Influence of three types of drinks on the surface of human dental enamel: in vitro study

Influência de três tipos de bebidas sobre a superfície de esmalte dentário humano: estudo in vitro

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ABSTRACT

Objective
The aim of this study was to evaluate the erosive effect of three beverages which are considered to be acidic in vitro study.

Methods
We used 45 third molars. The teeth were cut to obtain 90 4x4 mm enamel blocks, obtained from the buccal and lingual surfaces of each tooth. The groups were divided by type of beverage (Coca-Cola®, Coca-Cola do Brasil, Rio de Janeiro, Brazil; Coca-Cola Zero®, Coca-Cola do Brasil, Rio de Janeiro, Brazil and Gatorade uva®, Ambev, Jaguariúna, Brazil) and number of cycles (7, 15 and 30 cycles). For each cycle, the specimens were immersed in the drink for 2.5 minutes by alternating with immersion in saliva for 1 hour. Each cycle simulates one day of intake. Statistical analysis, ANOVA and Tukey 5% for profilometry showed the statistical difference between the kind of beverages and between periods.

Results
Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) (3.99±1.25) differed from other kinds of beverage. All the periods differed and the period of 30 days saw the greatest structural loss. For microhardness, the statistical analysis showed greater loss of surface hardness for the group Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) within 30 days (126.02 ± 30.98) SHL.

Conclusion
It was concluded that the soft drink Coca Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) had the greatest erosive effects and the higher the number of cycles the greater the influence on the process of dental erosion.

***Indexing terms**: Beverages. Dental enamel. Hardness test.*

RESUMO

Objetivo
Avaliar o efeito erosivo de três bebidas ácidas sobre o esmalte dentário num estudo in vitro.

Métodos
Foram utilizados 45 terceiros molares humanos. Noventa blocos de esmalte de dente humano (4X4 mm) foram obtidos a partir das faces vestibular e lingual de terceiros molares. Os blocos de esmalte foram divididos de acordo com o tipo de bebida testada (Coca-Cola®, Coca-Cola do Brasil, Rio de Janeiro, Brasil; Coca-Cola Zero®, Coca-Cola do Brasil, Rio de Janeiro, Brasil e Gatorade uva®, Ambev, Jaguariúna, Brasil) e quantidade de ciclos (7, 15 e 30 ciclos). Cada ciclo simulava um dia de ingestão, o qual compreendia a imersão dos corpos de prova (n=10/tipo de bebida e quantidade de ciclos) nas bebidas (2,5 minutos), alternados por imersão em saliva (1 hora). Para quantificação do efeito erosivo, foi avaliada a perfilometria (µm±DP) e a perda de dureza superficial. Os dados foram analisados por ANOVA 2 fatores e Tukey 5%.

Resultados
A perfilometria demonstrou diferença estatística entre as bebidas e entre os períodos, sendo que a Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brasil) (3.99±1,25) diferiu das outras bebidas. Todos os períodos diferiram entre si sendo que para o período de 30 dias ocorreu a maior perda de estrutura. Para a microdureza a análise estatística demonstrou maior perda de dureza superficial para o grupo Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brasil) no período de 30 dias (126,02 ± 30,98) perda de dureza superficial.

Conclusão
Concluiu-se que a Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brasil) apresentou os maiores efeitos erosivos e quanto maior número de ciclos, maior a influência no processo dentário erosivo.

Termos de indexação: Bebidas. Esmalte dentário. Testes de dureza.

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INTRODUCTION

Dental erosion is defined as the irreversible loss of dental structure due to a chemical process and without the involvement of microorganisms[1-2]. This process is the result of the action of acids whose pH is lower than 4.5. This value is below the critical pH, both for hydroxylapatite (critical pH of 5.5) and for fluorapatite (critical pH around 4.5), which causes the dissolution of these minerals present in the enamel, resulting in a surface lesion[3].

Dental erosion may be triggered by both intrinsic and extrinsic factors. Intrinsic factors include patients who present with anorexia nervosa, bulimia, gastric problems with frequent regurgitation, situations in which the gastric juices are often present in the oral cavity (pH of gastric juices = 2.3)[4]. Xerostomia may also have an impact on the appearance of the lesion, due to the reduced production of saliva responsible for a balanced pH5. Extrinsic factors include acids originating from foods (seasoning), beverages (juices, teas, sodas and energy drinks), medication (Vitamin C) and acidic products emanating from the work environment (chlorine from swimming pools, working in the fertilizer industry), which reduce oral pH, thereby helping the process to take place[6-8].

