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The effect of coconut sugar on cariogenic traits in *Streptococcus mutans*

Efeito do Açúcar de Coco na cariogenicidade do *Streptococcus mutans*

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ABSTRACT

Aim: The consumption of foods rich in sugar is linked to several non-communicable diseases, including dental caries. Coconut sugar has systemic benefits due to its lower glycemic indexes (GI) than other table sugars. However, there is currently no data regarding its cariogenic potential. This study aimed to evaluate the effect of coconut sugar on acidogenicity and adhesion of *Streptococcus mutans in vitro,* compared to sugarcane products. **Materials and methods:** Aliquots of cultures of S. mutans UA159 were resuspended in a buffer solution enriched with coconut sugar, crystal sugar (refined sugar), and minimally processed sugarcane (demerara light brown sugar and maskavo dark brown sugar), as well as positive (sucrose) and negative controls. The decrease in pH and its corresponding area under the curve (AUC; cm^2) were evaluated for the analysis of acidogenicity. *S. mutans* was incubated in BHI supplemented with each sugar and the percentages of microbial adhesion were calculated. After testing data normality, the one-way ANOVA test (Bonferroni post hoc) was used to compare the AUC and the proportion of adhesion of each group. **Results:** Regarding the acidogenic potential, statistical differences were found only between the negative control *versus* all other groups ($p<0.001$). Likewise, no significant difference in adhesion was found between the tested sugars ($p>0.05$). **Discussion:** Although the tested sugars are marketed as "healthy products," their amount and frequency of usage should be controlled. **Conclusion:** Coconut sugar presents a similar cariogenic potential to that of sugarcane products when acidogenicity and adhesion

RESUMO

Objetivo: O consumo de alimentos ricos em açúcar está associado a diversas doenças não transmissíveis, incluindo a doença cárie. O açúcar de coco tem benefícios sistêmicos devido aos seus índices glicêmicos (IG) mais baixos do que outros açúcares de mesa. No entanto, atualmente não há dados sobre seu potencial cariogênico. Esse estudo teve como objetivo avaliar o efeito do açúcar do coco na acidogenicidade e adesão de *Streptococcus mutans in vitro*, em comparação com produtos derivados da cana-de-açúcar. **Materiais e métodos:** alíquotas de culturas de *S. mutans* UA159 foram suspensas em solução tampão enriquecida com açúcar de coco, açúcar cristal (açúcar refinado) e cana-de-açúcar minimamente processada (açúcar mascavo demerara claro e açúcar mascavo), além de controles positivo (sacarose) e negativo. A diminuição do pH e correspondente área sob a curva (AUC; cm^2) foram avaliadas na análise de acidogenicidade. *S. mutans* foi incubado em BHI suplementado com cada tipo de açúcar e as porcentagens de adesão microbiana foram calculadas. Após testar a normalidade dos dados, o teste ANOVA de uma via (Bonferroni post hoc) foi utilizado para comparar a AUC e a proporção de adesão de cada grupo. **Resultados:** Em relação ao potencial acidogênico, diferenças estatísticas foram encontradas apenas entre o controle negativo *versus* todos os outros grupos ($p <0,001$). Não foi encontrada diferença significativa na adesão entre os açúcares testados ($p> 0,05$). **Discussão:** Apesar dos açúcares testados serem comercializados como "produtos saudáveis", sua quantidade e frequência de consumo deve ser controlada. **Conclusão:** O açúcar do coco apresenta potencial cariogênico semelhante à dos produtos da ca-

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are evaluated. Coconut sugar is not indicated as a substitute for sucrose in the control of cariogenic activity.

Keywords: Dental caries. Sugars. Sweetening agents. Cariogenic agents. Bacterial adhesion.

na-de-açúcar quanto a acidogenicidade e a adesão. O açúcar de coco não é indicado como substituto da sacarose no controle da atividade cariogênica.

Palavras-chave: Cárie dentária. Açúcares. Edulcorantes. Cariogênicos. Aderência bacteriana.

