In vitro Comparative Study on the Effect of Carbonated Beverages on Dental Enamel

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In recent decades, significant worldwide increase in the consumption of acidic beverages, such as soft drinks and fresh fruit extended to all categories of consumers but especially to young people. The purpose of this study is to demonstrate the effect of some of the most consumed beverages on dental enamel in order to draw an alarm signal. The study was performed in vitro on 18 extracted teeth. Half of the coronary enamel was covered with a nail polish, then the teeth were submerged in hermetic containers with the most commonly used beverages: Coca Cola, sugar-free Coca Cola, orange juice, red wine, green tea, Red Bull for one hour, one day, 3 days and 7 days. Evaluation of the samples at one hour, one day and 3 days was performed by examination at the optical microscope. After 7 days, the insulating nail polish was removed and the microstructure of the enamel exposed and not exposed to acidic action, was examined under the scanning electronic microscope (SEM). Results showed that because of the high acidity of the beverages used in the study, the surface of the enamel showed prisms changes, structural loss of the enamel, which can lead to cracks and structural disintegration with the reduction of the physical and mechanical properties of the enamel. The results obtained in this in vitro study should be reinterpreted and associated with the in vivo situation where biological factors such as bacterial plaque and saliva buffering capacity are likely to reduce the potential for erosion of beverages. Consequently, enamel erosion may be lower compared to the measurements found in this study.

Keywords: enamel erosion, acidic beverages, soft drinks, optical microscope, SEM analysis

Dental erosion (referred to in WHO classification as K03.2) [1] is a chronic, non-bacterial chemical process, caused by external or internal factors. External factors are consumption of foods and acidic beverages, and internal factors include regurgitation and gastric reflux. It is worth mentioning that the action of these factors is evident only when they act for a long period of time.

Consumption of soft drinks favors the appearance of tooth decay and enamel erosion. The production of dental caries is caused by the high sugar content of these drinks associated with deterioration of oral hygiene patterns. In order to reduce the risk, sugar substitutes foods and beverages have been recommended but sugar-free soft drinks have often been found to have a high potential for erosion.

Compared to cavities, dental erosion appears to be more strongly influenced by consumption of soft drinks.

In recent decades, there has been a significant worldwide increase in the consumption of acidic beverages, such as soft drinks and fresh fruit and vegetables. In fact, the latter are considered a healthy diet, which is being used by a growing number of patients.

Carbonated beverages have been reported in research studies as having a low *p*H (93% having *p*H <4) and a high buffering capacity [2]. They are sweetened with refined carbohydrates or sugar substitutes and contain additives that together can contribute to the dissolution of the enamel surface.

Lussi et al. reported that any *p*H below 4.5 is critical. They also reported that any *p*H above 4.5 will cause fluorine pathy formation and reduction of erosion potential [3].

These are sweetened with refined carbohydrates or sugar substitutes and contain additives that together can contribute to the dissolution of the enamel surface. Other factors involved in the dissolution of the enamel surface and the occurrence of erosion include the type, concentration and amount of acid, chelating properties of ingredients, the frequency and duration of exposure, the temperature [4].

Sugar-free drinks, marketed as *zero* or dietetic, are promoted as healthier alternatives. But, although sugar is out of the list of ingredients, the acidity is the same. Research shows that acidic beverages with and without sugar also generate similar levels of tooth erosion [5].

Frequent and excessive consumption of some acidic beverages such as lemon juice, orange juice, cola refreshments and citrus-flavored drinks has been reported as a risk factor for dental erosion [6].

Although prevalence and incidence of erosion is relatively high, a survey based on a questionnaire showed that only one third of practitioners observed erosion at the consultation, and the majority underestimated the prevalence of the disease [5].

Therefore, it is necessary to establish a diagnostic protocol aimed at examining dental structures by applying specific criteria.

However, in spite the fact that the prevalence of dental erosion is high and rising in many developed countries, its relevance to oral health is not comparable to marginal cavities and periodontitises [7].

