Effect of Various Sweeteners on Cariogenicity Features of Streptococcus Mutans: In-Vitro Study

[1]Srudhy.R, [2]Nijesh.J.E, [3]Preetha Elizabeth Chaly

[1]Postgraduate Student, [2]Associate Professor, [3]Professor and HeadDept. of Public Health Dentistry,Faculty of Dentistry - MeenakshiAmmal Dental College and Hospital, Meenakshi Academy of Higher Education and Research, Chennai

Abstract

Background: Dental caries is a global pandemic health crisis, that is caused by diet containing refined sugar. Many alternative sugars in the market have been used as a substitute for table sugar. But there is paucity in literature regarding the cariogenicity properties of these.

Aim: To assess the effect of various alternative sweeteners on *Streptococcus mutans* growth and acidogenicity. Study Design: Laboratory based In-Vitro Study.

Materials and Method: Ten percentage solutions of refined sugar, brown sugar, jaggery, palm jaggery, palm sugar, honey, Rare Sugar Sweet were used as test products to assess the growth of *Streptococcusmutans* in terms of the optical density using a spectrophotometer, Colony forming units(CFU) in Mutans-sanguis agar (MSA)and acidogenicity by measuring the pH. Statistical Analysis Used: Non-parametric test of significance with p value <0.05 was performed. Results: S. mutans growth in the honey and D-Psicose were significantly lower than that in the sucrose, while greater than that of xylitol and placebo group ($p < 0.05$). pH after 48 hours was statistically significant between the groups and dropped below the 5.5 in all the test groups except honey and D-Psicose. Honey had least cariogenicity in terms of growth of S.mutans and acidogenicity. Rare sugar sweet had S.mutans growth and acidogenicity less than sucrose.

Conclusion: Honey had least S. mutans growth and acidogenicity hence, can be regarded as safe alternatives for other commercial sweeteners in the market.

Keywords: *D-Psicose, Cariogenicity, Acidogenicity, Optical density, Colony Forming Units.*

Introduction

Disaccharide sucrose commonly referred to as 'Sugar' is a common household ingredient that is easily available and most commonly consumed in the human diet as it adds sweetness and enhances the taste of food. [1] Global consumption of sugar is constantly increasing and is projected to grow at around 1.48% per annum and is expected to reach 198 million tons in 2027. [2] India, is in fact, is the largest consumer of sugar world-wide and there is about a three to a four-fold rise in sugar availability and consumption. Easy availability, access, and utilization of free sugar is a threat to health, as this increases the risk of non-communicable diseases including obesity, diabetes and dental caries. [3]

Untreated dental caries in permanent teeth are the most common health condition according to the Global Burden of Disease 2011 and more than 530 million children suffer from dental caries of primary teeth. [4] Saccharolytic bacteria like *Streptococcusmutans* produce acids and lead to direct demineralization of the enamel of teeth which initiates dental caries. [5] Sucrose is the most cariogenic sugar and long-term restriction of sugar from diet results in the reduction in dental caries.

Corresponding Author:
Nijesh J.E.
Associate Professor, Department of Public Health Dentistry, Meenakshi Ammal Dental College and Hospital, Chennai, India
e-mail: nijesh_phd@madch.edu.in

[6] It seems impractical for the complete restriction of sugar given the human preference for the sweet food item. Instead, cariogenic sugar can be substituted with other sweetening agents that might be less cariogenic.

Honey could be an alternative for sucrose as it is found to be non-cariogenic or less cariogenic. [7] Brown sugar, jaggery, palm jaggery, palm sugar are alternatives to refined sugar that are functional foods and have health benefits. [8] The presence of reducing sugars, significant quantities of minerals and other minor constituents makes 'alternative sugar' looks promising.

