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Influence of Arenga pinnata solution on salivary pH and salivary microbiology Astha Bramhecha¹, Jogikalmat KrithikaDatta^{2*}

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ABSTRACT

Background: Palm sugar is extracted from the Palmyra palm. It is a rich source of calcium and phosphorus. It has been found to be less cariogenic and has less demineralization potential compared to refined sugar.

Aim: The aim of the study is to assess the effect of Arenga pinnata solution and refined sugar solution on salivary pH and salivary microbiology.

Material and Methods: 5 g of palm sugar and 5 g of refined sugar solutions were prepared, respectively. The participants were allocated to two equal groups, i.e., Group A: refined sugar solution (n = 10), and Group B: palm sugar solution (n = 10). Saliva samples are collected at baseline and 30 minutes after rinsing with each sugar solution. Salivary pH level and total microbial load were evaluated by salivary pH meter and bioluminometer. The Streptococcus mutans count was assessed by the culture plate method.

Results: A statistically significant difference was seen in salivary pH between the palm sugar and refined sugar groups after 30 minutes (p = 0.018). The increase in salivary microbial count after consumption of refined sugar solution was higher compared to palm sugar solution (p = 0.02).

Conclusion: A higher pH reduction was seen with refined sugar compared to palm sugar. The total microbial load was higher after consumption of the refined sugar solution, while there wasn't any significant change in Streptococcus mutans between the two groups.

Keywords: dental caries. Dietary sugars. Salivary pH. Salivary Microbiology

INTRODUCTION

Oral diseases, although preventable, are a huge health burden for many countries. They have a lifetime impact on people, causing pain, anxiety, deformity, and even death. Oral problems afflict 3.5 billion individuals worldwide, according to estimates. The most common disease, according to the Global Burden of Disease 2019 report, is untreated dental caries (tooth decay) in permanent teeth. (1) Dental caries is the localized destruction of organic and inorganic components of the enamel and dentin by acids formed as an end product of the bacterial fermentation of carbohydrates. (2)

J Popul Ther Clin Pharmacol Vol 30(14):e151–e158; 20 May 2023. This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al. In the last ten years, research has shown that eating habits, especially the consumption of free sugars, are crucial to the development of dental caries, acting as the required trigger for its occurrence and influencing other factors, including dental biofilm. Monosaccharides and disaccharides that manufacturers add to food and drinks, in addition to free sugars, include those found naturally in honey, fruit juice concentrates, syrups, and fruit juices (3).

Sucrose is the primary source of sugar in our diet, and it can contribute to tooth decay through two main processes. Firstly, it is easily disseminated in dental plaque and is efficiently metabolized by oral bacteria, lowering the pH of the plaque and demineralizing the enamel. Secondly, it is involved in the synthesis of extracellular glucans (soluble and insoluble), which are produced by enzymes with a significant affinity for sucrose. This property is unique to sucrose. (3)

In a healthy and balanced diet with low sugar intake, the abundant streptococci in the mouth can metabolize carbohydrates and produce acids, which can initiate demineralization. However, physiological mechanisms such as saliva can help restore the pH, stop the progression of lesions, and mineralize the tooth tissues at the crystal level, preventing cavities from forming. (4)

When sugars are consumed frequently and in high amounts in the diet, an ecological imbalance, known as dysbiosis, can occur in the oral microbiota. This dysbiosis can lead to increased virulence of the microorganisms in the dental biofilm due to bacterial competition, creating an unfavorable environment for oral health. (5)(6)

The authentic Indian diet traditionally consisted of a lot of sweets, but this was less of a health risk because the sugar came from whole foods (like fruit, which contains fiber, water, antioxidants, and other compounds that buffer the sugar load) and was frequently consumed in prehistoric rural South Asian environments where obesity and sedentary lifestyles were essentially nonexistent. carbohydrates In contrast, account for approximately 66-70% of total calorie intake in the modern Asian Indian diet, with refined sugar, white flour, and white rice accounting for the majority of this. (7)

