

Comparative Effects of Selected Sweeteners on Tooth Surface Demineralization and pH Variation In Vitro

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Abstract - Dental caries is a chronic communicable disease of the teeth that has been linked to the metabolism of sugars by the bacteria present in the mouth, particularly *Streptococcus mutans*. Lactic acid is produced as these two variables interact with each other, together with all the components necessary for demineralization to occur. Part of the purpose of this study is to determine which type of sugar is more capable of producing an acidic environment that could favor the development of dissolution of tooth surface. Sweeteners such as sucrose, fructose, stevia, and xylitol were the selected sweeteners used in this study. Teeth samples were acquired and soaked in different solutions containing sterile water, a colony of *Streptococcus mutans*, and the aforementioned sweeteners for 10 days. The pH values for each solution and the degree of dissolution formed were gathered and statistically treated using repeated measures ANOVA to determine and compare the mean values. Results showed that fructose obtained the lowest pH value ($\bar{x} = 5.68$), while the greatest tooth surface dissolution was seen on the teeth samples soaked in sucrose solutions ($\bar{x} = 1.57$ mm). Stevia also produced a high pH value and the third sweetener to produce a significant dissolution. These effects may be caused by the possible ingredients added to the stevia powder bought in the market. Therefore, recommendations include using pure stevia sweeteners and adding more test sets to produce a more favorable data. Proper selection of teeth samples along with the correct choice of tooth surface to be sectioned is also important to minimize measuring areas with already existing incipient lesions.

Keywords: Sucrose, Fructose, Stevia, Xylitol, pH, Dissolution

I. INTRODUCTION

Dental caries is one of the most common chronic oral diseases globally, particularly in populations with high sugar consumption. It is primarily caused by acidogenic bacteria, such as *Streptococcus mutans*, that metabolize dietary sugars to produce lactic acid. This acid weakens the enamel by dissolving calcium and phosphate ions, leading to lesion formation (Du et al., 2020). The continued dominance of acid-producing bacteria in the oral biofilm further suppresses beneficial alkali-generating species, resulting in a sustained acidic environment conducive to enamel demineralization and cavitation.

In the Philippines, the high intake of sweetened and processed food has contributed to a serious public health burden related to dental caries. According to the National Monitoring and Evaluation for Dental Survey (NMEDS), 87.4% of Filipinos suffer from dental caries—an issue that has been intensified by behavioral and dietary shifts during the COVID-19 pandemic (International Trade Administration, 2022). Understanding the effect of different sweeteners on enamel integrity is crucial, especially in countries with elevated sugar intake.

Among dietary sugars, sucrose is considered the most cariogenic due to its ability to enhance acid production and support the formation of extracellular polysaccharides by *S. mutans* (Du et al., 2020). Fructose, though naturally occurring and perceived as healthier, may still contribute to enamel demineralization when consumed excessively (Wei, 1995). In response to these risks, alternative sweeteners such as stevia and xylitol have gained attention for their potential oral health benefits. Pure stevia extracts have demonstrated minimal impact on plaque acidity (Siraj et al., 2019), although commercial variants may include additives like dextrose that alter this effect (Nascimento et al., 2021). Xylitol, in contrast, is widely supported by research for its anticariogenic properties and ability to raise plaque pH, inhibit bacterial growth, and promote remineralization (Mansour, 2018; ALHumaid & Bamashmous, 2022).

Despite these developments, there is limited comparative evidence on how these sweeteners differ in their potential to promote tooth surface dissolution under identical experimental conditions. This study addresses that gap by evaluating and comparing the pH levels and enamel demineralization effects of sucrose, fructose, stevia, and xylitol in an in vitro setting. The findings aim to inform dental professionals and consumers about safer sweetener choices for maintaining oral health.

The central problem investigated in this study is: Which among the selected sweeteners (sucrose, fructose, stevia, and xylitol) produces the most acidic environment conducive to the development and progression of tooth surface dissolution? So, the general objective of this study is to determine and compare the efficacy of selected sweeteners in the development and progression of tooth surface dissolution. Specifically, the study aims to measure and differentiate the pH levels of Sucrose, Fructose, Stevia, and Xylitol solutions. Additionally, it seeks to assess and compare the degree of tooth surface dissolution caused by these sweetener solutions on teeth samples.