Xylitol, another alternative, is a naturally occurring non-fermentable five-carbon sugar alcohol, which cannot be converted to acids by oral bacteria, thus it helps to restore a proper alkaline/acid balance in the mouth. [9]

With current advancements in food processing technology, many attempts have been made to find a novel "caries-preventing sweetener" or "anti-cariogenic sweetener". One such recent product manufactured in Japan is D-psicose. D-Psicose, a Japanese product is a low-calorie sweetener, which claims to controls bacterial growth and acid production (anti-cariogenic), even in combination with existing sweeteners. [10]

There are very few studies published to explore the cariogenic potential of these sweeteners. Hence an in-vitro study was conducted to assess the effect of various alternative sweeteners such as refined sugar, brown sugar, jaggery, palm sugar, palm jaggery, honey and D-Psicose on cariogenicity features of *Streptococcusmutans.*

Methodology

An in-vitro study was conducted on *S.mutans* MTCC 890 strain in the Central Research Laboratory, after obtaining the ethical approval. The cariogenic potential of Refined white sugar, Brown sugar, Jaggery, Palm sugar, Palm jaggery (Karupatti), Commercial honey, D-Psicose with sucrose (Rare sugar sweet®, Japan), Sucrose as Positive control (Lab grade) and Xylitol as a negative control (Lab Grade) was assessed.

a. **Preparation of Test solutions:** Ten percent of each test solution was prepared by mixing 10 grams or 10 mL of the test product in 100mL of distilled water and solutions were then filtered, sterilized through a 45- micron size pore filter and stored at 4°C. This solution was used for the preparation of agar media using Mutans Sanguis Agar

b. **Bacterial growth as a measure of Optical Density:** Sterile 96-well microtiter plates were taken and wells were labeled as Tests and controls. In the test wells, 10 microliters of *S. mutans* from the overnight culture were added along with 290 microliters of MSA-sweeteners. In the control wells, 300 microliters of the MSA-sweetener dilution without bacteria were added. The absorbance of each wellwas read at 540 nm in a spectrophotometer after an incubation period of 48 hours at 37°C.[11] The test was done in triplicate. The final test optical density was calculated by subtracting the optical density of the test minus the optical density of the control of each product used.

$OD_{final} = (OD_{test} - OD_{control})$ for each sweetener

c. **Bacterial viability in terms of Colony-forming units (CFU):** The effect of sweeteners on *S. mutans* viability was assessed. Bacterial cultures of all sweeteners from the microplate wells were plated on the MSA agar plate. The number of CFU for each sweetener was measured using an automated colony counter after incubating it for 48 hours at 37°C. The test was done in triplicate.

d. **Sweeteners influence on S. mutans acidogenicity:** The pH of the test solutions in the micro-titre plate was measured at baseline and the end of 48 hours using a digital pH-meter. The test was done in triplicate. To know the media characters at the end of 48 hours, a separate media solution with a microbe was prepared and kept as control.

Statistical tests: The colony count was transformed into $\log_{10}$ CFU for analysis. The data were analyzed using SPSS software version 19.0. by a statistician who was blinded to the groups. Non-parametric tests of significance were performed. In all tests, a P-value of <0.05 was considered as the level of statistical significance.

Results

a. **Inter-group comparison of optical densities:** The ascending order of the optical density of the sweeteners is as follows, xylitol, honey, palm sugar, refined sugar, brown sugar, rare sugar sweet, jaggery, palm jaggery and sucrose. In the post-hoc analysis, the optical density of palm jaggery was higher when compared to other test sweeteners used in this study and this difference was statistically significant ($p<0.05$). (Table 1)

Table 1: Inter-group comparison of Optical density, log_{10} CFU and pH

Group	Optical Density (Final) Mean±SD	Log_{10} CFU/mL Mean±SD	pH before Mean±SD	pH After Mean±SD
Refined sugar	0.59±0.08	5.00±0	7.2± 0.1	5.4±0.2
Brown Sugar	0.62±0.03	4.89±0.13	7.36±0.2	5.4±0.2
Palm Jaggery	1.11±0.48	4.99±0.01	7.2±0.1	5.2±0.1
Palm sugar	0.57±0.09	4.99±0.01	7.23±0.2	5.2±0.1
Jaggery	0.97±0.07	4.97±0.04	7.2±0.1	5.4±0.2
Honey	0.57±0.04	4.52±0.11	7.2±0.2	5.9±0.2
D-Psicose	0.69±0.06	4.79±0.08	7.2±0.1	5.6±0.2
Sucrose	1.51±0.07	5.00±0	7.2±0.1	5.2±0.2
Xylitol	0.16±0.05	3.35±0.05	7.3±0.2	6.6±0.1
Placebo	0.03±0.01	0	7.2±0.2	6.8±0.1
p-value	0.001*	0.002*	0.802	0.003*