Introduction

Sugars contribute to the positive energy that is fundamental for maintaining healthy body weight and nutritional balance[1]. However, there is a growing body of literature that recognizes the contribution of high levels of sugar intake to the incidence of several chronic non-communicable diseases[2]. Furthermore, the increased sugar intake unquestionably impacts the development of dental caries[3,4]. Sugar consumption drives ecological unbalance between members of the microbial community of dental biofilms by locally creating a low pH, and subsequent selective pressure for some members of the microbiota leading to a dominance of acid-producers pathobiontes[3]. If maintained, this unbalance can break the functional stability of the oral microbiome leading to caries.

To prevent chronic non-communicable diseases, the World Health Organization (WHO) recommends the consumption of a more natural, minimally processed and healthier diet[1]. In this context, the use of minimally processed sugar and food with low glycemic indices (GI) has been adopted. However, it is still not known whether these substitutes for refined sugar could possess lower cariogenic potential. Low GI foods are metabolized slowly and have played an important role in the dietary management of diseases such as diabetes, weight loss, and reduced the risk of heart disease and hypertension[5]. In addition, they have shown to contribute to a superior performance during sporting activities. Among the low GI index foods, the use of coconut sugar as a sweetening agent in foodstuffs has been increasing[6]. It is produced using the sap of the coconut tree (normally using a method where the sap is heated at a high temperatures) and it is similar to brown, granulated sugar in physical characteristics and flavor[7]. While refined sugar from sugarcane generally has a high GI ranging from 56 to 69, coconut sugar (or coconut palm sugar), produced using the sap of the coconut tree, has a much lower glycemic index (GI ≤ 55, on average, 35)[8,9]. However, its composition is essentially sucrose (approximately 70%), with 3–5% of glucose and 5–9% of fructose[10]. Those components are easily metabolized by saccharolytic bacteria, which leads to the frequent exposure of dental biofilms to a low pH. Consequently, inhibition of the growth of acid-sensitive species and a selection of organisms with aciduric physiology are expected. This condition can cause an imbalance in the microenvironment, and, consequently, can lead to the development of caries lesions[11–13]. However, despite the systemic benefits[6], there are still no studies on the behavior of this sugar related to dental caries markers.

To date, there are few studies that have investigated the association between the composition and form of obtaining added sugars with their cariogenicity. Examples of added sugars include: white sugar, brown sugar, raw sugar, corn syrup, malt syrup, fructose sweetener, honey, molasses, dextrose, and dextrin[10]. Fructose-rich corn syrup and inverted sugar syrup are half fructose and half glucose, and are expected to have a lower cariogenicity than sucrose, due to the lack of extracellular polysaccharide production capacity[14]. However, evidence showed an increased acidogenicity of *S. mutans*, and a decreased adhesion in biofilms formed in the presence of fructose-rich corn syrup compared to sucrose[15]. Another study suggests that sugar which has been minimally processed preserves its mineral content, and that during the metabolism of sugar by bacteria from the biofilm, this mineral content may interact in the demineralization/remineralization process and lead to a different cariogenic response[16]. It was previously shown that cane juice and molasses protect against decalcifi-

cation due to the presence of calcium and potassium, with a significantly lower incidence of caries with minimally processed sugar than refined ones in *in vitro* and animal model studies. The cariostatic effect was also evident when brown sugar was used to bake bread and a synergistic effect was exerted on the brown sugar by adding phosphate[16]. Regarding sugars derived from origins other than sugarcane, studies comparing fresh sugar beets with refined sugarcane did not demonstrate any caries-inhibitory property of the beets, although sugar beet, sugarcane-produced juices, and brown sugar have very different compositions[17]. Interestingly, a correlation has been demonstrated between GI and the area under the curve for plaque pH for breads, suggesting that 'low GI breads' present less accentuated cariogenic potential, but no such information was found regarding the low GI sugars[18].

This lack of studies in the literature indicates a need to understand the cariogenic potential of minimally processed sugar and sugars derived from origins other than sugarcane with a low GI, such as coconut sugar derivates. Thus, the aim of this study was to evaluate the effect of coconut sugar compared to other sugar derived from sugarcane (minimally processed sugar and refined crystal sugar) on the cariogenic traits of *S. mutans in vitro*.