Studies on the prevalence of dental erosion have demonstrated increasing numbers of children and adolescents9. The prevalence of erosion in adolescents, in the permanent dentition, is very varied when analyzing cross-sectional studies, attaining values of 13% to 95% in enamel and 0 to 26% in the dentin[5,10-11]. In the population of children aged between 15 and 17, the main etiological factor associated with dental erosion is the high consumption of acidic beverages, predominantly fruit juices and sodas, to the detriment of milk and water[6].

Excessive consumption of drinks with an acid pH, such as sodas, tends to cause the demineralization of the dental enamel, though this effect may be reversible given the saliva's ability to remineralize[12-13] the teeth. Individuals consuming citric fruits more than twice a day have a 37 times greater risk of developing lesions through erosion than those who do not. Similar risks appear to occur with the consumption of apple vinegar (10 times higher), sports drinks (4 times higher) or sodas (4 times higher), when consumed every day. The advancing loss of dental structure through erosion could be as much as around 1 μm per day[14].

It was shown by Grando et al.[15] that the average pH values of lemon, cola drinks and the Brazilian soft drink Guaraná, respectively, are 2.5, 2.6 and 3.36. By performing an in vitro evaluation of erosion caused by these drinks in the deciduous teeth, they concluded that all the products tested are potentially erosive, with lemon juice causing the greatest loss of calcium and inorganic phosphate, followed by the cola drinks and then Guaraná.

Bearing in mind the damage caused by some drinks, the aim of this study was to evaluate the erosive effect of three acidic beverages on the dental enamel in an in vitro study.

METHODS

Obtaining the teeth and preparing the blocks

A total of 45 human, erupted and partially erupted third molars were used, clinically free of caries, having been extracted by dental surgeons in private clinics, and approved by the Ethics Committee (n. 021/2009-PH/CEP); they were sterilized in a buffer solution of formaldehyde at 2%, pH 7.0, at room temperature, for a maximum period of 30 days.

The teeth were then sectioned in order to obtain 90 4X4 mm blocks of enamel, acquired from the vestibular and lingual surfaces of each tooth. Then the blocks were subjected to the flattening of the enamel, inserted in acrylic resin and polished using 600, 800 and 1200 grit wet sandpaper at high speed until the surface assumed a vitreous appearance. A sonic bath was performed for 5 minutes while the sandpapers were being changed. For the final polishing, a felt cloth was used, moistened in a diamond solution and applied for 3 minutes.

The study was divided into 9 groups, each with 10 specimens. The groups were divided according to the type of drink: Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil), pH=2.38, Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil), pH=2.8 and grape Gatorade® (Ambev, Jaguariúna, Brazil), pH=2.89; and the number of cycles (7, 15 and 30 cycles). All the specimens were immersed in the drinks for 2.5 minutes, alternating with immersion for 1 hour in artificial saliva (pH=6.73: Sodium benzoate - 1g; Magnesium chloride - 0.05g; Sodium chloride - 0.825g; Sorbitol - 42.74g; Dibasic potassium phosphate - 0.8035g; Calcium chloride - 0.166g; Carboxymethyl cellulose 10g; Potassium chloride - 0.62g; Distilled water - 940.6205ml; Monopotassium phosphate - 0.326g). The immersion time of 2.5 minutes was adopted to simulate the average time that the drink would be in contact with the teeth during the consumption of one can of drink per day. Accordingly, the consumption of drinks was represented by 7, 15 and 30 days.

Firstly the initial microhardness was evaluated (FM Digital Microhardness Tester, Future-Tech) with five indentations in all the blocks in a region that would not be subsequently used, serving as the average microhardness of enamel for each specimen. A diamond pyramid penetrator was used with a static load of 25g, applied for 5 seconds. The average microhardness values were used to carry out the division of the specimens into groups. Of the 120 specimens, 15 above the average and 15 below the average were eliminated, leaving a total of 90 specimens.

One half of each block was protected by nail varnish (control), with the aim of making profilometric analysis possible. The blocks were kept in a physiological solution at 37°C until used.

