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Comparative Evaluation of Enamel Demineralization Depth by Five Sweeteners: An *In-Vitro* Study

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Abstract:

Background: Dental caries is the most common infectious diseases found in human beings. When a fermentable dietary carbohydrate undergoes bacterial action, they produce acids that lead to the demineralization of tooth structure and ultimately form the dental caries. Among dietary products, sugar substances are the main cause of dental caries. Among today's diet, an increased amount of fermentable carbohydrates including highly processed starch-containing foods, and food products that contain novel synthetic carbohydrates such as sucralose and aspartame also follows the etiology. Coupled with this, there now exists a wide range of non-cariogenic sweeteners such as natural and synthetic that have an important role to play in caries control.

Materials and Methods: A total of 100 extracted single-rooted premolars were taken. Five groups of sugar solution were prepared using honey, palm sugar, sucralose, glucose, and sucrose, respectively, and the teeth were immersed. 1.5×10^8 cells of *Streptococcus mutans* were inoculated into each group for 21 days. Teeth were sectioned buccolingually into 300 µm using hard tissue microtome followed by evaluation under a stereomicroscope.

Results: The mean depth of enamel demineralization for all groups was calculated at three points. The mean depth of enamel demineralization was least for sucralose followed by honey, palm sugar, and glucose and the highest was with sucrose group. One-way ANOVA test was used to compare the mean depth of demineralization between all five experimental groups and Bonferroni test to compare between individual groups.

Conclusion: Within the limitations of the present study, it may be concluded that even though sucralose group exhibited the least mean depth of enamel demineralization, it cannot be statistically proved better than the honey group.

Key Words: Artificial sweeteners, enamel demineralization, natural sweeteners, sucralose, sucrose

Introduction

Dental caries is one of the most common contagious diseases found among human beings.[1] Fermentable dietary carbohydrates undergo bacterial action that leads to the production of acids which demineralizes the tooth structure and ultimately leads to the formation of dental caries.[1]

Diet plays an important role in the advancement of dental caries. About 50 years back dietary sugars were concerned as the major cause for dental caries. Sugars are mostly responsible for these; indeed, the most important dietary factor in the etiology of dental caries, today's diet contains more number of fermentable carbohydrates, these includes highly processed starch-containing foods, and food products that contain synthetic carbohydrates such as sucralose and aspartame. Coupled with this, there now exists a wide range of non-cariogenic sweeteners that plays a key role in caries control.[2] Currently, a large number of investigations are on their way, focused on identifying various foods and factors that defend and control dental caries.

Several studies have been published claiming a non-cariogenic or an apparent anticaries potential of sweeteners. Saccharine was the first and most used artificial sweetener. From there, several other sweeteners have been introduced over time. Currently, five sweeteners have been approved Food and Drug Administration (FDA): Aspartame, saccharine, acesulfame, sucralose, and neotame.[3]

One of the most investigated artificial sweeteners is sucralose. It is a non-caloric sweetener that has been approved by the FDA.[3] In its pure form, sucralose has been regarded as non-cariogenic and when combined with bulking ingredients is less cariogenic than sucrose.[3]

Palm sugar is a natural sweetener and a rich source of calcium, phosphorus, and other nutritional supplements. It is known to have medicinal qualities and is widely used in Indian Medical Systems. However, its cariogenic effect has not been studied.

Honey is carbohydrate-rich syrup produced by honeybees, derived from floral nectars and other plant secretions. It is

reported to contain 181 substances and is considered as part of traditional medicine.[4] One of the intrinsic features of honey is its antibiotic property. The therapeutic potential of honey is gradually growing, and scientific evidence for the effectiveness of honey in several experimental and clinical conditions is beginning to emerge.[4] Recently, studies have demonstrated that Manuka honey with a high antimicrobial activity is likely to be non-cariogenic.[5] Several studies have proven that honey has got excellent antimicrobial property. The natural antioxidants, especially flavonoids, exhibit a wide range of biological effect including antibacterial, anti-inflammatory, and antiallergic.[6] Honey has promising effects on periodontal therapy, prevention of infection in wounds after extraction, erosion of mucosa, radiotherapy-induced stomatitis, and oral ulcers.[7,8]

Sucrose and starches are the predominant dietary carbohydrates in modern societies. Sucrose has been traditionally considered a highly cariogenic substrate for the oral biofilm. Upon fermentation by oral bacteria, sucrose molecules are transformed into energy and large amounts of acids. Thus, frequent exposure to this carbohydrate creates conditions for caries onset by promoting tooth demineralization. While the causal relationship between sucrose and dental caries development is indisputable, the relationship between food starch and dental caries continues to be debated.[9]

The aim of this investigation was to test the cariogenic potential of commercial sweeteners against these natural and artificial sugar substitutes on enamel.

