

Carbohydrate-electrolyte drinks exhibit risks for human enamel surface loss

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Objectives: The aim of this investigation was to give insights into the impact of carbohydrate-electrolyte drinks on the likely capacity of enamel surface dissolution and the influence of human saliva exposure as a biological protective factor. **Materials and Methods:** The pH, titratable acidity (TA) to pH 7.0, and buffer capacity (β) of common beverages ingested by patients under physical activity were analyzed. Then, we randomly distributed 50 specimens of human enamel into 5 groups. Processed and natural coconut water served as controls for testing three carbohydrate-electrolyte drinks. In all specimens, we measured surface microhardness (Knoop hardness numbers) and enamel loss (profilometry, μm) for baseline and after simulated intake cycling exposure model. We also prepared areas of specimens to be exposed to human saliva overnight prior to the simulated intake cycling exposure. The cycles were performed by alternated immersions in beverages and artificial saliva. ANOVA two-way and Tukey HDS tests were used. **Results:** The range of pH, TA, and β were 2.85 - 4.81, 8.33 - 46.66 mM/L and 3.48 - 10.25 mM/L \times pH, respectively. The highest capacity of enamel surface dissolution was found for commercially available sports drinks for all variables. Single time human saliva exposure failed to significantly promote protective effect for the acidic attack of beverages. **Conclusions:** In this study, carbohydrate-electrolyte drinks usually consumed during endurance training may have a greater capacity of dissolution of enamel surface depending on their physicochemical properties associated with pH and titratable acidity. (*Restor Dent Endod* 2016;41(4):246-254)

Key words: Carbohydrate-electrolyte drinks; Dental enamel; Dental erosion; Profilometry; Surface microhardness

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Introduction

Today, sports drinks are wide-ranging within the recreational, fitness, and sporting professions and appear to be the beverage of choice for most serious athletes. It is worth to cite that the oral health of sports athletes can impact their performance.¹ Carbohydrate-electrolyte (CE) drinks are designed to help athletes rehydrate, and also restore electrolytes, carbohydrates, and other nutrients, which can be depleted after training or competition.² Current guidelines for athletes competing in intermittent sports recommend ingesting CE drinks at a rate of 30 - 60 g/hr during more than 1 hour of training and competition to provide fuel to the muscle and central nervous system.³ The delay fatigue, as well as the potential non-metabolic central effects related to reward and motivation, were reported to be related to the intake of CE drinks.⁴ These

drinks are commonly recommended for intense exercise.⁵

Most CE drinks, also called sports drinks, have pH levels below the critical pH of 5.5 for enamel demineralization, and consequently, may contribute to enamel surface dissolution upon conditions where there is an unbalance of calcium and phosphate in the oral environment around the enamel.⁶ In addition, the negative effect of acidic and high-carbohydrate nutrition might be increased by the reduced saliva flow rate during exercise.⁷ This potential for dental erosion promoted by consumption of these drinks is an important consideration for the field of oral health and dental hygiene, and thus the dental team must be aware to advise adequately.

We have found that endurance capacity during exercise is improved when sufficient CE fluid is ingested.⁸ Does taking CE drinks rather than water have added negative outcomes as dental erosion? To help answer this question, the small alterations that occur in the initial stages of erosion in the superficial layer of enamel can be evaluated by determination of surface hardness, and more aggressive outcomes can be measured by contact stylus surface profilometry.^{9,10}

Comprehensive information is available on the erosive risk of soft drink intakes by overall average non-exercising population, but there is still a lack of information for those who have unique training and nutritional habits and the interplay of salivary components. According to the National Health Interview Survey 2010, intake of sports and energy drinks have increased dramatically among US adults. Nationwide, 31.3% of adults were sports and energy drink consumers during the past 7 days.¹¹ Several investigations evaluated the correlation between sports drinks and dental erosion revealing controversial results for erosive tooth risk.^{12,13}

Enamel is the first target tissue in the erosive process, and its loss may have a multifactorial etiology linking exposure to exogenous acids with dental erosion.¹⁴ Extrinsic factors are related to environmental acids, dietary factors, medications, and lifestyle with most notably of frequent intake of acidic drinks.¹⁵ Association between diet and dental erosion have received considerable attention by dental research community.¹⁶⁻¹⁸ In addition, the increased consumption of sports and energetic drinks could increase the risk of dental erosion occurrence.¹⁹

Additionally, the presence of the salivary pellicle is one of the patient-related factors in the protection against enamel erosion. Salivary pellicles begin to form as soon as the enamel comes into contact with saliva, and its presence can modulate the erosive attack to enamel surface.²⁰ This distinction should be considered to evaluate the likely capacity of enamel surface dissolution when exposed to CE drinks *in vitro* studies.