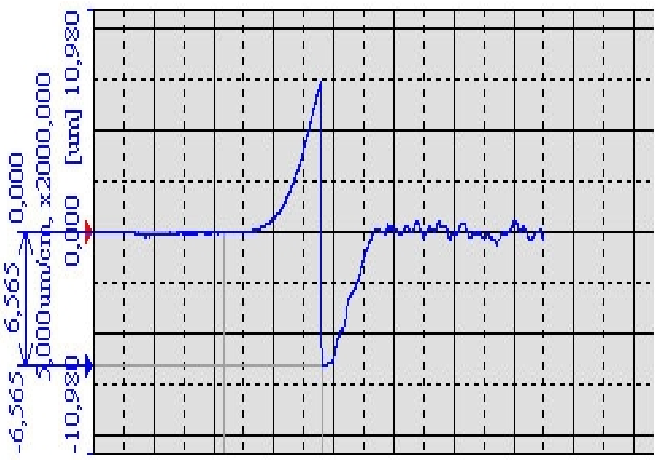

Figure 1. Roughness reading.

Evaluation of microhardness and profilometry

The profile of the enamel surface was evaluated based on a roughness tester (Perthometer S8P, Mitutoyo, Tokyo, Japan), with a stylus which passes over the surface, connected to a unit whose function it is to process and communicate the information quantitatively, and supply the results.

For this study, the roughness tester was connected to a microcomputer which processed and stored all the information relevant to the tests. The profile plotted by the roughness tester passed over the surface of the specimens, moving from the area of healthy enamel (control) towards the area of enamel subjected to the beverages. As the untreated, polished enamel (control) had a smoother surface, it was very close to being a straight line and became easy to identify during the test of the region subjected to the acid challenge test, a fact which can be corroborated using the reading from the roughness meter which quantified the wear through the numerical difference between the baseline and the peak of the drop (Figure 1).

After profilometry, the Vickers microhardness test was performed on the region of the acid challenge (final microhardness), with 5 indentations being carried out in the same way as with the initial microhardness and the final average was obtained for each specimen. The difference between the initial and final microhardness demonstrated a loss of surface hardness (SHL).

A two-way ANOVA Variance Analysis and a Tukey test were carried out to evaluate microhardness and profilometry.

RESULTS

Profilometry

Table 1 shows the average and standard deviation of each group in respect of the roughness meter reading which quantified wear via the numerical difference between the control surface and the surface subjected to the acid challenge test.

The 5% variance analysis revealed a statistical difference between drinks and between periods (p=0.00001), there being no interrelation between these factors. Accordingly, the Tukey test was conducted for the Beverages which revealed a statistical difference for Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) (3.99±1.25 µm) versus the groups Gatorade (2.9082±1.03 µm) and Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) (2.5318±0.54 µm). The Tukey test and the test for the Period demonstrated a statistical difference between the three periods, 7 cycles (2.0815 µm), 15 cycles (3.1798 µm) and 30 cycles (4.1783 µm).

Microhardness

Table 2 shows the average and standard deviation of each group in respect of the loss of surface hardness (SHL).

By means of the 5% variance analysis, a statistical difference was found between the beverages and between the periods and there was a relationship between them. The Tukey test was carried out accordingly. The Tukey test demonstrated that the 30 cycle Coca-Cola® group differed statistically from the 7 cycle Coca-Cola® (Coca-Cola do

Brasil, Rio de Janeiro, Brazil) group, all periods of Coca-Cola® Zero (Coca-Cola do Brasil, Rio de Janeiro, Brazil) and the 15 cycle Gatorade® (Ambev, Jaguariúna, Brazil) group.

Table 1. Average and standard deviation of wear obtained from enamel subjected to erosion by different types of beverage and cycle time with regard to profilometry.

Beverage	Period (cycles)	Average (μm)	Standard deviation
Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	7	2.471	0.400
Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	15	4.353	0.828
Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	30	5.175	1.197
Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	7	1.706	0.428
Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	15	2.349	1.044
Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	30	3.541	1.436
Grape Gatorade® (Ambev, Jaguariúna, Brazil)	7	2.068	0.508
Grape Gatorade® (Ambev, Jaguariúna, Brazil)	15	2.838	0.916
Grape Gatorade® (Ambev, Jaguariúna, Brazil)	30	3.819	1.341

Table 2. Average and standard deviation of loss of surface hardness of enamel subjected to erosion by different types of beverage and by cycle time.

