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Evaluation of salivary buffer capacity in dental biofilms in vivo exposed to acidic drinks

Evaluación de la capacidad tampón salival en biopelículas dentales in vivo expuestas a bebidas ácidas
Avaliação da capacidade tampão salivar em biofilmes dentais in vivo expostos a bebidas ácidas

ABSTRACT
Objective: to evaluate the cariogenic and erosive potential in vivo of different acidic drinks. Method: the pH of the dental biofilm was measured by a micro-electrode type BEETRODE® (WPI Inc., England) coupled to a potentiometer (Orion 230 A). The measurement moments were in the "baseline", 5, 10, 15, 20, 25 and 30 minutes. The data were analyzed and the level of significance was 5%. Results: all beverages analyzed have a minimum pH below 5.5, characterizing their cariogenic potential, 75% have a minimum pH below 4.5, also confirming their erosive potential. Conclusion: all acid drinks analyzed have cariogenic and erosive potential. Soft drinks cause a more pronounced drop in pH immediately, except in the absence of sucrose, where the drop in pH is less prolonged. The industrialized liquid grape juice was the one that came closest to water, being the product of the group less cariogenic and erosive.
DESCRIPTORS: pH, Fruit juices, Acid drinks, Caries, Dental erosion.

RESUMEN
Objetivo: evaluar el potencial cariogénico y erosivo in vivo de diferentes bebidas ácidas. Método: se midió el pH del biofilm dental mediante un microelectrodo tipo BEETRODE® (WPI Inc., Inglaterra) acoplado a un potenciómetro (Orion 230 A). Los momentos de medición fueron en la "línea base", 5, 10, 15, 20, 25 y 30 minutos. Los datos fueron analizados y el nivel de significancia fue del 5%. Resultados: todas las bebidas analizadas tienen un pH mínimo por debajo de 5.5, caracterizando su potencial cariogénico, el 75% tiene un pH mínimo por debajo de 4.5, lo que también confirma su potencial erosivo. Conclusión: todas las bebidas ácidas analizadas tienen potencial cariogénico y erosivo. Los refrescos provocan inmediatamente una caída más pronunciada del pH, excepto en ausencia de sacarosa, donde la caída del pH es menos prolongada. El mosto líquido industrializado fue el que más se acercó al agua, siendo el producto del grupo menos cariogénico y erosivo.
DESCRIPTORES: pH, Jugos de frutas, Bebidas ácidas, Caries, Erosión dental.

RESUMO
Objetivo: avaliar o potencial cariogênico e erosivo in vivo de diferentes bebidas ácidas. Método: o pH do biofilme dental foi medido por um micro-eletrodo tipo BEETRODE® (WPI Inc., Inglaterra) acoplado a um potenciômetro (Orion 230 A). Os momentos de medição foram no "baseline", 5, 10, 15, 20, 25 e 30 minutos. Os dados foram analisados e o nível de significância foi de 5%. Resultados: todas as bebidas analisadas apresentam pH mínimo inferior a 5,5, caracterizando seu potencial cariogênico, 75% apresentam pH mínimo inferior a 4,5, confirmando também seu potencial erosivo. Conclusão: todas as bebidas ácidas analisadas possuem potencial cariogênico e erosivo. Os refrigerantes causam uma queda de pH mais acentuada de imediato, exceto na ausência de sacarose, em que a queda do ph é menos prolongada. O suco de uva líquido industrializado foi o que mais se aproximou da água, sendo o produto do grupo menos cariogênico e erosivo.
DESCRITORES: pH, Sucos de frutas, Bebidas ácidas, Cárie, Erosão dental.

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INTRODUCTION

Dental caries has accompanied man for thousands of years, and is still considered a public health problem, despite the decline in its prevalence. [1] It is a multifactorial disease, as it results from the interaction of three primary factors in the individual: microorganism, substrate and host.

As the dental surface is constantly exposed to external products from the diet, microbial metabolism and hygiene and therapeutic products, the enamel can undergo a demineralization process caused by acids resulting from the metabolism of sucrose by oral microorganisms, in addition to acids from diet or intrinsic due to regurgitation.