Thus, the purpose of the present study was to compare a group of CE drinks with a group of processed and natural

isotonic drink in a standardized *in vitro* enamel erosive setup to determine the potential impact of the intake of these drinks in the enamel dissolution and the influence of human saliva exposure as a biological protective factor. Chemical factors associated with enamel dissolution were also evaluated. It was hypothesized that CE drinks intake could affect oral health with special regard to dental erosion.

Materials and Methods

An *in vitro* design was used to compare physicochemical properties (pH, titratable acidity [TA], and buffer capacity [β]) and erosion potential of commercially available CE drinks. The description of the chemical composition of tested CE drinks is shown in Table 1. Processed and natural isotonic drinks (coconut water) were used as controls. Fifty enamel slabs were randomly divided into 5 groups and designed as:

- (1) CE drink Gatorade (referred as 'G');
- (2) CE drink SUUM (referred as 'S');
- (3) CE drink Taea (referred as 'T');
- (4) CE drinks - Commercial coconut water (referred as 'CC');
- (5) CE drinks - Natural coconut water (referred as 'NC').

This experiment was conducted according to the randomized complete block design ($n = 10$) for each group. Enamel demineralization (surface softening) and tooth wear were the response variables, analyzed by surface hardness change and stylus profilometry, respectively.

Chemical properties

The pH, TA, and β of each beverage were measured in duplicate using a pH meter (Model Tec 3MP, Tecnal, Piracicaba, SP, Brazil). The TA was measured by adding 1 N sodium hydroxide to 30 mL beverage until the pH reached 7.0. The β was measured according to previous study,²¹ based on the formula: $\beta = \Delta C / \Delta \text{pH}$, where ΔC is the amount of titrator used (base) and ΔpH is the change in pH caused by the addition of the titrator.

Human enamel specimen's preparation

Fifty enamel specimens were obtained from twenty-five extracted, sound third molar teeth, following approval from the Research and Ethics Committee of the Federal University (protocol No. 08/2012). The teeth were stored in a 0.01 wt% thymol solution at 4°C for thirty days and refrigerated until use. Enamel slabs (5 x 5 x 2 mm) were obtained from the coronal buccal surfaces of each tooth and prepared using a water-cooled diamond saw and a cutting machine (IsoMet, Buehler, Lake Bluff, IL, USA). The enamel slabs were embedded in Pre-30 self-polymerized acrylic resin cylinders (Arotac SA Ind. e Com., Cotia, SP, Brazil) to facilitate handling, and were serially flattened

Table 1. Beverages composition used in this study, with overall description of their composition

Group	Beverage	Manufacturer	Composition
G	Gatorade	The Gatorade Co., Chicago, IL, USA	Water, sucrose syrup, glucose-fructose syrup, citric acid, natural lemon/lime flavors, natural flavours, salt, sodium citrate, monopotassium phosphate, ester gum, yellow dye No. 5
S	SUUM	Biotik Ltda, São Paulo, SP, Brazil	Sodium chloride, magnesium oxid, ascorbic acid, calcium pantothenate, cyanocobalamin, pyridoxine, dextrose-maltose, polietilenoglicol, citrus acid, sodium bicarbonate, potassium bicarbonate, natural flavours, lime flavour, calcium carbonate, silicon Dioxide.
T	Taeq	Pão de Açúcar Ltda, São Paulo, SP, Brazil	Water, sucrose syrup, monobasic sodium phosphate, cornflour, sodium chloride, citrus acid, orange flavor, sodium benzoate, potassium sorbate, sodium citrate buffer, ascorbic acid, EDTA, calcium disodium, yellow dye
CC	Commercial coconut water	Ducoco Produtos Alimentícios S/A, São Paulo, SP, Brazil	Coconut water, fructose (less than 1.0%), preservative INS 223 (sodium bisulfite).
NC	Natural coconut water	Liquid part of coconut from coconut palm (Cocus nucifera L.)	Glucose, sucrose, fructose, sodium, potassium, chloride, ascorbic acid, vitamin C, total ascorbic acid, thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folate, total, folic acid, folate, food, DFE, biotin, nicotinic acid (Niacin), magnesium, manganese, dietary fibers ³⁴