Beverage	Period (cycles)	Average (SHL)	Standard deviation	Homogeneous groups
Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	7	27.94	8.34	B
Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	15	76.08	20.55	A B
Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	30	126.02	30.98	A
Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	7	49.71	15.56	B
Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	15	32.56	9.23	B
Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil)	30	60.15	17.91	B
Grape Gatorade® (Ambev, Jaguariúna, Brazil)	7	82.55	23.75	A B
Grape Gatorade® (Ambev, Jaguariúna, Brazil)	15	43.50	10.23	B
Grape Gatorade® (Ambev, Jaguariúna, Brazil)	30	72.47	21.39	A B

DISCUSSION

When selecting the beverage, it should be borne in mind that the dissolution of enamel with erosion depends on the pH, the buffer capacity, length of exposure to the acid and the temperature, as well as the concentrations of calcium, fluorine and phosphate around the fluid[16].

Lussi et al.[17] listed three factors that influence the erosive potential of acidic beverages: Chemical factors: pH and buffer capacity of the beverage, type of acid, adhesion of the product to the dental tissue, chelation properties, presence of calcium, presence of phosphorous and presence of fluorine. Behavioral factors: Eating habits, lifestyle, high intake of fruit and vegetables, excessive consumption of acidic foods and drinks, the habit of plying children with acidic drinks at nighttime and oral hygiene practices. Biological factors: flow, composition and buffer capacity of the saliva, acquired pellicle, composition and dental structure, anatomy of the soft tissue in relation to the tooth and the physiological movement of the soft tissue.

One important factor in the methodology in this research study was the use of artificial saliva as the buffer agent in the acid challenge test[18].

Devlin et al.[19] conducted a study to determine the microhardness of the enamel in permanent teeth exposed to Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil). A commercially available, artificial saliva was used as the buffer and compared to a control group with water. The drinks were applied for 1, 2 and 3 hours, and also during the night (15 hours), washed in water and the microhardness was tested after each interval. In the treatment with Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil), the hardness of the enamel was significantly reduced with the passing of time, unlike water which remained similar to at the start. The main conclusion to be drawn from this study was that the use of artificial saliva after the acid challenge partially restored the initial hardness of the enamel, 18% of the initial hardness. This fact bears witness to the buffer capacity of saliva, even when changes occur in the microhardness after contact with carbonated drinks.

In the work of Araujo et. al.[20], they studied the acid challenge test with clarifying agents and a carbonated drink (Coca-Cola®, Coca-Cola do Brasil, Rio de Janeiro, Brazil) in bovine teeth restored with composite resin. After 14 days of the chemical challenge, none of the groups showed any alteration in the microhardness of the composite resin. Classic works of literature such as Morrier et. al.22 already demonstrated that acid challenge tests with Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) behave differently with enamel than with composite resin.

The higher roughness values, i.e. the greater demineralization of the enamel, may be explained by the pH of the drinks and their buffer capacity. Owens[21], by way of a study of the acid challenge test of different beverages, reached the conclusion that some drinks, such as Gatorade® (Ambev, Jaguariúna, Brazil) and Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil), possess a greater potential for enamel erosion when compared to drinks like Coca-Cola Diet® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) and the control group with water. These results are consistent with the present study in which the present study in which the highest enamel roughness values were found in the Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) group. The pH of the Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) is lower than that of Gatorade® (Ambev, Jaguariúna, Brazil) and Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil), however the factor that led to the statistical difference between them is not just the Ph, but also the presence of phenylalanine in the Coca-Cola Zero® (Coca-Cola do Brasil, Rio de Janeiro, Brazil). The amino acid phenylalanine is present due to the hydrolysis of aspartame by the saliva. This amino acid acts by neutralizing the acids. Another possibility is the formation of an amino acid based layer on the tooth surface. This layer could reduce dental erosion, acting as a barrier by preventing direct contact between the acids and the surface of the tooth[22].

Similar results were also found by Owens & Kitchens[23] using a methodology using electron scans of the surface using light microscopy to evaluate the demineralization of the enamel, using the same beverages. All the drinks produced surface wear, the most apparent being Gatorade® (Ambev, Jaguariúna, Brazil) and Coca-Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil), followed in third place by Coca-Cola Diet® (Coca-Cola do Brasil, Rio de Janeiro, Brazil).

Rytömaa et. al.[24] found, as in this study, that sodas produced more dental erosion than the isotonic drinks.

