Carbonated Soft Drinks Induced Erosive Changes on Enamel Surfaces of Primary Teeth: SEM-EDS Analysis

Penphong Sooksompien[1], Varisara Sirimaharaj[2]*, Sitthichai Wanachantararak[3]

1. Candidate of Master Degree Program in Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand.
2. Assistant Professor, DDS, MDSc, PhD, Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand.
3. Professor, DDS, MS, PhD, Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand.

Abstract

This study used scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) to evaluate morphological and atomic percentage (at.%) changes on the enamel surfaces of primary teeth after exposure to carbonated soft drinks.

Forty-five primary molars were extracted from healthy 4-12-year-old children. The crown of each molar was divided into 4 pieces along central and buccal-lingual grooves, and then each piece was randomly immersed in 3 commercial carbonated soft drinks (Coca-Cola Original, Coca-Cola No Sugar, or sparkling water) or deionized water for 15 minutes. SEM images were taken to describe the enamel surface morphological changes based on Silverstone classification. The at.% changes of 12 elements on the surfaces caused by the tested solutions were analyzed using one-way ANOVA, Tukey's post hoc test.

The results showed that the acidic pH of carbonated soft drinks caused morphological and elements concentrations changes on the enamel surfaces. After exposure to both Coca-Cola drinks, type 3 erosive patterns were observed on the enamel surfaces, while slightly and non-specific erosive pattern was seen after exposure to sparkling water. The at.% levels of calcium and phosphorus were significantly decreased (p<0.05) after exposure to carbonated soft drinks. However, no significant changes were observed for the other elements.

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Introduction

Tooth enamel, the most highly mineralized tissue in the body, serves as a protective layer for underlying dentin and pulp.[1,2]

By weight, it is made up of 96% inorganic material, 4% organic material, and a small amount of water. Hydroxyapatite nanocrystal enhances mechanical and physical properties of enamel, including its acid resistance.[3,4]

A critical pH for dissolution, ranging between 4.5 and 5.5,[5] helps enamel to resist certain levels of acid from dental caries (caused by bacterial products) and dental erosion (caused by both internal and external factors).[6,7] Even though the prevalence of dental caries is decreasing, the prevalence of dental erosion among children and adolescents has increased, presumably due to changing dietary habits and lifestyles.[8,9] Acidic diets including industrially processed foods and drinks[10,11] are the major cause of concern in terms of extrinsic factors impacting dental erosion.[6,7] Moreover, the low pH levels of some carbonated soft drinks have been associated with dental erosion and the promotion of dental caries.[10-13]

Both quantitative changes in elements composition and qualitative changes in morphology of enamel surfaces were observed after tooth exposure to soft drinks.[11,14] Calcium and phosphorus on enamel surfaces were found to be affected after induced erosion by immersion in acidic drinks.[8,15,16] Carbonated soft drinks caused dissolution in highly mineralized areas of

Corresponding author:
Varisara Sirimaharaj,
Assistant Professor, DDS, MDSc, PhD, Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand.
E-mail: varisara.s@cmu.ac.th, piiporxp@gmail.com

enamel prism of permanent teeth, producing a honeycomb appearance due to the hollowing of prism centers compared to peripheral areas[13, 17] or a wavy roughened appearance.[18] One report[19] found that sparkling water produced pitting erosive pattern.

Most past reports have focused on the erosive potential of soft drinks on permanent teeth[6,8,10,13] by evaluating the chemical compositions of drinks, surface hardness, and percentage of enamel weight loss of teeth.[6,9,10,17] However, little was previously known about the erosive effects, both in terms of element concentrations and morphological changes, of carbonated soft drinks and sparkling water on the enamel surfaces of primary teeth. Therefore, we aimed to investigate the morphological and atomic percentage (at.%) changes on the enamel surfaces of primary teeth after exposure to different carbonated soft drinks (Coca-Cola Original, Coca-Cola No Sugar, or sparkling water) using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) analyses.

Materials and methods

Forty-five sound or minimally carious primary molars were collected from healthy 4-12-year-old children with the consent of the patients and their parents at the Pedodontics Clinic, Faculty of Dentistry, Chiang Mai University. The experimental protocol, including the use of human tissue, was approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University (No.82/2020). Oral hygiene, tooth brushing habits, and nutritional preferences were controlled to be comparable for the collected teeth.

