

HETAFU Cut G: The World's First Soft Drink Redefining Oral Hygiene by Revolutionizing the Oral Microbiome

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Abstract

Background: Maintaining a balanced oral microbiome is crucial for preventing dental caries, periodontal diseases, and oral infections. HETAFU Cut G drink, formulated with probiotics, prebiotics and sugar alcohols has been developed as a natural and bioactive oral health supplement. This study evaluates its effects on key oral microorganisms, including *Streptococcus mutans*, *Lactobacillus* species, *Actinomyces* species, and *Candida* species, over an 8-week period.

Methods: A total of 250 participants were randomly assigned to consume HETAFU Cut G drink daily, with microbial levels assessed at baseline, 2 weeks, 4 weeks, and 8 weeks using microbiological techniques. The statistical significance of microbial reduction was analyzed using Z-scores and p-values.

Results: The study found a statistically significant reduction in the levels of pathogenic oral microbes, particularly *Streptococcus mutans* and *Candida* species, by the 8th week ($p < 0.05$). These findings indicate that HETAFU Cut G drink helps modulate the oral microbiome, promoting a healthier microbial balance.

Conclusion: The results suggest that HETAFU Cut G drink could serve as a novel adjunct to traditional oral hygiene practices, offering a non-invasive, convenient approach to improving oral health.

Keywords: *HETAFU Cut G drink, probiotics, prebiotics, sugar alcohol, oral microbiome.*

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Introduction

Oral health is a critical component of overall well-being, influencing not only dental integrity but also systemic health. The human oral cavity harbours a diverse and dynamic microbiome consisting of both commensal and pathogenic microorganisms.¹ A well-balanced oral microbiome is essential for maintaining oral homeostasis, whereas dysbiosis — an

imbalance in microbial populations — can lead to a range of dental diseases, including dental caries, periodontal disease, and halitosis.² Among the primary contributors to oral dysbiosis are *Streptococcus mutans*, *Lactobacillus* species, *Actinomyces* species, and *Candida albicans*, which play key roles in the initiation and progression of oral infections.³

Traditional approaches to oral hygiene, such as mechanical plaque removal through brushing and flossing, are effective but may not fully address microbial imbalances. In recent years, dietary interventions have gained attention as an adjunctive strategy for modulating the oral microbiome.⁴ Among these, functional ingredients such as sugar alcohols, prebiotics, and probiotics have demonstrated significant potential in promoting oral health.⁵

Sugar alcohols, including xylitol (INS 967), sorbitol (INS 420), and erythritol (INS 968), have been extensively studied for their ability to inhibit cariogenic bacteria. Xylitol and erythritol, in particular, interfere with bacterial metabolism, reducing acid production and inhibiting the adhesion of *Streptococcus mutans* to tooth surfaces. Additionally, these compounds help maintain salivary flow, which is crucial for neutralizing acids and remineralizing enamel.⁶

Prebiotics, such as fructooligosaccharides (FOS), serve as substrates for beneficial bacteria, fostering a microbial environment that supports oral and systemic health. By selectively stimulating the growth of beneficial bacteria while suppressing pathogenic species, prebiotics contribute to a more stable and resilient oral microbiome. Emerging evidence suggests that prebiotics may help mitigate halitosis, reduce inflammation, and enhance the natural defenses of the oral cavity.⁷

Probiotics, including *Bacillus coagulans*, *Lactobacillus reuteri*, and *Streptococcus salivarius*, have been shown to actively combat harmful microorganisms through competitive inhibition, antimicrobial production, and immune modulation.⁸ Probiotic supplementation has been linked to reduced levels of *Streptococcus mutans* and *Candida albicans*, improvements in periodontal health, and a decrease in volatile sulfur compounds responsible for bad breath.⁹

In response to the growing demand for holistic oral care solutions, HETAFU Cut G has been developed as an innovative functional soft drink designed to promote oral and gut health. By incorporating a scientifically formulated blend of sugar alcohols, prebiotics, and probiotics, HETAFU Cut G offers a novel, non-invasive approach to oral health maintenance. Unlike traditional oral care products that focus solely on mechanical plaque removal, this beverage aims to naturally modulate the oral microbiome, reducing harmful bacteria while supporting beneficial species.

This study aims to evaluate the impact of HETAFU Cut G on the oral microbiome by assessing changes in the populations of *Streptococcus mutans*, *Lactobacillus*, *Actinomyces* species, and *Candida albicans* over an eight-week period. By providing scientific evidence on the efficacy of functional dietary interventions in oral health, this research has the potential to contribute to a more integrative and preventive approach to dental care.