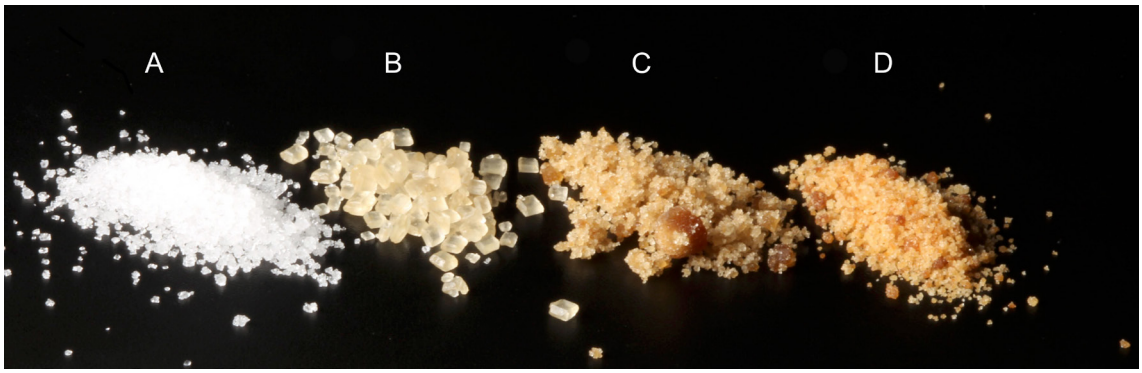
Materials and methods

Sugars

Figure 1 shows the physical characteristics of the sugars tested in this study. Saline was used as a negative control (Saline; NaCl 0.9%) and pure sucrose (*Vetec*®, Brazil) was used as a positive control. Four different sugars were used, and concentrations varied according to the experiment (all sugars including the pure sucrose were tested at same concentrations in each experiment):

- crystal sugar – sugarcane product with large white crystals (União®, lot t053, Brazil) (Figure 1a);
- demerara light brown sugar – raw sugar, a light brown sugar, sucrose sugarcane product with large golden crystals (*Foco Alternativo*®, Lot number 0084, Brazil) (Figure 1b);
- *maskavo* dark brown sugar – sucrose sugarcane product with a distinctive brown color due to the presence of molasses (*Saúde da Terra Produtos Orgânicos*®, Lot number 03/16, Brazil) (Figure 1c);
- coconut sugar – sucrose, glucose, and fructose sugar product extracted from coconut (Copra®, lot 615433015, Brazil) (Figure 1d).

Figure 1: Physical characteristics of sugarcane and coconut sugar. A = crystal refined sugarcane; B = demerara light brown sugarcane; C=*maskavo* dark brown sugarcane; D=coconut sugar.



Microorganism and culture conditions

The bacterial strain *Streptococcus mutans* UA159 used in this study was kept at -20 °C in sterile skim milk 10% (wt/vol; Difco, Dublin, Ireland). First, 50 µl of frozen bacterial stock was cultured on 10 mL Brain Heart Infusion (BHI) broth supplemented with 0.5% sucrose and incubated at 37 ºC overnight (18 h). Afterwards, 50 µl of this suspension was inoculated on BHI agar (Kasvi, Curitiba, Brazil) at 37 ºC for 48 h, and this subculture was used for the experiments described below.

Acidogenic potential analysis

The protocol that was used to determine acidogenic potential was previously described in earlier studies[19,20]. Briefly, approximately 10^8 CFU/mL (two loops of 1 µL, corresponding to the tube 0.5 in the McFarland scale) aliquots of the *S. mutans* culture were inoculated into 30 mL of BHI broth supplemented with 1% glucose and incubated at 37 ºC for 18 h. The cultures were centrifuged, and the pellets were resuspended in 10 mL of a buffer solution (50 mM KCl/1 mM MgCl2). The pH of the solution was adjusted to around 7.0 and each sugar was added to each tube at a final concentration of 5%. The decrease in pH was evaluated for 180 min using a glass electrode previously calibrated with pH standards (pH 4.0 and 6.8). Values of pH at each time point and for each sugar were tabulated, plotted in a standardized way (same length and width), and then individually imported into UTHSCSA ImageTool® software, version 3.0 (Image Tool Software Copyright). The area under the curve (AUC) was manually delimitated by drawing tools. Spatial measurements were calibrated previously to the AUC delimitation. AUC was calculated considering pH 7.0 as a cutoff point. The same examiner (AGR) performed all the analysis, in order to avoid divergences. All delimitation was done three times for each tested condition. The acidogenic potential was expressed as the AUC (cm^2). Each analysis was performed in triplicates.