The crown of each tooth was separated from the root by cutting along the cementoenamel junction using a tapered round-end diamond bur (D8 Intensive®, Swiss Dental Product, Switzerland) attached to an airotor handpiece with water-spray. The remaining pulp tissue was removed through the cut opening with a barbed broach and spoon excavator. In order to compare pieces of the same tooth, the crown was divided along the central and buccal-lingual grooves into 4 parts, mesio-buccal, mesio-lingual, disto-buccal, and disto-lingual parts, which were then randomly immersed in 10 ml of freshly opened tested solutions for 15 minutes.[17] Four experimental groups were composed of group A: Coca-Cola Original (The Coca-Cola Company, Thailand), group B: Coca-Cola No Sugar (The Coca-Cola Company, Thailand), group C: sparkling water (Carbonated Natural Mineral Water Source Perrier, Nestle Waters France), and group D: deionized water served as control. The pH levels of the tested solutions were measured at room temperature with a calibrated Mettler Toledo pH meter (model MP225, Switzerland) using 50 ml of each solution immediately after freshly opened the beverage containers and then at 5, 10, and 15 minutes.

After immersion, each specimen was washed in deionized water for 5 minutes and then transferred to a screw cap containing deionized water and ultrasonically cleaned for 10 minutes to clear any debris on the tooth surface. It was then processed for examination in a low-vacuum scanning electron microscope (JSM-5910LV, JEOL Ltd., Tokyo, Japan) system equipped with an energy dispersive spectral X-ray analyzer (Oxford Instruments, UK) by being kept in a closed jar with silica gel at room temperature for 3 days and coated with a gold sputtering machine (SPI-Module™ Sputter Coater). Two SEM images of enamel surface were taken at two magnifications (x2000 and x5000) for ultrastructure examination. The concentrations of 12 mineral elements, calcium (Ca), phosphorus (P), carbon **(**C**)**, nitrogen **(**N**)**, oxygen **(**O**)**, fluorine **(**F**)**, magnesium **(**Mg**)**, sodium **(**Na**)**, zinc **(**Zn**)**, chloride **(**Cl**)**, potassium **(**K**)**, and manganese **(**Mn**),** were analyzed using EDS at 5000x magnification.

Descriptive analysis was used to qualitatively compare enamel surface morphologies to determine the erosive potentials of carbonated soft drinks. The at.% changes of the elements on enamel surfaces measured by EDS were quantitatively compared using one-way ANOVA and Tukey's test. A p value less than 0.05 was considered a significant difference.

Results

The acidic carbonated soft drinks investigated in this study caused erosion on the enamel surfaces of the primary teeth. More specifically, the ultrastructures of the enamel surfaces of the primary teeth showed coral-, network-, and map-like appearances with non-homogeneous variations in pitting after being exposed to the Coca-Cola soft drinks, with these effects matching the type 3 erosive pattern of Silverstone classification (Figure 1 A, B, C, & D). Milder and shallower but otherwise similar erosion characteristics were observed on the enamel surfaces exposed to the sparkling water (Figure 1 E & F). Meanwhile, the enamel surfaces stored in deionized water had no signs of erosion (Figure 1 G & H).

