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In vitro investigation of erosive effect of carbonated beverages on enamel and dentin

In vitro studija o erozivnom uticaju gaziranih napitaka na gleđ i dentin

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Abstract

Background/Aim. Excessive consumption of acidic dietary substances, such as carbonated beverages, increased the chances of dental erosion. The aim of this study was to determine influence of carbonated beverages on enamel and dentin, during different intervals. **Methods.** Sixty samples were obtained from fifteen impacted third molars. Tooth crown was divided into four parts. One part was a control sample, immersed in destilled water and other three parts were the experimental samples, stored in the following tested beverages: carbonated water, Coca-Cola and Schweppes Bitter Lemon. For each beverage, pH was measured before immersion of the samples. The first group of twenty samples were analysed and photographed, using a Scanning Electron Microscope (SEM), after 60 minutes, the second group after 24 hours and the third group after 7 days of exposure to drinks. The enamel was analysed on the outer surface of the cusps and longitudinal section. Dentin was analysed on longitudinal section. An individually adopted scale was used for determination of the degree of erosive changes of these dental tissues. The data were analysed using the analysis of varance (ANOVA). **Results.** The pH levels of the tested beverages was bellow the critical pH for enamel demineralisation. The SEM images showed different intensity of erosive changes caused by the tested beverages. The analysis by ANOVA revealed a significant difference between all groups of the treated samples, after 60 minutes of exposure to beverages. The highest values of erosive changes showed the samples that were immersed in Schweppes Bitter Lemon. The analysis of the samples after 24 hours and 7 days showed that the samples immersed in Coca-Cola and Schweppes Bitter Lemon can be classified as one group that was statistically significantly different compared with the control samples and samples immersed in carbonated water. **Conclusion.** Prolonged exposure of dental tissue to carbonated beverages cause erosive changes and a loss of surface profile.

Key words:
carbonated beverages; immersion; enamel microabrasion; dentin.

Apstrakt

Uvod/Cilj. Prekomerno konzumiranje kiselih namirnica, uključujući i gazirane napitke, povećava mogućnost za nastanak dentalnih erozija. Cilj ovog rada je da se ispita uticaj gaziranih napitaka na gleđ i dentin tokom različitih vremenskih intervala. **Metode**. Šesdeset uzoraka je dobijeno od 15 impaktiranih trećih molara. Krunica zuba je podeljena na četiri dela. Jedan deo bio je kontrolni uzorak, potopljen u destilovanu vodu, a preostala tri dela su bili eksperimentalni uzorci, koji su potapani u ispitivane napitke: gaziranu vodu, Coca-Cola-u i Schweppes Bitter Lemon. Za svaki napitak izmerena je pH vrednost pre potapanja uzoraka. Prva grupa od 20 uzoraka je analizirana i fotografisana pomoću skenirajućeg elektronskog mikroskopa (SEM) posle 60 minuta, druga grupa posle 24 sata i treća grupa posle sedam dana izloženosti delovanju napitaka. Gleđ je analizirana na spoljašnjoj površini kvržice i na uzdužnom preseku. Dentin je analiziran na uzdužnom preseku. Za određivanje stepena nastalih erozivnih promena pomenutih zubnih tkiva primenjena je individualno prilagođena skala. Za analizu podataka korišćena je analiza varijanse (ANOVA). **Rezultati.** Izmerena pH vrednost ispitivanih napitaka bila je niža od kritične pH vrednosti pri kojoj dolazi do demineralizacije gleđi. Na SEM mikrofotografijama uočen je različit stepen erozivnih promena uzrokovanih delovanjem ispitivanih napitaka. Analizom podataka primenom ANOVA dobijena je statistički značajna razlika između svih grupa tretiranih uzoraka, nakon 60 minuta izloženosti delovanju napitaka. Najveći intenzitet erozivnih promena bio je prisutan na uzorcima koji su po-

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topljeni u Schweppes Bitter Lemon. Analiza uzoraka nakon 24 sata i sedam dana izloženosti delovanju napitaka pokazala je da uzorci potopljeni u Coca-Cola-u i Schweppes Bitter Lemon mogu biti svrstani u jednu grupu koja je statistički značajno različita u odnosu na kontrolne uzorke i uzorke potopljene u gaziranu vodu. **Zaključak.** Povećano izlaganje zubnih tkiva delovanju gaziranih napitaka uzrokuje erozivne promene i gubitak površinske strukture.

Ključne reči:
gazirana pića; imerzija; gleđ, mikroabrazija; dentin

Introduction

The consumption of soft drinks has increased enormously in the last few years [1, 2]. Soft drinks are non-alcoholic, flavored, carbonated beverage, usually commercially prepared and sold in bottles and cans [3]. Although not necessarily common to all types of carbonated beverages, the principal properties of note are carbonation, acidity and high levels of sugar or artificial sweeteners [4]. The effects of these beverages on dental hard tissues were extensively investigated in the last decade. Meta-analysis of the studies assessing tooth erosion and diet showed that high consumption of soft drinks, including carbonated beverages, was associated with an increased chance of tooth erosion. In fact, some studies considered soft drinks as the main cause of the perceived rise in the occurrence of dental erosion in young people [5–7]. The erosive potential of these drinks is thought to involve several factors: the types of acid content, pH value, titratable acidity, and ion concentration [8]. Intraoral pH, after drinking an acidulated drink, decreases to below pH5 within 2 to 3 minutes [9]. After acidic attact, pH takes about 25 minutes to change the acid environment, as further stimulated saliva neutralises any residual acid [9]. A single acidic attack is therefore of a minor importance, but if repeated, the ability of saliva to deal with the acid reduces. Hence, the danger is the frequent use of soft drinks over time [10].