The in vitro study by Carvalho Sales-Peres et. al.26 evaluated the capacity of different sodas (Coca-Cola®, Coca-Cola do Brasil, Rio de Janeiro, Brazil; Coca-Cola Light®, Coca-Cola do Brasil, Rio de Janeiro, Brazil); Guaraná® (Ambev, Jaguariúna, Brazil), Pepsi Twist® (Ambev, Jaguariúna, Brazil), Sprite Light®, Coca-Cola do Brasil, Rio de Janeiro, Brazil), to wear down the dental enamel, listing the percentage changes in surface microhardness (% SHL) for concentrations of fluorine and phosphate, buffer capacity and the pH of these drinks. The results showed that for all five sodas, there was a surface demineralization of the enamel. In relation to the chemical variables tested, despite not being statistically significant, the pH seems to have more influence on the erosive potential of these drinks. These results corroborate the findings of the present study and justify the pH as the main factor causing enamel demineralization.

In vitro erosion is greater than in situ erosion, precisely because the saliva protection factor is not present, even with the use of artificial or collected saliva[26]. Despite this, the data are similar for both methods, which lends support to the use of the in vitro model, on account of its simplicity. Nevertheless, many doubts still exist as to the saliva's capacity to reverse the process, precisely on account of the use of this model which does not succeed in extrapolating the results to the in vivo situation because of the lack of influence of variables such as buffer capacity, acquired pellicle, saliva flow and the fluorine concentration in the appearance of erosion. The in situ model is accepted as the benchmark between a natural, uncontrollable situation (in vivo) and a highly controllable laboratory situation (in vitro)[27].

The results of loss of dental structure in the present study corroborate the data found in the study developed by Kichens & Owens[28], in which carbonated and non-carbonated drinks were used in an acid challenge of enamel in order to test the erosive power of these types of beverage. In this study, a fluoridated varnish was used on the enamel but this was not a factor of significant impact, however beverage (type) and exposure time were variables that had a significant impact, as in the present study. The results demonstrate that both types of drink, carbonated and non-carbonated, produce a significant erosive effect on dental enamel, however treatment with fluoridated varnish did not show a significant protective influence on enamel surfaces.

van Eygen et al.[29] concluded that the drinking of sodas, even for a short time, as in the present study, could reduce the microhardness of dental enamel.

In vivo studies should be undertaken to better explain the effect of the acid and the buffer capacity of human saliva, in relation to the types of beverage most commonly consumed as well as the frequency of consumption.

CONCLUSION

The soda Coca Cola® (Coca-Cola do Brasil, Rio de Janeiro, Brazil) exhibited the greatest erosive effects on the enamel and the more often the intake the greater the influence on the dental erosion process.

Collaborators

ETC YAMAMOTO, A VANDERLEU and R AMARAL were responsible for carrying out the project design, statistical analysis, discussion, results and composition of the article. RD NICOLÓ, JC ROCHA, MAM ARAÚJO were responsible for the conception and organization of the project design and the composition of the article.

REFERENCES

1. Auad SM, Waterhouse PJ, Nunn JH, Steen N, Moynihan PJ. Dental erosion amongst 13- and 14-year-old Brazilian schoolchildren. Int Dent J. 2007;57(3):161-7.

2. Kazoullis S, Seow WK, Holcombe T, Newman B, Ford D. Common dental conditions associated with dental erosion in schoolchildren in Australia. Pediatr Dent. 2007;29(1):33-9.

3. El Aidi H, Bronkhorst EM, Truin GJ. A longitudinal study of tooth erosion in adolescents. J Dent Res. 2008;87(8):731-5. doi: 10.1177/154405910808700813.

4. Lussi A. Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci. 2006;20:1-8. doi: 10.1159/000093343.

5. Scheutzel P. Etiology of dental erosion: intrinsic factors. Eur J Oral Sci. 1996;104(2):178-90.

6. Eccles JD, Jenkins WG. Dental erosion and diet. J Dent. 1974;2(4):153-9. doi: 10.1016/0300-5712(74)90044-X.

7. Litonjua LA, Andreana S, Bush PJ, Cohen RE. Tooth wear: attrition, erosion, and abrasion. Quintessence Int. 2003;34(6):435-46.

8. Amaechi BT, Higham SM, Edgar WM. Techniques for the production of dental eroded lesions in vitro. J Oral Rehabil. 1999;26(2):97-102. doi: 10.1046/j.1365-2842.1999.00349.x.

9. May J, Waterhouse PJ. Dental erosion and soft drinks: a qualitative assessment of knowledge, attitude and behavior using focus groups of schoolchildren. A preliminary study. Int J Paediatr Dent. 2003;13(6):425-33. doi: 10.1046/j.1365-263X.2003.00500.x.