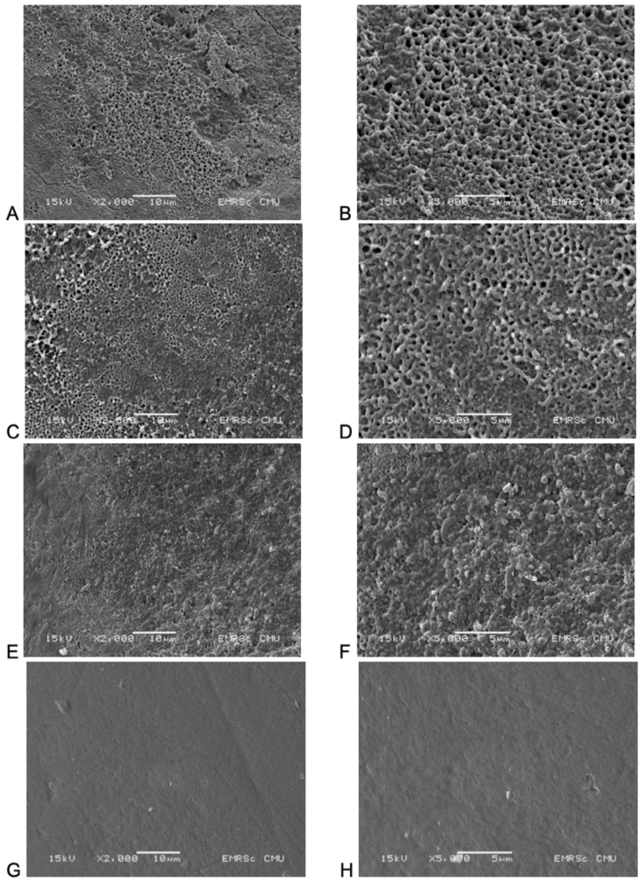

Figure 1. SEM images (at x2000 and x5000 magnifications) showing the microstructures of enamel of primary teeth following exposure to carbonated soft drinks, Coca-Cola Original (A, B), Coca-Cola No Sugar (C, D), sparkling water (E, F), and deionized water (G, H).

The element analysis using EDS found higher at.% levels of five major elements, C, Ca, P, O, and N, than the other elements in every sample. The mean±SD at.% levels of C in the specimens exposed to Coca-Cola Original, Coca-Cola No Sugar, and sparkling water were 36.33±7.51, 37.03±6.96, and 36.32±8.19, respectively, which were slightly but not significantly greater than the control groups (33.87±5.28), while the mean±SD at.% levels of N and O were less than those of C but with a similar trend (Figure 2). No significant differences were found for F, Mg, Na, Zn, Cl, K, and Mn elements. The mean±SD at.% levels of F and Mg in control group were 0.54±0.64 and 0.16±0.21, respectively (Figure 3).

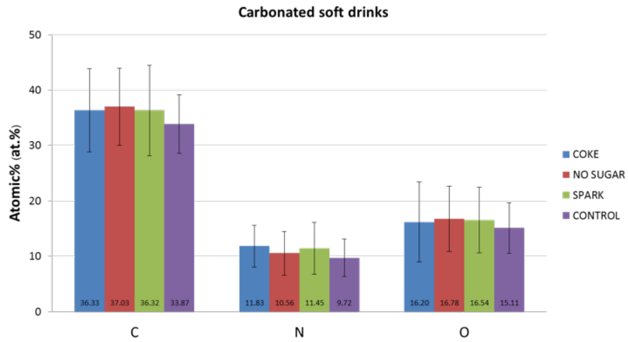

Figure 2. The at.% levels of C, N, and O element concentrations in the enamel surfaces of primary teeth after exposure to carbonated soft drinks and deionized water. The levels were slightly increased but not significantly different among the groups.

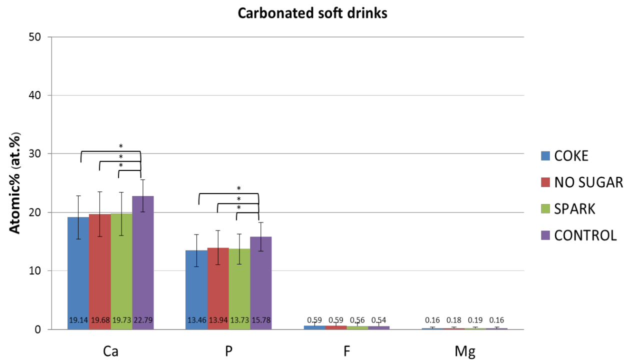

Figure 3. The at.% of Ca, P, F, and Mg element concentrations in the enamel surfaces of primary teeth after exposure to carbonated soft drinks and deionized water. There were significant decreases in the at.% levels of Ca and P on the enamel surfaces stored in carbonated soft drinks compared to the control group. The at.% levels of F and Mg were minute and not significantly different among the specimen groups.
*Indicates a significant difference for an element among the different drinks.

The concentrations of two elements, Ca and P, were significantly decreased after 15 minutes of immersion in three carbonated soft drinks compared to deionized water (Figure 3). The mean±SD at.% levels of Ca after immersion in Coca-Cola Original, Coca-Cola No Sugar, sparkling water, and deionized water were 19.14±3.70, 19.68±3.84, 19.73±3.70, and 22.79±2.76, respectively. Similarly, P levels were also significantly decreased after immersion in the carbonated soft drinks, with the mean±SD at.% levels of P after immersion in Coca-Cola Original, Coca-Cola No Sugar, sparkling water, and deionized water being 13.46±2.74, 13.73±2.56, 13.94±2.93, and 15.79±2.38, respectively. However, the ratio of Ca to P remained unchanged at 1.44±0.15 after immersion in the carbonated soft drinks.

