IMPACT OF LIGHT AND SUGAR-FREE BEVERAGES ON DENTIN EROSION - AN IN VITRO STUDY

ABSTRACT

AIM: To compare the erosive effect of different light and sugar free beverages in relation to their regular version, on dentin. **MATERIALS AND METHODS:** Bovine root dentin samples were randomized into 9 different types of drinks (each n=12): Coca-cola®, Light Coca-cola®, Coca-cola® Zero, Guaraná Antartica®, Guaraná Antartica® Zero, Sprite®, Sprite® Zero, Del Valle® Passion fruit juice and Del Valle® Light Passion fruit juice. During each one of the 10 experimental days, the samples were subjected to 3 erosive demineralization (by immersion of the sample in the studied drink) and remineralization (by immersion of the sample in artificial saliva) cycles. Profilometry was evaluated at the end of the 1st, 5th and 10th days. For each type of drink, the data were tested using 2 way ANOVA and Bonferroni test ($p<0.05$). **RESULTS:** Erosive cycling with regular cola resulted in a higher wear on the first day, on the 5th day the regular drink was similar to zero and greater than light version, on the 10th day there was no difference between the versions of cola drink. For the Sprite drink there was difference between the versions just in the 10th day, in which the higher wear was promoted by the zero version. For guaraná and juice beverages there was no differences between versions in relation to the resulted erosive wear. **CONCLUSION:** Erosive tooth wear promoted by light and sugar-free or regular versions of soft drinks and juices showed distinct behaviours over the cycling time.

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INTRODUCTION

Erosion is usually described as an irreversible loss of dental hard tissue resulting from acid exposure of extrinsic and / or intrinsic sources.[1-6] However, the terminology "dental erosion" was recently divided into "erosion" and "erosive tooth wear." Erosion describes the process of loss of structural integrity by demineralization, resulting in softening of the dental hard tissue. The subsequent process of loss of tooth structure induced by prolonged erosive challenge with repeated events of softening corresponds to erosive tooth wear.[7,8]

Dental erosion and erosive tooth wear is currently receiving attention from clinicians and researchers. There are sufficient evidences that erosion has appeared increasingly prevalent.[9]

Despite its multifactorial etiology, involving interaction between chemical (composition of the acid solution), biological (salivary flow rate and acquired pellicle) and behavioral factors (acid exposure followed by tooth abrasion),[10] extrinsic acids have been considered an important source of acid related to dental erosion occurrence. The changing of dietary habits of the general population, who has consumed more frequently acidic foods/ beverages seems to the responsible for this phenomenon.[5,6]

In vitro studies suggest that several properties of acidic drinks such as pH, buffer capacity and calcium, phosphate and fluoride concentrations influence their erosive potential.[11] Furthermore, the literature reports that light cola drink is less erosive than the regular one on enamel.[12] However, the impact of the type of sweetener, contained in acidic beverages, on the dentin erosion is unknown.

Therefore, this in vitro study aimed to identify the erosive effect of different acidic beverages containing sugar compared with their respective light/zero versions on dentin.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN:

The factors under investigation were type of sweetener (regular, light or zero) and erosive cycling time (1st, 5th and 10th days) on dentin. Bovine radicular dentin blocks (4x4mm) were selected by the initial surface hardness (SH) and randomized into 9 drinks: G1 – Coca-cola®, G2 – Light Coca-cola®, G3 – Coca-cola® Zero, G4 – Guaraná Antartica®, G5 – Guaraná Antartica® Zero, G6 - Sprite®, G7 - Sprite® Zero, G8 - Del Valle® Passion fruit juice, G9 - Del Valle® Light Passion fruit juice. Blocks were immersed in artificial saliva for 24 h. Then, the samples were subjectted to erosive cycling for 10 days. Each day cycling consisted of 3 cycles of erosive demineralization (5 min) - remineralization (120 min). The profilometry was assessed at the end of the 1st, 5th and 10th days of the experiment.

DENTIN BLOCKS PREPARATION:

The dentin blocks were obtained from bovine incisors roots. The blocks were cut using a ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4 mm thickness spacer. Dentin surfaces were planed with water-cooled silicon carbide discs (320 grade paper; Buehler, Lake Bluff, IL, USA). Then, the dentin external surfaces were polished with water-cooled silicon carbide discs (600, and 1200 grade papers; Buehler, Lake Bluff, IL, USA) and felt paper wet by diamond spray (1 mm; Buehler, Ltd., Lake Bluff, IL, USA).