Methodology

The study was designed to evaluate the effects of HETAFU Cut G drink, a groundbreaking soft drink that is the world's first beverage formulated to cleanse the teeth. This innovative drink combines a unique blend of probiotics, prebiotics, sugar alcohols, xylitol, erythritol, sorbitol, fructose oligosaccharide (FOS), and *Bacillus coagulans*, specifically engineered to promote oral health. The formulation aims to modulate the oral microbiome by encouraging the growth of beneficial bacteria while inhibiting harmful pathogens, ultimately supporting the maintenance of oral hygiene and preventing dental issues such as cavities and plaque buildup. Through this study, we sought to determine the impact of this novel drink on the microbial composition of the oral cavity, particularly its potential to reduce

the presence of cariogenic bacteria and support a healthy, balanced oral microbiome.

Study Design: This study is designed as a single-group interventional trial to evaluate the effects of HETAFU Cut G on the oral microbiome over eight weeks. A control group is not included, as the primary objective is to measure the within-subject changes in bacterial populations before and after intervention. Given that each participant serves as their own control, this approach minimizes inter-individual variability and enhances statistical power by focusing on longitudinal microbiome changes.

Participants: A total of 250 healthy adults aged 18– 60 years will be recruited for the study. Participants will be screened based on specific eligibility criteria to ensure uniformity in baseline oral health status.

Inclusion Criteria: Individuals with no diagnosed oral health conditions, No recent use of antibiotics, probiotics, or antifungal treatments (within the past three months), Non-smokers, Willingness to maintain their regular oral hygiene routine without introducing additional probiotic or antibacterial products

Exclusion Criteria: Individuals with allergies to study ingredients, Participants undergoing active dental treatments Pregnant or lactating women, Individuals with systemic conditions affecting oral health (e.g., diabetes, immunodeficiency disorders)

Intervention: Each participant will consume one serving of HETAFU Cut G daily for eight weeks. Participants will be instructed to consume the beverage at a consistent time each day and refrain from eating or drinking for 30 minutes afterward to maximize its oral effects.

Outcome Measures: Oral Microbiome Assessment:

- Quantification of Streptococcus mutans, Lactobacillus, Actinomyces species, and Candida albicans in saliva samples collected at baseline, four weeks, and eight weeks.
- Microbiological analysis using culture-based methods and quantitative PCR (qPCR) to assess bacterial load and shifts in microbial composition.

Statistical Analysis: Descriptive statistics summarized baseline characteristics. One way ANOVA was employed to assess longitudinal changes in bacterial counts, and Multiple Comparisons Using Dunnett T3 Test for Oral Microbial Counts Across Different Time Points. A p-value of <0.05 will be considered statistically significant.

This study aims to provide scientific evidence on the role of HETAFU Cut G in modulating the oral microbiome, promoting beneficial bacteria, and reducing pathogenic species. By offering a novel dietary strategy for oral health maintenance, this intervention has the potential to complement existing preventive dental care measures and contribute to a more holistic approach to oral hygiene.

Results

The results section presents an analysis of the data from 250 participants, focusing on the effects of HETAFU Cut G on oral microbiome populations, including Streptococcus mutans, Lactobacillus species, Candida albicans, and Actinomyces species. Changes in bacterial composition were assessed at baseline, four weeks, and eight weeks. Statistical tests were conducted to evaluate the significance of these changes. The findings aim to demonstrate the potential of HETAFU Cut G in promoting oral health by modulating the oral microbiome.

The study included a total of 250 participants, with a nearly equal distribution of gender; 126 (50.4%) were male, and 124 (49.6%) were female. The participants' ages ranged from 19 to 58 years, with a mean age of 38.62 years and a standard deviation of 11.357, indicating moderate variability in age distribution.