The aim of this study was to investigate, *in vitro*, the influence of different types of carbonated drinks on the dental hard tissues, enamel and dentin during time and to identify which types of these drinks are potentially the most aggressive toward the dental hard tissues.

Methods

Sixty samples for this study were obtained from 15 impacted third molars. After extraction, the soft tissue that remained was removed from the teeth surface, the teeth were washed thoroughly under running water and then immersed in 5.25% solution of sodium hypochlorite for one hour. The teeth were stored in the saline solution at the room temperature for no more than one month until the experiment started. A tooth crown was separated from the root using a water-cooled diamond saw, that was also used for the preparation of cutting lines on occlusal surfaces. Two cutting lines, one with vestibulo-oral and other with mesio-distal direction, were prepared through fissures, around peaks of the cusps. The chisel was placed on an intersection point of cutting lines and hit with a hammer, causing breakage of tooth crown in four parts. One part of every tooth was a control sample, immersed in destilised water, and three were experimental samples, stored in carbonated beverages: carbonated water, Coca-cola and Schweppes Bitter Lemon. The pH level was measured for each beverage, using the digital pH meter (Economic pH009) at room temperature of 23ºC, on opening of the bottle, before immersion of these samples into the adequate beverage. To ensure measurement accuracy the pH meter was calibrated each time before use, at pH 4, 6.9 and 9.2, using standard buffer solutions for all levels. The samples were divided into three groups of twenty samples, obtained from five teeth. The first group was stored in beverages for 60 minutes, the second group for 24 hours and the third group for 7 days. The beverages for the third group were replaced every 24 hours. After taking out from beverages, the samples were washed thoroughly under running water. Each quarter of the teeth was dried in a vacuum apparatus, fixed to the respective carriers and sputter-coated with gold. The prepared samples were examined using the Scanning Electron Microscope (SEM) JEOL, JSM 6460 LV, at a magnification of ×500, ×5000 and ×10000. The enamel was observed on the outer surface of cusps and longitudinal section. Dentin was also observed on the longitudilnal section. The SEM is one of the most frequently used devices for the qualitative assessing ultramicroscopic surface alterations associated with erosion both on enamel and dentine [11, 12]. Grading of the severity of surface alteration could be done on the individually adopted scales [13]. In this study, the individual scale with four values was used to evaluate the degree of dissolution of the dental hard tissues: 0 – unchanged morphology of the tissues with all basic structural units of the observed surface; 1 – morphological units were changed, but still recognized; 2 – loss of obvious lines between morphological units, they merged with each other; 3 – complete loss of structure, the morphological units were not recognisable.

Detemination of morfology changes was done for all images at magnification of ×500, ×5000, ×10 000. The choosen figures of the samples, at magnification of ×500, are presented below. The dissolution values of enamel and dentin were analyzed by the one-way ANOVA and the post hoc Scheffé test, a statistical significance was set at $p \leq 0.05$.

Results

Measuring of pH showed the lowest value for Coca-Cola and Schweppes Bitter Lemon was also highly acidic while carbonated water had the highest pH level.

The SEM images of samples that were stored in beverages for 60 minutes indicate that all experimental beverages caused erosive changes of morfology of enamel and dentin. Outer surface of control enamel was smooth and unchanged, with an intact aprismatic surface layer. The perikymata lines

could be observed (Figure 1a). Enamel surface of sample stored in carbonated water showed partial loss of aprismatic layer and formation of the pores and superficial irregularities (Figure 1b). The alterations the surface morphology after exposure to Coca-Cola and Schweppes Bitter Lemon became much more pronounced. The aprismatic layer was almost completely removed. An increased number of porosities on the enamel surface could be noticed. The localized areas of distinctive honeycomb structure could be observed, which was due to the formation of micropores with preferential dissolution of the center of the enamel prisms and interprismatic enamel became convex and protruded (Figures 1c, and 1d). After 24 hours of exposure to beverages the enamel surface showed a marked visible effect (Figure 2a). The superficial irregularities of samples treated with carbonated water were more expressed (Figure 2b). The samples treated with Coca-Cola and Schweppes Bitter Lemon showed enhanced change of morfology, with marked signs of dental erosion, craters, grooves and cracks (Figure 2c, and 2d). The areas of type II demineralisation, where peripheral zones of enamel prisms were dissolved and cores were protruding, were visible on the sample treated with Schweppes Bitter Lemon (Figure 2d). After seven days, carboneated water caused the enamel surface looked quite rough (Figures 3a and 3b). The surfaces of enamel, after immersion into Coca-Cola and Schweppes Bitter Lemon, became grooved, with deep cracks and craters and complete loss of morfology (Figures 3c and 3d).

The SEM images of longitudinal section of control enamel showed the groups of enamel rods distinctly defined and unchanged, with almost linear orientation (Figure 4a). The localized areas of demineralization could be noticed at the samples stored in carbonated water, where the enamel rods were not obvious as in the control sample (Figure 4b). Coca-Cola caused the alterations that were more significant; increased dissolved regions were present where the morphological units were not clearly visible and the breakage lines became rounded (Figure 4c). The morfology of enamel exposed to Schweppes Bitter Lemon was completly lost, breakage lines were not visible and the orientation of enamel rods could not be defined (Figure 4d). These alterations of surface enamel, caused by the experimental beverages increased and became more pronounced as the exposure time increased (Figures 5, and 6).