This study focuses on identifying the differences in the ability of Sucrose, Fructose, Stevia, and Xylitol to promote demineralization of tooth surfaces. Extracted human teeth samples were collected and immersed in five different solutions.

This study is particularly relevant for high-caries risk patients, as it aims to provide alternative sweetener options beyond the commonly used sugars in the market, potentially reducing their susceptibility to dental caries. For dental professionals and students, the research offers valuable insights into the cariogenic potential of both traditional and alternative sweeteners, helping determine if one is more beneficial for oral health than the others. On a broader scale, the findings may influence the public and the food and beverage industry by promoting the use of less cariogenic sweeteners. This shift could contribute to lowering the prevalence of dental caries in the community, while also raising public awareness about the impact of various sweeteners on oral and overall health.

II. LITERATURE REVIEW

Dental caries, a multifactorial disease characterized by enamel demineralization, continues to be a major public health issue globally, particularly in regions with high sugar consumption. Research over the past few decades has extensively investigated the relationship between dietary sugars, oral microbiota, pH changes, and the progression of caries. This literature review synthesizes findings from prior studies on the cariogenicity of various sweeteners—focusing specifically on sucrose, fructose, stevia, and xylitol—and how they influence pH levels and tooth surface dissolution.

The Role of Sugars in Demineralization and Caries Progression

Sucrose has long been identified as the most cariogenic sugar. Du et al. (2020) demonstrated that sucrose promotes significant microbial imbalance in oral biofilms, enriching acid-producing bacteria like *Streptococcus mutans*, while suppressing alkali-generating species such as *S. sanguinis*. Although fructose is considered less cariogenic than sucrose, research presents mixed results. Gupta et al. (2013) cited a Swedish study in which children consuming invert sugar (a mixture of fructose and glucose) showed slightly lower caries incidence than those consuming sucrose, though the differences were not statistically significant. Wei (1995) argued that the fructose content in certain fruits and vegetables can exceed that of common carbonated beverages, suggesting that the cariogenic potential of natural sugars may be underestimated in the literature. It is a monosaccharide, and together with glucose, forms the disaccharide sucrose (Sollid, 2020).

Non-Cariogenic Sweeteners: Stevia and Xylitol

Recent studies have shifted attention to sugar alternatives such as stevia and xylitol due to their potential to reduce caries risk while preserving sweetness. Stevia, derived from *Stevia rebaudiana*, contains steviol glycosides known for their intense sweetness and non-glycemic properties (Petruzzello, 2022). Siraj et al. (2019) conducted a randomized in vivo trial among young adults and found that rinsing with aqueous stevia solutions did not significantly lower plaque pH compared to sucrose, suggesting limited acidogenicity. However, stevia's effectiveness appears to depend heavily on its purity. Nascimento et al. (2021) observed that commercial stevia products may be as cariogenic as sucrose due to added compounds like lactose or maltodextrin, while pure stevia exhibited strong non-cariogenic characteristics.

Xylitol, a five-carbon sugar alcohol, has been extensively documented for its anticariogenic effects. Mansour (2018) emphasized that xylitol interferes with *S. mutans* metabolism, reducing acid production and bacterial adherence to the enamel surface. Benahmed et al. (2020) added that xylitol promotes an alkaline oral environment, unsuitable for acidogenic bacteria. A recent meta-analysis by ALHumaid and Bamashmous (2022) supported these findings, reporting a 35–60% reduction in caries development among children consuming xylitol-supplemented chewing gum and candies. These results reinforce the

recommendation of xylitol as a preferred sugar substitute for caries prevention. However, some adverse effects are associated with Xylitol such as severe allergies, including skin reactions and oral ulcers. Urticarial reactions have also been associated with Erythritol, a form of Xylitol. (Ghusn, 2023) Additionally, xylitol may pose long-term dangers to cardiovascular health. Erythritol and xylitol were both linked to an increased risk of blood clots. This might thereby raise the chance of having a heart attack or stroke. More research on the safety of sugar alcohol as an artificial sweetener is necessary in light of these findings. (Witkowski, 2024)