The baseline surface hardness (SH) was determined using the average values of five indentations performed at distances of 100 μm from each other (Knoop diamond, 25 g, 10s, Hardness tester from Shimadzu, Tokyo, Japan) and 108 blocks were selected by the initial surface hardness (Mean SH value = 43,23 ±8,64 KHN). The selected blocks were randomized into 9 groups (n=12) according to the type of beverage. Prior to the experiment, two layers of nail varnish were applied on half of the surfaces of each block to maintain a reference surface for dentin loss determination.

EROSIVE CYCLING:

After preparation, the samples were stored in artificial saliva [0.2 mM glucose, 9.9 mM NaCl, 1.5 mM CaCl2.2H2O, 3 mM NH4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K2HPO4, 3.3 mM urea, 2.4 mM NaH2PO4 and ascorbic acid (pH 6.8)], for 24h. Then, blocks were subjected to an erosive cycling for 10 days. One complete cycle consisted of the following steps: 1. Demineralization by the acidic beverage for 5 min under gentle agitation 2. Remineralization in artificial saliva for 120 min, at 37°C. The samples were consecutively cycled through this regimen three times/day. At the end of each cycling day, the blocks were immersed in artificial saliva, overnight, at 37 º C.

CHEMICAL PROPERTIES OF THE STUDIED BEVERAGES:

The pH of beverages was measured using a pH electrode. Fluoride concentrations were analyzed using a fluoride sensitive electrode (Orion, 96-06) connected to a digital analyzer apparatus pH/F (Procyon, SA-720), previously calibrated with standard solutions. The buffer capacity was assessed using a pH electrode by adding a base (0.2M NaOH) in constant quantities until the pH rise to 7.0.

EROSIVE DENTIN WEAR:

At the end of the 1st, 5th and 10th cycling days the nail varnish on the reference surfaces was carefully removed with acetone-soaked cotton wool and the wear was measured at the interface erosion-control by a contact profilometer (Hommel Tester T1000, VS, Schwenningen, Germany). The diamond stylus was moved from the control to the experimental area. Three profile measurements (2mm) were performed in the center of each block. The software of the device installed on a computer connected to the profilometer converted each profile in a chart that allowed quantifying the difference (in height) between the 2 parts (erosion-control) in microns.[13,14]

STATISTICAL ANALYSIS:

Statistical analysis was performed with GraphPad Instat. The assumptions of equality of variances and normal distribution of errors were checked using Shapiro-Wilk test. Since the assumptions were satisfied, two-way ANOVA and Bonferroni's test were applied. The significance limit was set at 5%.

RESULTS

The results of ph, buffer capacity and fluoride concentrations are given on tables 1, 2 and 3, respectively.

The means of dentin wear are given on tables 4, 5, 6 and 7 for each beverage group, considering the type of sweetener and the erosive cycling time.

Cola drinks promoted a progressively greater wear according to the cycling days. At the 1st day, the regular version resulted in an increased wear on dentin compared to zero and light versions. At the 5th day, the wear of samples exposed to regular drink was similar to zero version, both were greater than the light one. At the end of erosive cycling, at the 10^{th} day, these differences were not significant. The type of sweetener of guarana drinks did not influence the wear pattern. However, the regular version promoted a progressive wear according to cycling time, while the guarana zero at the fifth day cycling was similar to that of the tenth day.

The wear promoted by regular version of Sprite drink was lower on the tenth day, there was no significant difference between groups in the first and fifth days of erosive cycling. Sprite zero did not differ in relation to the erosive wear at the first, fifth and tenth days. For the regular version there was an increase in the wear of the samples between the first and fifth day of cycling.

Regarding to the juice there was no difference between regular and light versions. The wear of the samples exposed to regular version was similiar in the fifth and tenth day, so that for the light version there was no difference between the first, fifth and tenth days.

DISCUSSION

The knowledge of light cola drink being less erosive than the regular one on enamel,[12] provided the basis for the formulation of the present study. The tested hypothesis was that light version of the tested drinks promotes lower erosion on dentin, compared to the regular version of the same drink.

Table 1. Ph of the studied beverages.

	Cola	*Light Cola*	*Cola Zero*	*Guaraná*	*Guaraná Zero*	*Sprite*	*Sprite Zero*	*Juice*	*Light Juice*
Ph	2,95	3,5	3,37	3,06	3,55	3,0	3,1	2,29	2,57

Tabela 2. Buffer capacity of the studied beverages.

