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Comparative Evaluation of Splenda Sweetener and Sucrose on Salivary Ph and *Streptococcus mutans* Growth: An In Vivo Study

Kawthar ALharran[1], Dunia Aldulaimy[1,2]

[1,2]Department of Pedodontics, Orthodontics and Preventive Dentistry, College of Dentistry Mustansiriyah University, Baghdad, Iraq, E-mail[1]: kawtharalharran@uomustansiriyah.edu.iq, E-mail[2]: drduniaahmed13@uomustansiriyah.edu.iq.

Correspondence: *Kawthar ALharran*
Email: kawtharalharran@uomustansiriyah.edu.iq
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Abstract

Background: Dental caries is a common chronic disease that is intricately associated with dietary sugars and oral microbial activity, especially *Streptococcus mutans* (*S. mutans*). The purpose of this study was to compare the impact of Splenda (sucralose) and sucrose on both salivary pH and *S. mutans.* **The aim of the study**: The current study sought to comparatively assess the impacts of sucralose (Splenda) and monk fruit, on salivary pH and salivary Streptococcus mutans counts, relative to sucrose, after a standardised mouth-rinse protocol. **Methods**: Ninety healthy dental students from Mustansiriyah university aged from 20-24 they were randomly grouped into two groups (n=45 each): Splenda, and sucrose.se. Each participant rinsed with 10g of the assigned sweetener dissolved in 90ml of distilled water. Then the unstimulated saliva samples were taken at the start, right after rinsing, and again 15 and 30 minutes after rinsing (the rinsing took one minute). We used a digital pH meter to measure the pH of saliva and serial dilution and culture on selective media to count the number of *S. mutans*. **Results**: the findings indicated that sucrose markedly reduced salivary pH over time ($p<0.001$), whereas Splenda maintained near-neutral pH levels. Additionally, Splenda significantly reduced *S. mutans* counts ($p<0.001$), while sucrose increased bacterial growth ($p=0.004$). Statistically significant differences between the groups were observed at 30 minutes ($p<0.001$). **Conclusion**: Splenda have a cariostatic effect by preserving salivary pH and reducing *S. mutans,* supporting their use as sugar substitutes in caries prevention.

Key words: Splenda, *Streptococcus mutans*, salivary pH, dental caries, sugar substitutes, sucralose.

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Introduction

Dental caries is a common long-term disease that affects a lot of people around the world and is still a major public health problem. It entails the gradual demineralisation and degradation of dental hard tissues resulting from an imbalance between tooth minerals and the oral biofilm environment (Fejerskov et al., 2015). Caries that without treatment can cause pain, tooth sensitivity, and problems in the overall systemic health (Selwitz et al., 2007). Up to 60% of children and 80% of the general population around the world have dental caries (PE, 2005).
Dental caries has a complicated etiology both external and internal. The extrinsic factors, such as socioeconomic status and parental education, influence the possibilities of children to receive the dental care (Kumar et al., 2016). Bad oral hygiene habits, like not brushing enough and not using fluoride, facilitate dental caries forming (Marinho et al., 2016). Food is also very crucial. Consumption of high amounts of sugar especially before bedtime causes oral bacteria to produce more acid and accelerate the rate of enamel loss (Moynihan and Kelly, 2014). Cavities form the most frequently in the period between 6 and 8 when mixed dentition and permanent teeth are in place. Subsequently, the prevalence of cavities decreases with the increase in oral health and maturing of the teeth (Tinanoff and Reisine, 2009).

The intrinsic factors are largely related to the cariogenic bacteria such as *Streptococcus mutans* and *Lactobacillus* species. These bacteria decompose sugars in food to organic acids which reduce the PH in plaque and make enamel lose minerals (Kleinberg, 2002). Such aciduric bacteria thrive in acidic environments, and accelerate caries progression (Bowen and Koo, 2011). The host factors such as saliva help to protect the body by neutralising acid, clearing of food particles, as well as supplying minerals to the remineralisation (Dodds et al., 2005). The pH of saliva must remain at about 6.7 to safeguard enamel. If it drops below 5.5, it starts to lose minerals (Rusu et al., 2022).
Streptococcus mutans adheres to the pellicle of the enamel and other plaque bacteria. They produce acids and can survive in acids, thus becoming a threat to tooth cavities (Ibrahim et al., 2023).
S. mutans uses sucrose to create extracellular glucans, which assist in biofilm formation and maintain acid production, which degrades the enamel (Koo et al., 2013). There is a strong association between early colonisation and high MS counts and increased caries risk. The development of caries may occur without the presence of MS, but their presence is a reliable indicator of cariogenic potential (Burne and Marquis, 2000).
Saccharine in food, the most prevalent one of them, is sucrose, a significant source of cavities. The global population is consuming more sugar, with the

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proportion of its daily energy supply currently ranging between 9-27% (Popkin and Hawkes, 2016). Excess sugar may not only cause cavities, but also other health issues such as obesity, diabetes, heart disease and cancer (Malik et al., 2010). The mouth bacteria digest sucrose into acids that erode the tooth enamel and promote the growth of cariogenic biofilm (Takahashi and Nyvad, 2011). Moreover, rapid absorption of sugars leads to glycaemic spikes thus complicating the regulation of metabolism particularly those with diabetes (Livesey, 2003).