pH Dynamics and Demineralization Thresholds

A critical mechanism in caries development is the drop in oral pH following sugar intake. The normal salivary pH ranges from 6.2 to 7.6 (Pallepati et al., 2017), and any sustained drop below the critical threshold of 5.5 initiates enamel demineralization. In an experimental study, Pallepati et al. (2017) found that sucrose consumption caused a sharp pH decline that persisted for over an hour, while stevia and unsweetened controls returned to baseline pH within 60 minutes. These results suggest that certain sugar substitutes may exert minimal impact on plaque acidity. However, conflicting evidence exists. In the current study, stevia solutions produced acidic pH values similar to fructose and sucrose, likely due to impurities in the commercial stevia powder used. This inconsistency highlights a gap in the literature: the need to distinguish between pure and commercially processed sweeteners in assessing their cariogenic potential.

Gaps in Existing Research

While numerous studies have examined individual sweeteners' effects on pH and caries progression, few have conducted direct comparative in vitro analyses of multiple sweeteners under standardized laboratory conditions. Moreover, there is limited data on how commercially available sweetener blends—especially those marketed as “natural”—affect oral health. The variable formulation of these products (e.g., stevia with dextrose or sucralose) complicates their evaluation and may yield misleading conclusions about their cariogenicity.

Additionally, most existing research focuses either on plaque pH or bacterial counts, rather than integrating both with physical measurements of enamel dissolution. This limits our understanding of how biochemical changes translate into structural damage over time.

III. MATERIALS AND METHODS

- 1. Research Design** - This study employed a quantitative experimental research design, appropriate for comparing the effects of selected sweeteners on the pH level and enamel dissolution of tooth samples under controlled conditions. The experimental approach was chosen because it allows for precise manipulation of independent variables (types of sweeteners) and measurement of dependent variables (pH and degree of tooth surface dissolution), enabling causal inferences. This design ensures systematic observation and statistical analysis, essential for evaluating differences in acidogenic potential among sucrose, fructose, stevia, and xylitol.
- 2. Data Sources:** The study did not involve human participants directly but used extracted human teeth specimens as the biological sample for testing. A total of 50 natural teeth were

obtained from a private dental clinic in Lucena City, Quezon Province, and from senior clinicians of the Adventist University of the Philippines (AUP) College of Dentistry. Teeth with minimal to no caries or fractures were selected to ensure uniformity and minimize confounding variables. All samples were anonymized, stored hygienically, and handled according to standard ethical laboratory practices. The bacterial strain used in this study was *Streptococcus mutans*, a known cariogenic bacterium, sourced from the Philippine National Collection of Microorganisms (PNCM). Standard microbiological protocols were followed in acquiring and culturing the bacteria for experimental use.



Figure 1. Brand of sweeteners (Sucrose, Fructose, Stevia, and Xylitol) used

3. **Tools and Instruments:** A) Sweeteners: Sucrose (premium raw sugar), Fructose (high-fructose corn syrup), Stevia (commercial powder form), Xylitol (commercial powder form), B) Sterile water served as the control solution. C) pH Meter: The pH levels of all solutions were measured using a calibrated digital pH meter from the AUP College of Science and Technology, Chemistry Department. D) Microscopy: Tooth demineralization was assessed using a compound light microscope at 4x magnification. E) Digital imaging software was used to calibrate measurements and document lesion depths. F) Temperature-controlled incubator: Samples were maintained at 37°C during the immersion period. G) Statistical software (SPSS or equivalent) was used to conduct statistical analysis (Repeated Measures ANOVA).

4. **Procedures:** The research procedure followed a structured series of steps:

4.1 Preparation of Solutions: Each experimental solution consisted of 10 mL sterile water, 0.2 g of the respective sweetener (sucrose, fructose, stevia, xylitol), and a standardized colony of *S. mutans*. A total of five solutions were prepared: Control (sterile water only), Sucrose solution, Fructose solution, Stevia solution, Xylitol solution.

4.2 Sample Immersion and Incubation: Each tooth sample was immersed in one of the five test solutions and incubated at 37°C. The immersion period lasted 10 days, simulating prolonged exposure to sugar-rich environments.