10. Kim JW, Jang KT, Lee SH, Kim CC, Hahn SH, Garcia-Godoy F. In vivo rehardening of enamel eroded by a cola drink. ASDC J Dent Child. 2001;68(2):122-4.

11. Morrier JJ, Duprez JP, Boulet O. [Enamel, composites and Coca-cola]. Rev Odontostomatol (Paris). 1989;18(2):93-8.

12. Jarvinen VK, Rytomaa, II, Heinonen OP. Risk factors in dental erosion. J Dent Res. 1991;70(6):942-7. doi: 10.1177/00220345910700060601.

13. Sognnaes RF, Wolcott RB, Xhonga FA. Dental erosion. I. Erosion-like patterns occurring in association with other dental conditions. J Am Dent Assoc. 1972;84(3):571-6.

14. O'Sullivan EA, Curzon MEJ. A comparison of acidic dietary factors in children with and without dental erosion. ASDC J Dent Child. 2000;67(3):186-92.

15. Grando J, Gabilan N, Petry A. Erosão dental: estudo in vitro da erosão causada por refrigerantes e suco de limão no esmalte de dentes decíduos humanos - análises bioquímicas. Rev Odontopediatr. 1995;4(1):1-10.

16. Larsen MJ, Nyvad B. Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. Caries Res. 1999;33(1):81-7.

17. Lussi A, Jaeggi LT, Zero D. The role of diet in the aetiology of dental erosion. Caries Res. 2004;33(1):34-44. doi: 10.1159/000074360.

18. Gurdal P, Guniz Akdeniz B, Hakan Sen B. The effects of mouthrinses on microhardness and colour stability of aesthetic restorative materials. J Oral Rehabil. 2002;29(9):895-901. doi: 10.1046/j.1365-2842.2002.00924.x.

19. Devlin H, Bassiouny MA, Boston D. Hardness of enamel exposed to Coca-Cola and artificial saliva. J Oral Rehabil. 2006;33(1):26-30.

20. Araújo RM, Torres CRG, Araújo MAM. Microdureza de restaurações de resina composta expostas a agentes clareadores e coca cola. Rev Odonto. 2007;15(30):27-33.

21. Owens BM. The potential effects of pH and buffering capacity on dental erosion. Gen Dent. 2007;55(6):527-31.

22. Rios D, Honório HM, Magalhães AC, Wiegand A, de Andrade Moreira Machado MA, Buzalaf MA. Light cola drink is less erosive than the regular one: an in situ/ex vivo study. J Dent. 2009;37(2):163-6. doi: 10.1016/j.jdent.2008.11.004.

23. Owens BM, Kitchens M. The erosive potential of soft drinks on enamel surface substrate: an in vitro scanning electron microscopy investigation. J Contemp Dent Pract. 2007;18(7):11-20.

24. Rytomaa I, Meurman JH, Koskinen J, Laakso T, Gharazi L, Turunen R. In vitro erosion of bovine enamel caused by acidic drinks and other foodstuffs. Scand J Dent Res. 1988;96(4):324-33. doi: 10.1111/j.1600-0722.1988.tb01563.x.

25. Sales-Peres SHC, Magalhães AC, Machado MA, Buzalaf MA. Evaluation of the erosive potential of soft drinks. Eur J Dent. 2007;1(1):10-3.

26. West NX, Hughes JA, Parker DM, Newcombe RG, Addy M. Development and evaluation of a low erosive blackcurrant juice drink 2: comparison with a conventional blackcurrant juice drink and orange juice. J Dent. 1999;27(5):341-4. doi: 10.1016/S0300-5712(98)00070-0.

27. Hall AF, Buchanan CA, Millett DT, Creanor SL, Strang R, Foye RH. The effect of saliva on enamel and dentine erosion. J Dent. 1999;27(5):333-9. doi: 10.1016/S0300-5712(98)00067-0.

28. Kitchens M, Owens BM. Effect of carbonated beverages, coffee, sports and high energy drinks, and bottled water on the in vitro erosion characteristics of dental enamel. J Clin Pediatr Dent. 2007;31(3):153-9.

29. van Eygen I, Vannet BV, Wehrbein H. Influence of a soft drink with low pH on enamel surfaces: an in vitro study. Am J Orthod Dentofacial Orthop. 2005;128(3):372-7. doi: 10.1016/j.ajodo.2004.03.036.

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