Brown sugar is dissolved and then purified with phosphoric acid to create refined sugar, which is the most widely used type of sugar. Depending on where the processing takes place, it is then further decolorized by filtration through a bed of activated carbon or bone char. Refined sugar is typically sold as dried granulated sugar to prevent clumping. Due to its purity, refined cane sugar is preferred over palm sugar and jaggery powder in daily use. (8)

In place of refined cane sugar, palm sugar and jaggery powder, which contain a variety of beneficial minerals, vitamins, and antioxidants, can be used in everyday products and for nutraceutical purposes. (9) Individuals with a high caries index are more likely to consume a lot of refined sugar. Finding a sugar substitute that is equivalent to refined sugar and replacing it may minimize the risk of caries and increase quality of life. Previously our team had a rich experience in working on various research projects across multiple disciplines (10–17). Now the growing trend in this area motivated us to pursue this project. Thus, the aim of this study was to assess the salivary pH and salivary microbiology after consumption of palm sugar solution.

MATERIAL AND METHODS

This experimental study included adults between the ages of 18- 35 who were enrolled at the Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Chennai. Ethical clearance was granted by the ethical research clearance board of Saveetha Dental College, Chennai (IHEC/SDC/ENDO-2002/22/093). The effect of the consumption of two different sugars on salivary microbiology and salivary pH values was investigated. 20 adults between the ages of 18 and 35 who had at least one active carious lesion and a DMFT score of 3 were chosen as a convenient sample size for the study. The participants provided consent for participation in the study. The study excluded adults who were taking medication, had a systemic ailment, had periodontal issues, or were receiving orthodontic treatment. The subjects were informed of the study protocol.

Two varieties of sugar, namely, refined sugar and palm sugar, or *Arenga pinnata* sugar, were used in the study. The subjects were divided into two groups: the refined sugar group (Group A) (n = 10), and the palm sugar group (Group B) (n = 10). The salivary pH, salivary total microbial count, and *S.mutans* count were assessed.

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Preparation of the sugar solutions

For Group A, 5 g of refined sugar was dissolved in 100 ml of water, and 5 g of *Arenga pinnata* sugar was dissolved in 100 ml of water for Group B. Thus, a sugar solution with a 5% concentration was administered.

Salivary Sample Collection

The whole amount of saliva was then collected in a sterile container after participants had washed their mouths with water. Following the collection of baseline saliva, participants were instructed to rinse their mouths with either the refined sugar solution or the palm sugar solution, depending on to group A or group B. After 30 minutes, saliva samples were collected in a similar manner.

Measurement of Salivary pH

The pH level of the collected saliva was immediately assessed using litmus paper. To determine the salivary pH level, the litmus paper was immersed in the sterile container containing the collected saliva for 1 to 2 seconds and then applied to the scale designed as a colour table after 5 seconds. The procedure was repeated twice: once at baseline and once after 30 minutes of rinsing with the sugar solution. (Refer to fig. I)



FIGURE 1: Measurement of salivary pH

Measurement of salivary total bacterial count

A bioluminometer was used to determine the total bacterial count (SystemSURE Plus by Hygenia,USA) (refer to Fig II). A rapid method for detecting microorganisms is to use adenosine triphosphate (ATP) bioluminescence to detect microbial intracellular ATP. In living cells, ATP serves as an intermediary transporter of chemical energy. ATP reacts with the enzyme luciferase and the substrate luciferin to produce ATP bioluminescence, which can be used to measure the amount of ATP and thus help quantify the microorganisms. (18) Each participant was

instructed to rinse their mouths with water. To collect the saliva sample, a swab (UltraSnap by Hyginia, USA) was taken from the test tube and rubbed along the buccal mucosa. The swab was reinserted into the test tube. The Snap valve at the top of the swab was broken by bending the bulb. To allow the reagent to go down the shaft, the bulb was compressed. The tube was shaken for 5 seconds and inserted in the luminometer to get a reading. The reading is given in the unit RLU, or relative light units. After 30 minutes of rinsing the mouth with either sugar solution, the procedure was repeated.