Artificial sweeteners such as sucralose, approved by the FDA in 1998, are synthetic compounds about 600 times sweeter than sucrose and non-caloric (Magnuson et al., 2016). Sucralose itself is non-cariogenic; however, some sucralose-based products contain fermentable carbohydrates that may carry some caries risk (Takahashi and Nyvad, 2011). Overall, sucralose is considered a beneficial sugar substitute in caries prevention strategies (Takahashi and Nyvad, 2011). The objective of the study is to assess the changes in salivary pH and streptococcus mutans growth among a group of population aged 20-24 years old, after mouth rinsing with water containing different types of sweeteners. Alternative hypothesis: The use of Splenda sweetener significantly affects salivary pH levels and the counts of Streptococcus mutans in participants compared to baseline levels. Null hypothesis: The use of Splenda sweetener does not significantly affect salivary pH levels or the counts of Streptococcus mutans in participants compared to baseline levels.

Methodology:

Ethical consent

The Research Ethics Committee at the educational institution of Dentistry, University of Mustansiriyah, has evaluated the research project for ethical approval.

The decision was sanctioned based on the items presented, which have been received and evaluated by the committee. Reference number: REC145 Date: 01 December 2023, Project No. MUPRV010, the trial was registration in clinical Trail .gov under registration number (NCT06921434).

Sample size calculation:

Using G power 3.1.9.7 (Program written by Franz-Faul, University of Kiel, Germany) With power of study=80%, alpha error of probability=0.05 two-sided, effect size of F is 0.25 (medium effect size), three groups and four measurements, all these conditions the sample size is about 90 subjects (45 subjects for each group).

Effect size F are: Small=0.1, medium=0.25, large=0.4.

Inclusion and exclusion criteria

Ninety pre-graduate dental students, ages 20 to 24, from Mustansiriyah University's College of Dentistry, were participated in the study.

All the participants were in good overall health and did not have any active dental

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conditions or carious lesions. Continuous dental care, drugs that interfere with salivary flow, frequent mouthwash use, the existence of painful oral or systemic conditions, and difficulty understanding study instructions were among the exclusion criteria.

Methods:
Over the course of two months, an average of five participants were gathered every other day. In order to enable prompt culture media setup and sample inoculation, this schedule was designed in tandem with laboratory preparation. Samples were usually collected among multiple individuals at a time, and every sampling session took about 30 minutes per individual. All the samples were transported to the lab within two hours after collection using a cooling box to ensure the integrity of the samples during transportation.

Saliva Collection:
Students in the College of Dentistry at Mustansiriyah University were advised to avoid eating and drinking within an hour of the time the samples of saliva were to be collected by use of the passive drool technique. Before using the mouthwash, right after, fifteen minutes later, and thirty minutes later, each participant gave four samples. The effects of the sweeteners on saliva were consistently and reliably assessed thanks to this procedure.

Solution preparation:
The solution was made by dissolving 10 grams of each sweetener powder in 90 ml of distilled water and then stirred (10) seconds till it completely dissolved in the water.

Measurement of salivary pH:
The salivary pH was measured to observe how the things were before and after using mouth washes that contained various sweeteners. We used a calibrated digital pH meter (Hanna checker plus HI98100) to collect and analyse baseline (initial) saliva samples. We calibrated using standard buffer solutions of 4.0 and 7.0 pH. We inserted the electrode directly in the saliva about ten seconds to obtain a stable reading. The salivary pH was measured three times (10 to 30 seconds after rinsing with the test solution, 15 minutes after, and 30 minutes after). Each of the measurements was made in the same fashion to ensure that they were not different. This approach enabled us to examine the short-term and immediate impact of the sweeteners on how the salivary pH varies, which enabled us to comprehend how they could influence mouth acidity and the chance of cavities.

Preparation of culture media:
The culture media used in this study were all made according to the instructions from the manufacturer and were bought in freeze-dried form. For *Mitis Salivarius* Agar (MSA), 90.07 g of powder was dissolved in 800 ml of distilled water using a magnetic stirrer with heat. The volume was changed to 1000 ml and the pH was set to 7.0 ± 0.2.