4.3 pH Evaluation: On the 5th day, pH measurements were taken from each solution using the digital pH meter. These values reflect the acidogenicity resulting from bacterial metabolism of the sweeteners.

4.4 Demineralization Assessment: On the 10th day, tooth samples were sectioned to expose the enamel. Cross-sections were examined under a light microscope to measure the depth of incipient lesions. Digital imaging software was used for precise measurements in millimeters or microns.

4.5 Replication and Test Sets: The experiment was conducted using 10 test sets (n = 10 per group) to ensure statistical robustness.

5. **Analysis Techniques:** Data collected for pH and demineralization depth were analyzed using *Repeated Measures Analysis of Variance (ANOVA)*, a statistical method suitable for comparing means across multiple groups within a repeated-measure framework. This test was used to evaluate: A) Differences in pH values among the five solution groups, B) Differences in enamel dissolution measurements

All statistical computations were performed with the support of a university statistician, ensuring accuracy and validity. Significance was set at $p < 0.05$.

IV. RESULTS AND DISCUSSION

This chapter presents the key findings of the study and interprets their implications in relation to the research objectives. Data are presented in both tabular and graphical formats, and are followed by critical discussion that relates the findings to existing literature. The main areas of focus are the pH level of sweetener solutions and the degree of tooth surface dissolution, measured in millimeters after 10 days of exposure to each solution.

1) pH level of selected sweeteners

The first objective of this study was to evaluate and compare the pH values of sucrose, fructose, stevia, and xylitol solutions after bacterial fermentation. A pH value below 7 is considered acidic, while values above 7 are alkaline (Environmental Protection Agency, 2023). Lower pH indicates higher acid production and greater cariogenic potential.

a. Mean pH levels - Table 1 shows that fructose resulted in the lowest mean pH (5.69), followed by stevia (5.82), sucrose (6.16), xylitol (6.91), and control (7.66). This suggests that fructose, although natural, can contribute to significant acidity in the oral environment.

Table 1. Mean pH Values of Control, Sucrose, Fructose, Stevia, and Xylitol

Solution Type	Mean pH	Std. Deviation	N	Interpretation
Control (pH)	7.66	.21	10	Alkaline
Sucrose (pH)	6.16	.36	10	Acidic
Fructose (pH)	5.69	.37	10	Acidic
Stevia (pH)	5.82	.63	10	Acidic
Xylitol (pH)	6.91	.30	10	Neutral

b. Statistical Comparison of pH Values - Repeated Measures ANOVA indicated statistically significant differences in mean pH levels across all five groups ($p < 0.001$). Post-hoc pairwise comparisons showed that xylitol had a significantly higher (more neutral) pH than all other sweeteners ($p < 0.001$), while fructose and stevia did not differ significantly from each other ($p = 0.467$). These findings suggest that while both sucrose and fructose lower pH levels to acidic ranges, xylitol helps maintain oral neutrality, thereby minimizing cariogenic risk (Benahmed et al., 2020; Mansour, 2018).

2) Degree of Tooth Surface Dissolution

The second objective was to measure enamel dissolution caused by each sweetener over a 10-day exposure period. Dissolution was quantified by measuring incipient lesion depth in millimeters.

A. Mean Demineralization Values - Table 2 presents the mean demineralization values. Sucrose caused the greatest enamel loss (1.57 mm), followed by fructose (1.24 mm), stevia (1.10 mm), xylitol (0.53 mm), and control (0.27 mm).

Table 2. Mean pH Values of Control, Sucrose, Fructose, Stevia, and Xylitol

Solution Type	Mean Dissolution (mm)	Std. Deviation	Rank of Cariogenicity
Sucrose	1.57	1.17	Highest
Fructose	1.24	0.50	Highest
Stevia	1.10	1.29	Moderate
Xylitol	0.53	0.75	Low
Control	0.27	0.36	None

These results align with prior studies identifying sucrose as the most cariogenic sugar, capable of triggering deeper and more extensive enamel lesions (Du et al., 2020). Fructose and stevia also showed measurable demineralization, though less severe. The presence of additives in the commercial stevia used likely altered its anticipated performance (Nascimento et al., 2021).