The longitudinal section of dentin in the control sample and the sample exposed to carbonated water for 60 minutes showed morfology of sound dentin, with the unchanged morfological units (Figures 7a, and 7b). The tubules were surrounded by sound peritubular dentin that was clearly devided from interubular dentin. The samples stored in Coca-Cola and Schweppes Bitter Lemon represented the tubules with increased lumen, because of dissolution of peritubular dentin. The areas with undefined morfology could be observed (Figures 7c, and 7d). The samples treated with carbonated water for 24 hours and 7 days showed the localised areas of demineralisation, where dental tubules were not visible (Figures 8, 9a and 9b). Samples immersed in Coca-Cola and Schweppes Bitter Lemon showed the recognizable dentinal tubuls, but with lost sharp borders between them. Peritubular and intertubular dentin was not visible (Figures 8, and 9c, and 9d).

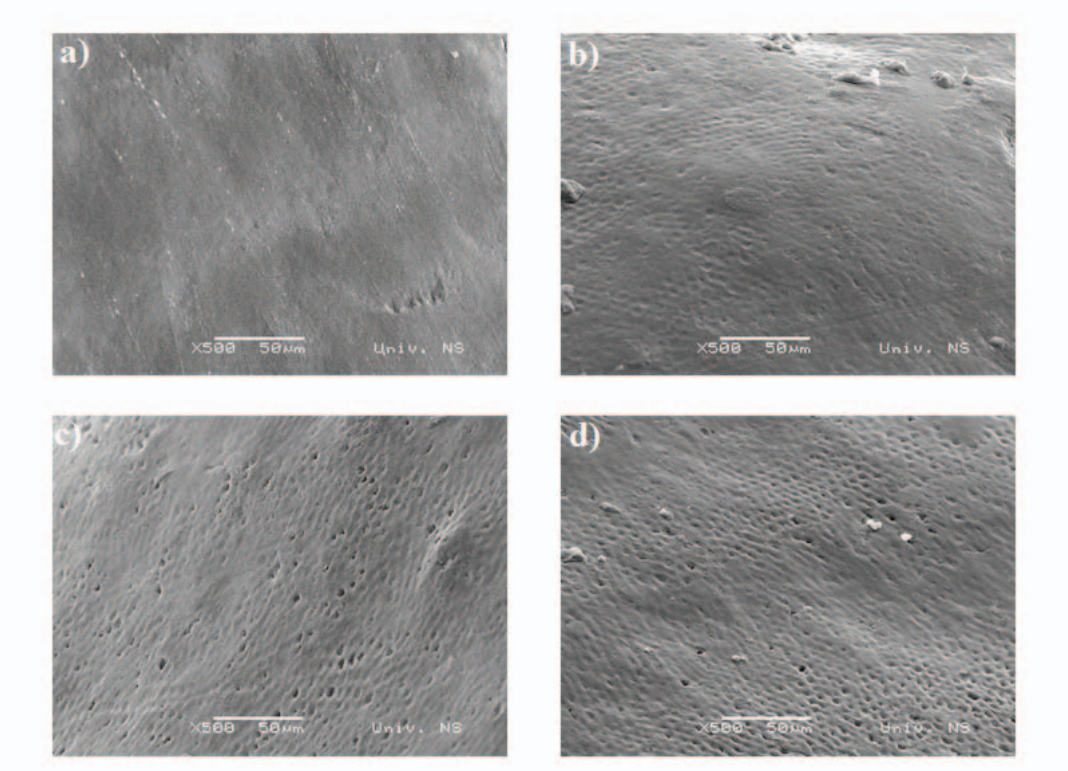

Fig. 1 – Scanning electron microscope (SEM) image of enamel surface after 60 minutes of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