EDTA, ethylenediaminetetraacetic acid; DFE, dietary folate equivalent.

with water-cooled abrasive discs (320, 600, and 1,200 grit Al₂O₃ papers, Buehler) and polished with felt paper and diamond spray (1 µm, Buehler).

The surface hardness was determined by performing five indentations (Knoop diamond, 50 g, 5 seconds, FM 100, Future Tech, Tokyo, Japan) in the center of the enamel surface, for selection and randomized distribution purposes. Enamel slabs presenting values ranging from 314.1 - 383.9 Knoop hardness number (KHN) (349.6 ± 3.4 KHN) were randomly assigned according to a computer-generated randomization list. This step had established a baseline evaluation prior to immersion in the beverage media. Next, two third of the exposed enamel surface was protected with tape while the remaining surface was exposed to *in vitro* whole-saliva based pellicle.

Human saliva exposure

To simulate this condition, human whole saliva was used to promote pellicle formation on the samples. Fresh saliva was collected 1 hour after breakfast from 6 volunteers without active carious lesions, erosions, or salivary dysfunction. Salivary secretion was stimulated by chewing a block of paraffin for 5 minutes.²² The saliva from the first minute of chewing was swallowed and the rest was collected and subsequently introduced into a centrifuge tube. The saliva samples were centrifuged for 20 minutes at 2,000 rpm in a pre-cooled centrifuge (4°C, 5415R, Eppendorf, Sao Paulo, Brazil) and filtered by vacuum sterile filtration device

with 0.45 µm pore size (Stericup Express, Millipore Corp., Billerica, MA, USA). The clear fluid above sediments was pooled and used for *in vitro* whole-saliva based pellicle. Each group was immersed independently in clarified saliva and incubated overnight under agitation at 100 rpm (3 mL per slab) at room temperature (24 ± 1°C) prior erosive challenge according to the protocol described in the previous study.²³

Simulated intake cycling exposure model

Before the immersion in beverages, part of the tape was removed leaving one-third of enamel surface as a control. The beverages used during this study were purchased at local grocery stores and markets in Fortaleza, Ceara, Brazil. Average of 5 coconuts were used to obtain the coconut water which was mixed before used. For the CE beverage SUUM, an effervescent solution made by diluting one tablet in 500 mL of distilled water were prepared. The coconut waters in both liquid presentations were kept cool in a refrigerator (4°C) until use. The specimens were alternately immersed manually, 5 minutes each, in 3 mL of each drink and in artificial saliva (1.45 mM Ca, 5.4 mM PO₄, 0.1 M Tris buffer, 2.2 g/L porcine gastric mucin, pH 7.2, Sigma-Aldrich, St. Louis, MO, USA) for 4 hours (24 cycles) at room temperature. The simulated intake cycling exposure model was based on average intake of CE drinks during exercise reported in the literature.^{2,3}

Physical properties: enamel surface hardness/surface enamel loss

The enamel surface hardness was measured as described earlier, and an average of each group was obtained. Five indentations on each specimen were made at a distance of 100 μm each, five initially on sound enamel surface (SH₀) and five after the erosive stage (SH₁). Using these measurements, the percentage of surface hardness change (%SHC) was calculated by the formula: %SH_{change} = ((SH₁ - SH₀) / SH) x 100.²³ The enamel wear was determined in relation to the reference surfaces (covered by the tape), using a profilometer (Hommel Tester T1000, Hommelwerke GmbH, Villingen-Schwenningen, Germany). At intervals of 100 μm, five profile traces (1.5 mm in length) were recorded on each specimen. These profilometric traces were taken by moving the stylus from the reference surface to the exposed surface. For each sample, the mean values obtained from 5 traces were calculated. In addition, one representative slab of each group was analyzed by scanning electron microscopy (SEM). They were immersed in Karnovsky's fixative for 24 hours. They were then dried in desiccators, mounted on aluminum stubs, and sputter-coated with gold/palladium. Samples were examined by a scanning electron microscopy (VEGA/XMU series, TESCAN, Brno, Czech Republic) with different magnifications at an accelerating voltage of 20 kV.

