sciendo

10.2478/bjdm-2019-0024

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

In Vitro Assessment of Erosive Effects of Some Common Soft Drinks on Dental Hard Tissues*

SUMMARY

Background/Aim: *The chemical nature of soft drinks is acidic, so they possess the capacity to cause hard tissue erosion. The aim of this study is to assess the potentially erosive effect linked to pH and titratable acidity of some common soft drinks on dental hard tissues in prolonged time exposure.* ***Material and Methods:*** *Seven types of soft drinks in Romanian market were selected for this study. The pH and Titratable acidity were determined. Twenty one human dental hard tissue specimens were immersed in 5 ml solution each for 336h. Every 48h weight loss was determined for each specimen and after 336h volume changes were calculated. Enamel microhardness was determined using a Microhardness Tester model HV-1000 and compared to normal enamel microhardness.* ***Results:*** *The pH values of beverages ranged from 2.37 to 3.1, showing no significant differences. All specimens presented color and surface texture modifications, weight loss and volume reduction. There were found significant differences between the mean values of weight loss and enamel microhardness of each type of specimens and type of beverages tested. The most representative findings were achieved by indentations done in specific zones on Sprite® immersed specimen. This result suggest that microhardness near the eroded surface was significant lower than in other zones.* ***Conclusions:*** *Data obtained have shown that all the soft drinks tested had an erosive effect, causing dental hard tissue dissolution. For prolonged time exposure titratable acidity may be a major predictor for erosive potential. Dental erosion may affect different levels of enamel causing decreased enamel microhardness.*

Key words: Dental Erosion, Soft Drinks, Ph, Titratable Acidity, Weight Loss, Microhardness, Differences

Enea Bitri[1], Lucian Petcu[2], Gabriela Mocanu[3], Doina Paula Balaban[4]

[1] University "OVIDIUS", Faculty of Dental Medicine, Aleea Universitatii 1, Constanta, Romania
[2] University "OVIDIUS", Faculty of Dental Medicine, Department of Biophysics & Biostatistics, Aleea Universitatii 1, Constanta, Romania
[3] University "OVIDIUS", Faculty of Dental Medicine, Department of Endodontics, Dental Practice, Aleea Universitatii 1, Constanta, Romania
[4] University "OVIDIUS", Faculty of Dental Medicine, Department of Biochemistry, Aleea Universitatii 1, Constanta, Romania

ORIGINAL PAPER (OP)
Balk J Dent Med, 2019;132-140

Introduction

Dental erosion is the chemical dissolution of dental hard tissues caused by the action of acids that are not produced by dental plaque[1,2]. The chemical nature of soft drinks combined with a prolonged contact with dental hard tissues may destroy the hard tissues due to erosion[3,4]. The erosive capacity of the soft drinks was found to be related to their pH and total acidity or titratable acidity[5-7].

*Note: 24th BaSS Congress awarded paper for oral presentation

Numerous studies conducted in European countries have concluded a relationship between diet and the incidence of dental erosion[8,9]. Abuse of acidic beverages and food are among the behavioral factors that can install dental erosion[10]. High consumption of citric fruits and juices, can also increase the risk of dental erosion[11,12]. Soft drinks consumption has increased over the last decades. In Romania average consumption of soft drinks in 2016. was 87.7 l per capita and in 2017. was 99.4 l per capita[13]. The consumption of soft drinks is especially high among children. According to World Health Organization Europe®, 30% of fifteen year old male teenagers in

Romania consume at least once a day soft drinks. High consumption rates in Balkan Countries was reported also in Albania, Bulgaria, Croatia and North Macedonia but low consumption among teenagers in Greece and Rep. Moldova[14]. Most researchers have reported that juices, carbonated and non-carbonated drinks, from both Europe and the United States are potentially erosive[15-18].

Several factors contribute in the process of tooth wear. The understating of interactions between chemical and biological factors may explain why some individuals develop more dental erosion than others with the same exposure of dietary acids[19]. Phosphoric and citric acid are the most used in soft drinks production, but also other acids such as maleic or tartaric acid are used. Studies on animal enamel have shown that phosphoric acid is very erosive at pH 2.5 but much less so at pH 3.3. Citric, maleic and tartaric acids because of their acidic nature have the ability to chelate calcium at higher pH[20]. Some in vitro studies have shown that cola drinks, have lowest pH at opening but the pH was neutralize easier than the pH of fruit juices and non-cola drinks[21-24]. Some in vitro studies have shown that citric acid caused far more erosion than phosphoric acid[25]. The results of prolonged time contact with acidic solution, shows not only visible clinical defects, but also changes of surface microhardness. It is recognized that the hard tissue demineralization is related to a reduced microhardness[26].

The purpose of this study is to evaluate the erosive potential of some common soft drinks, assess the effects of soft drinks on dental hard tissue by assessing the weight loss, volume reduction, and enamel microhardness changes of human dental hard tissue specimens in *in vitro* experimental study.