b. Inter-group comparison of log_{10} of Colony Forming Units per mL: The ascending order of the optical density of the sweeteners is as follows, xylitol, honey, rare sugar sweet, jaggery, brown sugar, palm sugar, refined sugar and sucrose. Honey and rare sugar sweet had log_{10} CFU/mL when compared to refined sugar, brown sugar, palm sugar, palm jaggery, jaggery and sucrose, while it was higher in both these sweeteners when compared to xylitol. This difference in colony-forming units in honey and rare sugar sweet with other test products was statistically significant($p<0.05$) (Table 1).

c. Intra and Inter-group comparison of pH: The pH at baseline was around 7.2 for all the sweeteners, and even though there was a slight difference in pH, this was not statistically significant (p=0.802). The pH of media without sweetener added (Control) was 8.6 at the end of 48 hours, suggesting that the sweeteners were responsible for the change in pH. The pH after 48 hours for various sweeteners were 5.4±0.2 for refined, brown sugar and jaggery, 5.2±0.1 for palm jaggery, palm sugar, 5.9±0.2 for honey, 5.6±0.2 for rare sugar sweet, 5.2±0.2 for sucrose, 6.6±0.1 for xylitol and 6.8±0.1 for placebo. The difference in pH at end of 48 hours between the sweeteners was statistically significant(p=0.003). All the test products except xylitol, honey and rare sugar sweet had a pH less than 5.5, which is regarded as the critical pH for demineralization of enamel.

Figure 1: Preparation of Test solutions

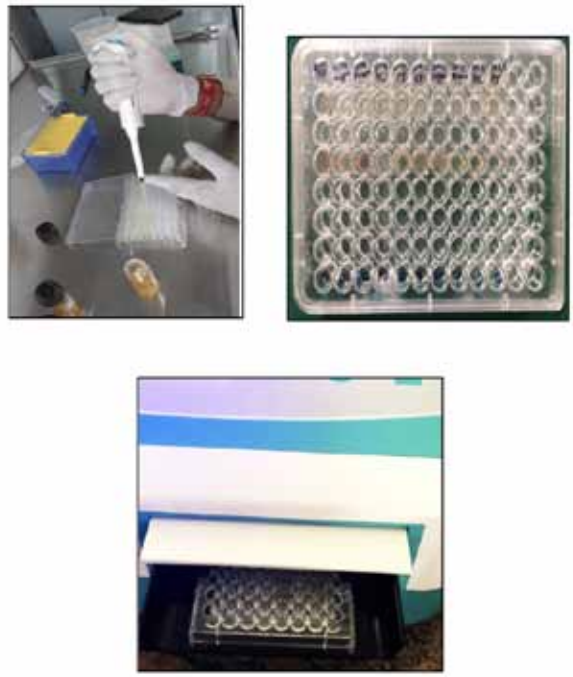

Figure 2: Bacterial growth as a measure of Optical Density using spectophotometer

Figure 3: Bacterial viability in terms of Colony forming units (CFU)

Figure 4: Sweeteners influence on S. mutans acidogenicity

Discussion

Dental caries is caused by the interaction of the agent, host and substrate. The substrate is particularly refined carbohydrates in the diet. The refined carbohydrates in the diet are easily metabolised by S. mutans into inorganic acids, which causes demineralization, eventually leading to cavitation. The nature of the substrate, the frequency and amount of sugar consumed affects the initiation and progression of dental caries.[12] This has led to an intensive search for an alternative sweetener without cariogenicity. Advancement in food technology and a rise in health consciousness has led to the launch of many new alternative sweeteners.