Statistical analyses were performed using SPSS version 17.0 software (SPSS, Chicago, IL, USA). The percentage of SHC and wear in each group was calculated and the data were analyzed as the dependent variables by a two-way analysis of variance (ANOVA) and *post hoc* Tukey tests. The effects of the tested drinks and *in vitro* whole-saliva pellicle were considered. The correlation between the acidity of the tested drinks and (1) wear and (2) SH_{change} was analyzed by simple linear regression. The strength of the association between these two variables (acidity vs wear and acidity vs SH_{change}) was estimated with the Pearson correlation statistic (α = 5%). The correlation between the β means and wear/SH_{change} was also analyzed in a similar manner. Statistical significance was preset at α = 0.05.

Results

The physiochemical properties of beverage categories are presented in Table 2. The quantity of base (1 N NaOH) required neutralizing the beverages upon opening were high for sports drinks, followed by control groups.

Regarding dependent variables, there was a statistically significant difference between the %SHC of samples exposed to different drinks (p < 0.05), where the highest values were related to groups G and T. The S group revealed a median hardness loss followed by CC and NC groups, sequentially (Figure 1). The effect of *in vitro* whole-saliva pellicle was considered not significant (p = 0.73).

For wear in micrometers (μm), a two-way ANOVA found a tested drink effect (p < 0.001) but no significant *in vitro* whole-saliva pellicle effect (p = 0.40) or interaction effects (p = 0.12) were observed. A Tukey *post hoc* test (p < 0.05, Figure 2) revealed the following significant differences: G and T were greater than those produced during exposure to Suum; these 3 beverages were more erosive than both control groups.

In addition to the comparative results of %SHC and wear, regression estimates were used to describe data and to explain the relationship between variables. The linear relationship was observed between the acidity and %SHC, and a negative correlation was significant (r = -0.92, p < 0.05). A linear relationship and a significant positive correlation (r = 0.69, p < 0.05) were observed between the β - %SHC and between TA - %SHC (r = 0.89, p < 0.05).

In relation to wear, a linear regression analysis detected a moderate relationship between the wear and pH values (r = -0.61, p = 0.0001) with negative correlation, and between wear - β (r = 0.49, p = 0.0001) and wear - TA (r = 0.66, p < 0.05) a similar disposition but with positive correlation.

SEM of specimens submitted to tested drinks confirmed clearly demarcated erosion steps between exposure and covered enamel, exception for the control groups (CC and NC), where it was not possible to clearly identify an interface between the exposure and covered areas by SEM, due to the absence of wear (Figures 3a and 3b). This interface was clearly visible for G, S, and T groups (Figures

Table 2. Initial pH, the average amount of base necessary to raise the pH to 7.0, and average buffer capacity at pH 5.5 of the carbohydrate-electrolyte drinks analyzed

Group	NC	CC	S	T	G
Initial pH	4.71	4.81	4.26	2.73	2.85
Titrateable acidity to pH 7.0 (mM/L)	8.33	10.60	28.30	41.66	46.66
Buffer capacity pH 5.5 (mM/L x pH)	3.48	3.99	10.25	6.55	10.25

NC, natural coconut water (control); CC, commercial coconut water (control); S, carbohydrate-electrolyte drink (SUUM); T, carbohydrate-electrolyte drink (Taeq); G, carbohydrate-electrolyte drink (Gatorade).