Material and Methods

Seven soft drinks 1L or more package from Romania market were selected. The aim of selection was to include as more as possible representatives types of soft drinks in Romanian market. In this selection were represented international brands/local brands, carbonated/ne-carbonated beverages, cola type/other types of drinks (Table 1).

For the determination of pH and Titratable acidity was used the principle of potentiometric titration with standard volumetric solution of NaOH. Reactives used were standard volumetric solution NaOH 1N and standard buffer solution for device calibration. pH and Titratable acidity were measured using an automatic titrator Titroline – Easy – SCHOTT – Instruments Germany® according to International Standard ISO – 750 – 1998 – Fruit and vegetable products – Determination of titratable acidity®[27].

Determinations were done following these steps:

- Device calibration for domain pH 0-14;
- Determination of initial pH of beverage;
- Start of magnetic stirrer;
- Automatic adding in small portion NaOH 1N solution until pH reach 7.

Ten determinations were done for each beverage and the results of Titratable acidity are expressed in mL NaOH/L beverage. Mean values of pH and Titratable acidity are expressed in average values ± Standart Deviation.

Table 1. Soft drinks

Beverages	Brand/Origin	Type
Coca Cola®	Coca Cola®/ International	Carbonated/Cola type
Pepsi Cola®	PepsiCo®/International	Carbonated/Cola type
Sprite®	Coca Cola®/ International	Carbonated/Other
Prigat SourCherry®	PepsiCo®/International	Ne-carbonated/Other
Frutti Fresh Tutti Frutti®	EuropeanDrinks®/ Local	Carbonated/Other
Cola Carrefour®	Carrefour®/ International	Carbonated/Cola type
Giusto Pineapple®	RomAquaBorsec®/ Local	Ne-carbonated/Other

Dental hard tissue preparation

Twenty three human caries free teeth extracted for periodontal or orthodontic reasons collected at “Social Center” belonging to University “OVIDIUS” were used in this study. This teeth were divided in three groups each of seven and two witnesses:

- Maxillary anterior;
- Mandibular anterior;
- Premolar.

After disinfection and cleaning with NaOCl 5% solution, blocks of hard tissue with approx. dimensions of 6.5mm x 5mm x 2.5mm from the vestibular section were prepared using diamonded discs with medium granulation on a high speed hand piece[28]. Specimen dimensions were measured using a Digital Caliper® with a precision of 0.01mm and their initial volumes were calculated. Weight was measured using an analytical electronic balance type Shimadzu – Japan®. Each specimen was randomly distributed to a beverage.

Erosive process

Erosive effects of soft drinks tasted were assessed using gravimetric methods by calculating weight loss of human enamel specimens during a prolonged erosive process[28]. Hard tissue specimens were immersed in 5ml beverage in polyethylene flacons for 336 h. After each 48 h their weight was measured, weight loss was calculated and the beverage was replaces with a fresh one. Before each measure specimens was washed with deionized waster and dried with absorbing papers. Last weigh determination was done after 96 h in scope to assess if the erosive process may stop at some point. At the end of the erosive process, final volumes was calculated and compared to initial volumes. Hard tissue dissolution data were analyzed using one-way analysis of variance ANOVA and quantitative data analysis methods.

Enamel microhardness test

Another method used to assess the effects of erosion was by surface microhardness measurements. The basic principle of micro- and nano indentation require the indentation of a diamond tip of tetra-pyramidal for a given load and duration[29,30]. Because this method needs hard surface for determinations and the specimens selected presented softened surface caused by erosion in this study the indentations were done on the enamel level beneath the total eroded surface[31,32]. Four specimen from the mandibular group immersed in Coca Cola®, Pepsi Cola®, Sprite®, Prigat SourCherry® and two witnesses were selected for enamel hardness test. Specimens were packed in acrylic resin and cut transversally before testing. Enamel microhardness was determined using a Microhardness Tester model HV-1000® with Vickers indenters. Different indentation Loads & Time were applied (F=100 gf/mm², F=200gf/mm² & t=10s, t=15s, t=30s) on the enamel level underneath the total eroded enamel. Three indentations were done for each Load & Time and the values were expressed in main values. On the specimen immersed in Sprite® were realized indentations on three different zones: near eroded surface, medium and near DEJ. Statistical analysis were realized using one-way analysis of variance ANOVA and descriptive graphics.

Results

The data obtained from pH and Titratable acidity (Table 2) determinations have shown that the pH levels ranged in interval 2.37-3.10 with no significant differences between beverages. Although there were no significant differences according pH levels there were significant differences according to Titratable acidity between beverages. Cola type drinks presented comparable Titratable acidity and at least three times lower than other types of soft drinks. Prigat SourCherry® presented the most significant higher level of Titratable acidity. The analysis of pH and Titratable acidity has shown that there is no direct correlation between pH and Titratable acidity levels.