Materials and Methods

This study was conducted in Amrita School of Dentistry in collaboration with Unibiosis Laboratory and National Center for earth studies-Trivandrum, India.

Selection of teeth and preparation of specimen

About 100 caries-free human premolars were selected and stored in 10% formalin solution to disinfect them and prevent the growth of bacteria, or else it would remain viable within the root canals of the teeth.[10,11]

Exclusion criteria

- Teeth with developmental defects
- Cracks
- Caries
- White spots.

All the remaining soft tissues were removed using a razor blade; the teeth were cleaned using non-fluoridated pumice and polished with prophylactic rubber cups. All surfaces of the teeth were covered with nail polish except the buccal surface.

Mueller-Hinton medium (MH) was prepared and sterilized in an autoclave for 15 min at 121°C and 15 (pounds) of pressure. Teeth was placed in a conical flask with 100 ml MH media and then incubated at 37°C. After 24 h, the samples was evaluated to ensure the absence of contamination by evaluating the cloudiness of those samples; thereafter, teeth were removed from the conical flask with the help of sterile forceps and randomly divided into five groups.

Groups in the study:

Group 1	Honey (Local bee Keeper, Wayanad, India)
Group 2	Palm sugar (Local store, Palakkad, India)
Group 3	Sucralose (Nice chemicals, Kochi, India)
Group 4	Glucose (Nice chemicals, Kochi, India)
Group 5	Sucrose (Nice chemicals, Kochi, India)

Each group was put into new flasks containing 100 ml of five different solutions prepared as follows.

Tube 1-20 g of honey+80 ml sterilized MH media
Tube 2-20 g of palm sugar+20 ml distilled water+80 ml sterilized MH media
Tube 3-20 g of sucralose+20 ml distilled water+80 ml sterilized MH media
Tube 4-20 g of glucose+20 mdl distilled water+80 ml sterilized MH media
Tube 5-20 g of sucrose+20 mdl distilled water+80 ml sterilized MH media

About 1.5×10^8 cells of *Streptococcus mutans* ATCC 2517 (equivalent to 0.5 McFarland units) was added to each flask. On alternate days, 2 ml of the solution was replaced by 2 ml of a previously prepared solution for 21 days. The extracted solutions from each group were cultured in nutrient agar plates to make sure the absence of any bacteria other than *S. mutans*.

The samples were mounted in self-cure acrylic resin and sectioned buccolingually using a hard tissue microtome (Buehler isomet 5000-USA) (Figure 1) to obtain sections of approximately 300 mm in thickness. A final polishing was done to get a thickness of 100 mm using high-capacity grinding microtome (Struers, USA) for histological examination. Histological sections were evaluated under a stereomicroscope (Leica Microsystems, Germany). The demineralization depth of each section was measured at three points, and the average of the three representative measurements was taken and considered as the lesion depth.

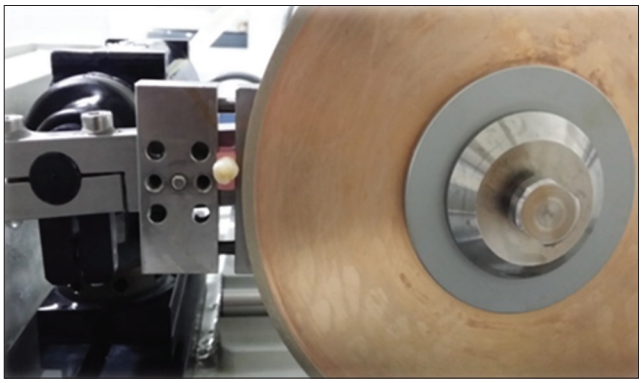


Figure 1: Sectioning using (Buehler Isomet 5000-USA).