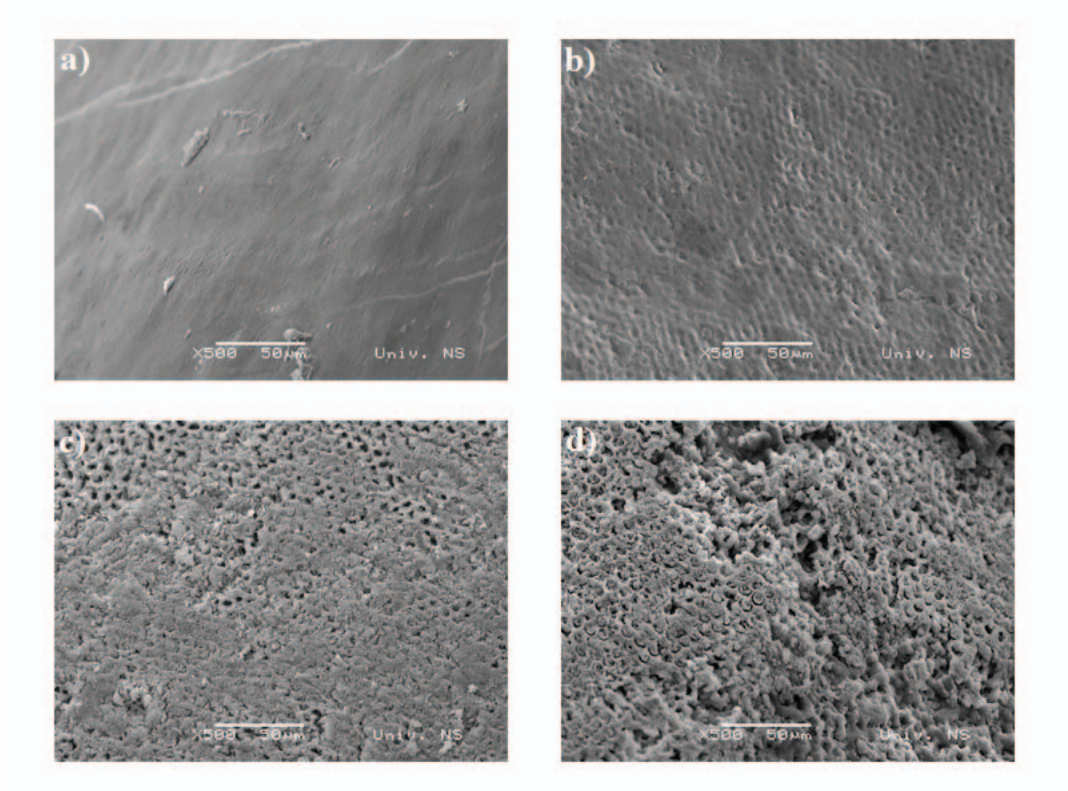

Fig. 2 – Scanning electron microscope (SEM) image of enamel surface after 24 hours of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

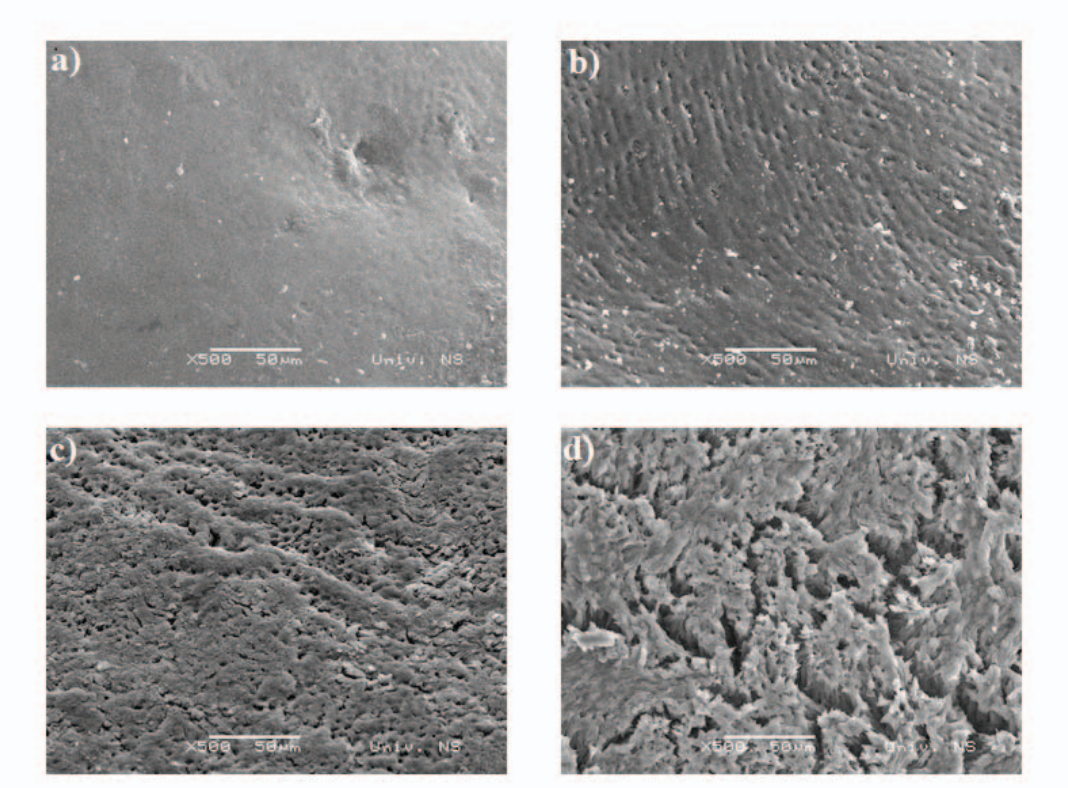

Fig. 3 – Scanning electron microscope (SEM) image of enamel surface after 7 days of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