FIGURE 2: Measurement of salivary total bacterial count with bioluminometer

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Measurement of salivary S.mutans count

Two samples of saliva were taken: once before the test solutions were used and once 30 minutes later. Saliva samples were serially diluted and plated on selective media (Mitis Salivarius agar) and incubated for 24 h at 37 $^{\circ}$ C. (Refer to figure III)



FIGURE 3: Measurement of Salivary s.mutans count through culture plate method

RESULTS

The influence of Arenga pinnata solution on salivary pH, salivary total bacterial count, and salivary *Streptococcus mutans* count was studied in a sample population (refer to Table I). A paired sample t-test was applied to assess the difference in salivary pH, total bacterial count, and S.mutans count at baseline and after 30 minutes of consuming either refined sugar or palm sugar.

The results of the t-test showed that there was a significant difference in salivary pH at baseline and after 30 minutes in both the refined sugar and palm sugar groups. Additionally, there was a significant difference in salivary microbial count at baseline and after 30 minutes of consuming refined sugar, but no significant difference in the palm sugar group. Similarly, there was a

significant difference in salivary *S.mutans* count at baseline and after 30 minutes of consuming refined sugar, but no significant difference in the palm sugar group.

An independent sample t-test was also applied to compare the differences between the refined sugar group and the palm sugar group. The results of this t-test showed that there was a significant difference in salivary pH after 30 minutes between the two groups. There was also a significant difference in salivary microbial count after 30 minutes of consuming refined sugar and palm sugar. However, there was no significant difference in salivary *Streptococcus mutans* count after 30 minutes of consuming refined sugar and palm sugar.

TABLE 1: Statistical analysis of pH, salivary microbiology and streptococcuS.mutans count at baseline and 30 mins after refined sugar and arenga pinnata sugar solution consumption

	Salivary pH			Salivary microbial count			Salivary S.mutans count		
Groups	Baseline	After 30 mins	p-value	Baseline	After 30 mins	p-value	Baseline	After 30 mins	p-value
Refined Sugar	10.6 ± 1.64	6.8 ± 0.78	0.00	7077 ± 517	8561 ± 865	0.005	10780 ± 2195	18900 ± 6603	0.037
Palm Sugar	10.1 ± 1.79	8.4 ±0.84	0.006	7226 ±329	7408 ± 221	0.165	18320 ± 13228	19480 ± 19675	0.199
p-value	0.524	0.00		0.602	0.02		0.244	0.217	

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DISCUSSION

Multiple factors occurring simultaneously lead to the formation of caries. The dissolution of the dental hard tissue as a result of the acid produced by the oral microorganisms through the breakdown of dietary carbohydrates or sugars leads to caries. The loss of minerals (demineralization) during the caries process occurs when the pH drops below a critical pH, and redeposition (remineralization) occurs when the pH rises again. Depending on the rate of demineralization and remineralization, if the latter is slower, a carious lesion develops. (19)

In the historic study conducted by Stephan R. rinsing the mouth with glucose solution led to an increase in the acidity of the bacterial biofilm. However, the time and extent of this increase varied depending on the degree of caries activity, with the most significant increases observed in cases with high levels of caries activity. Only in some cases with caries activity, the pH dropped below 5.0, and these low pH readings were more common in cases with the most severe levels of caries activity. The difference between the pronounced drop in pH on the labial surfaces of the upper and lower teeth was consistent with the relative vulnerability of these tooth surfaces to caries. (20)(Stephan R, 1944) The study evaluated salivary pH as it plays a crucial role in the development of caries, considering that the acidity of the oral environment, as indicated by pH levels, is a significant factor in the caries process.

The primary natural defense mechanism for the oral cavity is saliva, which is crucial for defending the exposed tooth surfaces. Mechanical rinsing, antibacterial activity, buffering ability, and the production of antimicrobial peptides are a few ways in which saliva can reverse the demineralization of the exposed tooth surface. (21,22) Hence, saliva samples were assessed in the study.