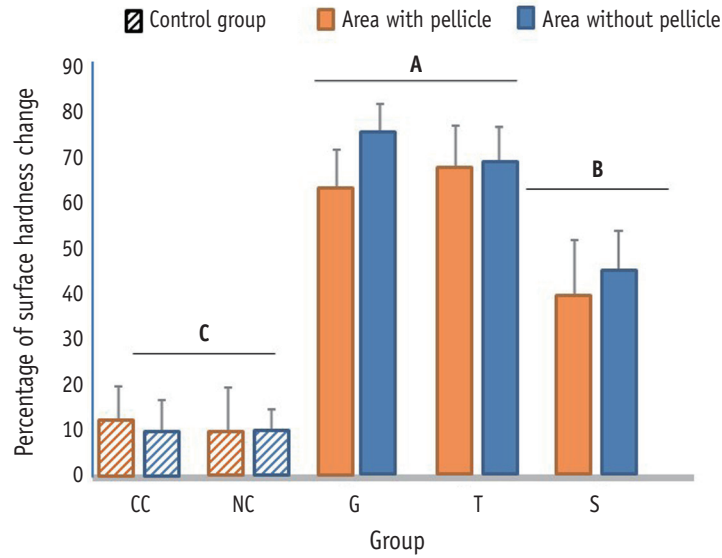


Figure 1. Mean and standard deviation of %SHC considering the presence and absence of acquired pellicle. Different letters correspond to a significant statistic difference among the groups of tested carbohydrate-electrolyte drinks ($p < 0.05$) and the bars represents that results are statistically similar when the comparison of the area covered with or without *in vitro* whole-saliva based pellicle. CC, commercial coconut water (control); NC, natural coconut water (control); G, carbohydrate-electrolyte drink (Gatorade); T, carbohydrate-electrolyte drink (Taeq); S, carbohydrate-electrolyte drink (SUUM).

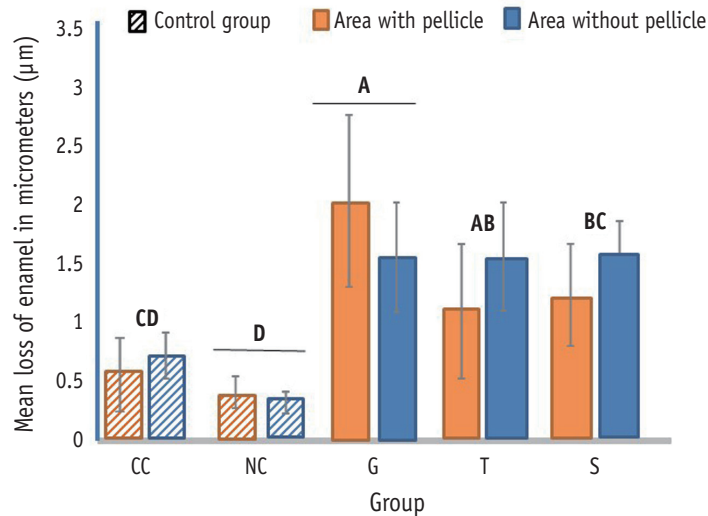


Figure 2. Mean and standard deviation of wear (μm) considering the presence and absence of acquired pellicle. Different letters correspond to a significant statistic difference among the groups of tested carbohydrate-electrolyte drinks ($p < 0.05$) and the bars represents that results are statistically similar when the comparison of the area covered with or without *in vitro* whole-saliva based pellicle. CC, commercial coconut water (control); NC, natural coconut water (control); G, carbohydrate-electrolyte drink (Gatorade); T, carbohydrate-electrolyte drink (Taeq); S, carbohydrate-electrolyte drink (SUUM).

3c, 3d, and 3e). The observation of the surfaces of eroded areas shows that some influence morphological alterations on enamel with *in vitro* whole-saliva pellicle. There was

localized surface loss showing the early degradation of enamel prismatic structure.

