, this study aims to provide insights into how such an innovative, non-invasive solution could complement or even substitute traditional oral hygiene practices, especially for those who face challenges in maintaining conventional oral care routines.

Our findings reveal several key insights into the efficacy and potential mechanisms by which Hetafu's unique formulation may contribute to improved oral health outcomes, offering a new avenue for caries prevention and management. The consistent gender distribution across the 2 Candies and 5 Candies groups, with 45.0% males and 55.0% females in each group, likely reflects a deliberate sampling strategy to balance gender representation. Methods such as gender stratification during randomization or quota sampling may have been used to ensure an equal gender ratio, thereby minimizing potential confounding effects due to gender differences. This balanced distribution allows for a more reliable comparison between groups, making the findings on the candy formulation's effects more generalizable across genders.

The similar age distributions in the 2 Candies and 5 Candies groups may stem from careful randomization or a study design aimed at achieving age balance. This approach helps control for age as a potential confounding variable, ensuring that any observed effects are not influenced by age differences between groups. A balanced age distribution strengthens the reliability of the findings, allowing for a clearer comparison of outcomes related to the candy formulation. The P-value of 0.9 further confirms that there is no significant age difference, supporting the validity of comparisons.

Irrespective of whether participants consumed 2 or 5 candies, the results indicate that the candy formulation did not have a significant impact on key beneficial oral bacteria, including *Streptococcus salivarius*, *Lactobacillus paracasei*, *Bifidobacterium species*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, and *Lactobacillus reuteri*, all of which were present at baseline levels of 10^5 CFU/ml. This finding suggests that the candy does not alter or negatively affect the levels of these important probiotics, which are known to contribute significantly to oral health.

Streptococcus salivarius is a key oral microbe that is considered beneficial due to its role in maintaining oral health by preventing the growth of harmful pathogens. It has been shown to produce antimicrobial substances that can help inhibit the growth of pathogenic bacteria, such as those associated with dental caries and periodontal disease. The fact that the candy did not affect the population of *Streptococcus salivarius* indicates that the formulation is gentle on this protective microbe, ensuring that it continues to play its role in the oral microbiome without disruption.

Similarly, *Lactobacillus* species, including *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, and *Lactobacillus reuteri*, are beneficial bacteria that are frequently found in the human oral cavity and gastrointestinal tract. These bacteria are involved in the fermentation of carbohydrates, producing lactic acid, which lowers the pH and creates an environment that is hostile to harmful bacteria, thus helping to prevent cavities and other oral diseases. The absence of any significant changes in the levels of these *Lactobacillus* species further supports the idea that the candy formulation does not interfere with the beneficial roles of these microbes in the oral cavity.

Bifidobacterium species are another group of beneficial bacteria that contribute to maintaining gut and oral health. These bacteria play a key role in the digestion of fiber and the production of short-chain fatty acids, which are essential for maintaining the integrity of the intestinal lining and preventing oral infections. The fact that the candy did not alter the levels of *Bifidobacterium* suggests that the formulation is not harmful to this group of microbes, and could, therefore, support a healthy oral and gastrointestinal microbiota.

The data presented in this study demonstrate that, irrespective of the number of candies consumed-whether 2 or 5-there were no statistically significant differences in the levels of *Streptococcus salivarius* across the groups at any of the measured time points (baseline, 2 weeks, 4 weeks, and 8 weeks). At baseline, the mean rank of *Streptococcus salivarius* was nearly identical between the two groups, with similar trends continuing at each subsequent time point. This suggests that the quantity of candy consumed does not significantly impact the levels of *Streptococcus salivarius*, which is a key beneficial bacterium in the oral cavity. *Streptococcus salivarius* plays a vital role in maintaining oral health by producing antimicrobial substances that help protect the mouth from harmful bacteria and pathogens, thereby supporting a balanced microbiome. The lack of observed differences in the bacterial levels between the groups implies that the candy consumption, regardless of its amount, does not disrupt the presence or function of this beneficial organism.

In addition to *Streptococcus salivarius*, the study also measured the baseline levels of several other beneficial bacteria, including *Lactobacillus paracasei*, *Bifidobacterium* species, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, and *Lactobacillus reuteri*, all of which were recorded at 10^5 CFU/ml at the start of the study. The results revealed that these important probiotics were unaffected by candy consumption, further supporting the conclusion that the candy formulation does not alter the levels of good oral bacteria. These beneficial microorganisms, particularly *Lactobacillus* species, are integral to maintaining a healthy oral microbiome. They contribute to the prevention of harmful microbial overgrowth, enhance oral immune responses, and aid in the prevention of dental caries and other oral diseases. Since the candy did not negatively affect these beneficial bacteria, it suggests that the product is safe for oral health and does not interfere with the protective functions of these microorganisms.

Overall, the findings indicate that the consumption of either 2 or 5 candies does not significantly alter the levels of *Streptococcus salivarius* or other beneficial bacteria in the oral cavity. This suggests that the candy does not disrupt the natural balance of the oral microbiome, particularly the good bacteria that help maintain oral health. As a result, this candy formulation appears to be a safe and potentially beneficial addition to oral health regimens. It does not interfere with the presence or activity of the beneficial bacteria in the mouth and may be considered a suitable option for maintaining oral hygiene without compromising the microbial balance critical for overall oral health.