The diet plays a central role in the development of caries disease, studies show the causal relationship between the consumption of fermentable carbohydrates and the development of carious lesions. [2] Sucrose plays an important role in the formation of dental biofilm, promoting greater induction of caries. [3] Dietary factors related to both products, in the case of diet, such as the type of carbohydrate, carbohydrate concentration, adhesiveness, with emphasis on the retention time and protective components present; as for the factors related to the individual, such as the frequency of ingestion and time of removal in the mouth, influence the greater or lesser risk of developing caries or dental erosion. [4]

Concomitantly with the decline of caries, there have been changes in the eating habits of the populations with an increase in the intake of acidic foods and drinks that are recognized as etiological factors for tooth erosion.

In addition to diet, another factor of great influence in caries disease is saliva. Salivary buffers are mainly derived from the bicarbonate and phosphate systems, correcting the pH changes that occur by the formation of acidic and basic ions, for example, by the fermentation of sugars. [5]

Much knowledge is already established and can elucidate the etiology of tooth decay and erosion, however, the effect of unmodified acidic and sugary foods, such as soft drinks and industrialized orange, guava and grape juices, on the Biofilm pH in vivo.

Given the above, the objective of the study was to evaluate the buffer capacity of saliva in dental biofilms in vivo exposed to the liquid foods mentioned to estimate the risk of dental demineralization (caries and erosion) as well as to evaluate the biofilm pH variation as a function of time and estimate the risk of caries and dental erosion according to the results obtained.

METHODS

The present study was developed at the Laboratory of Oral Biology (LABIAL - Laboratório de Biologia Bucal) of the Health Sciences Center (CCS - Centro de Ciências da Saúde) of the Federal University of Paraíba (UFPB) in two stages: laboratory and clinical. In the laboratory stage, the pH of the selected acidic drinks was determined in vitro and in the clinical stage these drinks were tested in vivo, when the pH of the dental biofilm was measured in vivo when exposed to such drinks.

The selection of liquid foods was carried out from the database of the research "First Diagnosis of the Food and Nutritional Situation of the population of the Municipality of João Pessoa/PB" or, in the original language, "Primeiro Diagnóstico da Situação Alimentar e Nutricional da população do Município de João Pessoa/PB". [6]

The foods were selected from three categories or groups: a) carbonated acidic drinks (cola-based drinks - with and without sugar - and the Paullinia cupana base - with and without sugar); b) liquid and powdered industrialized juices (orange, guava, grape flavors); c) positive and negative controls, respectively fresh lemon juice and water.

The study included 8 volunteer patients, of both sexes (4 males and 4 females), aged between 18 and 27 years old, who attended the UFPB Clinic of Cariology in the first semester of 2012. The sample was pre-selected based on the assessment of oral hygiene and the presence of dental biofilm, and patients with good oral hygiene standards (OHI-S <1.1) and without evident white spot lesion, who had at least 20 teeth, were selected natural with two index teeth (molars present), who agreed to sign the informed consent form, were not taking any medication and who agreed with the study protocol.

The products were kept at room temperature until the moment of analysis. Powdered juices were prepared with filtered water (without deionization process) to simulate domestic use. Preparations and dilutions followed the manufacturers' recommendations (1,5g/50 ml.).

First, the electrodes were calibrated with standards of 4,0 and 7,00. The readings were performed in triplicate on the specific electrode (model 91-02 Orion Research Corp. Cambridge Mass, USA) coupled to a potentiometer (model 290A +, Orion Research Corp., Cambridge, Mass., USA), obtaining the average and standard deviation of the pH of each drug and drink. Before analysis, they were subjected to agitation of the material in a magnetic stirrer (model MA-162, Marconi, Brazil).

The procedures for the research

complied with the guidelines and regulations governing research involving human subjects, approved by the Research Ethics Committee of the Health Sciences Center (CCS) UFPB under the CAAE No. 0005.0.426.126-11, according to the ethical standards for research involving human beings, based on Resolution 196/96 of the National Health Council/National Commission for Ethics in Research.