Due to the fact that salivary secretion is influenced by a number of circumstances, the study only included participants who were free of any systemic illnesses as well as those who were not taking any medications. (22)Moreover, salivary secretion is influenced by circadian rhythms, systemic illnesses, certain drugs, and psychological issues. (23,24)Although none of these variables can be completely controlled, we tried to control a few in the current investigation, including systemic illnesses, time, and olfactory variables.

Increased bacterial populations in dental plaque, S.mutans streptococci such as and lactobacilli, can lead to higher rates of acid production, disturbing the balance of the biofilm ecosystem and exacerbating demineralization. Mutans streptococci are particularly well-suited to the cariogenic environment with high sugar and low pH, as they possess traits that allow them to thrive in an acidic environment and produce acid. Under similar conditions, other bacteria may also produce acid, but at a slower rate. In the absence of higher cariogenic species, these bacteria may contribute to the early stages of demineralization or result in lesions, especially in a vulnerable host. The absence of total specificity in the microbiological aetiology of caries disease and the documented patterns of bacterial succession during the evolution of lesions may be explained by this series of events. (25)(26)

Upon examination of the study's results, it was noted that there were no notable differences in the baseline values for all three parameters between the groups. However, there was a significant discrepancy in pH and total bacterial count after a 30-minute interval in both groups. This indicates a greater decline in pH after the consumption of refined sugar solution and an overall increase in the total bacterial count, suggesting a higher risk of caries development with increased intake of refined sugar. Additionally, a noteworthy discovery was the significant increase in Streptococcus mutans count in the refined sugar group after 30 minutes, unlike the palm sugar group. This implies that palm sugar may be less cariogenic compared to refined sugar and may be considered a potential alternative.

A 3-year longitudinal study by Spunzar et al. (1995) suggested that a higher sugar intake overall was also linked to an increase in overall caries incidence. The likelihood of developing caries increases by 1% for every additional 5 g of sugar consumed daily. (27) Palm sugar causes less biofilm formation and enamel demineralization depth than sucrose (28)

Palm sugar is composed of sucrose, glucose, and fructose, with percentages of 89.94%, 3.61%, and 3.50%, respectively, whereas sugar cane only contains sucrose at 94.75%. (29) According to Ishak et al. (2013), palm sugar is considered more

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nutritious than refined sugar since it contains minerals and vitamins. (30) Palm sugars are minimally processed and can contain other components besides sugars, such as the dietary fiber inulin, which is present in substantial amounts. (31,32) This may lower the glycemic index (GI) of palm sugar compared to refined sugar, which contains almost 99% sucrose. Additionally, palm sugar contains a higher amount of vitamin C and antioxidants. Refined cane sugar is more commonly used due to its purity and ability to provide sweetness without any unwanted taste. (8)(33) An in-vitro study by Jayadevan et al. (March 2019) found that palm sugar can reduce biofilm formation and enamel demineralization depth compared to sucrose (28). Considering the health concerns associated with increased sugar consumption, aren sugar, or arenga pinnata sugar, can be used as a healthy alternative. (34)

Our institution is passionate about high quality evidence based research and has excelled in various fields.(35)(11–13,15,35–42).This is the first clinical investigation that examines the cariogenic potential of refined sugars and Arenga pinnata sugar, as far as the author is aware. The limited sample size, which is regarded as an important limitation of the study, could be the cause of the lack of a significant difference in the Streptococcus mutans count.

CONCLUSION

Overall, these results suggest that the consumption of refined sugar may have a more detrimental effect on salivary pH and microbial count compared to the consumption of palm sugar. Further research is needed to fully understand the mechanisms behind these differences and to determine the potential benefits of using Arenga pinnata solution as a means of mitigating the negative effects of refined sugar on oral health.

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Data Availability

Upon request to the corresponding author,

datasets related to this article will be available.

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