Table 2. pH & Titratable acidity values

Beverages	pH Mean ± SD	Titratable acidity mL NaOH 1N/L beverage
Coca Cola®	2,50 ± 0,38	9,83 ± 2,06
Pepsi Cola®	2,37 ± 0,36	10,93 ± 2,08
Sprite®	2,85 ± 0,51	27,68 ± 6,37
Prigat SourCherry®	2,70 ± 0,41	87,68 ± 24,55
Frutti Fresh Tutti Frutti®	2,93 ± 0,44	31,33 ± 6,58
Cola Carrefour®	2,49 ± 0,37	9,38 ± 1,50
Giusto Pineapple®	3,10 ± 0,50	47,43 ± 10,43

Average dimensions results were:

- Mandibular Anterior Group
  6.00mm x 5.04mm x 2.61mm;
- Maxillary Anterior Group
  6.13mm x 4.97mm x 2.58mm;
- Premolar Group
  6.95mm x 5.33mm x 2.66mm.

The first information we obtained after the erosive process was that all the specimens immersed in soft drinks had changed their color and surface texture. From the visible assessment all the specimens presented significant or less significant sings of erosion.

Data obtained after erosive process had shown that the beverages had an erosive effect on dental hard tissue specimens causing hard tissue dissolution more or less significant depending on the time duration and the type of soft drink. Specimen immersed in Prigat SourCherry® presented the highest weight loss 46.4 % of the initial weight. This also was the of the smallest specimen samples. Comparable weight loss evolution recorded for Coca Cola®, Pepsi Cola®, Sprite®, Giusto Pineapple®. Cola Carrefour® caused no significant hard tissue dissolution (Table 3 and 4) with evident volume reduction of the specimens (Table 5).

Table 3. Weight loss (g) during 48 hour intervals Mandibular Anterior Group

Beverage	0-48h	48-96h	96-144h	144-192h	192-240h	240-336h
Coca Cola®	0.0005	0.0036	0.0024	0.0022	0.0024	0.0041
Pepsi Cola®	0.0004	0.0026	0.0029	0.0028	0.0022	0.0064
Sprite®	0.0018	0.0034	0.003	0.0029	0.003	0.0072
Prigat SourCherry®	0.0106	0.0074	0.0119	0.0075	0.0082	0.0117
Frutti Fresh Tutti Frutti®	0.0027	0.0037	0.0039	0.0049	0.0041	0.0084
Cola Carrefour®	0.0006	0.0004	0.0005	0.0002	0.0002	0.0008
Giusto Pineapple®	0.0032	0.0029	0.0035	0.0032	0.0047	0.0063

Table 4. Weight loss (%) Mandibular Anterior Group percentage reported to initial weight

Beverage	48h	96h	144h	192h	240h	336h
Coca Cola®	0.36%	3.00%	4.39%	6.00%	7.76%	10.76%
Pepsi Cola®	0.29%	2.21%	4.35%	6.42%	8.04%	12.76%
Sprite®	1.48%	4.29%	6.77%	9.17%	10.82%	16.77%
Prigat SourCherry®	8.58%	14.57%	24.21%	30.28%	36.92%	46.39%
Frutti Fresh Tutti Frutti®	2.01%	4.76%	7.66%	11.31%	14.37%	20.62%
Cola Carrefour®	0.43%	0.71%	1.07%	1.21%	1.36%	1.93%
Giusto Pineapple®	2.03%	3.87%	6.09%	7.74%	10.72%	14.72%

Table 5. Volume Mandibular Anterior Group. Before and After immersion (mm^3)

Beverage	Before	After
Coca Cola®	82.16	70.81
Pepsi Cola®	70.75	62.90
Sprite®	80.25	71.92
Prigat SourCherry®	80.35	52.53
Frutti Fresh Tutti Frutti®	82.52	68.02
Cola Carrefour®	81.89	78.54
Giusto Pineapple®	77.31	72.70

Wight loss of Maxillary Anterior Group hard tissue specimens presented similar dissolution rates as Mandibular Anterior Group. Prigat SourCherry® also caused the most hard tissue dissolution but less than on specimen from the Mandibular Group. Cola type drinks had caused less hard tissue dissolution compared to other types but compared weight loss as Frutti Fresh Tutti Frutti®, Sprite® & Giusto Pineapple®. Specimen immersed in Cola Carrefour® had presented insignificant weight loss (Table 6, 7).Volume changes of Maxillary Anterior Group was in correlation with weight loss assessments (Table 8).