Statistical analysis

For statistical analysis, SPSS Version 2.0 software (Chicago, IL, USA) was used, and all the experimental groups were compared statistically using one-way ANOVA test. Bonferroni test was done to find the statistical significance within the groups.

Results

In the present study, the mean depth of enamel demineralization for all groups was calculated at three points (Tables 1-3). The mean depth of demineralization for Group I (honey) was 160.01 µm, Group II (palm sugar) 196.07 µm, Group III (sucralose) 145.47 µm, Group IV (glucose) 235.72, and Group V (sucrose) 287.78 µm (Tables 1-3). Therefore, the lowest mean depth of demineralization was observed in Group III (sucralose) followed by Group I (honey), and the highest was observed with Group V (sucrose).

One-way ANOVA test was used to compare the mean depth of demineralization between all five experimental groups. A statistically significant difference between all the groups was observed ($P < 0.001$) (Table 4).

Intergroup comparison was done using Bonferroni test (Table 5), no significant difference was observed between Group III (sucralose) and Group I (honey) ($P > 0.05$).

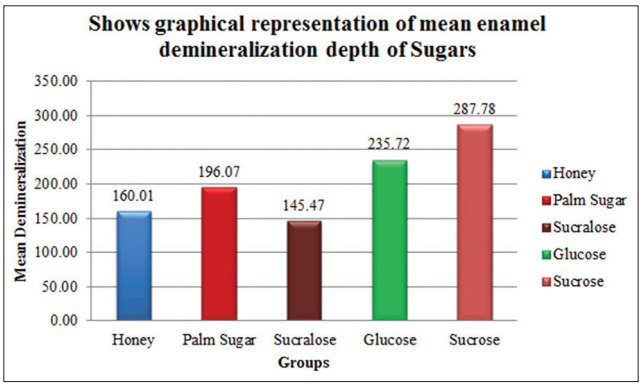
However, comparison between Group 1 (honey) and Group III (sucralose) with other three groups (Group II - palm sugar, Group IV - glucose, and Group V - sucrose) showed a statistically significant difference ($P < 0.001$) (Table 5).

A graphical representation of the mean depth of demineralization for all the five experimental groups is shown in Graph 1.

In the present study, sucralose group exhibited the least depth of enamel demineralization, followed by honey, palm sugar, glucose, and sucrose groups.

Discussion

Sweeteners are one of the most common causes of dental caries.[12] Artificial and natural sweeteners such as honey, palm sugar, sucralose, glucose, and sucrose are commonly used in food industry to sweeten beverages and confectioneries. Honey is super-saturated, delicious, and naturally sweet nectar popular worldwide and is collected by bees from a wide variety of plants. As a sweetener honey does not cause untoward effects.



Graph 1: Graphical representation of mean enamel demineralization depth of sugars.

Table 1: Depth of demineralization induced by honey and palm sugar.

Specimen number	Group I Honey Depth of demineralization				Group II Palm sugar Depth of demineralization			
	1st	2nd	3rd	Average	1st	2nd	3rd	Average
1	103.8	102.2	106.7	104.23	108.5	135.3	152.7	132.17
2	170.3	169.3	173.7	171.10	146.7	152.3	160.3	153.10
3	201.1	200.3	203.7	201.70	160.3	163.7	169.9	164.63
4	196.6	194.9	197.3	196.27	171.3	173.4	178.1	174.27
5	180.3	181.4	183.6	181.77	165.9	169.1	173.4	169.47
6	185.7	186.7	188.3	186.90	185.1	190.3	194.1	189.83
7	219.3	221.2	222.1	220.87	196.1	199.3	203.1	199.50
8	110.3	109.2	112.7	110.73	201.1	206.6	210.1	205.93
9	119.2	120.3	122.3	120.60	222.3	230.1	235.9	229.43
10	141.2	142.4	144.7	142.77	235.9	241.1	244.2	240.40
11	150.1	151.3	153.6	151.67	210.3	211.4	215.6	212.43
12	130.2	131.1	133.4	131.57	213.1	218.2	225.1	218.80
13	170.9	169.1	173.8	171.27	146.9	152.7	160.9	153.50
14	110.1	109.9	112.3	110.77	235.4	241.7	244.1	240.40
15	110.7	109.4	112.5	110.87	210.6	211.9	215.3	212.60
16	185.8	186.3	188.1	186.73	185.7	190.8	194.4	190.30
17	196.4	194.1	197.8	196.10	222.9	230.2	235.6	229.57
18	201.5	200.2	203.3	201.67	235.8	241.1	244.1	240.33
19	119.9	120.1	122.2	120.73	160.7	163.9	169.2	164.60
20	180.5	181.5	183.8	181.93	196.9	199.9	203.8	200.20
Mean±SD				160.01±37.757				196.07±32.67

SD: Standard deviation

Table 2: Depth of demineralization induced by sucralose and glucose.