One such novel alternative is D-Psicose which is marketed as 'Rare Sugar sweet' in Japan which is a C-3 epimer of D- fructose, with higher solubility, smooth texture, desirable mouthfeel and has 70% of the sweetness of sucrose with only 0.2 kcal/gm.[13] It has a low glycaemic index and prevents postprandial hyperglycaemia. [14]

In India, many traditional sugars such as brown sugar, jaggery, palm jaggery, palm sugar and honey have been consumed regularly for centuries. Parents substitute these sugars in common food items and drinks for their children's well-being, as they are regarded as a safer alternative to that of refined sugar. Hence, these sugars were tested for cariogenicity in this study.

Sucrose has been regarded as arch-rival for dental caries hence used in this study as a positive control. [15] Xylitol was used as a negative controlas it is analternative sugaras it is not metabolised by *S.mutans* to produce acids. [16]

Cariogenicity of food items can be assessed by four parameters viz cariogenic plaque forming ability of the substrate, acid forming potential, clearance time and ingestion time. [17] Since this is an in-vitro study only, the plaque forming ability of the substrate and the acid forming potential was recorded.

Plaque forming ability of the substrate was assessed by measuring the growth of *Streptococcusmutans*. Triangulation was done between optical density and colony forming units to obtain a good calibrated growth estimate. The acid forming ability was assessed using a calibrated digital pH meter.

In this study, ten percentage of solutions were prepared, for all the test products. This was done to ensure that the results are comparable to the study conducted by Stephen in 1940, where 5.5, which is the critical pH of demineralization of enamel was determined using 10% solutions of glucose/sucrose.[18]

In this study, natural honey, had lower OD as well as CFU when compared to that of the positive control and the pH did not drop below 5.5. This suggests that honey can be a potential alternative to refined sugar. Natural honey contains various amino acids, vitamins, minerals and enzymes along with 95–99% sugar, which is predominantly fructose and glucose. [19] Honey has anti-inflammatory, antibacterial, anti-viral, anti-fungal, anti-oxidant properties and promotes wound healing, protects from gastrointestinal infections. [20] The topical application of honey caused less fall in pH when compared to sucrose solution among orthodontic patients. [21]

Both refined sugar and other sweeteners except honey which are tested in-vitro might be cariogenic as they contain fermentable carbohydrates. [22] But, the tested alternative sweetenerscontain reducing sugars with significant quantities of minerals like Calcium, Copper, Iron, Manganese, Chromium, Magnesium, Potassium, Niacin, Vitamin B_6 and other minor constituents, which makes them, nutritionally and functionally different from refined sugar. [23] Besides, the processing of food in the oral cavity depends on its properties and oral physiology. [24] The cariogenic potential of these traditional sugars is a subject of debate and further in-vivo evidence is needed to determine its cariogenicity.

The novel product D-Psicose has bacterial growth less than that of positive control and the pH was above 5.5. Hence might be a potential alternative to refined sugar, for the prevention of dental caries. The study is the first of its kind to assess the cariogenicity features of the tested sweeteners using two key determinants of cariogenicity and triangulation did to assess the *S.mutans* growth increases the reliability of the results. This is a preliminary In-vitro study, hence the results have to be interpreted with caution. To conclude the cariogenicity of the sweeteners in humans in-vivo studies is required.

CONCLUSION:

Among the tested traditional sugars, honey seemed to have a less cariogenic effect when compared to that of refined sugar. Besides, the novel sweetener D-Psicose as well as the potential for being suggested as an alternative. The other tested traditional sugars though seem as cariogenic as refined sugar, the cariogenicity is still debatable. Hence, further In-vivo studies are necessary to determine the cariogenicity of these sugars in humans by measuring all four parameters for the cariogenicity of food.