Analysis of the microbial adhesion

Microbial adhesion in the presence of each sugar was analyzed as previously standardized and described elsewhere[21]. Two loops of colony growth (approximately 10^8 CFU/mL aliquots) of *S. mutans* UA159 were transferred to a clean test tube (tube A), containing 3 mL of BHI supplemented with 0.5% of each sugar. The cultures were incubated for 18 h at a 30º angle, in an anaerobic atmosphere (95% N2, 5% CO2) at 37º C. After incubation, the tube (A) was rotated twice around to read its long axis, and the detached bacterial cells were transferred to a second tube (B) for according tube single hum movement. Next, 3 mL of a phosphate buffer (0.05 M PBS, pH 6.8) was added to tube A, and this tube was rotated again. The released bacterial cells were transferred to a third tube (C). Tubes B and C were centrifuged at 3000 rpm for 15 min and the supernatants were discarded. The buffer solution was added (3 mL) to all tubes and the cells were dispersed by vortexing. The optical density (OD) was determined by a spectrophotometer at 540 nm. Percentages of microbial adherence with different sugars were calculated according to the following formula: proportion of adherence = ODa/(ODa + ODb + ODc)*100.

Statistical analysis

A normal distribution was tested and confirmed by the Kolmogorov–Smirnov and Shapiro-Wilk tests, as well as with a histogram. One-way ANOVA was used to determine whether there was any difference in the AUC (in cm^2) and proportion of adherence within groups (%), with Bonferroni post hoc tests. The significance level was set at 5%. All statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

These experiments *in vitro* showed that there are no differences in the acidogenic potential (Figure 2 and Table 1) and adhesion (Table 2) of *S. mutans* UA159. Regarding the acidogenic potential, the AUC showed a range from 33 to 34 cm^2 in tested sugars and statistical differences were found only when comparing the negative control to other groups (difference of approximately 15 cm^2 from negative control to tests; $p<0.001$). The pH curve showed that all sugars had sufficient acidity to demineralize the dental enamel surface, achieving the critical pH for enamel demineralization between 5 and 10 minutes (pH lower than 5.5), although there was no significant difference between the groups (Figure 2; $p>0.05$). The curve reached values lower than pH 4 after 30 minutes and a plateau can be observed after 120 min. In terms of microbial adhesion, the percentage of adherent cells of *S. mutans* was similar in the presence of coconut sugar in comparison to the other sugarcane products (Table 2). The proportion of microbial adherence ranged from 38 to 46% for each sugar, with a total average of 42%. A minimum adherence of 20% and maximum adherence of 66% was found in the presence of the tested sugars.

Figure 2: Acidogenic potential represented by the pH curve of *S. mutans* UA159 on refined sugarcane ("crystal sugar"), minimally processed sugarcane ("demerara" and "*maskavo*"), and "coconut sugar". Positive control (sucrose) and negative control (saline) are also represented. X-axis: time, in minutes ; Y-axis: pH.