Depth of demineralization							
Group III				Group IV			
Sucralose				Glucose			
1st	2nd	3rd	Average	1st	2nd	3rd	Average
92.3	93.6	95.1	93.67	181.5	189.9	205.9	192.43
95.7	96.3	99.1	97.03	195.3	204.2	210.1	203.20
101.1	111.9	114.2	109.07	197.2	199.1	205.3	200.53
120.1	122.2	124.9	122.40	201.2	205.6	213.2	206.67
132.1	134.1	135.7	133.97	200.2	206.2	215.6	207.33
150.6	151.7	153.9	152.07	204.2	207.1	210.1	207.13
162.1	163.4	165.1	163.53	290.1	294.3	295.1	293.17
178.1	179.1	181.2	179.47	282.3	288.1	292.7	287.70
162.1	163.4	165.1	163.53	260.7	267.1	269.9	265.90
191.1	193.3	194.6	193.00	250.1	156.7	269.9	225.57
195.9	196.7	199.2	197.27	245.7	248.1	256.1	249.97
110.2	111.9	114.2	112.10	270.8	273.2	278.9	274.30
162.9	163.1	165.6	163.87	195.6	204.1	210.5	203.40
95.5	96.1	99.3	96.97	197.7	199.9	205.9	201.17
162.3	163.6	165.8	163.90	200.5	206.1	215.8	207.47
132.8	134.6	135.2	134.20	282.1	288.4	292.6	287.70
195.1	196.3	199.9	197.10	290.4	294.9	295.8	293.70
191.5	193.2	194.4	193.03	270.4	273.1	278.4	273.97
101.7	111.1	114.4	109.07	250.9	156.5	269.9	225.77
132.6	134.8	135.2	134.20	204.1	207.4	210.2	207.23
Mean±SD			145.47±35.883				235.72±37.57
SD: Standard deviation							

Table 3: Depth of demineralization induced by sucrose

Group V			
Sucrose			
Depth of demineralization			
1st	2nd	3rd	Average
235.2	236.3	239.4	236.97
242.1	246.3	248.4	245.60
252.9	253.3	258.7	254.97
268.9	269.8	273.1	270.60
281.3	283.4	285.7	283.47
284.2	287.1	288.4	286.57
290.1	292.3	296.7	293.03
299.9	301.2	303.7	301.60
305.7	306.9	308.1	306.90
310.9	311.1	312.2	311.40
320.7	321.8	323.9	322.13
332.9	333.1	335.1	333.70
242.9	246.6	248.1	245.87
299.8	301.9	303.7	301.80
332.3	333.3	335.6	333.73
290.9	292.8	296.9	293.53
284.1	287.6	288.8	286.83
320.4	321.1	323.1	321.53
252.8	253.3	258.7	254.93
268.9	269.1	273.3	270.43
Mean±SD			287.78±29.74
SD: Standard deviation			

It inhibits the growth of a wide range of bacterial species *in vitro* as per the study of Mandal and Mandal[13] Lin *et al.*, and Molan *et al.* reported that honey has antibacterial activity against cariogenic bacteria such as *S. mutans* and *Lactobacillus.*[14,15]

Table 4: One-way ANOVA.

Groups	*n*	Demineralization	95% Confidence interval for mean		*P* value
		Mean±SD	Lower bound	Upper bound	
Honey	20	160.01±37.76	142.34	177.68	<0.001
Palm sugar	20	196.07±32.67	180.78	211.36	
Sucralose	20	145.47±35.88	128.68	162.27	
Glucose	20	235.72±37.57	218.13	253.30	
Sucrose	20	287.78±29.74	273.86	301.70	

SD: Standard deviation, ANOVA: Analysis of variance

Table 5: *Post-hoc* Bonferroni test.