Table 6. Weight loss (g) during 48 hour intervals Maxillary Anterior Group

Beverage	0-48h	48-96h	96-144h	144-192h	192-240h	240-336h
Coca Cola®	0.0002	0.0023	0.0025	0.0023	0.0022	0.0046
Pepsi Cola®	0.0001	0.0012	0.0026	0.0026	0.0026	0.0075
Sprite®	0.0010	0.0017	0.0036	0.0025	0.0027	0.0060
Prigat SourCherry®	0.0059	0.0036	0.0067	0.0078	0.0067	0.0075
Frutti Fresh Tutti Frutti®	0.0020	0.0029	0.0030	0.0031	0.0027	0.0022
Cola Carrefour®	0.0005	0.0004	0.0003	0.0002	0.0002	0.0006
Giusto Pineapple®	0.0050	0.0033	0.0036	0.0034	0.0049	0.0076

Table 7. Weight loss (%) Maxillary Anterior Group percentage reported to initial weight

Beverage	48h	96h	144h	192h	240h	336h
Coca Cola®	0.13%	1.67%	3.44%	4.88%	6.35%	9.43%
Pepsi Cola®	0.05%	0.77%	2.31%	3.86%	5.41%	9.86%
Sprite®	0.58%	1.59%	3.71%	5.19%	6.78%	10.32%
Prigat SourCherry®	4.35%	6.93%	11.88%	17.63%	22.43%	27.97%
Frutti Fresh Tutti Frutti®	1.49%	3.66%	5.9%	7.98%	10.24%	13.89%
Cola Carrefour®	0.4%	0.73%	0.98%	1.14%	1.31%	1.8%
Giusto Pineapple®	2.97%	4.94%	7.08%	9.11%	12.03%	16.55%

Table 8. Volume Maxillary Anterior Group. Before and After immersion (mm^3)

Beverage	Before	After
Coca Cola®	78.62	71.43
Pepsi Cola®	83.54	79.26
Sprite®	84.36	76.20
Prigat SourCherry®	76.33	56.00
Frutti Fresh Tutti Frutti®	72.02	65.02
Cola Carrefour®	67.40	63.21
Giusto Pineapple®	90.36	77.08

Data obtained from the erosive process of Premolar Group are comparable with Mandibular Anterior & Maxillary Anterior Groups showing a continuous linear weight loss evolution. Specimen immersed in Prigat SourCherry® presented 34.33% of weight loss, Coca Cola®, Pepsi Cola®, Sprite®, Frutti Fresh Tutti Frutti® and Giusto Pineapple® had comparable dissolution ranged from 10.03% to 17.11% weight loss. Also insignificant dissolution compared to other beverages was recorded for Cola Carrefour® (Table 9 and10).

Table 9. Weight loss (g) during 48 hour intervals Premolar Group

Beverage	0-48h	48-96h	96-144h	144-192h	192-240h	240-336h
Coca Cola®	0.0027	0.0025	0.0025	0.0044	0.0028	0.0053
Pepsi Cola®	0.0007	0.0025	0.0023	0.0025	0.0028	0.0058
Sprite®	0.0033	0.003	0.0031	0.003	0.0033	0.0051
Prigat SourCherry®	0.0102	0.0048	0.0090	0.0116	0.0093	0.0147
Frutti Fresh Tutti Frutti®	0.0019	0.0031	0.0036	0.0032	0.0034	0.0069
Cola Carrefour®	0.0005	0.0006	0.0002	0.0003	0.0004	0.0015
Giusto Pineapple®	0.0060	0.0046	0.0030	0.0028	0.0030	0.0074

Table 10. Weight loss (%) Premolar Group percentage reported to initial weight

Beverage	48h	96h	144h	192h	240h	336h
Coca Cola®	1.67%	3.23%	4.78%	7.52%	9.26%	12.55%
Pepsi Cola®	1.0%	1.93%	3.32%	4.83%	6.52%	10.03%
Sprite®	1.97%	3.76%	5.61%	7.4%	9.36%	12.42%
Prigat SourCherry®	5.87%	8.64%	13.82%	20.5%	25.86%	34.33%
Frutti Fresh Tutti Frutti®	1.12%	2.95%	5.08%	6.32%	8.39%	12.46%
Cola Carrefour®	0.28%	0.62%	0.73%	0.9%	1.13%	1.98%
Giusto Pineapple®	3.83%	6.76%	8.68%	10.47%	12.38%	17.11%

Table 11. Volume Premolar Group. Before and After immersion (mm^3)

Beverage	Before	After
Coca Cola®	88.79	82.25
Pepsi Cola®	111.3	102.39
Sprite®	102.47	95.76
Prigat SourCherry®	104.89	83.07
Frutti Fresh Tutti Frutti®	100.09	87.97
Cola Carrefour®	92.75	92.07
Giusto Pineapple®	98.34	93.71

Statistical data analysis using one-way ANOVA showed that there were no significant differences between Groups of specimens (p>0.5), but there exist significant differences between beverages (p<0.05, Figure 1).

The most indentations values obtain from the eroded specimens were below the range of witness Vickers Hardness. Lowest Vickers Hardness were meassured for specimen immersed in Prigat SourCherry®. No significant differences were observed for the cola type drinks. Witness specimen in average had Vickers Hardness in the range of normal enamel hardness presented in other international studies[31,32]. Vickers Hardness near the eroded surface tasted on the specimen immersed in Sprite® was significant lower than other levels of enamel (Figure 2). From the microscopic images we had observed that the indentations near the eroded surface tend to collapse the enamel structure below (Figure 3 and 4).