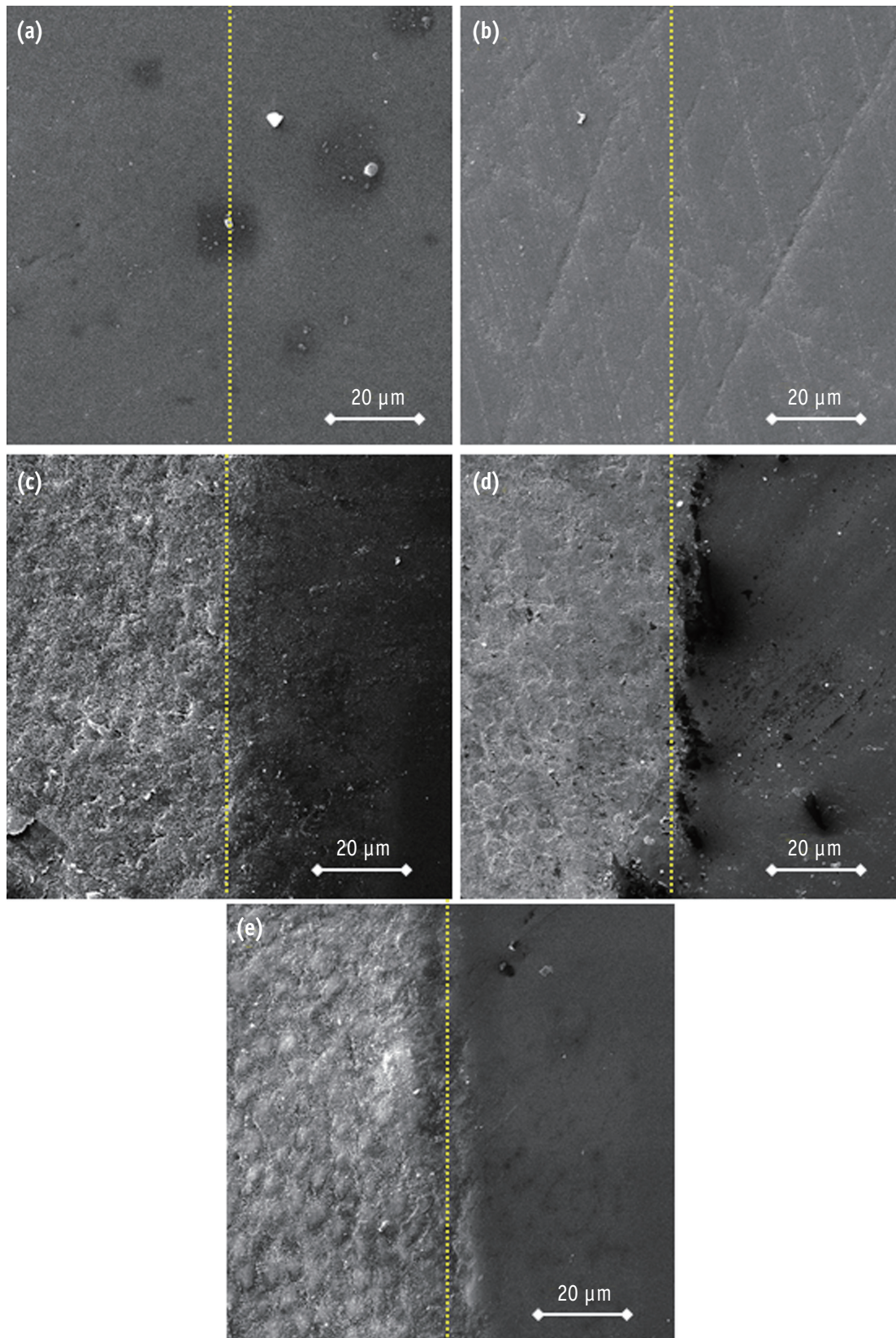


Figure 3. Representative scanning electron micrographs (SEM) of the enamel interface between control (right-hand side) and eroded slab surfaces (left-hand side) with pellicle presence in the each group (x1,500).

Discussion

This study provides a broad view in that the CE drinks are used by athletes for adequate hydration during sports and exercises and how they may cause significant short term enamel erosion.^{1,24,25} Although, we have an extensive literature related to dental erosion, the contributions of the associated diet factors to dental erosion are still unclear to establish guidelines for athletes or those that participate in training and intense exercises. However, it must be considered that the teeth of those individuals engaged in physical activity are exposed to CE drinks for hours every day and might be affected by an increased risk of dental erosion.