Refreshments containing acids and sugars have acidic potential, many studies have shown a positive relationship

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between the occurence of dental erosion and the consumption of acidic beverages [4, 8-10].

Acid meals and drinks are a significant component of many diets. A drink becomes harmful to dental enamel when its *p*H is below 5.5.

Due to the fact that in developed countries consumption of carbonated juices as well as fast food foods with acidic activity is extended to all categories of consumers but especially to young people, the purpose of this study is to demonstrate the effect of some of the most consumed beverages on dental enamel in order to draw an alarm signal.

Experimental part

Material and method

The study was performed in vitro on 18 extracted teeth, which were washed and dried for 24 h on an absorbent surface. Half of the coronary enamel was covered with a nail polish, then the teeth were submerged in hermetic containers with the most commonly used beverages: Coca Cola, sugar-free Coca Cola, orange juice, red wine, green tea, Red Bull for one hour, one day, 3 days and 7 days. Each time, the beverages were replaced with new ones.

The initial *p*H of each beverage was measured using a calibrated *p*H meter at the beginning of each determination using 5.5 *p*H as the standard.

The composition and *p*H of the beverages used is shown in table I.

The acidic beverages were changed in each cycle to ensure the presence of phosphoric acid and the containers were sealed because the removal of the beverage gas increases the *p*H and decreases the potential for dissolution of the hydroxyapatite [11].

Evaluation of the samples at one hour, one day and 3 days was performed by examination at the optical microscope.

After 7 days, the insulating nail polish was removed and the microstructure of the enamel exposed and not exposed to acidic action, was examined under the scanning electronic microscope (SEM). For SEM analysis, the samples were dehydrated, fixed on a STAB-type aluminum support by gluing a copper conducting strip to discharge the load. The new system was introduced into the Quorum type coat and covered with a 9nm gold coat to conduct the investigated conductivity of the SEM for 60sec. Sample investigation was performed using the QUANTA INSPECT F scanning microscope with field emission FEG (field emission gun) of 1.2nm resolution and an energy dispersive X-ray spectrometer (EDS) with the resolution at MnK de 133eV.

Results and discussions

The appearance of the enamel after removing the teeth from the selected beverages for study at 1h, 24 h, 3 days and 7 days is shown in figure 1-6.

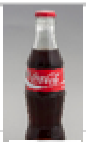
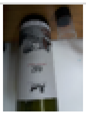
The SEM images show the microstructure of the enamel exposed to the action of the analyzed beverages (fig.7-18).

This study shows the effect of six of the most consumed soft drinks on the enamel. The results that are revealed in this study indicate that the soft drinks used cause changes in the superficial enamel morphology.

By evaluating samples of soft drink at 1h, 24 h, 3, and 7 days we could observe the rhythm and the gradual action on the enamel of the six beverages.

Macroscopic and microscopic morphology shows a degree of enamel dissolution in descending order of the

Table 1
DRINKS USED IN THE STUDY

Beverages		pH	Composition
Coca cola		2.36	Carbonated water, sugar, dye (150d), acidifier (338 *), flavorings, caffeine
Coca Cola light		2.97	Fizzy water, coloring agent (150d), acidifying agent (338, 331*), flavorings, caffeine, sweeteners (951*, 950*), preservatives (211*)
Orange juice		2.8	Oranges (citric acid)
Red Wine		3.5	Water, ethyl alcohol 8.5-18% vol., Tartaric acid, malic, citric, succinic, pyruvic, glucose, fructose, polyhydric alcohols, phenolic compounds, minerals, vitamins
Green tea		2.93	Green tea (Japan); infusion 2 tea bags/400ml water
Red Bull®		3.23	Water, sucrose, glucose, sodium citrate, taurine, gluconolactone, caffeine, inositol, niacinamide, calcium pantothenate, HCL pyridoxine, vitamin B12, flavors and artificial dyes.