Groups	*P* value
Group I	
Palm sugar	0.015
Sucrose	<0.001
Glucose	<0.001
Group II	
Sucrose	<0.001
Glucose	0.005
Group III	
Palm sugar	<0.001
Sucrose	<0.001
Glucose	<0.001
Group IV	
Sucrose	<0.001

However, there have been only a few reports about the efficacy of honey against these bacteria.[14]

Factors that are effective in antimicrobial activity of honey include the osmotic effect, enzymatic glucose oxidation reaction, production of hydrogen peroxide, high osmotic pressure, a low pH, and the presence of phenolic acids, lysozyme, flavonoids, phytochemicals, antioxidants, beeswax, nectar, pollen, and propolis as reported by Hashizume *et al.*, Al-Mamary *et al.*, Lin *et al.*, and Patel *et al.*[4,5,14,16] In addition to these antimicrobial activities, honey has beneficial effects in the oral cavity and for many oral diseases.

Sela *et al.*, 1998, suggested that honey contains factors that may reduce the solubility of exposed enamel in an acid buffer solution, compared to pure sucrose. In addition to the solubility-reducing substances, honey contains factors that may also reduce bacterial effects on dental caries.[17] The results of this study indicated that honey has fewer caries activity than sucrose, glucose, and palm sugar. The antibacterial activity of honey against cariogenic bacteria and its other beneficial properties may reduce its caries activity compared to other sugars. Hence, honey can be used instead of other cariogenic sugars as a sweetener and as a material in toothpaste, gum, candy, chocolate, and so on.[12,14,15] In the present study, the effect of honey on enamel demineralization confirms with study by Ahmadi-Motamayel *et al.*[1]

The antibacterial activity and composition vary with the source of the honey and the way it is processed. Honey should be protected from light to maintain its antibacterial effect.[17] In the present study, we used fresh, natural honey extracted from the honeycomb. Different types of honey from various sources may have different properties as reported earlier by Patel *et al.*[5]

The result of the previous study by Cury *et al.*, in 2000, evaluated the cariogenicity formed in the presence of sucrose, glucose, and fructose, and the results showed that fructose and glucose had lower caries production than sucrose.[18] When we compared our study results with Cury *et al*. study, we can infer that sucrose is more cariogenic when compared with honey and glucose. So, it is important that honey has fewer caries activity than other sugars such as fructose and sucrose. However, the lower caries activity of honey depends on many good properties of this material.

Sucrose for years was billed as the "arch criminal" in dental caries because it was considered to be much more cariogenic than other sugars reported by Newbrun.[19] However, after many types of research, it was found out that the differences between sucrose and the various monosaccharides in terms of cariogenic potential is less than what was originally believed.[20] Picton and Wiltshear in 1970, conducted a study in Sweden that included a small number of pre-school children who were found to have invert sugar (a mixture of glucose and fructose) instead of sucrose was found to have a lower caries incidence in 2 years, although it did not produce any marked difference in the statistical significance. Picton and Wiltshear study was comparable with the present study; glucose caused the least demineralization when compared with sucrose.[21]

Sugars can be readily metabolized by many bacterial species resulting in dental biofilm formation, thereby producing acids that can lead to demineralization of the tooth structure. The unique property of sucrose as a cariogenic substrate includes the production of extracellular glucans by mutans streptococci that in turn increase its concentration in plaque. Sucrose was given special consideration as a cariogenic substrate owing to its unique ability to for the synthesis of extracellular (water-soluble and water-insoluble) glucans by mutans streptococci and enhancing its accumulation in plaque. Bowen and Pearson in 1992, conducted studies on rats infected with *S. mutans* and had found that increased cariogenicity of sucrose compared to other sugars.[22] Recent clinical studies by Zero have indicated that the extracellular glucan alters plaque ecology by increasing its porosity, which permits deeper penetration of dietary sugars and greater production of acid on tooth surface and thereby causing enamel demineralization.[23]

Picton and Wiltshear 1970, von der Fehr *et al.*, 1970, and Kashket *et al.*, in 1991 reported that no sweetener was capable of demineralizing the enamel at levels comparable to those induced by sucrose, these findings confirm the higher cariogenic potential of sucrose when compared with any other carbohydrate.[21,24,25] Although reduced with respect to sucrose, the products tested did induce enamel demineralization. Hence, artificial and natural sweeteners appear to induce less demineralization than sucrose, but preserving some demineralizing potential.