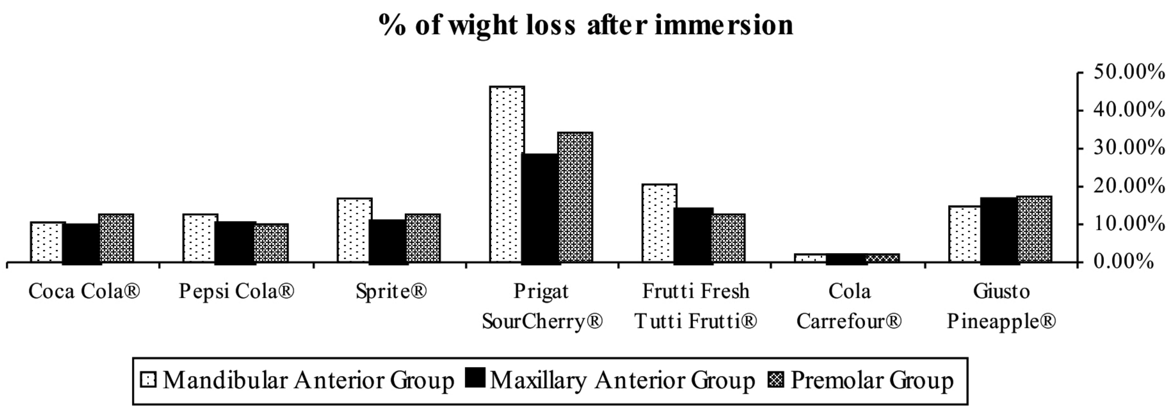

Figure 1. Weight loss (%) of the three Group specimens after erosive process

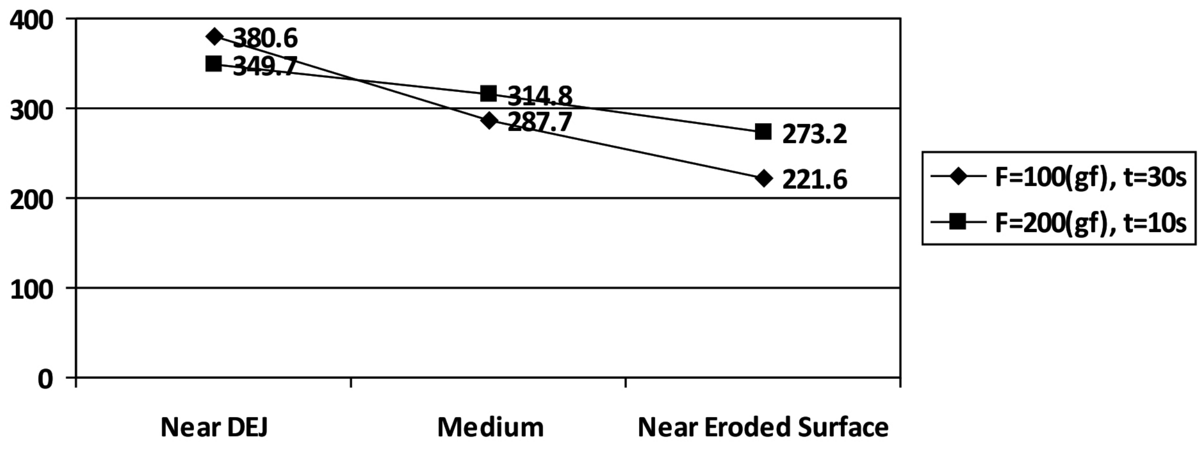

Figure 2. Indentations in three different zones. Sprite® specimen. F=100(gf), t=30s, F=200(gf), t=10s. (Kgf/mm2)

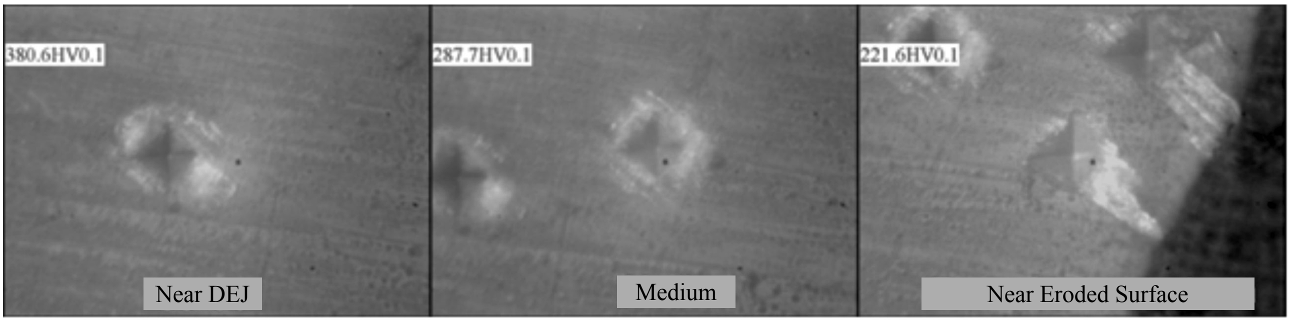

Figure 3. Microscopic images of indentations in three different zones. Sprite® specimen, F=100(gf), t=30s