For data collection, a clinical record containing data on the volunteer (date of birth, age and sex) and table for recording the pH values of the dental biofilm were used.

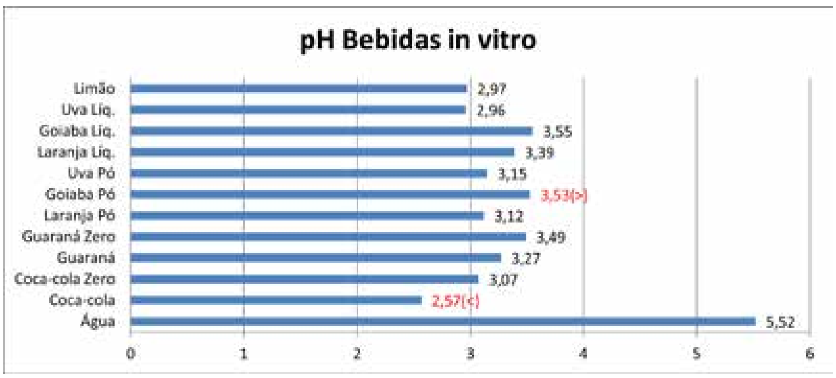
All volunteers were instructed not to brush their teeth 24 hours before the experiment. On the day of collection, a 2 mL sample of each tested drink was dripped onto the buccal surface of the tooth with the greatest presence of dental plaque. The dripping of the product was performed only once in order to observe its effect on dental biofilm in vivo. It is therefore characterized as a short-term project. All 8 volunteers were submitted to the 12 tested products.

To measure the pH of the biofilm, a micro-electrode type Beetrode (WPI Inc., England) coupled to a potentiometer (Orion 230 A) was used. The calibration of the device was constantly performed with pH 4,01 and 7,00 standards. The measurement moments were in the "baseline" (0 - before dripping), 5, 10, 15, 20, 25 and 30 minutes. Only the proximal and cervical thirds of anterior teeth were measured. The data were recorded

in a clinical form. This electrode has the advantage of the precision of the measured values. In the presence of defects, it was replaced with pH tape.

For statistical analysis, data were transferred to a computerized database and analyzed using descriptive statistics techniques, using absolute and percentage distributions and statistical techniques, using the one-way ANOVA and Wilcoxon test. The pH values were tabulated to obtain AUC (under curve area) following the mathematical formula of the Riemann integral. The level of significance adopted was 5%. The statistical package SPSS (Statistical Package for Social Science) version 16.0 was used.

Graph 1 - Distribution of acidic drinks according to the average pH in vitro.



Source: In vitro analysis performed at LABIAL, 2012.

Table 1 - minimum pH, mean pH and total auc of the beverage groups

Bebidas	pHmin	meanpH	auctotal
Água	5,40a	5,82a	31,67a
Refrig.	4,28b	4,98b	57,24b
Sucos	4,23b	4,93b	58,72b
Limão	3,83b	4,49b	74,50c

Source: SPSS 16.0

RESULTS

In the in vitro analysis of the 12 researched drinks, all had a pH lower than the critical pH for enamel demineralization, which is 5,5, and the average for the group of carbonated acid drinks was 3,1 (± 0,020), for the group of industrialized juices it was 3,28 (± 0,043), for lemon juice it was 2,97 (± 0,045) and for mineral water it was 5,42 (± 0,027). The cola-based drink, the normal version, had the lowest pH (2,57) while the powdered guava juice had the highest pH (4,24) (Graph1).

Table 1 shows that there is a statistically significant difference between the pH of the water in relation to the soft drink and juice groups and to the lemon, but the same significance is not present between the soft drink group in relation to the juice group.