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Then, the medium was put in an autoclave for 15 minutes at 15 psi and 121°C. Once the mixture had cooled to 50°C, 200 units of bacitracin and 1 millilitre of sterile 1% potassium tellurite solution were added without any germs. The prepared medium was stored at 4°C in sterile Petri dishes until it was ready to be used.

The number of *Streptococcus mutans*

A vortex mixer was used to mix the saliva samples for one minute. To make tenfold serial dilutions, 0.1 ml of the sample was put into 0.9 ml of sterile saline in nine Eppendorf tubes. Using a sterile glass spreader, 0.1 ml samples from each dilution were put on MSB agar. Plates were put in an anaerobic jar with a gas pack and left at 37°C for 48 hours. After that, they were kept in an aerobic environment for 24 hours at the same temperature. After counting the colonies, we picked plates with 30 to 300 colonies to use in the CFU/ml calculation.
OCD = CFU volume plated × dilution factor\text {OCD} = \frac {\text {CFU}} {\text {volume plated} \times \text {dilution factor} OCD = volume plated × dilution factor CFU, where OCD stands for initial colony density.

Mutans streptococci identification

A. Morphology of Colonies

The convex shape, adherence to agar, light blue colour, and frosted glass appearance of *S. mutans* colonies on MSB agar were used to identify them (Emilson, 1983).
B. Staining with Gram

After being smeared onto slides, the colonies were heat-fixed and stained according to the Gram stain protocol. Gram-positive cocci, which are typical of *S. mutans*, were indicated by the appearance of blue or purple cells.

C. Test for Catalase

A drop of 3% hydrogen peroxide was added to a slide containing pure isolates. The lack of bubbling demonstrated that *S. mutans* is catalase negative.

Statistical Analysis
SPSS version 22, Chicago in press, Illinois, USA is a statistical package for the social sciences. Descriptive statistics Minimum, maximum, mean standard deviation while Inferential statistics are Shapiro Wilk test, Mixed design Two-way ANOVA for salivary PH with Bonferroni, and Paired T test for PCR, Level of significance is when p value is less than 0.05.

Results:
The results of this study revealed notable differences between Splenda (sucralose) and Sucrose in terms of their effects on salivary pH and *Streptococcus mutans* counts.

1. Salivary pH in the sucrose group significantly decreased from 6.74

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at baseline to 6.22 after 30 minutes ($p < 0.001$).

2. In contrast, Splenda maintained near-neutral pH levels with minor fluctuations, showing an increase from 6.77 to 7.15 immediately after rinsing, followed by a slight decrease to 7.03 and 7.04 at 15 and 30 minutes respectively ($p < 0.001$). Both main effect of groups, time and interaction of them have significant effect on salivary PH.
3. Regarding bacterial count, Sucrose exposure resulted in a rise in *S. mutans* levels from 74.70 to 101.73 CFU/mL ($p = 0.004$).
4. Splenda showed a significant reduction from 62.40 to 39.70 CFU/mL ($p < 0.001$).

Table (1) Descriptive statistics of salivary PH among treatment and groups.

Groups		Before	Immediately	15-mins	30-mins
Splenda	Minimum	5.60	6.40	6.50	6.60
	Maximum	7.20	7.70	7.50	7.30
	Mean	6.77	7.15	7.03	7.04
	±SD	0.32	0.28	0.25	0.22
Sucrose	Minimum	6.60	6.30	5.10	4.90
	Maximum	6.80	7.40	7.30	7.10
	Mean	6.74	6.90	6.36	6.22
	±SD	0.08	0.31	0.53	0.48

Table (2) Mixed design two ANOVA using Main and interaction effect of groups and time in salivary PH.

	F	P value
Groups	56.913	**0.000**
Time	23.877	**0.000**
Groups*time	21.713	**0.000**

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Table (3) Multiple pairwise comparison of salivary PH among groups using Bonferroni post hoc

Groups	Time		Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
Splenda	Before	Immed.	-0.377	0.054	**0.000**	-0.523	-0.231
		15-mins	-0.257	0.068	**0.002**	-0.441	-0.072
		30-mins	-0.267	0.066	**0.001**	-0.446	-0.088
	Immed.	15-mins	0.120	0.061	0.317	-0.045	0.285
		30-mins	0.110	0.058	0.372	-0.047	0.267
	15-mins	30-mins	-0.010	0.028	1.000	-0.086	0.066
Sucrose	Before	Immed.	-0.163	0.054	**0.020**	-0.309	-0.017
		15-mins	0.380	0.068	**0.000**	0.195	0.565
		30-mins	0.523	0.066	**0.000**	0.344	0.702
	Immed.	15-mins	0.543	0.061	**0.000**	0.378	0.708
		30-mins	0.687	0.058	**0.000**	0.530	0.844
	15-mins	30-mins	0.143	0.028	**0.000**	0.067	0.220

Table 4: Descriptive statistics of count of *S. Mutans* among time and groups

Groups		Before	30-mins
Splenda	Minimum	15.000	13.000
	Maximum	183.000	132.000
	Mean	62.400	39.700
	±SD	38.833	26.372
Sucrose	Minimum	32.000	32.000
	Maximum	192.000	264.000
	Mean	74.700	101.733
	±SD	37.354	54.696

Table (5) Paired sample T test of salivary *S.Mutans* among phases using Paired t test.