	Cola	*Light Cola*	*Cola Zero*	*Guaraná*	*Guaraná Zero*	*Sprite*	*Sprite Zero*	*Juice*	*Light juice*
[NaOH]	1,75	1,75	1,75	2,75	2,25	4,75	3,75	9,75	7,75

Tabela 3. Fluoride concentrations of the studied beverages.

	Cola	*Light Cola*	*Cola Zero*	*Guaraná*	*Guaraná Zero*	*Sprite*	*Sprite Zero*	*Juice*	*Light Juice*
[F]	0,32	0,31	0,36	0,07	0,06	0,19	0,26	0,33	0,35

Table 4. Mean wear (μm) of dentin exposed to Coca cola® (p<0,05).

Beverage*	**1st day****	**5th day**	**10th day**
Coca cola	$1{,}59^{a,A}$	$2{,}53^{b,A}$	$2{,}83^{b,A}$
Light Coca cola	$0{,}36^{a,B}$	$1{,}88^{b,B}$	$2{,}51^{c,A}$
Coca cola Zero	$0{,}75^{a,B}$	$2{,}45^{b,A}$	$2{,}95^{c,A}$

* Values in the same column followed by distinct capital letters indicate statistical significant difference (p<0,05) among beverages.

** Values in the same line followed by distinct lowercase letters indicate statistical significant difference (p<0,05) among erosive cycling time.

Table 5. Mean wear (μm) of dentin exposed to Guaraná® (p<0,05).

Beverage*	**1st day****	**5th day**	**10th day**
Guaraná	$0{,}75^{a,A}$	$2{,}35^{b,A}$	$3{,}17^{c,A}$
Guaraná Zero	$0{,}53^{a,A}$	$2{,}88^{b,A}$	$3{,}18^{b,A}$

* Values in the same column followed by distinct capital letters indicate statistical significant difference (p<0,05) among beverages.

** Values in the same line followed by distinct lowercase letters indicate statistical significant difference (p<0,05) among erosive cycling time.

Table 6. Mean wear (μm) of dentin exposed to Sprite® (p<0,05).

Beverage*	**1st day****	**5th day**	**10th day**
Sprite	$0{,}67^{a,A}$	$2{,}49^{b,A}$	$2{,}97^{b,A}$
Sprite Zero	$0{,}46^{a,A}$	$3{,}09^{b,A}$	$3{,}85^{c,B}$

* Values in the same column followed by distinct capital letters indicate statistical significant difference (p<0,05) among beverages.

** Values in the same line followed by distinct lowercase letters indicate statistical significant difference (p<0,05) among erosive cycling time.

Table 7. Mean wear (μm) of dentin exposed to Del Valle® Passion fruit juice (p<0,05).

Beverage*	**1st day****	**5th day**	**10th day**
Juice	$0{,}63^{a,A}$	$2{,}43^{b,A}$	$2{,}56^{b,A}$
Light Juice	$0{,}63^{a,A}$	$2{,}36^{b,A}$	$3{,}01^{c,A}$

* Values in the same column followed by distinct capital letters indicate statistical significant difference (p<0,05) among beverages.

** Values in the same line followed by distinct lowercase letters indicate statistical significant difference (p<0,05) among erosive cycling time.

Several products have been tested in vitro to determine the extension of their erosive potential. These tests also provide information about the possible factors affecting the erosive potential of beverages, including pH, as a dominant factor, buffer capacity, degree of acids saturation, concentration of calcium and phosphate, as well as the presence of erosion inhibitors, such as fluoride.[17] In addition to the factors related to beverage's composition, other modifying factors, which vary between individuals and different occasions, such as the pattern and salivary flow rate, buffering capacity and the protective properties of the acquired pellicle might influence on the occurrence and progression of dental erosion.[11]

The applied in vitro protocol did not use mucin, which is an important component for salivary pellicle formation. Salivary pellicle presents an important preventive effect against erosion, since the presence of the pellicle might diminish the contact between the acid and the dental tissue.[15,16] Therefore, the present wear values should be higher than those found in clinical situations, because the human physiological characteristics for erosion protection were not fully reproduced in this study.