Table 1: Distribution of participants based on gender

Gender	Frequency	Percentage
Male	126	50.4
Female	124	49.6
Total	250	100.0

Table 2: Descriptive Statistics of Participants' Age

	N	Min.	Max.	Mean	Std. Deviation
Age	250	19	58	38.62	11.357

Table 3: Changes in Oral Microbial Counts after Consuming the HETAFU CUT Drink over Time

		N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Streptococcus mutans	Baseline	250	6.122	.5830	6.050	6.195	5.1	7.5
	2 weeks	250	5.273	.6326	5.194	5.352	4.2	6.8
	4 weeks	250	4.432	.6413	4.352	4.511	3.3	6.0
	8 weeks	250	3.048	.7314	2.957	3.140	2.0	5.1
	Total	1000	4.719	1.3073	4.638	4.800	2.0	7.5
Lactobacillus species	Baseline	250	16.142	.9150	16.028	16.256	14.1	17.5
	2 weeks	250	15.094	.9066	14.981	15.207	13.0	16.5
	4 weeks	250	12.402	1.7848	12.180	12.625	10.0	15.5
	8 weeks	250	9.529	1.3569	9.360	9.698	7.5	11.6
	Total	1000	13.292	2.8728	13.114	13.470	7.5	17.5
Actinomycetes species	Baseline	250	8.952	.3613	8.907	8.997	8.1	9.6
	2 weeks	250	6.578	.5095	6.515	6.641	5.7	7.8
	4 weeks	250	5.134	.3860	5.086	5.182	4.2	6.0
	8 weeks	250	2.640	.5394	2.572	2.707	1.7	3.7
	Total	1000	5.826	2.3356	5.681	5.971	1.7	9.6
Candida species	Baseline	250	10.964	.4534	10.907	11.020	10.1	12.1
	2 weeks	250	9.724	.5307	9.657	9.790	8.6	10.9
	4 weeks	250	7.900	.5322	7.834	7.967	6.9	9.1
	8 weeks	250	4.948	.7169	4.858	5.037	4.1	6.3
	Total	1000	8.384	2.3341	8.239	8.529	4.1	12.1

This table provides a comprehensive analysis of the changes in oral microbial counts for four key microorganisms — *Streptococcus mutans*, *Lactobacillus species*, *Actinomycetes species*, and *Candida species* — over an 8-week period following the consumption of a specific drink. Data was collected from 250 participants at each time point (baseline, 2 weeks, 4 weeks, and 8 weeks), with a total of 1000 observations. The parameters measured include the mean microbial count, standard deviation (indicating variability), 95% confidence intervals (reflecting statistical reliability), and the observed minimum and maximum values at each time point.

Streptococcus mutans: The mean *Streptococcus mutans* count demonstrated a consistent decline over time. At baseline, the mean was 6.122 with a standard deviation of 0.5830. After 2 weeks, the mean decreased to 5.273, followed by 4.432 at 4 weeks and 3.048 at 8 weeks, indicating a substantial reduction. The 95% confidence interval narrowed progressively, showing increased consistency in microbial reduction. Minimum values dropped from 5.1 to 2.0, reflecting a broader range over time.

Lactobacillus species: Similar to *Streptococcus mutans*, *Lactobacillus species* counts decreased over time. The baseline mean was 16.142 with a standard

deviation of 0.9150. By 2 weeks, the mean declined to 15.094 and continued to fall to 12.402 at 4 weeks and 9.529 at 8 weeks. The reduction suggests a gradual but significant impact of the drink on this bacterial population. Minimum values shifted from 14.1 at baseline to 7.5 at 8 weeks, indicating a wider range of response among participants.

Actinomyces species: The reduction in *Actinomyces species* counts was notable, with baseline values starting at a mean of 8.952 and a low standard deviation of 0.3613, indicating minimal variability. By 2 weeks, the mean dropped to 6.578, then to 5.134 at 4 weeks, and further down to 2.640 at 8 weeks. The 95% confidence interval consistently narrowed, demonstrating a reliable decline in counts. Minimum values decreased significantly from 8.1 to 1.7, reflecting the drink's pronounced effect.

Candida species: The mean *Candida species* counts showed a gradual but marked reduction over the 8-week period. At baseline, the mean was 10.964 with a

standard deviation of 0.4534. At 2 weeks, the mean decreased to 9.724, continuing to fall to 7.900 at 4 weeks and 4.948 at 8 weeks. Minimum values dropped from 10.1 to 4.1, showing a substantial reduction, while the 95% confidence intervals indicated consistent reliability in the decreasing trend.

Overall Trends: Across all four microorganisms, the total mean values across the 8-week period indicate a downward trend, suggesting that the drink had a significant impact on reducing oral microbial populations. *Streptococcus mutans* and *Actinomyces species* showed the most pronounced reductions, while *Candida species* and *Lactobacillus species* also demonstrated substantial declines. The consistent narrowing of the 95% confidence intervals over time suggests increasing reliability and uniformity in the reduction of microbial counts. These findings support the potential efficacy of the drink in improving oral health by reducing harmful bacterial and fungal populations.