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Conflict of Interest: NA

References

1. Gupta P, Gupta N, Pawar AP, Birajdar SS, Natt AS, Singh HP. Role of sugar and sugar substitutes in dental caries: a review. International Scholarly Research Notices. 2013;2013.
2. http://www.fao.org/docrep/i9166e/i9166e_Chapter5_Sugar.pdf; Accessed on 11.11.2018.
3. Kalra S, Kalra B, Gupta Y. Reduce free sugars for a healthy life. Journal of Medical Nutrition and Nutraceuticals. 2015 Jan 1;4(1):3.
4. United Nations, General Assembly. Political declaration of the High-level Meeting of the General Assembly on the Prevention and Control of Non-communicable Diseases, A/66/L. 1.
5. Gudkina J, Brinkmane A. The impact of salivary mutans streptococci and sugar consumption on caries experience in 6-year olds and 12-year olds in Riga. Stomatologija. 2010 Jan 1;12(2):56-9.
6. Moynihan P. The interrelationship between diet and oral health. Proceedings of the Nutrition Society. 2005 Nov;64(4):571-80.
7. Molan PC. The potential of honey to promote oral wellness. General dentistry. 2001;49(6):584-90.
8. Hotz C, Gibson RS. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. The Journal of nutrition. 2007 Apr 1;137(4):1097-100.
9. Muhlemann H. The effect on rat fissure caries of xylitol and sorbitol. Helv odontol acta. 1970;14:48-50.
10. Iida T, Ichihara T, Izumori K, Tokuda M, Ogawa T, inventors; Matsutani Chemical Industries Co Ltd, Kagawa University NUC, assignee. Noncarious material and anticarious agent containing rare sugar. United States patent US 8,496,915. 2013 Jul 30.
11. Chu J, Zhang T, He K. Cariogenicity features of Streptococcus mutans in presence of rubusoside. BMC Oral Health. 2016 Dec 1;16(1):54.
12. Gudkina J, Brinkmane A. The impact of salivary mutans streptococci and sugar consumption on caries experience in 6-year olds and 12-year olds in Riga. Stomatologija. 2010 Jan 1;12(2):56-9.
13. Chattopadhyay S, Raychaudhuri U, Chakraborty R. Artificial sweeteners–a review. Journal of food science and technology. 2014 Apr 1;51(4):611-21.
14. Chung MY, Oh DK, Lee KW. Hypoglycemic health benefits of D-psicose. Journal of agricultural and food chemistry. 2012 Feb 1;60(4):863-9.
15. Zero DT. Sugars–the arch criminal?. Caries research. 2004;38(3):277-85.
16. Aluckal E, Ankola AV. Effectiveness of xylitol and polyol chewing gum on salivary streptococcus

mutans in children: A randomized controlled trial. Indian Journal of Dental Research. 2018 Jul 1;29(4):445.

17. Matukubo T. Cariogenic potential index and classification of foodstuffs (author's transl). Shikai tenbo= Dental outlook. 1982(Spec Iss):140-5.
18. Stephan RM. Hydrogen ion concentration of the dental plaque. Journal of Dental Research. 1938 Jun;17(3):251-6.
19. El-Arab AM, Girgis SM, Hegazy EM, Abd El-Khalek AB. Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice. BMC Complementary and Alternative Medicine. 2006 Dec 1;6(1):6.
20. Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: a review. Iranian journal of basic medical sciences. 2013 Jun;16(6):731.
21. Mohammadi SN, Prashant GM, Kumar PN, Sushanth VH, Imranulla M, Rai R. Effect of Different Sugar Free Flavoured Chewing Gums on Salivary pH-a Double Blinded, Parallel Arm Randomized Clinical Trial. Journal of Advances in Medicine and Medical Research. 2016:1-6.
22. etty Im G. Elaine Gardner, British Dietetic Association (BDA) Spokesperson, discusses the sugar content in coconut sugar and provides related oral health advice. British Dental Journal. 2017 Nov 24;223(10):749.
23. Jaffé WR. Nutritional and functional components of non-centrifugal cane sugar: A compilation of the data from the analytical literature. Journal of food composition and analysis. 2015 Nov 1;43:194-202.
24. Feron G, Salles C. Food oral processing in humans: Links between physiological parameters, release of flavour stimuli and flavour perception of food. International Journal of Food Studies. 2018 Apr 13;7(1).