The pH levels of Coca-Cola Original, Coca-Cola No Sugar, and sparkling water, when measured immediately after opened the beverage container, were 2.39±0.01, 2.74±0.02, and 5.10±0.09, respectively. These levels were slightly but not significantly increased with time. The deionized water had a constant pH at about 7.00 throughout the measurements.

Discussion

The enamel surfaces of primary teeth investigated in this study after exposure to carbonated soft drinks, especially Coca-Cola soft drinks, showed coral-, network-, and map-like erosive patterns that could be classified as type 3 characteristic erosive patterns,[20] which result from a mixture of the hollowing of prism centers with peripheral parts being relatively preserved (type 1) and peripheral parts being dissolved with intact prism cores (type 2).

Consistent with the results of this study, when aprismatic structures in which the enamel rods are disoriented comprise the majority of primary tooth enamel, the efficacy of acid etching might be reduced, producing smooth-surfaced acid-resistant islands surrounded by porous acid-dissolved surfaces.[21,22] This is in contrast to permanent tooth enamel that showed pitting surface after being exposed to 36% phosphoric acid for 15 seconds.[19,22,23]

The Coca-Cola drinks in this study had acidic pH levels below the critical pH range required for enamel dissolution (pH 4.5-5.5)[6,11] and were similar to the other studies that found Coca-Cola Original to have pH between 2.16-2.76[16,24] and found alternative cola drinks, i.e., Coke Zero, Diet Coke, and Coca-Cola Light, to have pH between 2.60-3.16.[10,25] We found no difference between the enamel surfaces stored in the two Coca-Cola soft drinks. However, some studies have suggested that Coca-Cola Original causes deeper changes on enamel surfaces than sugar free Coca-Cola,[18,26,27] while another study found that Coca-Cola Original promoted less enamel loss than Coca-Cola Light and Diet Coke.[28] Sparkling water has been found to cause mild erosion of enamel,[29] as it has a pH ranging within the critical pH[19] and similar to other studies that found mild acidic pH between 4.35-6.30.[15,29]

The results of this study suggested that the pH levels of all investigated carbonated soft drinks were slightly increased, but not significant different, when measured 3 times at 5-minute intervals in comparison to after freshly opened the containers. In contrast, Bamise et al.[24] found the pH values of Coca-Cola drinks tended to decrease after opening the containers and repeated measurements every 30 seconds for 3 minutes.

According to the manufacturers' information, the acidic components of Coca-Cola soft drinks are composed of phosphoric acid and carbonic acid, while sparkling water mainly contains carbonic acid and other natural mineral elements.[5,10,12,13] Phosphoric acid in carbonated soft drinks is blamed as the primary cause of dental erosion because it has low pKa value[30] that classified as a triprotic acid containing three available protons to etch enamel surfaces[31] and cause permanent loss of enamel structure.[12,25,32] Meanwhile, carbonic acid is a diprotic acid with higher pKa value and is considered as a very weak acid.[30] The breakdown of this unstable acid after exposure to open air yields CO_2 and H_2O, causing a reduction in its concentration. This is consistent with the literature suggesting that the pH levels of 1, 10, and 100 mmol/l of carbonic acid were 4.68, 4.18, and 3.68, respectively,[33] which is corresponding with the results of this study indicating that the pH levels of the solutions were slightly increased, but not significantly different, with time after the containers were opened.

Many studies suggested that the acidic pH values of solutions appear to be primarily responsible for the early demineralization which softens enamel surfaces during the first few minutes of exposure.[12,13,24,31] The buffering capacity or titratable acidity is later responsible for the continuous demineralization during prolonged exposure.[10,12,13] However, some studies found no relationship between pH values and the erosive potential of solutions.[11,12]

The significant reductions in the Ca and P at.% levels on enamel surfaces found with EDS analysis in this study were consistent with the results of X-ray fluorescence spectroscopy and Raman microscopy scans.[14] Not only did an electron probe micro-analysis study find a similar reduction of up to 30 µm in depth from the enamel surface,[11] but an X-ray microanalysis (XRMA) also showed less quantities of Ca and P in demineralized lesions of enamel.[3] The Ca and P at.% contents of the enamel of the control group specimens in this study were 22.79±2.76 and 15.78±2.44, respectively, which were slightly higher than those found in a previous study[19] but higher than those of an XRMA,[32] which were 34.82±4.18 wt.% and 15.90±1.30 wt.% (equivalent to 18.90 at.% and 11.17 at.%, respectively).