Table 4: Analysis of Variance (ANOVA) for Oral Microbial Counts across Different Time Points

		Sum of Squares	df	Mean Square	F	Sig.
Streptococcus	Between Groups	1287.432	3	429.144	1018.038	≤0.001*
	Within Groups	419.854	996	.422		
	Total	1707.287	999			
Lactobacillus	Between Groups	6579.939	3	2193.313	1312.205	≤0.001*
	Within Groups	1664.785	996	1.671		
	Total	8244.724	999			
Actinomyces	Between Groups	5243.111	3	1747.704	8421.923	≤0.001*
	Within Groups	206.688	996	.208		
	Total	5449.799	999			
Candida	Between Groups	5122.895	3	1707.632	5317.944	≤0.001*
	Within Groups	319.823	996	.321		
	Total	5442.718	999			

This table presents the results of a one-way Analysis of Variance (ANOVA) performed to evaluate the statistical significance of changes in the counts of *Streptococcus mutans*, *Lactobacillus species*, *Actinomyces species*, and *Candida species* across four time points (baseline, 2 weeks, 4 weeks, and 8 weeks) after

consuming the specified drink. The analysis includes key metrics such as the sum of squares, degrees of freedom (df), mean square values, F-statistics, and significance (Sig.) values. These metrics help determine whether there are statistically significant differences between the microbial counts over time.

Streptococcus mutans: For *Streptococcus mutans*, the sum of squares between groups was 1287.432 with 3 degrees of freedom, resulting in a mean square value of 429.144. The within-group sum of squares was 419.854 with 996 degrees of freedom, indicating a mean square of 0.422. The F-statistic of 1018.038 is exceptionally high, with a significance value (Sig.) of $\leq 0.001^*$, confirming that the reduction in *Streptococcus mutans* counts over time is statistically significant.

Lactobacillus species: The ANOVA results for *Lactobacillus* species show a between-group sum of squares of 6579.939 with a mean square of 2193.313. The within-group sum of squares was 1664.785, with a mean square of 1.671. The high F-statistic of 1312.205 and a significance value of $\leq 0.001^*$ suggest that the observed differences in *Lactobacillus* species counts over time are highly significant. This confirms that the drink had a substantial impact on reducing *Lactobacillus* species counts.

Actinomyces species: The results for *Actinomyces* species revealed a between-group sum of squares of 5243.111, with a mean square of 1747.704. The within-group sum of squares was relatively low at 206.688, with a mean square of 0.208. The F-statistic was extraordinarily high at 8421.923, with a significance value of $\leq 0.001^*$, indicating a significant decline in *Actinomyces* species counts over time. The large F-value suggests that the between-group variance is considerably greater than the within-group variance.

Candida species: For *Candida* species, the ANOVA indicated a between-group sum of squares of 5122.895 with a mean square of 1707.632. The within-group sum of squares was 319.823 with a mean square of 0.321. The F-statistic was 5317.944, with a significance value of $\leq 0.001^*$, confirming that the reduction in *Candida* species counts

over the 8-week period is statistically significant. This result highlights the pronounced impact of the drink on fungal populations in the oral cavity.

Overall Interpretation: The ANOVA results for all four microorganisms (*Streptococcus mutans*, *Lactobacillus* species, *Actinomyces* species, and *Candida* species) demonstrate statistically significant differences in microbial counts across the four time points, with all p-values (Sig.) being $\leq 0.001^*$. The high F-statistics in each case indicate that the observed reductions in microbial populations are unlikely to have occurred by chance. These findings provide strong evidence of the efficacy of the drink in significantly reducing harmful oral microbes over time.

Table 5 presents the results of the Dunnett T3 post-hoc multiple comparison test for the microbial counts of *Streptococcus mutans*, *Lactobacillus* species, *Actinomyces* species, and *Candida* species at four different time points: baseline, 2 weeks, 4 weeks, and 8 weeks after consuming the specified drink. The Dunnett T3 test is suitable for unequal variances and provides a detailed comparison between each pair of time points. The parameters displayed include the mean difference between groups, standard error, significance (Sig.) value, and the 95% confidence interval (lower and upper bounds) for each comparison.

Streptococcus mutans: The comparisons indicate a consistent and significant reduction in *Streptococcus mutans* counts over time. From baseline to 8 weeks, the mean difference was 3.0740 ($p < .001$), reflecting a substantial decrease. Each subsequent time point showed significant differences when compared to the previous one, confirming the gradual decline in *Streptococcus mutans* counts.