S. mutans treated with sweeteners containing, sucralose, honey, palm sugar, and glucose showed less enamel demineralization than sucrose. Extracellular polysaccharides (EPS) are responsible for 40% of the composition of the dental biofilm, and they are one of the main virulence factors of the bacterial consortium, as they allow bacterial cell adhesion to the acquired pellicle, serve as scaffolds for biofilm maturation and increase the porosity of the structure allowing sugar diffusion within the biofilm.[26]

Mechanisms to explain the lower demineralizing potential of the commercial and natural sugars tested here may be due to lack of metabolization by *S. mutans*. Bowen *et al*. reported that sucralose has been deemed to be metabolically inert and thus non-cariogenic in animal models.[27] Since sucralose has been shown not to interfere with bacterial metabolism, a decrease in polysaccharide production is not expected to be observed by an inhibitory effect of the sweetener.[13] EPS production, however, did show a decrease when compared with sucrose. This may derive from a highly increased sucrose-induced polysaccharide production rather than from an inhibition caused by the sweeteners. As a matter of fact, an increased metabolic activity on sucrose exposure has been previously reported by Koo *et al*.[28] This regime of sweetener exposure intended to mimic a moderate and probably common consumption; cariogenicity could increase on an increased frequency of exposure.

By itself and as the sole source of carbon, sucralose does not support bacterial growth and could be, therefore, considered as antibacterial. Although our data do show a decreased enamel demineralization in sucralose group when compared with other groups, apparent contraindication may be explained by the other components contained in the commercial form of the products. Artificial sweeteners are in general 100 of times sweeter than sucrose reported by Roberts and Wright.[29] Products sold in the market and cannot be commercialized in their pure form. For instance, a tablet of a commercial product advertised as sucralose usually contains only about 10% of sucralose with higher proportions of other carbohydrates, usually lactose, starch, or starch hydrolysates. Like lactose, starches alone or in combination with simple carbohydrates are potentially cariogenic reported by Duarte *et al.* and Ribeiro *et al.*[30,31] Studies by Villegas *et al.* demonstrated that combination of starch and sucrose causes more demineralization than sucrose alone on dentine.[32]

It is important to highlight that the results of this study were obtained using an *in vitro* approach. Despite the fact

that, we used teeth immersed in different sugars to detect the demineralized area that intends to resemble the oral environment. In this study, teeth were sterilized in 10% formalin to disinfect the teeth to prevent bacterial growth, which may otherwise remain viable in the root canal.[10,11] Marsh stated that oral biofilm comprises metabolically active and organized consortium of hundreds of bacterial species.[33] *S. mutans* was chosen for this study because in another study conducted by Szpunar *et al*., in 1995, found that *S. mutans* bacterial species were predominantly responsible for enamel demineralization in tooth.[34] Moreover, the model used here does not include saliva. Stookey conducted a study and found that saliva has important anticaries properties that may modulate the mere effect of the nutrients presented to the oral biofilm.[35] Further *in vivo* studies must be conducted to actually replicate the complexity of the oral environment including the complete dental biofilm and the presence of saliva. Hence, the results from clinical studies with sweeteners might be different from those reported here.

Taken together, these results suggest that artificial (sucralose) and natural (honey) sweeteners have lower cariogenic potential than sucrose. The former seems to derive from the incapacity of *S. mutans* biofilms to metabolism the products with the same efficiency the bacterium ferments sucrose, despite the presence of other fermentable carbohydrates.

Results of the present study indicate that the artificial sugars such as sucralose and natural sweeteners like honey are less cariogenic, and the use of artificial sugars should be carefully recommended.

Conclusion

The present study compared the depth of demineralization of enamel caused by honey, palm sugar, sucralose, glucose, and sucrose. Within the limitations of the present study, it can be concluded that a statistically significant difference in the mean depth of enamel demineralization was observed in all groups except sucralose and honey.

Results of the present study demonstrated that least depth of enamel demineralization was seen in sucralose followed by honey group and the maximum was seen in sucrose followed by glucose and palm sugar.

The results signify the lesser enamel demineralization potential of sucralose and honey.

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