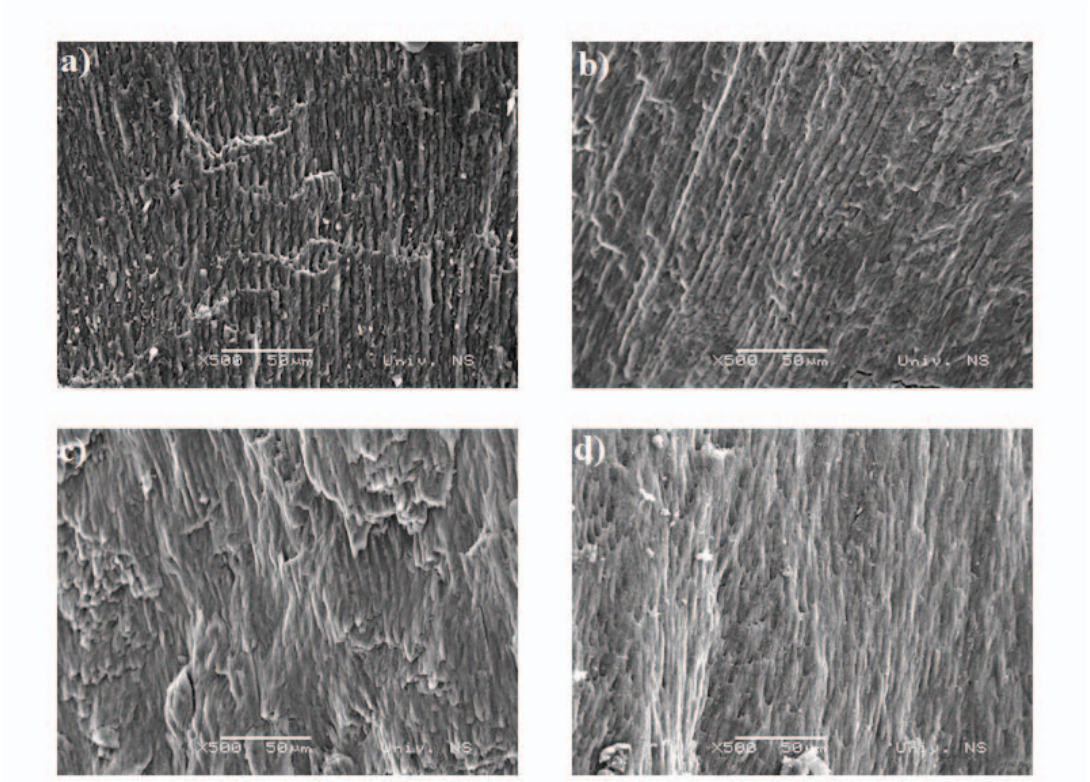

Fig. 4 – Scanning electron microscope (SEM) image of enamel, longitudinal section, after 60 minutes of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

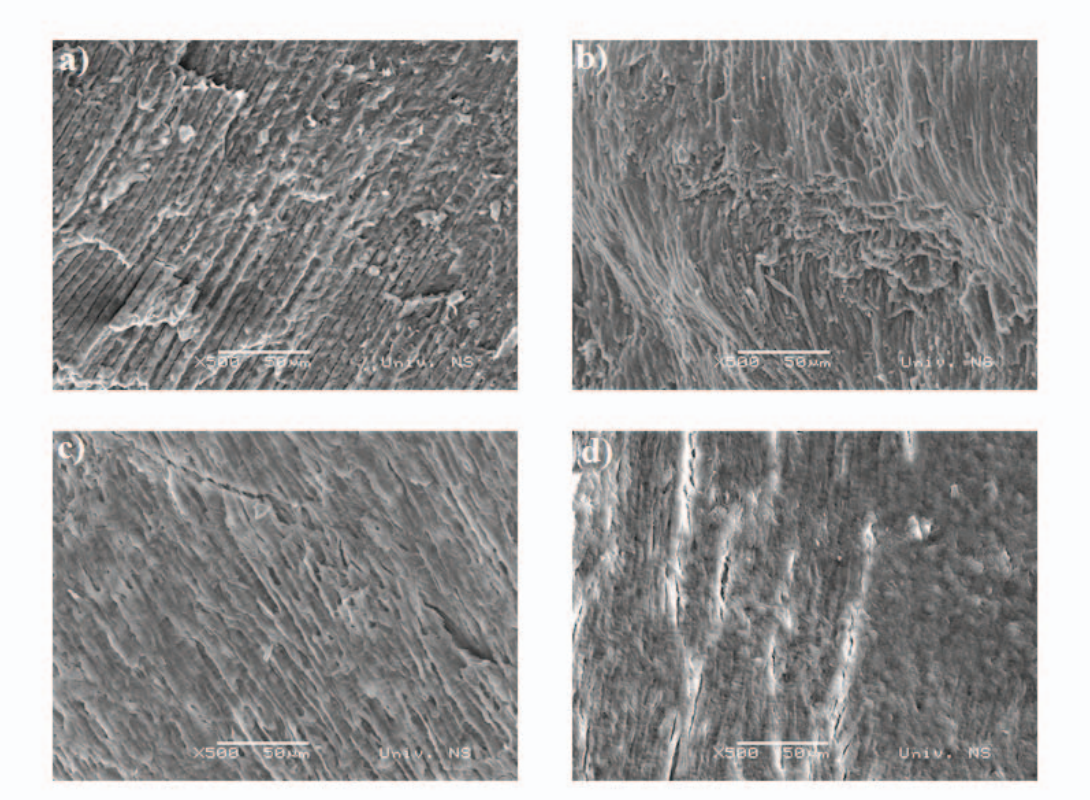

Fig. 5 – Scanning electron microscope (SEM) image of enamel, longitudinal section, after 24 hours of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