Table 5: Multiple Comparisons Using Dunnett T3 Test for Oral Microbial Counts across Different Time Points

Multiple Comparisons							
Dunnett T3							
Dependent Variable	(I) Timeline	(J) Timeline	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Streptococcus	Baseline	2 weeks	.8496*	.0544	≤0.001*	.706	.993
		4 weeks	1.6908*	.0548	≤0.001*	1.546	1.836
		8 weeks	3.0740*	.0592	≤0.001*	2.918	3.230
	2 weeks	Baseline	-.8496*	.0544	≤0.001*	-.993	-.706
		4 weeks	.8412*	.0570	≤0.001*	.691	.992
		8 weeks	2.2244*	.0612	≤0.001*	2.063	2.386
	4 weeks	Baseline	-1.6908*	.0548	≤0.001*	-1.836	-1.546
		2 weeks	-.8412*	.0570	≤0.001*	-.992	-.691
		8 weeks	1.3832*	.0615	≤0.001*	1.221	1.546
	8 weeks	Baseline	-3.0740*	.0592	≤0.001*	-3.230	-2.918
		2 weeks	-2.2244*	.0612	≤0.001*	-2.386	-2.063
		4 weeks	-1.3832*	.0615	≤0.001*	-1.546	-1.221
Lactobacillus	Baseline	2 weeks	1.0480*	.0815	≤0.001*	.833	1.263
		4 weeks	3.7396*	.1269	≤0.001*	3.404	4.075
		8 weeks	6.6128*	.1035	≤0.001*	6.339	6.886
	2 weeks	Baseline	-1.0480*	.0815	≤0.001*	-1.263	-.833
		4 weeks	2.6916*	.1266	≤0.001*	2.357	3.026
		8 weeks	5.5648*	.1032	≤0.001*	5.292	5.838
	4 weeks	Baseline	-3.7396*	.1269	≤0.001*	-4.075	-3.404
		2 weeks	-2.6916*	.1266	≤0.001*	-3.026	-2.357
		8 weeks	2.8732*	.1418	≤0.001*	2.499	3.248
	8 weeks	Baseline	-6.6128*	.1035	≤0.001*	-6.886	-6.339
		2 weeks	-5.5648*	.1032	≤0.001*	-5.838	-5.292
		4 weeks	-2.8732*	.1418	≤0.001*	-3.248	-2.499
Actinomycetes	Baseline	2 weeks	2.3744*	.0395	≤0.001*	2.270	2.479
		4 weeks	3.8188*	.0334	≤0.001*	3.730	3.907
		8 weeks	6.3128*	.0411	≤0.001*	6.204	6.421
	2 weeks	Baseline	-2.3744*	.0395	≤0.001*	-2.479	-2.270
		4 weeks	1.4444*	.0404	≤0.001*	1.338	1.551
		8 weeks	3.9384*	.0469	≤0.001*	3.814	4.062
	4 weeks	Baseline	-3.8188*	.0334	≤0.001*	-3.907	-3.730
		2 weeks	-1.4444*	.0404	≤0.001*	-1.551	-1.338
		8 weeks	2.4940*	.0420	≤0.001*	2.383	2.605
	8 weeks	Baseline	-6.3128*	.0411	≤0.001*	-6.421	-6.204
		2 weeks	-3.9384*	.0469	≤0.001*	-4.062	-3.814
		4 weeks	-2.4940*	.0420	≤0.001*	-2.605	-2.383
Candida	Baseline	2 weeks	1.2400*	.0441	≤0.001*	1.123	1.357
		4 weeks	3.0632*	.0442	≤0.001*	2.946	3.180
		8 weeks	6.0160*	.0536	≤0.001*	5.874	6.158
	2 weeks	Baseline	-1.2400*	.0441	≤0.001*	-1.357	-1.123
		4 weeks	1.8232*	.0475	≤0.001*	1.698	1.949
		8 weeks	4.7760*	.0564	≤0.001*	4.627	4.925
	4 weeks	Baseline	-3.0632*	.0442	≤0.001*	-3.180	-2.946
		2 weeks	-1.8232*	.0475	≤0.001*	-1.949	-1.698
		8 weeks	2.9528*	.0565	≤0.001*	2.804	3.102
	8 weeks	Baseline	-6.0160*	.0536	≤0.001*	-6.158	-5.874
		2 weeks	-4.7760*	.0564	≤0.001*	-4.925	-4.627
		4 weeks	-2.9528*	.0565	≤0.001*	-3.102	-2.804

*. The mean difference is significant at the 0.05 level

Lactobacillus species: For Lactobacillus species, there was a notable decrease over the 8-week period. The mean difference from baseline to 8 weeks was 6.6128 ($p < .001$). Similar significant reductions were observed at each interval, with a particularly sharp decline occurring between 4 and 8 weeks. This suggests that the drink had a progressively increasing impact over time.