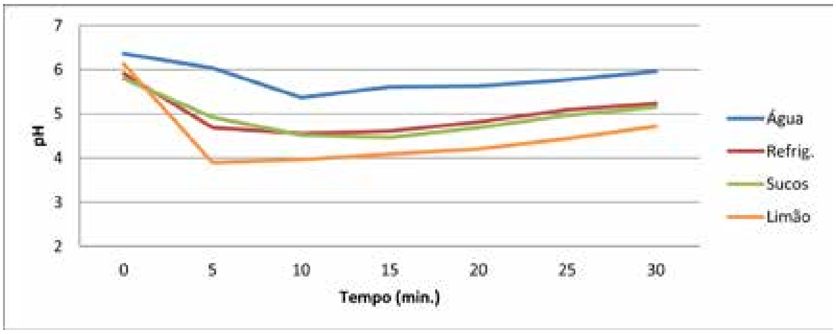
Table 1 shows that there is a statistically significant difference between the pH of the water in relation to the soft drink and juice groups and to the lemon, but the same significance is not present between the soft drink group in relation to the juice group.

In graph 2, it is possible to confirm the observations in table 1, when verifying the similarity between the juice and soft drink groups with regard to the pH average and the curve formed. In graph 3, it can be seen that the normal cola drink is the drink that takes the longest to recover the pH value, on the other hand, its zero sugar version causes the lowest pH drop with a faster recovery compared to other soft drinks

Graph 4 proves that liquid grape juice is the drink that leads to the lowest pH drop with a faster recovery than other juices, and that the juice that leads to the highest pH drop with slower recovery is guava juice in powder.

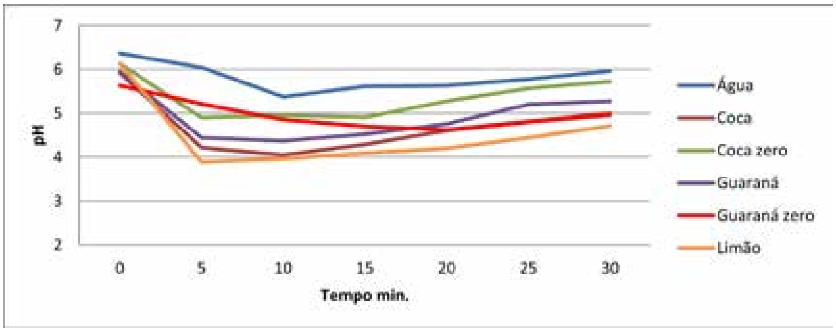
Graph 5 compared the measurements of the groups over time by repeated measures ANOVA, when it can be seen that, with the exception of water, all other groups showed little recovery of pH over time, which is statistically significant. It is also observed that all groups reached pH values below the critical value.

Graph 2 - Stephan curve of the control group (water), soft drinks, juices and positive control (lemon) with minimum pH.



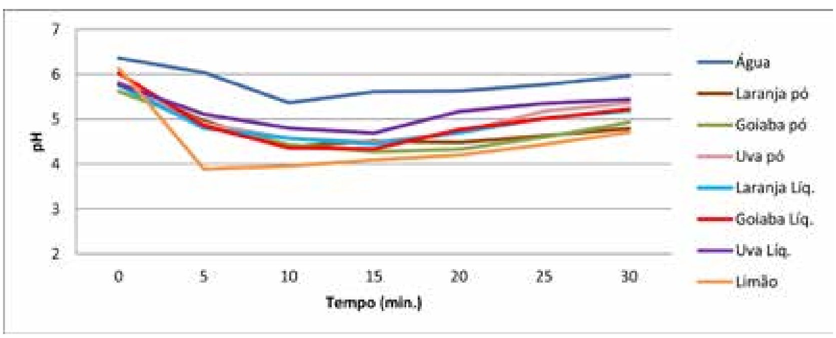
Source: Anova. In vivo analysis performed at the UFPB cariology clinic, 2012.

Graph 3 – Stephan curve of the control group (water), soft drinks, Normal and zero and positive control (lemon) with minimum pH values.



Source: Anova. In vivo analysis performed at the UFPB cariology clinic, 2012.

Graph 4 - Stephan curve of the control group (water), Orange Juice, Guava and Grape Powder and Liquid Orange, Guava and Grape and positive control (lemon) with minimum pH values over the time (0, 5, 10, 15, 20, 25, 30 min).