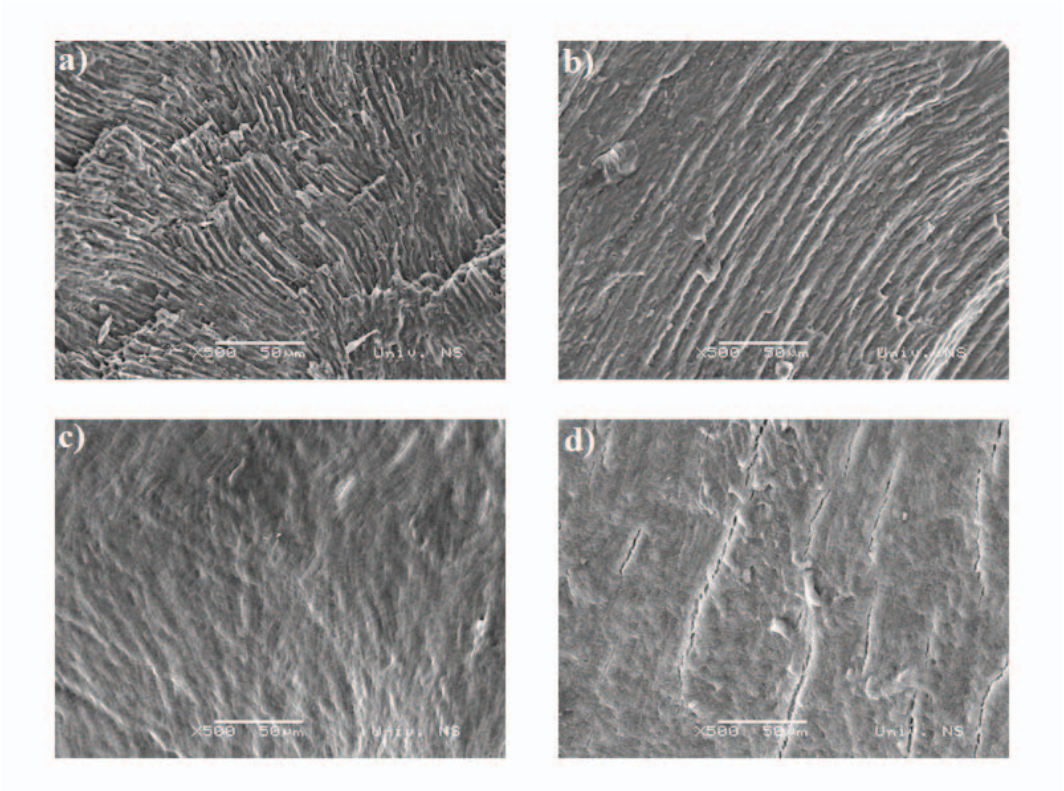

Fig. 6 – Scanning electron microscope (SEM) image of enamel, longitudinal section, after 7 days of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

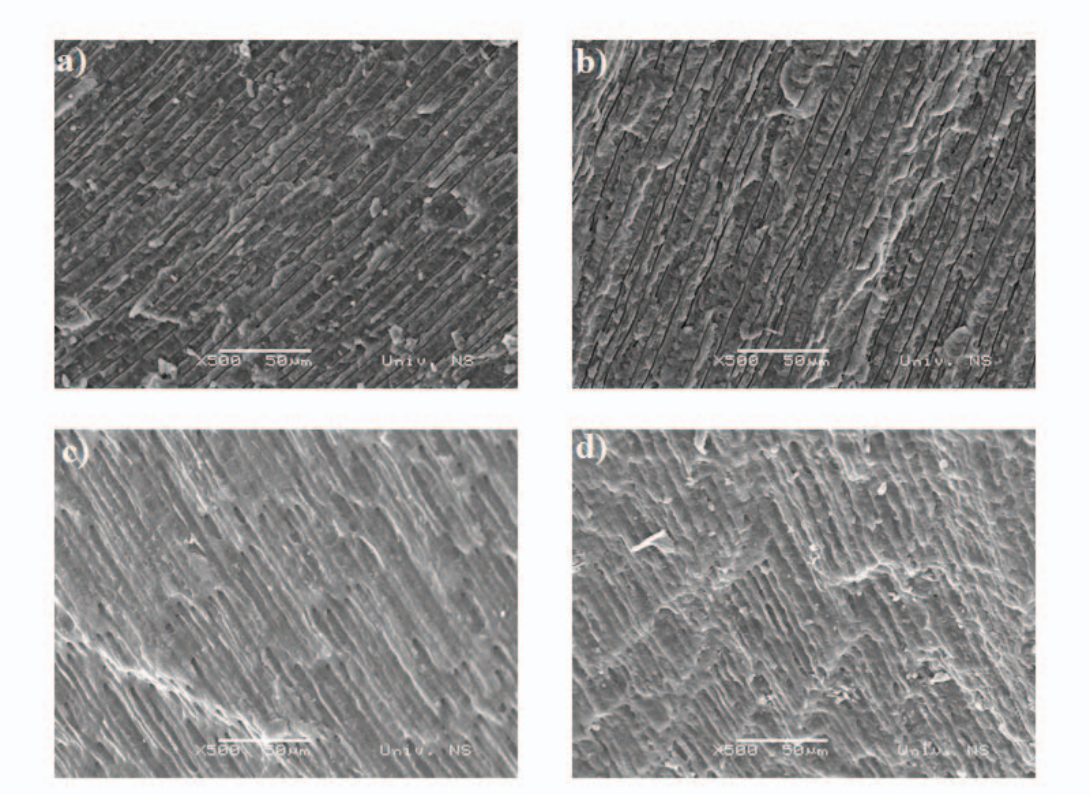

Fig. 7 – Scaning electron microscope (SEM) image of dentin, longitudinal section, after 60 minutes of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