Actinomycetes species: The Actinomycetes species counts exhibited one of the most pronounced declines among the four microbes. The mean difference from baseline to 8 weeks was 6.3128 ($p < .001$). Comparisons between other time points also showed significant decreases, with each interval reflecting a stepwise reduction, indicating consistent effectiveness of the drink throughout the 8-week period.

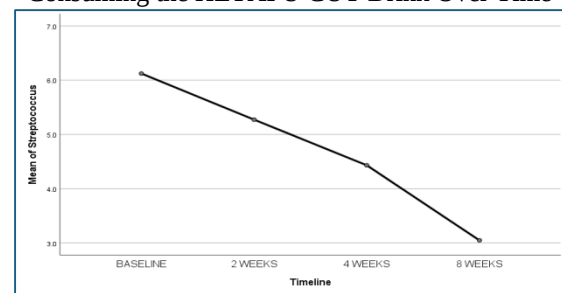
Candida species: The Candida species counts also declined significantly over time. The largest mean difference was observed between baseline and 8 weeks (6.0160, $p < .001$). All other comparisons between time points also showed statistically significant reductions. Notably, the reduction from 4 weeks to 8 weeks (2.9528, $p < .001$) reflects a marked impact in the later stages of the observation period.

Overall Interpretation: The Dunnett T3 test results reveal statistically significant differences across all time points for all four microbial groups. The consistent downward trend observed from baseline to 8 weeks suggests that the consumption of the specified drink had a profound and cumulative effect on reducing microbial counts. These findings support the drink's potential efficacy in managing oral microbial populations over time.

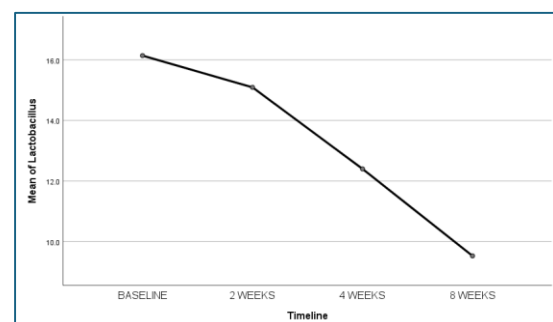
Discussion

The present study evaluates the effects of HETAFU Cut G on the oral microbiome,

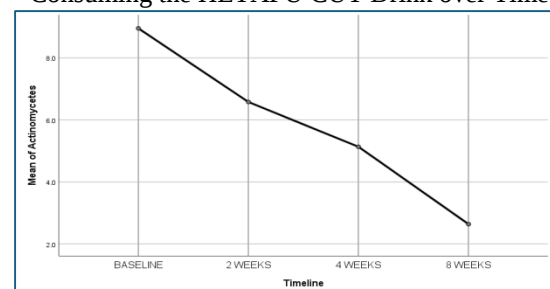
Graph 1: Changes in Streptococcus Counts after Consuming the HETAFU CUT Drink Over Time



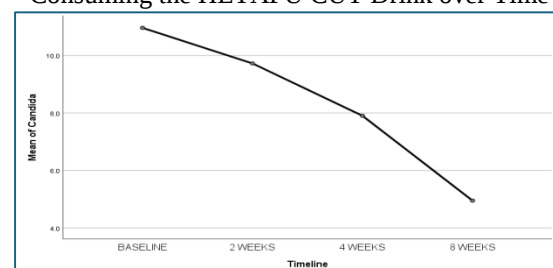
Graph 2: Changes in Lactobacillus Counts after Consuming the HETAFU CUT Drink over Time



Graph 3: Changes in Actinomycetes Counts after Consuming the HETAFU CUT Drink over Time



Graph 4: Changes in Candida Counts after Consuming the HETAFU CUT Drink over Time



focusing on its ability to modulate bacterial populations associated with oral health and disease. By analysing changes in Streptococcus mutans, Lactobacillus species, Candida albicans, and

Actinomyces over an eight-week period, this study provides insights into the potential role of dietary interventions in maintaining oral microbial balance. The nearly equal gender distribution and diverse age range of participants enhance the study's applicability to a broad adult population. Findings from this research contribute to the growing body of evidence supporting the use of probiotics, prebiotics, and sugar alcohols in oral health maintenance, offering a non-invasive alternative to conventional oral hygiene methods.