**330 = Citric Acid, 331 = Sodium Citrate, 338 = Phosphoric Acid; 150d = Caramel IV; 221 = sodium benzoate; 951 = aspartame, 950 = potassium acetosulfame.*

Appearance after 1 hour	Appearance after 24 hours	Appearance after 3 days	Appearance after 7 days	Final appearance

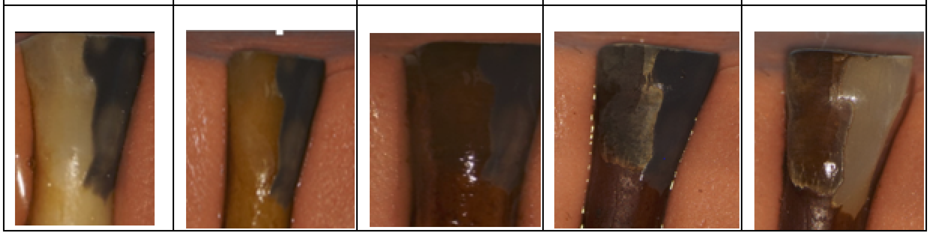


Fig. 1. The appearance of the enamel after holding the teeth in Coca-Cola

Appearance after 1 hour	Appearance after 24 hours	Appearance after 3 days	Appearance after 7 days	Final appearance

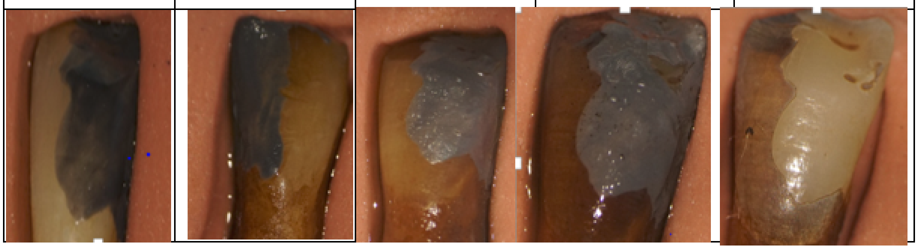


Fig. 2. The appearance of the enamel after holding the teeth in Coca Cola light

Appearance after 1 hour	Appearance after 24 hours	Appearance after 3 days	Appearance after 7 days	Final appearance

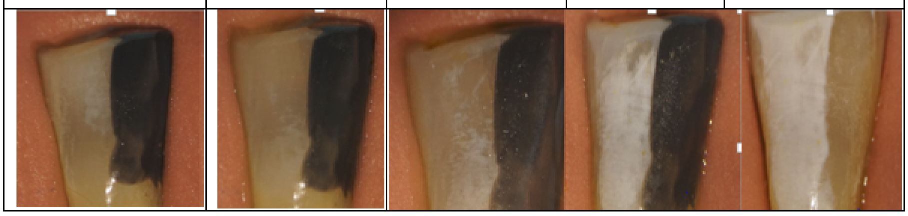


Fig. 3. The appearance of the enamel after holding the teeth in orange juice

Appearance after 1 hour	Appearance after 24 hours	Appearance after 3 days	Appearance after 7 days	Final appearance

Fig. 4. The appearance of the enamel after holding the teeth in red wine

Appearance after 1 hour	Appearance after 24 hours	Appearance after 3 days	Appearance after 7 days	Final appearance

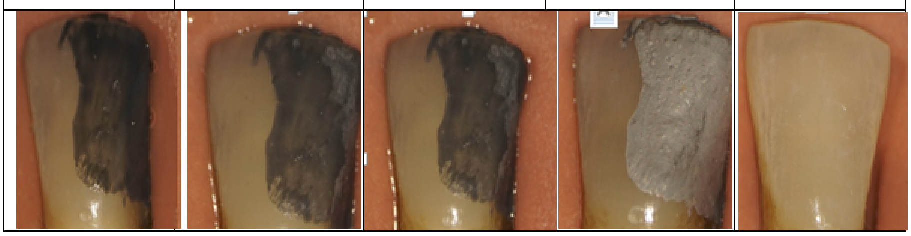


Fig. 5. The appearance of the enamel after holding the teeth in green tea

Appearance after 1 hour	Appearance after 24 hours	Appearance after 3 days	Appearance after 7 days	Final appearance