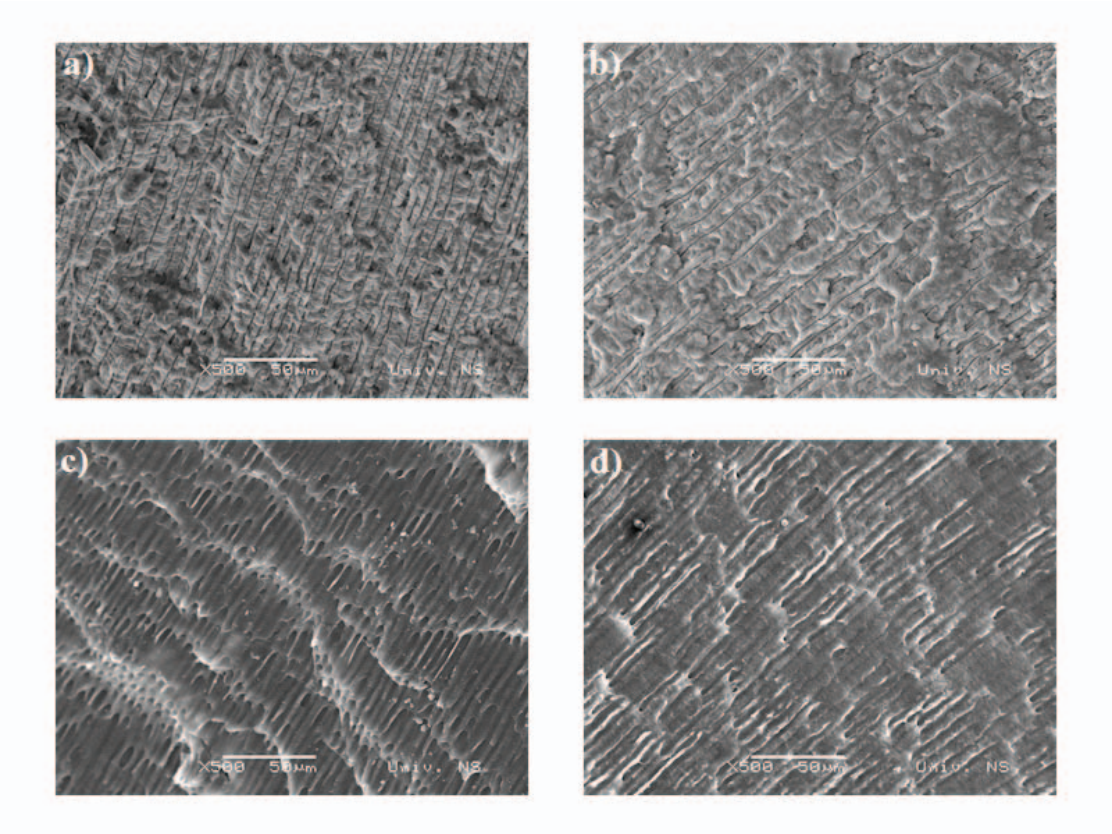

Fig. 8 – Scanning electron microscope (SEM) image of dentin, longitudinal section, after 24 hours of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

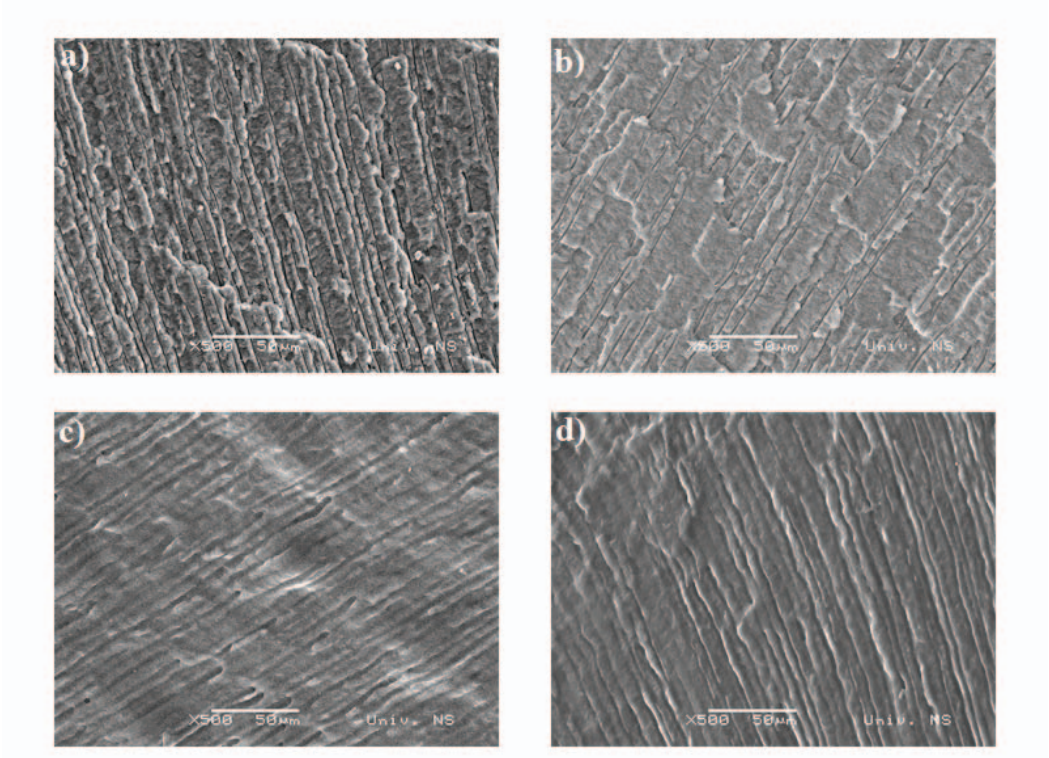

Fig. 9 – Scanning electron microscope (SEM) image of dentin, longitudinal section, after 7 days of exposure to beverages (×500): a) The sample exposed to destiled water; b) The sample exposed to carbonated water; c) The sample exposed to Coca-Cola; d) The sample exposed to Schweppes Bitter Lemon.