The gender distribution in the study was nearly equal, ensuring balanced representation of male and female participants, which enhances the generalizability of the findings. The age range of 19 to 58 years, with a mean of 38.62 years and a standard deviation of 11.357, reflects a moderately diverse adult population. This variability allows for a broader assessment of how HETAFU Cut G influences the oral microbiome across different age groups. The balanced demographic characteristics strengthen the reliability of the study's conclusions regarding the effectiveness of HETAFU Cut G in modulating *Streptococcus* mutans, *Lactobacillus* species, *Candida albicans*, and *Actinomyces* species over time.

The present study aimed to evaluate the impact of a specific drink on oral microbial populations over an 8-week period. The study focused on four key microorganisms: *Streptococcus*, *Lactobacillus*, *Actinomyces*, and *Candida*, all of which play a critical role in oral health. The results indicate a statistically significant reduction in microbial counts across all four species, as demonstrated by the ANOVA and Dunnett T3 post hoc tests ($p \leq 0.001$). The findings suggest that the drink has a substantial antimicrobial effect, leading to a progressive decline in harmful oral microbes over time.

Streptococcus species, particularly *Streptococcus mutans*, are primary contributors to dental caries and plaque formation. The observed decline in *Streptococcus* counts from a baseline mean of 6.122 to 3.048 at 8 weeks represents a 50.2% reduction. The narrowing 95% confidence interval (CI) over time indicates increasing reliability of the observed trend, suggesting a consistent reduction across participants. The observed minimum and maximum values also reflect a broader range of microbial suppression, with minimum counts decreasing from 5.1 to 2.0.

Lactobacilli are acidogenic and aciduric bacteria, playing a significant role in dental caries progression. While *Lactobacillus* species are part of the normal oral microbiome, their excessive presence is associated with low pH conditions that favor enamel demineralization. The reduction from 16.142 at baseline to 9.529 at 8 weeks (a 40.9% decrease) indicates that the drink may have an inhibitory effect on *Lactobacillus* overgrowth. However, since *Lactobacillus* also contributes to gut and oral health, a controlled decrease rather than total eradication may be beneficial.

Actinomyces species, particularly *Actinomyces naeslundii* and *Actinomyces viscosus*, are linked to early-stage plaque formation and root surface caries. The decline from 8.952 to 2.640 (a 70.5% reduction) suggests a strong suppressive effect of the drink. The consistently low standard deviation values indicate that the reduction was observed uniformly across participants. The significant drop in minimum values from 8.1 to 1.7 suggests that some individuals experienced near-complete microbial suppression.

Candida species, particularly *Candida albicans*, are opportunistic fungi that can lead to oral candidiasis. The study observed a reduction in *Candida* counts from 10.964 to 4.948 (a 54.9% reduction). The decrease

in minimum values from 10.1 to 4.1 suggests that while some individuals exhibited significant reductions, others retained residual fungal populations, which is expected as *Candida* is a commensal organism.

The ANOVA results indicate highly significant differences in microbial counts across time points ($p \leq 0.001$ for all species). The high F-statistics across all four groups indicate that between-group variance is much larger than within-group variance, confirming that the reductions are not due to random variability. The Dunnett T3 post hoc analysis further supports this, showing that each subsequent time point exhibited a statistically significant difference from the previous one.

The results of this study align with previous research highlighting the antimicrobial properties of bioactive compounds, probiotics, and polyphenols in oral health interventions. Similar studies have demonstrated that polyphenol-rich beverages, such as green tea and cranberry extract, reduce *Streptococcus mutans* and *Lactobacillus* counts by inhibiting bacterial adhesion. Probiotic formulations containing *Lactobacillus reuteri* have been found to selectively suppress Actinomycetes and *Streptococcus* without harming beneficial oral flora. Additionally, essential oils like thymol and eugenol have shown antifungal properties against *Candida albicans*, reducing the risk of oral candidiasis. The observed microbial reduction trends in this study suggest that the tested drink may contain bioactive ingredients with broad-spectrum antimicrobial effects. Further microbial sequencing studies may help identify the specific mechanisms by which the drink alters the oral microbiome.⁶⁻⁹

The findings of this study have important implications for oral health management, particularly in individuals prone to dental caries, periodontal disease, and oral

candidiasis. By effectively reducing pathogenic microbes, the drink could serve as a preventive or adjunctive measure in oral hygiene regimens. Additionally, the progressive decline in microbial counts over time suggests sustained antimicrobial activity, which may provide long-term benefits when incorporated into daily oral care routines.