In addition, the ratio of Ca to P remained constant after the specimens had been immersed in carbonated soft drinks, a finding similar to those of other studies,[11,22] suggesting that Ca and P were dissolved in the proportion to their formulas. The atomic ratio of Ca to P in the enamel of the control groups was 1.46±0.17, which was close to that of 1.45 (1.87±0.12 weight ratio) reported by Lakomaa and colleagues study.[34] These values were slightly less than the 1.67 value obtained for permanent teeth[35] and the ratio of hydroxyapatite crystal formula ($Ca_{10}(PO_4)_6(OH)_2$).[22,36,37] The higher P contents in the results might be related to the proteins substructure of enamel, such as amelogenin, enamelin, and ameloblastin, as these may be affected by phosphorylation during the calcification process.[38]

Large amounts of C, N, and O were observed in all the specimens with no significant changes in those amounts being observed after immersion in the drinks. These elements are commonly found in every human tissue, including enamel.[39,40] The concentrations of C and N were 33.87±5.28 at.% and 9.72±3.43 at.%, respectively, which were higher than those found in other studies of primary teeth[36,37] (11.25-23.09 at.% and 3.17-7.50 at.%). However, the concentration of O (15.11±4.57 at.%) was lower than the levels found in those two studies[36,37] (33.56-55.51 at.%). Besic and colleagues[40] proposed that acid-susceptible teeth contain more organic contents, including C (39%), H (42%), and O (6%), while acid-resistant teeth contain higher Ca (35%) and P (17%), whereas N and S contents were not considered.

The higher concentrations of C and N in the enamel of primary teeth than permanent teeth indicated the higher interprismatic space, making them more porous and permeable.[2,3] This space consists of organic material and calcium-deficient carbonate hydroxyapatite.[3] Carbonate can substitute for phosphate or hydroxyl ion in the crystal, resulting in decreased crystal stability and increased solubility that put this enamel at risk of dental caries and erosion.[41, 42] There was no significant change in the at.% of O in the present study, a finding that corresponded to the other studies.[37,42] This is because O is the major element of both organic and inorganic contents of hard tissue of the tooth, including hydroxyapatite crystal.[40]

The at.% levels of F and Mg were 0.54±0.64 and 0.16±0.21, respectively, which were lower than a previous study (1.19±0.34 and 0.23±0.08, respectively).[36] There were no significant changes in these levels after exposure to carbonated soft drinks, consistent with the findings of most previous studies.[42-44] It is possible that the amounts of F and Mg in this study were too minute to determine any difference using the EDS technique.

This in vitro study might not represent real-life intraoral conditions, which include various factors such as the salivary flow rate and differing dietary habits. The timing of holding the drinks in the mouth might led to prolonged contact to the teeth and promoted the pronounced pH drop of the tooth surface.[24] Moreover, the enamel specimens in this study were exposed to solutions and maintained at room temperature rather than at the typical intraoral temperature, which might increase enamel solubility.[28]

Prolonged exposure to carbonated soft drinks caused morphological changes and significant reductions of Ca and P levels in the enamel surfaces. It is thus important to include in dietary counseling for children and their parents that consumption of carbonated soft drinks, including sparkling water, may cause dental erosion and promote dental caries.

Conclusions

After the exposure of primary teeth specimens to three carbonated soft drinks, morphological changes on the enamel surfaces were observed. The enamel surfaces exposed to two Coca-Cola drinks were largely dissoluble, showing both a hollowed prism centers with preserved peripheral parts pattern mixed with intact prism cores and peripheral dissolution pattern. Sparkling water was shown to cause less dissolution of the enamel surfaces. In addition, the Ca and P elements were the only two elements that showed significant decreases in their in at.% levels after the specimens were immersed in the three carbonated soft drinks.

Declaration of Interest

The authors report no conflict of interest.