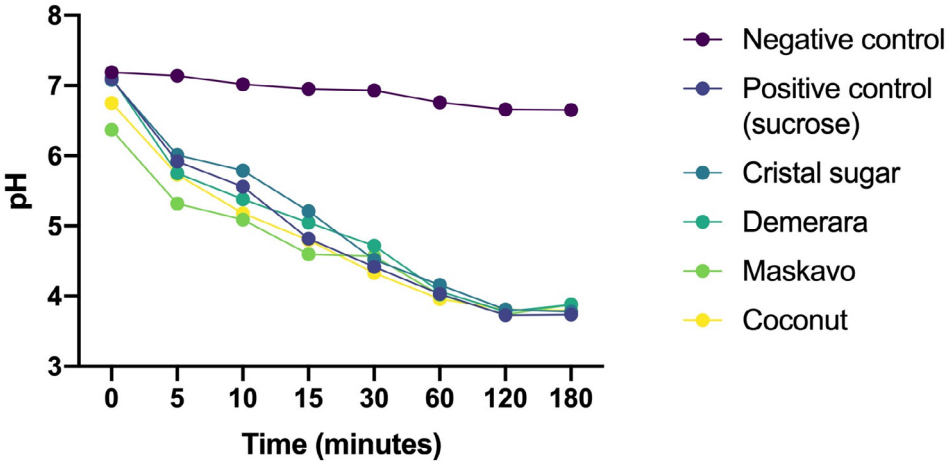


Table 1: Acidogenic potential of *S. mutans* UA159 represented by the area under the curve (AUC; cm^2) on refined sugarcane ("crystal sugar"), minimally processed sugarcane ("demerara" and "*maskavo*"), and "coconut sugar".

Sugar	AUC (Mean ± SD)
Negative control (Saline)	48.1 ± 1.4[a]
Coconut sugar	33.5 ± 1.4[b]
Mascavo dark brown sugar	33.0 ± 4.7[b]
Demerara light brown sugar	33.2 ± 3.3[b]
Crystal sugar	34.2 ± 1.2[b]
Positive control (Sucrose)	33.0 ± 1.5[b]

Different letters denote the significant difference between groups ($p<0.05$) by ANOVA.

Table 2: Percentage of adhesion of *S. mutans* UA159 on refined sugarcane ("crystal sugar"), minimally processed sugarcane ("demerara" and "maskavo"), and "coconut sugar".

Sugar	Mean	Minimum	Maximum	Std. Deviation
Coconut sugar	45.50	37	66	10.44
Maskavo dark brown sugar	37.50	21	56	11.88
Demerara light brown sugar	38.00	20	52	13.37
Crystal sugar	41.67	31	58	10.05
Positive control (Sucrose)	46.33	32	60	10.91
MEAN	41.8	28.2	58.4	11.33

ANOVA; p>0.05

Discussion

Sugar intake levels contribute to the incidence of several non-communicable diseases, including dental caries. Recently, coconut sugar has been gaining popularity due to its low glycemic index, which may have a systemic benefit. However, no evidence has been found regarding its cariogenic potential. In this study, the effect of coconut sugar on two cariogenic traits (acidogenicity and adhesion) of *S. mutans* was investigated *in vitro* and compared to minimally processed and refined sugarcane. It was found that *S. mutans* has a similar acidogenic potential and adhesion in solutions containing sucrose (positive control), white crystal sugar, demerara light brown sugar, *maskavo* dark brown sugar, and coconut sugar. Although benefits in terms of glycemic control indicate its use as a "table sugar" substitute for sucrose (in diabetic individuals, for example), the results of this study indicate that some caution should be taken regarding its cariogenicity.

Minimally processed sugars from sugarcane and coconut sugar could be healthy alternative sweeteners as they provide benefits in terms of GI values and the presence of various minerals. It was suggested that sugar, as it occurs in nature, are supplemented by a "protective agent" which is removed, in some or most part, during the sugar refining process[16]. Anti-cariogenic properties or 'cariostatic factors' of some foods, such as milk or cheese are suggested. The presence of inorganic phosphates has also been included in these protective factors[16]. This would result in a lower cariogenicity in the minimally processed sugars. In this context, we tested here Mascavo dark brown sugar and Demerara light brown sugars, and some previous experiments were carried out to test this hypothesis *in vitro*[20,21]. Properties of less refined mealie, from which only about 10% of the grain had been eliminated, have been tested. The results indicate that the degree of refinement does not significantly increase the decalcifying potency of the carbohydrate. Furthermore, these results showed that the naturally occurring carbohydrates of sugarcane, the mealie and, more specifically that of the wheat, also caused decalcification, reaching acidity of the order of pH 4–5, rejecting the hypothesis of these food items having a lower cariogenic trait[22,23]. Although new, healthier free sugars have been used as sweeteners, our study corroborates with their results, showing that minimally processed sugarcane (Mascavo and Demerara) had similar cariogenic properties to refined sugarcane like microbial adherence and acidogenic potential.