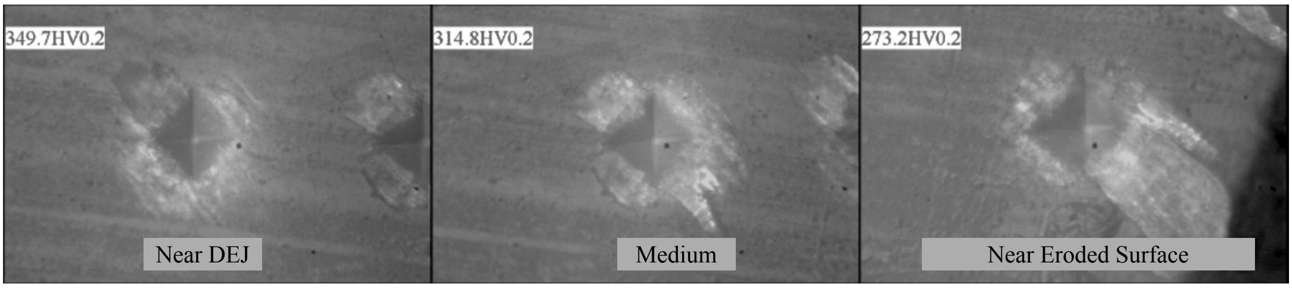

Figure 4. Microscopic images of indentations in three different zones. Sprite® specimen, F=200(gf), t=10s

By analyzing the statistical data with one-way ANOVA the result showed that for indentation with F=100(gf); t=10s, F=100(gf), t=30s; F=200(gf), t=10s; F=200(gf), t=30s, no significant differences were found ($p>0.05$), but for indentations F=100(gf), t=15s and F=200(gf), t=15s the analysis showed significant differences ($p<0.05$).

Discussions

From this study no certain conclusion can be drown regarding the erosive effects of soft drinks in oral cavity, because factors such as saliva buffer capacity or drinking habits are not included[34-36]. Besides pH and Titratable acidity, factors such as acid type (e.g. phosphoric acid or citric acid), buffer capacity, adhesion, chelating effect, phosphate-, fluoride- and calcium content of the beverages also contribute in erosive potential of soft drinks[34,36]. The total acidity of beverages is considered to be an important factor in the development of dental erosion, because it determines the amount of hydrogen ion that interact with the tooth surface[10,37]. In this study chemical factors such as pH, Titratable acidity and acid type of soft drinks tasted were used to explain the association between erosive potential of beverages and erosion effects. From the data collected all the beverages had pH ranged 2.37-3.1, with no significant differences and situated below the critical pH for enamel dissolution[33]. Titratable acidity measurements showed significant differences between beverages.

Cola type drinks (Coca Cola®, Pepsi Cola®, Cola Carrefour®) presented lower levels of total acidity compared to other drinks. Compared values were achieved also for Sprite®, Frutti Fresh Tutti Frutti® and Giusto Pineapple®. Prigat SourCherry® recorded the highest total acidity at least double compared to other drinks. Ne-carbonated beverages (Prigat SourCherry® & Giusto Pineapple®) had higher total acidity compared to other drinks. This may be explained from the number of acids used, added acids such as citric acid and from fruit content acids. Sour Cherry fruits and pineapple fruits contain high levels of acidity and the most representative is maleic acid[38]. There was no correlation between pH levels and Titratable acidity levels. Chemical factors results should not be considered representative for all soft drinks in the Romanian market due the limited number of soft drinks included in this study.

Due the two aspects of dental erosion, surface physical changes and chemical dissolution of hard tissues, various techniques have been used to evaluate dental erosion[1,30,39]. Erosive effects of soft drinks tasted were assessed using gravimetric methods by calculating weight loss of human enamel specimens during a prolonged erosive process[28]. This method had some limitations in assessing the erosive effects accurately. Extended length of immersion, tooth human age, specimen shapes and relatively small number of specimens might presented some errors for interpretation. All the hard tissue specimens immersed in soft drinks after 336 hours presented hard tissue dissolution more or less significant. All the soft drinks solution even after 48 hours remained undersaturated in respect of hard tissue mineral continuing the dissolution. The pH didn't predict the evolution of weight loss but can be considered as a condition for dental erosion. The beverage that caused the most significant weight loss was Prigat SourCherry®, also this beverage had the highest Titratable acidity. High levels of acidity and the acid types such as citric acid and maleic acid that are considered especially erosive, may explain the significant erosive potential of Prigat SourCherry®[10,20].