References

1. Ten Cate A. Oral Histology, Development, Structure, and Function. St. Louis: Mosby, New York: Inc; 1998.
2. De Menezes Oliveira MAH, Torres CP, Gomes-Silva JM, Chinelatti MA, De Menezes FCH, Palma-Dibb RG, et al. Microstructure and mineral composition of dental enamel of permanent and deciduous teeth. Microsc Res Tech. 2010;73(5):572-7.
3. Sabel N, Robertson A, Nietzsche S, Norén J. Demineralization of enamel in primary second molars related to properties of the enamel. Sci World J. 2012.
4. Wong F, Anderson P, Fan H, Davis G. X-ray microtomographic study of mineral concentration distribution in deciduous enamel. Arch Oral Biol. 2004;49(11):937-44.
5. West NX, Joiner A. Enamel mineral loss. J Dent. 2014;42:S2-S11.
6. Larsen M, 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.
7. Linnett V, Seow W. Dental erosion in children: a literature review. Pediatr Dent. 2001;23(1):37-43.
8. Rirattanapong P, Vongsavan K, Suratit R, Tanaiutchawoot N, Charoenchokdilok V, Jeansuwannagorn S, et al. Effect of various forms of calcium in dental products on human enamel microhardness in vitro. Southeast Asian J Trop Med Public Health. 2012;43(4):1053-8.
9. Haghgou HR, Haghgoo R, Asdollah FM. Comparison of the microhardness of primary and permanent teeth after immersion in two types of carbonated beverages. J Int Soc Prev Community Dent. 2016;6(4):344.
10. Lussi A, Megert B, Shellis RP, Wang X. Analysis of the erosive effect of different dietary substances and medications. Br J Nutr. 2012;107(2):252-62.
11. Willershausen B, Schulz-Dobrick B. In vitro study on dental erosion provoked by various beverages using electron probe microanalysis. Eur J Med Res. 2004;9(9):432-8.
12. Barac R, Gasic J, Trutic N, Sunaric S, Popovic J, Djekic P, et al. Erosive effect of different soft drinks on enamel surface in vitro: application of stylus profilometry. Med Princ Pract. 2015;24(5):451-7.
13. Wang YL, Chang CC, Chi CW, Chang HH, Chiang YC, Chuang YC, et al. Erosive potential of soft drinks on human enamel: an in vitro study. J Formos Med Assoc. 2014;113(11):850-6.
14. Cândido M, Silveira J, Mata A, Carvalho M, Pessanha S. In vitro study of the demineralization induced in human enamel by an acidic beverage using X-ray fluorescence spectroscopy and Raman microscopy. X-Ray Spectrometry. 2019;48(1):61-9.
15. Parry J, Shaw L, Arnaud M, Smith A. Investigation of mineral waters and soft drinks in relation to dental erosion. J Oral Rehabil. 2001;28(8):766-72.
16. Caneppele TMF, Jeronymo RDI, Di Nicoló R, Araújo MAMd, Soares LES. In Vitro assessment of dentin erosion after immersion in acidic beverages: surface profile analysis and energy-dispersive X-ray fluorescence spectrometry study. Braz Dent J. 2012;23(4):373-8.
17. Barac R, Gašić J, Popović J, Mitić A, Radenković G, Potić-Floranović M, et al. Evaluation of enamel surface roughness and morphological changes after exposure to Coca-Cola, orange and artificial gastric juice: An in vitro study. Acta Medica Medianae. 2018;57(3):33-40.
18. Owens B, Kitchens M. The Erosive Potential of Soft Drinks on Enamel Surface Substrate: An. J Contemp Dent Pract. 2007:011-20.
19. Brown CJ, Smith G, Shaw L, Parry J, Smith AJ. The erosive potential of flavoured sparkling water drinks. Int J Paediatr Dent. 2007;17(2):86-91.
20. Silverstone L, Saxton C, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res. 1975;9(5):373-87.
21. Kakaboura A, Papagiannoulis L. Bonding of resinous materials on primary enamel. Dental Hard Tissues and Bonding: Springer; 2005. p. 35-51.
22. Alcantara Galeana MdCZ, Contreras Bulnes R, Rodríguez Vilchis LE, Espinosa Pesqueira ME, Barrera Ortega CC, López Hurtado IM, et al. Microhardness, structure, and morphology of primary enamel after phosphoric acid, self-etching adhesive, and Er: YAG laser etching. Int J Opt. 2017;2017.
23. Fava M, Myaki SI, Arana-Chavez VE, Fava-de-Moraes F. Effects of a non-rinse conditioner on the enamel of primary teeth. Braz Dent J. 2003;14:168-71.
24. Bamise C, Ogunbodede E. Dental erosion: immediate pH changes of commercial soft drinks in Nigeria. Tanz Dent J. 2008;15(1).
25. Owens B, Mallette J, Phebus J. Effects of carbonated cola beverages, sports and energy drinks and orange juice on primary and permanent enamel dissolution. Austin J Dent. 2014:1004.
26. Rios D, Honório HM, Magalhães AC, Wiegand A, Machado MAdAM, Buzalaf MAR. Light cola drink is less erosive than the regular one: an in situ/ex vivo study. J Dent. 2009;37(2):163-6.
27. Rios D, Ionta F-Q, Rebelato R, Jordão M-C, Wang L, Magalhães A-C, et al. The effect of aspartame and pH changes on the erosive potential of cola drinks in bovine enamel: An in vitro study. J Clin Exp Dent. 2018;10(9):e933.
28. Korte A, Angelopoulou MV, Maroulakos G. Assessing the Effect of Low Calorie Soda Beverages on Primary Tooth Enamel: An In Vitro Study. J Clin Pediatr Dent. 2019;43(3):190-5.
29. Marchan SM, Bascombe K, Hector T, Smith WA, Ramnanansingh TG. The long-term effect of sparkling flavored water on human tooth enamel determined by gravimetric analysis: a preliminary evaluation. Braz Dent Sci. 2020;23(1):1-7.
30. Munegumi T. Where is the border line between strong acids and weak acids. World J Chem Educ. 2013;1(1):12-6.
31. Reddy A, Norris DF, Momeni SS, Waldo B, Ruby JD. The pH of beverages available to the American consumer. J Am Dent Assoc. 2016;147(4):255.
32. Weat N, Hughhes J, Addy M. The effect of pH on the erosion of dentine and enamel by dietary acid inviter. J Oral Rehab. 2001;28(9):860-4.
33. O'Neill M. The Merck Index. An Encyclopedia of Chemicals, Drugs and Biologicals. Whitehouse Station, New Jersey: Merck Research Laboratories, Division of Merck and Co. Inc; 2006.
34. Lakomaa EL RI. Mineral composition of enamel and dentin of primary and permanent teeth in Finland. Eur J Oral Sci. 1977;85(2):89-95.
35. Dusevich V, Melander JR, Eick JD. 13 SEM in dental research. Scanning Electron Microscopy for the Life Sciences. 2012:211.
36. Wongyai T, Sirimaharaj V, Wanachantararak S. Mineral Comparisons of Primary Teeth Among Different Caries Experience Groups in vitro. CM Dent J. 2019;40(3):135-45.
37. Sabel N, Robertson A, Nietzsche S, Norén J. Demineralization