Sugar-free soft drinks usually present sweeteners, which may interfere in their erosive effect, however, there is no consensus in the literature regarding the performance of this beverages in relation to the regular formulation (with sucrose). Some in vitro studies found no differences between the erosive potential of light soft drinks in relation to their regular version,[5, 17,18] while others have shown that diet cola causes less demineralization than the regular one.[19,20]

The present data demonstrated that at the first cycling day the regular cola drink resulted in higher dentin wear than light/zero versions. These results are in agreement with a previous study conducted by Rios et al.[12] (2009) in enamel substrate.

Nevertheless, there was no difference between the regular version in relation to light or zero versionof the studied juice and guaraná drinks, while for Sprite the greatest wear was promoted by its zero formulation, on the end of the tenth experimental day.

The artificial sweetener of light cola is aspartame, a synthetic dipeptide consisting of aspartate and phenylalanine aminoacids. Aspartame concentration in the light cola is 24 mg/100 ml, corresponding to a concentration of 2 mg/100 ml of phenylalanine, according to the manufacturer’s information. There is no report in the literature about the possible role of phenylalanine for dental erosion inhibition.

However, it must be considered that phenylalanine is an aromatic aminoacid, which has a pK of carboxylic grouping of 1.83 and 9.13 of the amino group.[21] Thus, at soft drink pH around of 3, protons could be taken by the carboxylic (COO-) or amine (NH_2) grouping of phenylalanine, nullifying its deleterious action on tooth dentin. Another protector mechanism of the phenylalanine could be its ability to form a film on the dentin surface, constituting a diffusion barrier, reducing the contact of the acid with the dentin. Recent in vitro studies have shown that some types of proteins, when present in the erosive agent, can inhibit the deleterious effects on hidroxiapatita.[22,23] It should be emphasized that the proposed mechanism is merely a supposition, and future studies are required to elucidate the mechanism by which light cola drink presents less erosive potential.

In relation to Guaraná and Sprite drinks, zero versions contain sodium cyclamate and saccharin as sweeteners, which appears to offer no protective effect against erosion, since the zero version of Sprite promoted increased wear when compared to the regular one.

Regarding the juice, comparing the compositions reported by the manufacturer, the light version presents no added sweetener and perhaps for this reason the wear values of both versions, regular and light, showed similar values.

The present study evaluated pH, buffering capacity and fluoride concentration of the different types of studied bdrinks. According to Hannig et al.[24] the erosive effect of different acids is pH dependent and varies significantly between pH 2 and 3. It means that even a small decrease in pH can result in significant increase in enamel loss,[17, 25-29] and the same could occur to dentin. The results of cola drinks are in accordance with this hypothesis. It was noted that the regular version of cola drink has the lower ph, which could explain the higher wear promoted by this soft drink, especially in the early days of erosive cycling, comparing to light /zero versions. However, for the guaraná drinks, there is a large difference in pH, between the different versions and no statistically significant difference was found between the wear of regular and zero formulations, regardless of cycling time. A similar condition was found for the evaluated juice, that despite showing difference in pH between the versions, also showed no difference for the promoted wear between light and regular formulations. In addition, for the Sprite drink the difference in pH between the regular and zero formulations is negligible, however, there were differences in the wear between the versions on the last day of cycling. In this way, it was observed that pH showed distinct influences on the erosive potential of the studied beverages.

Regarding the buffer capacity, for the cola drink, this factor did not contribute to elucidate the divergent results between different versions, since the values are the same for all three types of this beverage. The buffer capacity also did not influence the erosive potential of guarana-based drinks and juices, as they have behaved similarly with respect to wear promoted by regular or zero/ ligth versions, despite the buffer capacity is greater for regular formulation. In addition, for sprite drinks, the greater buffering capacity of the regular version is opposed to the increased wear promoted by zero sugar formulation.

However on the present study, the buffering capacity did not adequately influence the observed erosive wear, especially on the first day of erosion because the samples were exposed to a large volume of drink for only 5 minutes.[25] Therefore, the pH remained constant during this period of exposure time, with no effective interference on the buffer capacity.

In terms of fluoride concentration, all groups presented low values, confirming its minimal interference on the erosive potential.[30] It is important to emphasize, however, that other factors, not assessed in this study, such as the concentration of calcium and phosphate, might also present a role on the erosive potential of the studied drinks.

CONCLUSION

Under the conditions of the present in vitro study it can be concluded that the regular and light/zero versions of acidic beverages may act differently on dentin erosion. The erosive potential is related to beverages chemical properties which is different for each type of soft drink or juice.

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