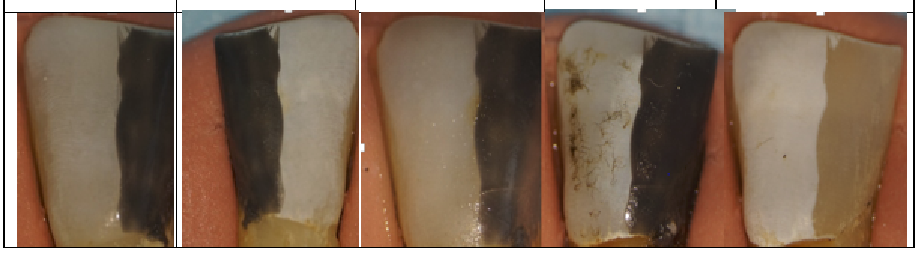


Fig. 6. The appearance of the enamel after holding the teeth in Red Bull

Sample 1 Coca-Cola

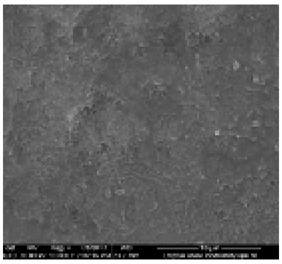


Fig.7 Coca-Cola dissolved the secondary enamel coating in the image, observing groups of prisms and inter-prismatic enamel

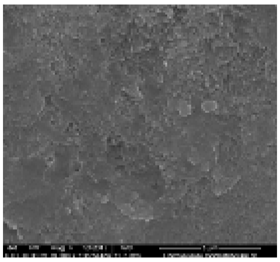


Fig.8 Partial enamel demineralization, with enamel stretched areas in which the prisms have completely disappeared and prism zones undergoing dissolution. This is due to the direction of the prisms in the groups but also because the demineralization initially affects the prism sheath

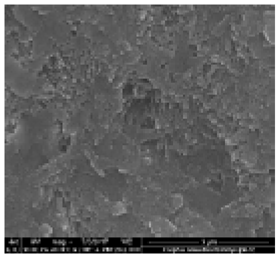


Fig.9 At magnification 40 000x an unstructured enamel mass is interrupted by the partially demineralized structures of the original prisms. At the level of some prisms, there are also partially circumscribed areas of prisms. The appearance is broken by prismatic structure with rare-cut crystals whose ends seem balloon (match-stick) and complete destroyed prisms.

Sample 2 Red Bull

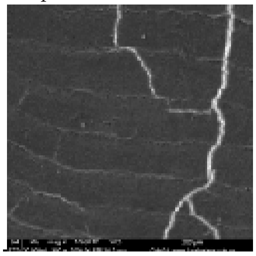


Fig. 10. Horizontal lines are Retzius strips that become visible as a result of the dissolution of the aprismatic enamel of the surface

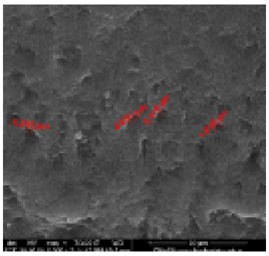


Fig. 11. Crystals with faded contours are noticed, indicating an increased degree of demineralization inside the prisms (similar to the appearance of Coca Cola); The number of hydroxyapatite crystals is very low

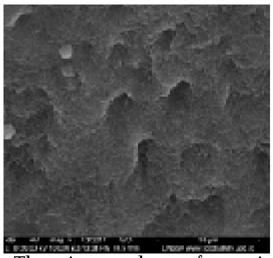


Fig. 12. The picture shows few prisms that maintain the appearance of hydroxyapatite crystals, areas where crystals are missing. Crystals are thinned and disordered