Source: Anova. In vivo analysis performed at the UFPB cariology clinic, 2012.

DISCUSSION

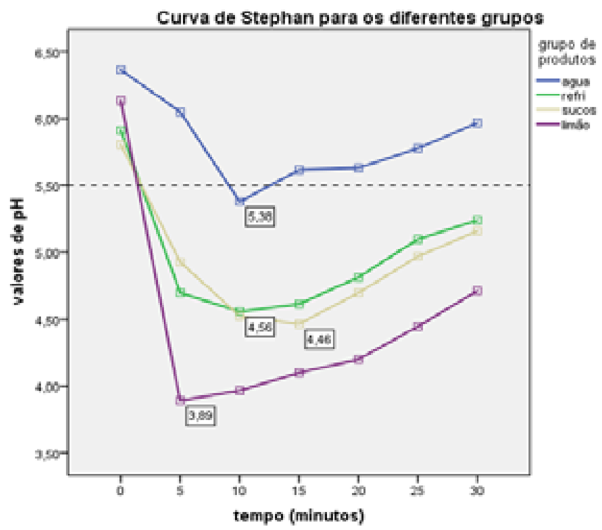
In this study, the pH recording of biofilms in vivo was performed using a palladium microelectrode technique with a 100 μm active sensitive tip and a glass half-cell reference electrode coupled to a potentiometer. Despite the possible discomfort due to the need for the volunteer to have to hold a finger in a solution of potassium chloride containing the reference electrode, this equipment is considered one of the most reliable for measuring pH fluctuations in this environment. The explanation for this credit is due to the fact that the pH determination is effectively performed by the precise [H +] in analytical conditions that reach a margin of error of 3% and 0,05 pH units. [7] The different techniques for measuring pH have always encountered problems with the reliability of the results since variations and fluctuations of pH in vivo are frequent in the oral cavity. [8,9,10]

The pH value of dental biofilms in vivo is considered one of the main indicators of dental demineralization. [7] The pH value in these conditions reflects the direct and indirect result of internal biofilm factors (for example: composition of the microbiota) as well as external factors such as salivary buffer capacity and acidity of food and drinks.

In the present study it was observed that the effect of soft drinks based on normal and zero cola, drinks based on normal and zero Paullinia cupana and of liquid and powdered industrialized juices of orange, guava and grape on the salivary buffer capacity was significant in the erosive and cariogenic.

Modern diets usually contain very acidic foods, and acidic fruits have been increasingly consumed by young people and children in the form of diluted pulps and juices. [11] Because they are tasty, soft drinks and fruit juices are well accepted, however, despite being sources of vita-

Graph 5 – Stephan curve of the control group (water), soft drinks, juices and positive control (lemon) with minimum pH values over the time (0, 5, 10, 15, 20, 25, 30 min).



Source: Anova. In vivo analysis performed at the UFPB cariology clinic, 2012.

mins, they can bring losses such as short stature, obesity, development of caries and dental erosion. [12].

Measuring pH is a practical method to access the erosive and cariogenic potential of acidic drinks. In the present study, the endogenous pH of soft drinks ranged from 2,57 to 3,49. Results similar to those obtained by Dantas [13] who found pH values ranging from 2,33 to 2,83 for cola-based soft drinks, as well as the findings of Duran, López and Cotrina [14] and Sobral et al. [15] who observed, respectively, variations of 2,53 to 3,07 and 2,36 to 3,54 in the pH of soft drinks.

Regarding the percentage of acids in the different drinks analyzed, it is observed that natural lemon juice (positive control) presented the highest percentage of acids among the analyzed drinks and mineral water (negative control) the lowest percentage. Considering that, according to Magalhães et al. [16] drinks with lower pH and greater acidity, such as juices and soft drinks, have greater erosive power, due to the presence of citric, acetic and phosphoric acids, it can be said that many analyzed drinks are in these conditions, which make them potentially erosive.