Compared weight loss were assessed for Sprite®, Frutti Fresh Tutti Frutti® and Giusto Pineapple® demonstrating a correlation with compared Titratable acidity values of these beverages. Regardless of that cola type drinks had lower pH than other drinks, those beverages caused less hard tissue dissolution comparing with other beverages tasted. These results can be correlated with low Titratable acidy of those drinks but also a positive correlation may be found with the type of acid used in cola type drinks. Phosphoric acid is less erosive than citric or maleic acid, due the lower pKa and the lower ability to chelate calcium[1,5,20]. Surprisingly Cola Carrefour® caused insignificant hard tissue dissolution to all of groups. Although this beverage had the second lower pH and compared Titratable acidity to other cola type drinks, none of the chemical factors didn't predict the evolution of weight loss of this beverage.

No study was found in international literature about the erosive potential of this beverage. By analyzing the data of weight loss, the results suggest that there are no significant differences between the three groups of specimens, but there were significant differences between soft drinks. Regardless that volume calculations can present errors due irregular shapes of specimens the changes achieved support a correlation between weight loss and volume reduction.

Another method used to assess the effects of erosion was by surface hardness measurements. The basic principle of micro- and nano indentation require the indentation of a diamond tip of tetra-pyramidal for a given load and duration[29,30,40]. Because this method needs hard surface for determinations and the specimens selected presented softened surface caused by erosion in this study the indentations were done on the enamel level beneath the total eroded surface[31,32]. The aim was to assess the microhardness of the enamel level underneath the softened surface. This method may present some errors and limitations, mostly due the irregular shapes of some indentations. The results showed that specimen immersed in Prigat SourCherry® presented lower Vickers Hardness at all the loads & time compared to other specimens. Witness specimens that was a normal enamel specimen presented Vickers Hardness values similar to other international studies[31,32]. Only in two loads at 15s time significant differences were found. The most representative findings were achieved by indentations done in specific zones on Sprite® immersed specimen. This result suggest that microhardness near the eroded surface was significant lower than in other zones. This hypothesis is supported also by the visual analysis of microscopic images that show the collapse of enamel structure due the effect of indentation. Due the small number of indentations in those zones no statistical significance can be achieved. After all the microhardness measurements the results showed that enamel level underneath the softened surface is also affected and the affection is more significant in the zones near the eroded surface.

Conclusions

Data obtained from this study had showed that all the soft drinks tasted had the pH below the critical pH of enamel dissolution and had an erosive effect causing hard tissue dissolution. Titratable acidity may be a major predictive factor for erosive potential in prolonged time exposure to acidic soft drinks. Dental erosion may affect different levels of enamel causing decreased enamel microhardness depending on the distance from the eroded surface.

We assume that the erosive effects achieved in this study are less probable to occur *in vivo* situation but this findings may help to understand and prevent better the erosive effects caused by acidic soft drinks. Further studies should be done considering more factors, larger beverage selection, better time assessment, and new innovative methods to assess the erosive potential of soft drinks.

References

1. Lussi A (ed): Dental Erosion. Monogr Oral Sci. Basel, Karger, 2006, vol 20, pp: 9-16.
2. 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;113:850-856.
3. Lussi A, Schaffner M, Hotz P, Suter P. Dental erosion in a population of Swiss adults. Community Dent Oral Epidemiol, 1991;19:286-290.
4. 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;31:153-159.
5. Zero DT, Lussi A. A erosion – chemical and biological factors of importance to the dental practitioner. Int Dent J, 2005;55:285-290.
6. Singh S, Jindal R. Evaluating the buffering capacity of various soft drinks, fruit juices and tea, J Conserv Dent, 2010;13:129-131.
7. Bamise C, Ogunbodede EO, Olusile AO, Esan AT. Erosive potential of soft drinks in Nigeria. World J Med Sci, 2007;2:115-119.

8. Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. Caries Res, 2004;38:34-44.
9. Murrell S, Marshall TA, Moynihan PJ, Qian F, Wefel JS. Comparison of in vitro erosion potentials between beverages available in the United Kingdom and United States. J Dent, 2010;38:284-289.
10. Zero DT. Etiology of dental erosion – extrinsic factors. Eur J Oral Sci, 1996;104:162-177.
11. Ganss C, Schlechtriemen MK. Dental erosions in subjects living on a raw food diet. Caries Res, 1999;33:74-80.
12. Smith BNG, Knight JK. A comparison of pattern of tooth wear with aetiological factors. Br Dent J, 1984;157:16-19.
13. Institutul National de Statistica, Consumul de bauturi, in anul 2017. Romanian. Available from: http://www.insse.ro/cms/ro/content/consumul-de-b%C4%83uturi-%C3%AEn-anul-2017.
14. World Health Organization Europe, European Health Information Gateway (2014): Soft Drinks Consumption (11, 13, 15 years): Percentage of young people 15 years who drinks soft drinks at least once a day. Available from: https://gateway.euro.who.int/en/indicators/cah_24-soft-drink-consumption-11-13-15-years/.
15. Owens BM. The potential effects of pH and buffering capacity on dental erosion. Gen Dent, 2007;55:527-531.
16. Mandel L. Dental erosion due to wine consumption. JADA, 2005;136:71-75.
17. Ren YF, Zao Q, Malmstrom M, Barnes V, Xu T. Assessing fluoride treatment and resistance of dental enamel to soft drink erosion in vitro: Applications of focus variation 3D, scanning microscopy and stylus profilometry. J Dent, 2009;37:167-176.
18. Larsen MJ, Nyvad B. Enamel erosion by some soft drinks and orange juice relative to their pH, buffering effect and contents of calcium phosphate. Caries Res, 1999;33:81-87.
19. Lussi A, Schaffner M, Jaeggi T. Dental erosion – diagnosis and prevention in children and adults, Int Dent J, 2007;57:385-398.
20. Rugg-Gunn AJ, Nunn JH. Diet and dental erosion. Nutrition, diet and oral health. Hong Kong: Oxford University Press; 1999.
21. Bamise CT, Kolawol KA, Oloyede EO. The determinants and control of soft drinks-incited dental erosion. Rev Clín Pesq Odontol, 2009;5:141-154.
22. Jensdottir T, Bardow A, Holbrook P. Properties and modification of soft drinks in relation to their erosive potential in vitro. J Dent, 2005;33:569-575.
23. Jensdóttir T, Thornórsdóttir I, Arnadóttir IB, Holbrook WP. Erosive drinks on the icelandic market. Laeknabladid. 2002;88:569-572.
24. Bamise CT, Ogunbodede EO, Olusile AO, Esan TA. Erosive potential of soft drinks in Nigeria. World J Med Sci, 2007; 2 (2):115-9.
25. West NX, Hughes JA, Addy M. The effect of pH on the erosion of dentine and enamel by dietary acids in vitro. J Oral Rehabil, 2001;28:860-864.
26. Attin T, Koidl U, Buchalla W, Schaller HG, Kielbassa AM, Hellwig E. Correlation of microhardness and wear in differently eroded bovine dental enamel. Arch Oral Biol, 1997;42:243-250.
27. International Organization for Standardization. ISO 750: 1998. Fruit and Vegetable Producs, Determination of Titratable Acidity. Avalaible from: https://www.iso.org/standard/22569.html.
28. von Fraunhofer JA, Rogers MM. Dissolution of dental enamel in soft drinks. Gen Dent, 2004;52:308-312.
29. Lussi A (ed): Dental Erosion. Monogr Oral Sci. Basel, Karger, (2006), vol 20, chapter 10. pp. 152-173.
30. Grenby TH. Methods of assessing erosion and erosive potential. Eur J Oral Sci, 1996;104: 207-214.
31. del Pilar Gutiérrez-Salazar M, Reyes-Gasga J. Microhardness and chemical composition of human tooth. Mat Res, 2003;6:367-373.
32. Chuenarrom C, Pojjanut B, Daosodsai P. Effect of indentation load and time on knoop and vickers microhardness tests for enamel and dentin. Mat Res, 2009;12:473-476.
33. Barron RP, Carmichael RP, Marcon MA, Sàndor GK. Dental erosion in gastroesophageal reflux disease. J Can Dent Assoc, 2003; 69:84-89.
34. Lussi A, Carvalho TS. Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 25: 1–15. (2014)
35. Jager DH, Vieira AM, Ruben JL, Huysmans MC. Estimated erosive potential depends on exposure time. J Dent 40: 1103–1108. 10. (2012)
36. Zimmer S, Kirchner G, Bizhang M, Benedix M. Influence of various acidic beverages on tooth erosion. Evaluation by a new method. PloS one, 2015;10:e0129462.
37. West NX, Hughes JA, Addy M. Erosion of dentin and enamel in vitro by dietary acids: The effect of temperature, acid character, concentration and exposure time. J Oral Rehabil, 2000;27:875-880.
38. Will F, Hilsendegen P, Bonerz, D, Patz C, Helmut D. Analytical composition of fruit juices. J Applied Bot Food Qual, 2005;79:12-16.
39. West NX, Jandt KD. Methodologies and instrumentation to measure tooth wear; future perspectives; in Addy M, Embery G, Edgar WM, Orchadson R (eds): Tooth Wear and Sensitivity. London, Martin Dunitz, 2000, pp 105–120.
40. Lussi A (ed): Dental Erosion. Monogr Oral Sci. Basel, Karger, 2006, vol 20, pp: 152-173.

Conflict of Interests: Nothing to declare.
Financial Disclosure Statement: Nothing to declare.
Human Rights Statement: None required.
Animal Rights Statement : None required.

Received on August 17, 2019.
Revised on September 25, 2019.
Accepted on September 27, 2019.

Correspondence:

Enea Bitri
Private Dental Practice, Lagjja "Xhevdet Nepravishta", Albania
e-mail: eneabitri1@gmail.com
Lucian Petcu
University "OVIDIUS", Faculty of Dental Medicine
Constanta, Romania
e-mail: petculucian@univ-ovidius.ro
Doina Paula Balaban
University "OVIDIUS", Faculty of Dental Medicine
Constanta, Romania
e-mail: dpbalaban@yahoo.com