Sample 3 Coca Cola light

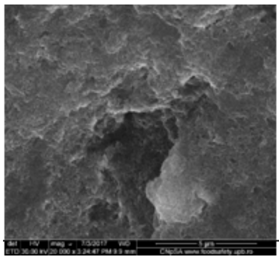


Fig. 13. Prisms with dense crystals are observed, the prisms remain full

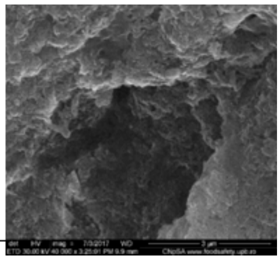


Fig. 14. There are dense crystals, there are no crystalline areas lacking at the level of the prisms

Sample 4 Green Tea

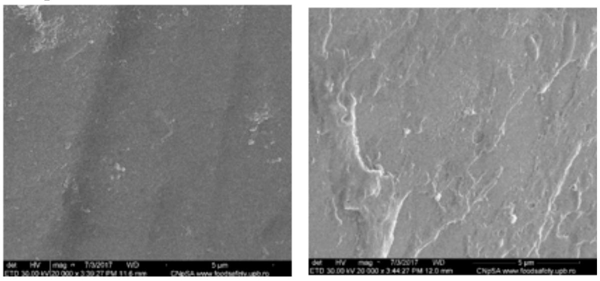


Fig. 15 a, b There are no changes in prism/crystal structure

Sample 5 red wine

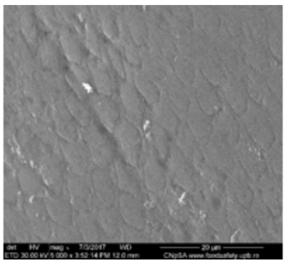


Fig. 16. Normal, compact enamel structure with normal appearance prisms. (Red wine stains, does not demineralize)

Sample 6 fresh orange juice

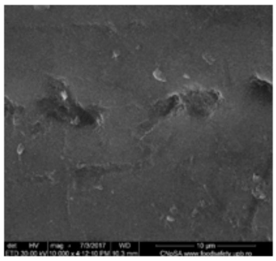


Fig. 17. In the picture we observe the compartmented demineralization of the enamel, similar to the teeth emerged in Coca-Cola

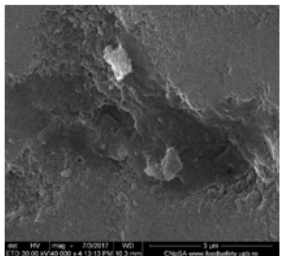


Fig. 18. Demineralization is lower than in Coca Cola, the power of acid penetration seems to be weaker, but the appearance also depends on prism orientation

following: Red Bull, Coca Cola, orange juice, Cola light, red wine, green tea. Despite the fact that the Red Bull *p*H value is over 3, it has the most intense demineralisation effect. This demonstrates that the effect of these beverages on enamel is also influenced by other factors outside the *p*H, such as exposure time, acid type (e.g., phosphoric acid or citric acid), salivary buffer, adhesion, chelating effect, phosphate, fluoride and calcium content of the beverage, age [12]. Besides these, there are individual patient-related factors such as drinking habits, salivary composition and flow, or oral hygiene measures (eg fluoride) which, although influencing the occurrence of erosion, are variable, should not be included as landmarks [2].

Energy drinks are potentially cariogenic and erosive because they contain sugar that feeds oral bacteria and have a low *p*H, which can have effects on both teeth and restorative materials [13].

In this study, Red Bull's SEM images show the mass demineralization of the hydroxyapatite crystals and the decrease in their number, the crystals being thinned and disordered. The appearance is one of a uniform demineralization, almost total of the exposed prisms. Red Bull had the highest buffering capacity (indicating the strongest enamel erosion potential).

In their recent study, Beltan and Cardona have shown that energy drinks produce enamel erosion based on exposure time [14].