The erosive potential of an acidic drink or food depends not only on the pH value

Analyzed by groups, soft drinks and juices obtained very similar pH values, which coincides with the results obtained by Hannan and Marreiro [17], who found no differences between these groups.

Cola-based soft drinks had the lowest pH values, corroborating the results found in the works of Fushida and Cury [18] with an endogenous pH value of 2,29 for the drink based on normal cola and by Rodrigues et al. [19] who found the minimum mean pH values for zero line refrigerants (2,85).

The curves of orange and guava juices were very close to each other, being at a disadvantage in relation to grape juice, which was less acidic and had no potential to cause dental erosion. In relation to powdered juices, the curves of the three drinks were closer to that of the lemon (positive control), being able to cause erosion. The most worrying situation is found with the drink based on normal cola, which has acidity similar to lemon. It is also noted that zero sugar soft drinks have a higher pH than those without sugar, however, the recovery of the pH drop occurs in less time than the sucrose versions.

The erosive potential of an acidic drink or food depends not only on the pH value, but is also determined by the buffering capacity. Thus drinks containing acids such as citric, ascorbic and tartaric in small quantities exhibit a low buffering capacity and are quickly neutralized by salivary plugs, which prevents the prolonged drop in oral pH, thus causing less loss of mineral from the dental structure. [20]

With the researched beverages, the pH of the biofilm showed a decline until the time of 15 minutes when exposed to industrialized and powdered grape juice, powdered guava and Guarana zero. The other drinks showed a drop in pH only for 10 minutes and then the pH shows an upward direction, with the exception of the zero-cola drink, which had a sudden drop in pH, but at 5 minutes the gradual increase in pH started. Grape juices, both liquid and powder, despite causing a pH drop for 15 minutes, were the drinks that most quickly raised the pH, demonstrating to be of low cariogenic and erosive power, due to the rapid recovery of pH. These results corroborate those found by Silva et al. [21] who evaluated the change in salivary pH in children from Paraná after ingesting industrialized fruit juice, measuring the pH before, immediately after ingestion and 5, 10 and 15 minutes after ingestion and concluded that fruit juice had a low pH and that 10 minutes after ingestion, the salivary pH of all participants was above 5.5, rising close to normal values over a period of 15 minutes.

The fact that the pH value of water is critical means that it has no buffering capacity like saliva, also due to the effect of momentary dehydration at the time of measurement. It is also possible to observe the times when the minimum pH was reached, and for lemon (positive control), this value is immediate and significant, different from the other groups.

CONCLUSION

All the acidic drinks analyzed reduced the pH of the dental plaque in all volunteers. The group of soft drinks caused a sharp drop in pH immediately when the pH value was analyzed. In the juice group, the liquid grape was the one that came closest to water, being the product of the group less cariogenic and erosive. The absence of sucrose in zero sugar soft drinks contributes to the pH drops being less prolonged than in the presence of this sugar. It is important to emphasize the role of saliva in the buffering effect at pH. Additional measures must be taken to reduce the impact of reducing the pH of the food in the oral cavity, among which would be the use of straw and tooth brushing after eating the food. ■

REFERENCES

1. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Secretaria de Vigilância em Saúde. SB Brasil 2010: Pesquisa Nacional de Saúde Bucal: resultados principais / Ministério da Saúde. Secretaria de Atenção à Saúde. Secretaria de Vigilância em Saúde. – Brasília: Ministério da Saúde, 2012. 116 p. : il.

2. Johansson AK, Sorvari R, Birkred D, Meurmen JH. Dental erosion in deciduous teeth – an in vivo and in vitro study. J. Dent. 2001; 29: 333-40.

3. Paes Leme AF et al. Effects of Sucrose on the Extracellular Matrix of Plaque-Like Biofilm Formed in vivo, Studied by Proteomic Analysis. Caries Res. 2008; 42:435-443.