of enamel in primary second molars related to properties of the enamel. Sci World J. 2012;2012.
38. Yamakoshi Y. Dental and Oral Biology, Biochemistry. 2014.
39. Brown CJ, Chenery SR, Smith B, Mason C, Tomkins A, Roberts GJ, et al. Environmental influences on the trace element content of teeth—implications for disease and nutritional status. Arch Oral Biol. 2004;49(9):705-17.
40. Besic F, Bayard M, Wiemann Jr M, Burrell K. Composition and structure of dental enamel: elemental composition and crystalline structure of dental enamel as they relate to its solubility. J Am Dent Assoc. 1975;91(3):594-601.
41. Sydney-Zax M, Mayer I, Deutsch D. Carbonate content in developing human and bovine enamel. J Dent Res. 1991;70(5):913-6.
42. Adabache-Ortiz A, Silva-Briano M, Campos-Esparza M, Ventura-Juárez J. Comparison of chemical elements on carious & normal premolar's enamel layers using energy dispersive X ray spectrometer (X Ray-EDS). Microscopy Research. 2014;2(04):81.
43. Derise NL, Ritchey S. Mineral composition of normal human enamel and dentin and the relation of composition to dental caries: II. Microminerals. J Dent Res. 1974;53(4):853-8.
44. Derise NL, Ritchey S, Furr A. Mineral composition of normal human enamel and dentin and the relation of composition to dental caries: I. Macrominerals and comparison of methods of analyses. J Dent Res. 1974;53(4):847-52.