While the results are promising, several limitations should be considered. First, the study focused on four microbial species, but the oral microbiome is complex. Future studies using 16S rRNA sequencing could provide a broader perspective. Second, a control group consuming a placebo beverage would strengthen the conclusions by ruling out confounding variables. Third, the study was limited to 8 weeks, and investigating whether microbial reductions persist beyond this period would help determine the long-term efficacy of the drink. Finally, individual variations in microbial reduction were observed, which could be influenced by dietary habits, oral hygiene practices, or genetic factors.

This study provides compelling evidence that the tested drink exerts a significant antimicrobial effect on key oral microorganisms over an 8-week period. The reductions in *Streptococcus*, *Lactobacillus*, Actinomycetes, and *Candida* counts were statistically significant, with progressive declines over time. These findings suggest that the drink may serve as a novel intervention for maintaining oral microbial balance and preventing oral diseases. Further studies with larger sample sizes, control groups, and microbiome analyses are needed to confirm its clinical utility and elucidate the mechanisms driving these effects.

Conclusion

The findings of this study indicate that HETAFU Cut G drink has a significant antimicrobial effect on key oral

microorganisms, including *Streptococcus mutans*, *Lactobacillus* species, *Actinomycetes* species, and *Candida* species, over an 8-week period. The consistent reduction in microbial counts suggests that this drink may help in modulating the oral microbiome, potentially lowering the risk of dental caries, periodontal diseases, and oral candidiasis. The bioactive ingredients in HETAFU Cut G drink, such as probiotics, prebiotics and sugar alcohols may contribute to its antimicrobial properties by inhibiting bacterial adhesion, reducing biofilm formation, and promoting beneficial microbial balance. These findings support its potential role as an adjunct to conventional oral hygiene practices, offering an innovative, non-invasive approach to improving oral health.

References

1. Baiju RM, Peter EL, Varghese NO, Sivaram R. Oral health and quality of life: current concepts. *Journal of clinical and diagnostic research: JCDR*. 2017 Jun 1;11(6):ZE21.
2. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K. Oral microbiome: a review of its impact on oral and systemic health. *Microorganisms*. 2024 Sep;12(9):1797.
3. Thomas C, Minty M, Vinel A, Canceill T, Loubieres P, Burcelin R, Kaddech M, Blasco-Baque V, Laurencin-Dalieux S. Oral microbiota: A major player in the diagnosis of systemic diseases. *Diagnostics*. 2021 Jul 30;11(8):1376.
4. Abranches J, Zeng L, Kajfasz JK, Palmer SR, Chakraborty B, Wen ZT, Richards VP, Brady LJ, Lemos JA. Biology of oral streptococci. *Microbiology spectrum*. 2018 Oct 30;6(5):10-128.
5. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A. Effects of Sweeteners on the Gut Microbiota: A Review of Experimental Studies and Clinical Trials. *Adv Nutr*. 2019 Jan 1;10(suppl_1):S31-S48. doi: 10.1093/advances/nmy037. Erratum in: *Adv Nutr*. 2020 Mar 1;11(2):468. doi: 10.1093/advances/nmz112. PMID: 30721958; PMCID: PMC6363527.
6. K. Mäkinen K. Sugar alcohols and prevention of oral diseases—comments and rectifications. *Oral Health and Care*. 2017;2(4).
7. Dou Y, Yu X, Luo Y, Chen B, Ma D, Zhu J. Effect of fructooligosaccharides supplementation on the gut microbiota in human: a systematic review and meta-analysis. *Nutrients*. 2022 Aug 12;14(16):3298.
8. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. *International journal of environmental research and public health*. 2014 May;11(5):4745-67.
9. Baxi S, Bhatia V, Tripathi AA, Kumar P, Tiwari A, Bagde H. Comparative Evaluation of Three Probiotics for *Streptococcus mutans* Prevention in Plaque Around Orthodontic Braces. *Cureus*. 2023 Apr 21;15(4):e37923. doi: 10.7759/cureus.37923. PMID: 37220436; PMCID: PMC10200068.

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