4. Silva Rita de Cássia Ribeiro, Silva Luce Alves da, Araújo Roberto Paulo Correia de, Soares Felipe Fagundes, Fiaccone Rosemeire Leovigildo, Cangussu Maria Cristina Teixeira. Dieta padrão obesogênica: o impacto na saúde bucal de crianças e adolescentes do Recôncavo Baiano - Brasil. Cafajeste. saúde colet. [Internet]. Junho de 2015 [citado em 18 de dezembro de 2020]; 23 (2): 198-205. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1414-462X2015000200198&lng=en. https://doi.org/10.1590/1414-462X201500020038

5. Thystrup A, Fejerskov O. Diferentes conceitos da cárie dentária e suas implicações. In: Thylstrup A, Fejerskov O. Cariologia clínica. 2.ed. São Paulo: Santos; 2011. p.209-17.

6. Lima FEL et al. Validade de um questionário quantitativo de frequência alimentar desenvolvido para população feminina no nordeste do Brasil. Revista Brasileira de Epidemiologia. 2007; 10(4): 483-490.

7. Preston AJ, Edgar WM. Developments in dental plaque pH modelling. J. Dent. 2005 mar; 33(3): 209-22.

8. Lussi A, von Salis-Marincek M, Ganss C, Hellwig E, Cheaib Z, Jaeggi T: Clinical Study Monitoring the pH on Tooth Surfaces in Patients with and without Erosion. Caries Res 2012;46:507-512. doi: 10.1159/000339783

9. Dong YM, Pearce EIF, Yue L, Larsen MJ, Gao X-J, Wang J-D. Plaque pH and Associated Parameters in Relation to Caries. Caries Research. 1999; 33(6).

10. Carléna A, Hassana H, Lingströmb P. The 'Strip Method': A Simple Method for Plaque pH Assessment. Caries Res. 2010; 44:341-344

11. Rampersaud G, Bailey L, Kauwell G. National survey beverage consumption data for children and adolescents indicate the need to encourage a shift toward more nutritive beverages. J. Am. Diet. Assoc. 2003; 103: 97-100.

12. Cavalcanti AL et al. Cariogenic and erosive potential of industrialized fruit juices available in Brazil. Braz. J. Oral Sci. 2010 july-sep; 9(3):351-57.

13. Dantas RVF et al. Características físico-químicas da dieta líquida cafeínada. Pesqui. Bras. Odontopediatria Clín. Integr. 2008; 8:333-6.

14. Duran CL, López AM, Cotrina LD. Evaluación in vitro del efecto erosivo de três bebidas carbonatadas sobre la superfície del esmalte dental. Rev. Estomatol. Hered. 2007; 17: 58-62.

15. Sobral MAP, Luz MAAC, Gama-Teixeira A, Garone Netto N. Influencia da dieta líquida ácida no desenvolvimento de erosão dentária. Pesqui. Odontol. Bras. 2000; 14: 406-10.

16. Magalhães AC et al. Insights into preventive measures for dental erosion. J. Appl. Oral. Sci. 2009; 17(2):75-86.

17. Hannan AS, Marreiro RO. Avaliação do pH de refrigerantes, sucos e bebidas lácteas fabricados na cidade de Manaus, Amazonas, Brasil. Pesq. Bras. Odontop. Clin. Integr. João Pessoa, 2009 set-dez; 9(3):347-353.

18. Fushida CE, Cury JA. Estudo in situ do efeito da frequência de ingestão de coca-cola na erosão do esmalte-dentina e reversão pela saliva. Rev. Fac. Odont. USP. 1999; 13(2):127-34.

19. Rodrigues JÁ, Lima-Arsati YBO, Vieira PLS, Tagata CS. Estudo do pH de bebidas consumidas pela sociedade brasileira. Rev. Assoc. Paul. Cir. Dent. 2007; 62(2):106-112.

20. Assad AM et al. Determinação do pH, capacidade de tamponamento, carboidratos totais e sacarose em sucos de frutas industrializados "zero açúcar" e light. Rev. Sul-Bras. Odontol. 2010 jul-set; 7(3):281-6.

21. Silva JYB, Brancher JA, Duda JG, Losso EM. Mudanças do pH salivar em crianças após ingestão de sucos de frutas industrializados. RSBO. 2008; 5(2):7-11.