Overall, the stability of these beneficial bacterial populations despite candy consumption is a positive outcome. It suggests that the candy may be formulated to target and reduce harmful oral microbes without disturbing the essential balance of beneficial bacteria. This is crucial, as maintaining a healthy balance of good bacteria in the oral microbiome is essential for preventing oral diseases, such as caries and periodontitis. The fact that these key probiotics remained unaffected by the candy is an indication that the candy is safe for long-term use in oral health without causing disruption to the natural microbiota.

Limitations

One of the key limitations of this study is the relatively short duration of observation. The effects of candy consumption on oral microbiota were measured at 2-week, 4-week, and 8-week intervals, but a longer duration might provide more insights into the long-term impact of candy on oral bacteria. Oral microbiota can change over extended periods, and a more prolonged study would help identify whether there are delayed or cumulative effects. Additionally, although the study had a reasonable number of participants in each group, a larger sample size would increase the statistical power and generalizability of the results. A control group consisting of participants who consumed no candy at all would have strengthened the study, as it would allow for a clearer comparison between the candy groups and help isolate the specific effects of candy consumption on oral bacteria. Another limitation is the specificity of microbial measurements.

Strengths

Despite these limitations, the study has several strengths. The inclusion of multiple time points—baseline, 2 weeks, 4 weeks, and 8 weeks—provided a dynamic view of microbial changes over time. This approach allowed for a more detailed understanding of the candy's effect on oral bacteria. Another strength is the focus on preserving beneficial bacteria, which are crucial for maintaining oral health. The study monitored *Streptococcus salivarius*, *Lactobacillus* species, and *Bifidobacterium* species, ensuring that the candy did not disrupt the oral microbial balance by affecting these good bacteria. This is an important aspect, as these beneficial microorganisms play a role in protecting against harmful microbes and maintaining oral health.

Furthermore, the study found consistent results across both Hetafu candy groups (2 and 5 candies), with no significant differences in microbial levels, suggesting that the quantity of candy consumed does not have a significant impact on the levels of beneficial bacteria. This outcome indicates that the candy formulation is likely safe for oral health, at least in terms of its effects on beneficial microorganisms. Lastly, the study's non-invasive nature makes it a practical and accessible way to promote oral health. The use of candy, a familiar and easy-to-consume form, suggests that such a method could be incorporated into daily oral hygiene practices without requiring major changes to individuals' habits and lastly hetafu sugar free candies are made completely sugar free,which makes an added advantage of preventing caries.

Future Recommendations

To build on the strengths of this study, future research should consider a longer duration to better understand the long-term effects of candy consumption on oral microbiota. Oral health is a gradual process, and the potential cumulative effects of the candy might not be apparent in a short-term study. Additionally, including a control group of participants who consume no candy at all would provide clearer insights into how the candy itself influences the microbiome, separate from other factors that may contribute to oral health.

Expanding the scope of microbial analysis would also be beneficial. Future studies should look at a broader range of oral bacteria, including pathogenic species, to evaluate the candy's effect on both beneficial and harmful microorganisms. This would help to ensure that the candy does not inadvertently promote the growth of harmful bacteria while supporting beneficial ones.

Finally, including a more diverse population in terms of age, oral hygiene practices, and diet would make the findings more widely applicable. Factors such as age and lifestyle habits can have a significant impact on oral microbiota, and understanding how these factors interact with candy consumption would help refine recommendations for specific populations.

Conclusion

In conclusion, this study provides valuable insights into the impact of Hetafu candy consumption on oral microbiota, specifically focusing on beneficial bacteria such as *Streptococcus salivarius*, *Lactobacillus* species, and *Bifidobacterium* species. The findings indicate that consuming 2 or 5 candies does not significantly alter the levels of these beneficial microbes, suggesting that the Hetafu candy formulation does not negatively affect the good bacteria essential for oral health. Despite variations in Hetafu candy quantity, no significant differences were observed in the microbial levels over the study period, supporting the notion that the candies are safe for oral health.

While the study highlights the non-disruptive effect of Hetafu candy on the oral microbiome, future research should explore longer study durations, include control groups, and broaden the range of microbial measurements to further confirm the Hetafu candy's safety and efficacy in maintaining oral health. Additionally, examining different Hetafu candy formulations and their impact on both beneficial and harmful bacteria could provide more comprehensive recommendations for incorporating such products into daily oral hygiene practices. Overall, the study contributes to understanding how functional candies could serve as a practical and beneficial addition to oral health management.

The Hetafu candy appears to be a promising product for promoting oral health, as it maintains the balance of beneficial oral bacteria such as *Streptococcus salivarius*, *Lactobacillus* species, and *Bifidobacterium species* while potentially targeting harmful pathogens. This highlights the Hetafu candy's potential role in supporting overall oral hygiene, preventing oral diseases, and promoting a healthy microbiome, making it a suitable addition to daily oral care routines without the risk of negatively affecting beneficial microorganisms.

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