As described before, the composition of coconut sugar is 70% sucrose, with a small proportion of glucose (3–5%) and fructose (5–9%). Although sucrose is well known as the most cariogenic sugar, these sugars are all capable of reducing the pH to levels needed to demineralize enamel[24]. However, it has been shown that dental biofilm formed in the pre-

sence of frequent exposure to sucrose was more cariogenic than dental plaque formed in the presence of glucose + fructose, due to the synthesis of insoluble extracellular polysaccharides and the lower concentrations of calcium, phosphate, and fluoride in dental biofilm formed in the presence of sucrose[24]. Although sucrose is predominant, different proportions of other sugars might influence metabolic pathways of microbial biofilms in contact with coconut sugar when compared to sugarcane derivatives. A recent study showed by advanced mRNA sequencing methods that the presence of sucrose and fructose significantly alters the gene expression of *S. mutans*, affecting its energy metabolism, acid production, stress tolerance, cell-to-cell communication, and bacterial evolution by lateral gene transfer[25]. Fructose modifies the expression of a great number of genes related to virulence[25]. Furthermore, as suggested before, high-fructose sugars could present a lower extracellular polysaccharide production; thus, the presence of fructose in the coconut sugar could lead to different cariogenic traits in *S. mutans*, but its presence in coconut sugar did not reduce the acidogenicity and adhesion of *S. mutans*. These observations on the connections between the type of commercialized sugar and its potential clinical impact could help advance the treatment of caries, based on diet evaluation and sugar consumption.

Our findings indicate that the GI of the coconut sugar could not infer any lower cariogenic trait, being important to alert patients with caries that they are not suitable as a sucrose substitute. Without this indication, the treatment of dental caries could be a failure. These results, therefore, need to be interpreted with caution. This is an *in vitro* analysis, considering only one planktonic microorganism. Dental biofilm response of these sugars should be better studied in order to observe whether there are different metabolic pathways in saccharolytic bacteria in the presence of particular molecules in its composition. Studies evaluating the transcription of microorganisms in biofilms might explain whether there is any influence of the glucose and fructose from coconut sugar composition in bacterial metabolism, as well as the role of each type of carbohydrate. Although these tested sugars are marketed as "healthy products," their amount and frequency of usage should be controlled. It is still critical to adopt a new policy for sugar reduction as a means of controlling dental caries, and primarily other systemic diseases, such as diabetes and obesity[26].

Conclusion

Based on the results of this study, *S. mutans* metabolizes coconut sugar, presenting an acidogenicity and adhesion similar to sugarcane products. Coconut sugar is not indicated as a substitute for sucrose in the control of cariogenic activity.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1 World Health Organization. Guideline: sugars intake for adults and children. Geneva: World Health Organization; 2015.

2 'Dietary sugars and human disease': conclusions and recommendations. Br Dent J. 1990 Jan 20;168(2):46. doi: 10.1038/sj.bdj.

3 Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology. 2003;149(Pt 2):279-94.

4 Sheiham A, James WP. Diet and dental caries: the pivotal role of free sugars reemphasized. J Dent Res. 2015;94(10):1341-7. doi:10.1177/0022034515590377.

5 Srikaeo K, Thongta R. Effects of sugarcane, palm sugar, coconut sugar and sorbitol on starch digestibility and physicochemical properties of wheat based foods. International Food Research Journal. 2015;22(3):923-9.

6 Drewnowski A, Tappy L, Forde CG, McCrickerd K, Tee ES, Chan P, *et al*. Sugars and sweeteners: science, innovations, and consumer guidance for Asia. Asia Pac J Clin Nutr. 2019;28(3):645–63.