Xylitol, by contrast, caused significantly less demineralization. This outcome is consistent with its well-documented role in promoting remineralization and inhibiting *S. mutans* growth (ALHumaid & Bamashmous, 2022; Mansour, 2018).

B. Statistical Significance - Repeated Measures ANOVA confirmed that the differences in enamel dissolution across groups were statistically significant ($p < 0.05$). Pairwise comparisons indicated:

- a) Sucrose dissolution was significantly greater than that of xylitol and control ($p = 0.046$, $p = 0.002$).
- b) Fructose and stevia had no significant difference between them ($p = 0.763$).
- c) Xylitol and control exhibited the least and statistically similar demineralization levels ($p = 0.335$).

C. Interpretation and Implications

The results of this study reaffirm that sucrose and fructose create acidic environments conducive to enamel demineralization, confirming their strong cariogenic potential. Stevia, although promoted as a non-cariogenic sweetener in its pure form, produced unexpected acidic pH levels and moderate enamel dissolution—likely due to additives in the commercial product used. This supports findings from Nascimento et al. (2021), who observed similar outcomes with market-based stevia variants. Xylitol, consistent with earlier studies (Benahmed et al., 2020; ALHumaid & Bamashmous, 2022), showed the least demineralizing effect and helped

maintain near-neutral pH conditions. These properties position xylitol as a preferable alternative for caries prevention.

The study contributes to academic and clinical understanding by offering direct comparative insights between common and alternative sweeteners under identical experimental conditions. It underscores the importance of evaluating sweetener composition—especially commercial blends—before making dental health recommendations.

V. CONCLUSION

This study aimed to compare the effects of sucrose, fructose, stevia, and xylitol on solution pH and tooth surface dissolution using an in vitro experimental setup. The goal was to identify which sweeteners promote enamel demineralization and which may serve as safer alternatives. Results showed that sucrose and fructose significantly lowered pH and caused the highest levels of enamel dissolution, confirming their strong cariogenic potential.

Stevia, despite its reputation as a non-cariogenic sweetener, also produced an acidic environment and moderate enamel damage. This outcome likely reflects the influence of additional ingredients in the commercial stevia product used, underscoring the importance of evaluating not only the sweetener type but also its formulation. In contrast, xylitol maintained near-neutral pH and caused the least demineralization, supporting its role as a protective agent against dental caries.

While the study offers valuable insights, its findings are limited by the in vitro design, the use of a commercial stevia blend, and a modest sample size. These factors may affect how well the results translate to real-life conditions, such as those influenced by saliva, diet, or hygiene habits.

Future research should involve pure stevia formulations, in vivo testing with human participants, and longer observation periods to assess cumulative effects. Based on current findings, xylitol is recommended as the most effective sugar alternative for reducing caries risk—an important consideration for dental professionals, food manufacturers, and public health policy makers alike.

AUTHORS' CONTRIBUTIONS

Yedd Andrea S. Azores contributed to the conceptualization of the study, literature review, and preparation of the original manuscript draft.

Mingu Lee was primarily responsible for the experimental methodology, laboratory investigation, and data collection.

Jeanneth I. Supangan conducted the statistical analysis, interpreted the results, and assisted in drafting the results and discussion sections.

Judith Clarisse N. Testa managed project coordination, supervised the research process, and contributed to the editing and final revision of the manuscript.

All authors participated in the design of the study, reviewed and approved the final version of the manuscript, and are accountable for its content.

ACKNOWLEDGEMENT

The researchers would like to express their heartfelt gratitude to their adviser, Dr. Lady Zarah Osano, for her unwavering support and guidance throughout the study. Sincere thanks are also extended to Dr. Herminiano Subido Jr., Dean of the College of Dentistry, for his valuable insights and encouragement, and to Dr. Luke Ivan Concel, Department Chair, for generously sharing his expertise. The group also appreciates Dr. Giovanni Mercado, their research professor, for his direction and constructive feedback. Special thanks go to the staff of the AUP Chemistry, Biology, and Medical Laboratory Science Departments for granting access to their laboratories and assisting in the research process. The researchers are deeply grateful to their families and friends for their unwavering belief and support. Above all, they thank the Almighty for granting them the strength, wisdom, and perseverance to complete this study.

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