Groups	**Paired Samples Test**		
	Paired Differences Mean	Paired T test	P value
Splenda	-22.70000	-4.481	**0.000**
Sucrose	27.03333	3.170	**0.004**

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Discussion:

Even though dental caries has gone down around the world, it is still a big health problem, especially for people with low incomes (Mota-Veloso et al., 2016). The use of sugar substitutes has become more popular as a way to prevent problems. Sucralose, which is 600 times sweeter than sugar. The Food and Drug Administration (FDA) of the U.S. has also indicated that sucralose is safe to all people including children and those with diabetes (Grotz and Munro, 2009).

This trial evaluated the effects of Splenda (sucralose) and sucrose on the salivary pH and *Streptococcus mutans (S. mutans)* levels among healthy participants. Results showed that the body reacted differently to all the sweeteners.

The pH of the saliva at the beginning of the study and immediately after rinsing and 15 and 30 minutes later was measured.

The pH of the saliva of the subject decreased to 6.22 thirty minutes after rinsing with sucrose, which had a pH of 6.74. This big change in acidity is in line with what we already know about how sucrose can cause cavities (Fejerskov et al., 2015). Bacteria that eat sucrose turn it into lactic acid, which then breaks down enamel. This decrease to below the critical level of pH (approximately 5.5) demonstrates the importance of sucrose in the initiation and propagation of tooth decay (Bowen and Koo, 2011).

On the other hand, Splenda kept the salivary pH stable and close to neutral for the whole measurement period, with only small changes. These results back up the idea that sucralose is a non-cariogenic compound that acidogenic oral bacteria can't break down, which means it doesn't pose much of a risk to the health of enamel (Moynihan and Kelly, 2014).

We determined the responses of *S. mutans* counts at the beginning and 30 minutes following rinsing. The number of S. mutans in the sucrose group increased significantly (74.70 to 101.73 CFU/mL) and this indicates that it is capable of promoting bacterial growth and biofilm development (Guan et al., 2020).

But people who rinsed with Splenda saw a big drop in *S. mutans* levels (from 62.40 to 39.70 CFU/mL). This implies that Splenda could alter cariogenic bacteria functioning by either preventing access to fermentable foods or disrupting other metabolic processes such as glycolysis. (Moynihan and Kelly, 2014).

In this discussion the role of sweets in caries prevention plans is focused. Cariogenicity of different types of sweeteners and influence on the oral microbial community are discussed, and their implications on dental health are considered.

Some of the studies have indicated that the consumption of sugar is closely associated with a decreased saliva pH that may result in enamel *demineralization* and increased risk of

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dental caries. On the other hand, non-cariogenic sweeteners have been shown to have little to no effect on microbial activity and salivary acidity (Zhu et al., 2021).

Limitation:

This research is significant in terms of its ability to inform on the influence of sucralose and sucrose on the salivary pH and *Streptococcus mutans* levels. However, it is important to keep in mind some of its limitations.

The observation time (30 minutes) might not be a good representation of long-term alterations in oral microbial environment and enamel demineralisation. Second, the bacterial species (*S. mutans*) was analysed only, whereas the oral microbiome is composed of different acidogenic and aciduric bacteria, which cause caries formation.

Third, the rate of salivary flow and buffering capability of which change the pH of the mouth were not directly measured.

Lastly, the single-rinse model might not be a good reflection of the real dietary habits, where people are subjected to sweeteners and for prolonged periods.

Future suggestion:

Future studies would include longer follow-up durations to discover permanent microbial changes.

Include a larger panel when assessing the plaque and oral microbial populations.

Test host genetic variables, buffering capacity, and salivary flow. Analyse the pharmacodynamics of sucralose and mogrosides and their impact on bacterial physiology.

Conclusion:

The results of this study show that Splenda (sucralose), a sweetener that doesn't have any nutritional value, is much less likely to cause acid and cavities than sucrose. People who took sucralose had a salivary pH that was almost neutral and a big drop in the number of *Streptococcus mutans*. This shows that sucralose might help keep cavities from forming. On the other hand, sucrose lowered the pH of saliva and sped up the growth of bacteria, which proved that it could cause cavities. These results endorse the incorporation of non-cariogenic sweeteners, such as sucralose, in dietary approaches designed to enhance oral health and diminish the prevalence of dental caries.

Conflict of interest

The authors reported that they have no conflicts of interest.

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