7 Asghar M, Yusof Y, Mokhtar M, Ya'acob M, Mohd Ghazali H, Varith J, *et al*. Processing of coconut sap into sugar syrup using rotary evaporation, microwave and open-heat evaporation techniques. J Sci Food Agric. 2020 Apr 26;100.

8 Augustin LS, Kendall CW, Jenkins DJ, Willett WC, Astrup A, Barclay AW, *et al*. Glycemic index, glycemic load and glycemic response: an international Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC). Nutr Metab Cardiovasc Dis.2015;25(9):795-815. doi:10.1016/j.numecd.2015.05.005.

9 Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values. Am J Clin Nutr. 2002;76(1):5-56.

10 Purnomo H. Sugar componentes of coconut, in indonesian. Asian food J. 2001;7-200.

11 Ervin RB, Ogden CL. Consumption of added sugars among U.S. adults, 2005-2010. NCHS Data Brief. 2013;122:1-8.

12 Marsh P, Nyvad B. The oral microflora and biofilms on teeth. Dent Caries Dis Its Clin Manag. 2008 Jan 1;163–87.

13 Marsh PD. Microbiologic aspects of dental plaque and dental caries. Dent Clin North Am. 1999;43(4):599-614, v-vi.

14 Takahashi N, Nyvad B. Caries ecology revisited: microbial dynamics and the caries process. Caries Res. 2008;42(6):409-18. doi:000159604.

15 Frostell G, Birkhed D, Edwardsson S, Goldberg P, Petersson LG, Priwe C, *et al*. Effect of partial substitution of invert sugar for sucrose in combination with Duraphat treatment on caries development in preschool children: the Malmö Study. Caries Res. 1991;25(4):304–10.

16 Ma R, Sun M, Wang S, Kang Q, Huang L, Li T, *et al*. Effect of high-fructose corn syrup on the acidogenicity, adherence and biofilm formation of Streptococcus mutans. Aust Dent J. 2013 Jun;58(2):213–8.

17 Jenkins GN. Natural protective factors of foods. Symp Swed Nutr Found. 1965;3:67–74.

18 Lingström P, Liljeberg H, Björck I, Birkhed D. The relationship between plaque pH and glycemic index of various breads. Caries Res. 2000;34(1):75–81.

19 Damé-Teixeira N, Arthur RA, Parolo CC, Maltz M. Genotypic diversity and virulence traits of Streptococcus mutans isolated from carious dentin after partial caries removal and sealing. Scientific WorldJ ournal. 2014;165201. doi:10.1155/2014/165201.

20 Stegues CG, Arthur RA, Hashizume LN. Effect of the association of maltodextrin and sucrose on the acidogenicity and adherence of cariogenic bacteria. Arch Oral Biol. 2016 May;65:72–6.

21 Arthur R, Cury A, Mattos-Graner R, Rosalen P, Vale GC, Leme A, *et al*. Genotypic and phenotypic analysis of S. mutans isolated from dental biofilms formed in vivo under high cariogenic conditions. Braz Dent J. 2011 Jan 1;22:267–74.

22 Osborn TWB, Noriskin JN, Staz J. A comparison of crude and refined sugar and cereals in their ability to produce in vitro decalcification of teeth. J dent res. 1937; 16(165). doi:10.1177/00220345370160030201.

23 Rothman KJ, Greenland S. Causation and causal inference in epidemiology. Am J Public Health. 200; 95 Suppl 1, S144-150. doi:95/S1/S144.

24 Cury JA, Rebelo A, Cury DBAA, Derbyshire MT, Tabchoury CP. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res. 2000;34(6):491-7. doi:16629.

25 Zen L, Burne RA. Sucrose- and fructose-specific effects on the transcriptome of streptococcus mutans, as determined by RNA sequencing. Appl Environ Microbiol. 2015;82(1):146-56. doi:10.1128/AEM.02681-15.

26 Srikaeo K, Thongta R. Effects of sugarcane, palm sugar, coconut sugar and sorbitol on starch digestibility and physicochemical properties of wheat based foods. International Food Research Journal. 2015;22(3):923-9.