The present findings showed that enamel under simulated intake of CE drinks had a significantly greater loss of surface hardness as well as tooth structure loss than the controls. The most probable explanation would be related to the acidic and buffering properties of these beverages. Our data showed that the tested CE drinks present pH values below critical one (pH 5.5), which allows us to expect an initial demineralization of enamel. The presence of various acids can explain their erosive potential. According to the information from the manufacturers, citric acid, and ascorbic acid are the components of tested CE drinks. These findings are in agreement with *in vitro* and *in situ* studies that reported the potential of drinks to demineralize enamel, leading to the development of dental erosion.^{26,27} Drinks containing higher concentrations of citric acid are more aggressive on enamel;^{28,29} also, comparable findings in the study support this statement since the sports drinks used in this study contain citric acid or citrates. Citric acid is a tribasic carboxylic acid and causes enamel dissolution by reaction with hydroxyapatite. The citrate ion is a chelating ligand, and forms a soluble complex with the calcium ion, promoting further dissolution.²⁶ However, coconut water did not show any evidence of erosion, either for %SHC or for wear analysis showing to be no harm to enamel. Previous report has suggested that the presence of ionic elements such as calcium fulfill functional roles in the chemical reactions during the erosive process.³⁰ Coconut water had low amounts of organics acids and it is highly rich in inorganic ions such as K, Na, Ca, Mg, and P.³¹ In addition, sports drinks contain additives and organic acids that are very erosive because of their ability to break down calcium on dental enamel.²⁸

Besides the role of high consumption of acidic drinks over erosive potential, factors such as pH, TA, β , and beverage composition can contribute to the erosive process acting like predisposing factors necessary for the erosion of enamel. Buffering capacity and titrations become important later when there is a longer contact between the enamel and the drink. The data from G, S, and T groups indicated this relation with the erosive process showing higher wear

and hardness loss values were related to the beverages that present higher pH, β , and TA values. TA and the ability of the drink to resist a change of pH, represented by buffering effect, are considered a better indicator of erosive potential than pH.³¹ Special attentions for β at 4.5 - 5.5, critical range pH, where enamel apatite dissolves without any mutual formation of surface fluorohydroxyapatite.^{29,32} The higher the buffering effect of the drink, the more apatite will be dissolved before neutral pH can be reached. The G, S, and T results represented high erosive potential by high buffering effect in low pH.

Regarding to our reference (control group), both the available presentations of coconut water were selected. The commercial presentation was submitted to previous pasteurization and potting processes. The in nature presentation was used without further industrial manipulation. Although, coconut water presents low pH (4.71 - 4.81), its high quantities of Ca (27.35 mg/100 g) and P (4.66 mg/100 g) inhibit erosive process.³³ In contrast, sports drinks such as Gatorade contains small quantity of Ca (0.13 mM/L) and P (2.98 mM/L). This suggests that chemical aspects can modulate the potential to cause dental erosion, including the concentration of calcium, phosphates, and fluoride. Hence, modifications in these parameters may lead to a reduction in the erosive potential of a given acidic beverage.³⁴

It is known that saliva plays a critical role in the removal of erosive compounds from the oral cavity and acts as a buffer against ingested acids.³⁴ In the present study, the pellicle alone did not promote protective effect against initial surface enamel loss. The pellicle was able to provide 20% less enamel loss considering the surface hardness change for the most detrimental sports drink evaluated (Gatorade), where the values for percentage of surface hardness change were 77% in the area without the pellicle versus 62% in the area where the pellicle was present. It may be assumed that the *in vitro* formed pellicle layer used was insufficient against cycling erosive process. In the oral cavity, the constant saliva flow, temperature, different enzymes, and microorganisms play a role in pellicle maturation and may affect the protective properties of the pellicle.²¹ In addition, the simulated time of exposure of enamel to the beverages was limited. Constant saliva exposure for neutralization and the remineralization potential should be considered in further studies. These are the critical considerations on weaknesses and limitations of the study.

Furthermore, considering our results, coconut water is unable to promote an erosive process in dental enamel, making it a healthy electrolyte drink of choice. The use of natural alternatives to the manufactured sports drinks can be indicated to help people replenish fluids and nutrients during exercise without the potential erosive risks.

Conclusions

In conclusion, this study demonstrated that the tested beverages promote surface loss on enamel and have detrimental influence exerted by their chemical properties and composition. The saliva exposure in our experiment might lead to a suggested protective effect of enamel under erosive attack. Further analyses in other erosive risk factors and CE drinks, together with the study of the role of salivary components, will be crucial to further delineate the early erosive risk on human enamel.

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Conflict of Interest: No potential conflict of interest relevant to this article was reported.

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