According to a study, classic and dietetic Coca Cola, red wine, orange juice have average potential for erosion, and Red Bull has high potential for erosion [12].

Through the SEM evaluation of teeth introduced into Coca-Cola type beverages, it is noted that they present a pattern of enamel partial demineralization, the stronger demineralization being produced by Coca-Cola. It dissolved the secondary enamel coating and caused the appearance of the partially demineralized enamel areas alternating with unstructured enamel areas.

Lussi et al., in a recent study, has shown that the erosive effect of the Coca Cola drink is manifested both in deciduous and permanent teeth and demineralisation of deciduous teeth enamel is significantly associated with the *p*H and calcium concentration of the beverage [15].

Studies show that enamel hardness is greatly reduced in the case of teeth submerged 5 min /day, 10 days in Coca-Cola type beverages and lemon drinks, moreover, remineralization treatment with fluoride products, after 10 days shows the existence of a partial recovery, only ¾ of enamel hardness [16].

The erosive capacity of Coca Cola is known and determined by research so that a recent study that wanted to demonstrate the power of remineralizing of an innovative preparation has used this beverage in artificial in-vitro teeth demineralization [17].

Fruits such as grapefruit, oranges and lemons are acidic and can erode the tooth enamel. When they are concentrated in juices are more harmful. Orange juice has the ability to reduce enamel hardness by 84%. But because juices also have beneficial effects, they should be consumed occasionally and completely at a single consumption rather than being drunk all day long [4].

In this study, orange juice causes enamel compartmented demineralisation, similar to that produced by Coca-Cola. In both Coca Cola and fresh orange juice, the microscopic appearance of partially demineralized prisms depends on their direction.

These results are in line with literature data showing that cola and orange juice have a high erosive potential [18- 22].

In the case of Coca Cola light on SEM images, there are prisms with dense crystals, there are no prism-free areas of crystals, but perhaps even in this case, the secondary enamel coating is destroyed.

This is in agreement with the study by Owens et al. which shows that Coca-Cola has caused erosive effects of higher enamel compared to dietetic coke. This finding suggests that the presence of refined carbohydrates (sucrose, fructose) in Coca-Cola compared to artificial sweeteners in dietary composition can contribute to the demineralisation of teeth [23].

Regarding tea and wine, in this study, there were no changes in the prisms/crystals structure in the case of samples introduced in these beverages, red wine proved to be more readily a factor in the production of enamal dyschromia.

Initially, the *p*H of the grape juice is approximately 3.0 and varies from *p*H 3.3 to *p*H 3.8. The main constituent acids are malic and tartaric acid, with a concentration of 5-8 g / L, and lactic acid in the concentration of 1-3 g / L, depending on the degree of lactic fermentation, together with lower amounts of succinic acid and citric acid.

Research has shown that the micro hardness of teeth enamel introduced into red wine 5 min /day, 10 days has a moderate effect and remineralization techniques produce total recovery of the initial enamel hardness properties [16].

This is inconsistent with a study that reported the presence of pronounced enamel demineralisation areas and a widening of dentinal canals as well as the presence of irregular polyhedrine deposits on the surface of enamel and dentine teeth kept in wine for 30 min [24].

The study by George R et al. demonstrated the presence of somelier erosion but also the linear relationship between the number of years of activity of the taster and the degree of erosion, which confirms the theory that, in order to cause erosion, the action of etiological factors must be constant for a long period of time [25].

Tea is often seen as a healthier alternative to juices and sweet drinks because of its antioxidants. In addition, it has been reported that tea has a complex composition and its consumption has beneficial effects on the teeth due to its rich content of fluoride. Although the tea is acidic, with a *p*H of 2.9, it reduces only 1 *p*H unit to the surface of the teeth, and the rest of the *p*H is restored about 2 min after consumption [26].

In this study, no changes were observed in the enamel crystals/prisms introduced into green tea.The poor erosive effect of tea is mentioned in various research [22, 27].