Table 1

The degree of erosive changes of enamel and dentin during different time intervals of exposure to beverages

Type of beverage	Degree of morphology changes, mean ± SD					
	60 min	F (p)	24 h	F (p)	7 days	F (p)
Distilled water	0.000 ± 0.000		0.000 ± 0.000		0.000 ± 0.000	
Carbonated water	0.733 ± 0.458	44.212	1.133 ± 0.458	44.212	1.467 ± 0.458	44.212
Coca-Cola	1.200 ± 0.561	(0.000)	2.000 ± 0.561	(0.000)	2.467 ± 0.561	(0.000)
Schweppes Bitter Lemon	1.733 ± 0.458		2.467 ± 0.458		2.800 ± 0.458	

Analysis by ANOVA reveals consistent significant difference among the groups of treated samples; SD – standard deviation.

The analysis by ANOVA revealed a consistent significant difference among the groups of treated samples. After 60 minutes of exposure to beverages ($F = 44.212$, $p < 0.01$), the post hoc Scheffé testing revealed a statistically significant difference among all groups of samples. The experimental beverages produced a statistically significant alteration in the dental hard tissue morfology. The highest values of erosive changes showed the samples that were immersed in Schweppes Bitter Lemon (Table 1). The analysis of the samples after 24 hours also showed a significant difference among the groups ($F = 73.373$, $p < 0.01$). The control group was statistically different from the other groups. There was also a statistically significant difference between carbonated water and other groups, while Coca-Cola and Schweppes Bitter Lemon produced the statistically similar changes of structural units of enamel and dentin (Table 1). After 7 days of immersion in the tested beverages, the assessed values of the sample groups were statistically different ($F = 95.734$, $p < 0.01$). The samples that were immersed in Coca-Cola and Schweppes Bitter Lemon presented the similar changes of morfology and they could be classified as one group that was statistically significantly different when compared with the control samples and samples immersed in carbonated water. There was a statistically significant difference between the changes produced by carbonated water and the changes produced by other beverages (Table 1).

Discussion

In vivo influence of soft drinks on dental health is in correlation with the exposure time to acidic challenges, which is determined by the frequency of consumption and drinking habits [12, 14]. Study about the association of frequency of intake of some drinks and dental erosion showed the proportion of students with increased dental erosion as the frequency of drink increased [14]. Moreover, dietary habits also had a significant association with dental erosion; keeping the drinks in mouth for a long time increased the risk of dental erosion by 2.7 times compared with those who swallowed the drinks immediately [14]. *In vitro* studies, including those with the SEM analysis, also showed a progressive destruction of the enamel ultrastructure with the increase of the exposure time [8, 15].

Based on an average daily consumption of 25 ounces of soft drink and a residence time in the mouth of 5 seconds, the total exposure time to beverages would equal 22,750 seconds (380 minutes or 6.3 hours) per year [16]. It is more likely that the exposure time for a beverage on the dentition is closer to 20 seconds before salivary clearance occurs; this would make the annual exposure of dental enamel to soft drinks approximately 90,000 seconds (that is, 1,500 minutes or 25 hours) per year [16]. Test periods of 24 hours and 7 days used in this study can be compared with one and 7 years, if soft drinks are consumed in a way that was indicated before.

The erosive potential of an acidic drink is not exclusively dependent on its pH value, but is also strongly influenced by its mineral content, its titratable acidity ('the buffering capacity') and by the calcium-chelation properties of food and beverages [13, 14]. The formulae and contents of pop-Cola beverages are closely guarded industrial secrets, and sparse data about acidity is provided on their consumer packages [17].

All carbonated beverages contain carbonic acid formed by carbon dioxide in solution [18]. Erosive potential of sparkling mineral water tested in this study is low because there is no added other acids to its content. Like in other studies, less demineralization was observed on the teeth that were exposed to sparkling mineral water compared with other carbonated beverages [19]. Even after 7 days of exposure to carbonated water, the stractural units of dental hard tissues were still recognized. Acidity and erosive potential of other tested drinks is influenced by added acids, such as phosphoric acid that is present in Coca-Cola and citric and ascorbic acids present in Schweppes Bitter Lemon.

From a theoretical point of view, both acids, phosphoric and citric, are very erosive because complete dissociation of one molecule results in the formation of three hydrogen atoms [20]. Most *in vitro* researches has shown that citric acid is more erosive than phosphoric acid [21]. Citric acid has double actions and is very damaging to the tooth surface. This means that citric acid at lower pH, provides hydrogen ions to directly attack the mineral surface and at higher pH, the citrate ion draws the calcium out of the crystal surface. Phosphoric acid provides hydrogen ions at low pH and binds calcium at higher pH. Citrate, however, forms a complex with calcium because of the relative sizes and three-dimensional shapes of the molecules [13]. The pH level is an important variable, and chelation has a striking effect on tooth tissue erosion at high pH [21]. The chelating action of citric acid and adition of ascorbic acid can be associated with a higher potential of Schweppes Bitter Lemon for the dissolution of the hy-

droxyapatite crystals. A different degree of erosive changes between the samples treated with Coca-Cola and Schweppes Bitter Lemon can be observed at samples after 60 minutes exposure. With the prolonged exposure and progression of acid etching, effects of both beverages became similar, resulting in the formation of a completely demineralized layer and a loss of structural units.

The erosion potential of popular beverages is important for clinical guidelines regarding beverage consumption practices and development of potentially "safer" beverages, especially for children [16].

Conclusion

Prolonged and repeated exposure of dental tissue to carbonated beverages caused the erosive changes at a microscopic level that can be associated with serious clinical problems. Due to the fact that beverage intake cannot be limited, it is important to identify the excessive consumption of carbonated beverages as etiological factor for dental erosion. Dietary advice and preventive care are mandatory for the patients at a risk of developing dental erosion, although modification of erosive potential and development of low erosive beverages are also possible.

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