But other authors have shown that unsweetened green and black tea are corrosive, but black tea was more aggressive than green tea. However, tea is not as bad as other acidic or sugary substances. Teeth introduced into substances such as lemon juice, vinegar and soda showed changes and injuries in the second week, while black tea did not erode teeth until the 16th week [12] .

A recent study showed that teeth held for 3 months in tea and coca cola are at risk for fractures and cracks when applying brackets to orthodontic treatments [28].

Regarding the moment of structural changes and their evolution (analyzed with the optical microscope at one hour, 24 h, 3, 7 days), Coca-Cola had a higher erosive potential as early as the first hour of exposure. Orange juice produced demineralization after longer exposures (3 days). The explanation is the presence of citric acid in orange juice, which has the ability to chelate calcium ion with the effect of accelerated calcium loss in the dental structure, thus demineralization of the enamel.

In addition to citric acid, orange juice contains many different weak organic acids, such as ascorbic acid, malic acid, tartaric acid and oxalic acid, which can be considered as buffer components [29].

In agreement with these data, Barac et al. in their study of how the teeth and orange juice exposure were 15, 30 and 60 min, 3/day for 10 days showed that Coca-Cola had the strongest erosion potential in the first 15 min of exposure, while at the 30- and 60 min exposures, the potential for erosion between Coca-Cola and orange juice was similar [30].

In the first exposure hour, Red Bull (*p*H 3.23) and Coca-Cola type beverages (*p*H 2) showed a higher erosion potential than orange juices (*p*H 2.8). After a day, however, the erosive potential of cola drinks slowed down compared to orange juice. This shows, once again, that only *p*H does not provide valid information about the erosive capacity of beverages.

Beverage ingredients can play an important role in their erosive capacity.

Red Bull's high level of enamel dissolution is due to the high concentration of refined carbohydrates (sucrose, glucose) and to the chelating properties of Red Bull sodium citrate [31].

Citric acid (also found in orange juice at a concentration of 1%) is an important constituent of various acidic beverages with erosive potential which cause demineralisation of enamel. Citric acid has a great potential for dissolving hydroxyapatite crystals by forming calcium citrate and chelating action (calcium binding) that removes Ca2 + from tooth enamel, resulting in an increased tendency to demineralize [22].

Citric acid has a higher erosive capacity than the phosphoric acid found in Cola drinks (0.1% concentration) and which is a weak mineral acid. Phosphoric acid produces minimal erosion at *p*H 3 for enamel and *p*H 4 for dentin.

Rios et al. (2009) conducted a study comparing the classic and dietetic Coca Cola erosive potential and concluded that Dietetic Cola has a 5-fold lower erosive effect compared to classic cola. The factors that determined this would be explained by the difference in *p*H between the two beverages and the presence of phenylalanine resulting from the hydrolysis of aspartame in the presence of saliva [32].

Coca Cola without sugar and wine contain sodium citrate (citric acid sodium salt), a buffering agent that can help maintain the *p*H level in carbonated beverages. Citrates can also bind calcium, favoring elevated levels of acidity.

EDS analysis has shown that when calcium has decreased on the exposed surface, phosphorus has also decreased, however, oxygen has increased, which confirms that early demineralisation acts at the structural level of hydroxyapatite [14].

High-quality refreshments have significantly lower erosive potential. Low *p*H and high citrate content may result in a higher loss of enamel coating. The erosive potential of soft drinks can be predicted on the basis of acid content, *p*H, titration, and ion concentration [33].

Conclusions

SEM results showed that because of the high acidity of the beverages used in the study, the surface of the enamel showed prisms changes, structural loss of the enamel, which can lead to cracks and structural disintegration with the reduction of the physical and mechanical properties of the enamel.

On the other hand, the results obtained in this in vitro study should be reinterpreted and associated with the in vivo situation where biological factors such as bacterial plaque and saliva buffering capacity are likely to reduce the potential for erosion of beverages. Consequently, enamel erosion may be lower compared to the measurements found in this study.

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