

In vitro effect of energy drinks on human enamel surface

Análise in vitro da ação de bebidas energéticas no esmalte dental humano

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Resumo

Introdução: O potencial erosivo das bebidas energéticas (BE) devido ao baixo pH e à presença de ácido cítrico pode estar relacionado ao aumento dos índices de erosão dental da população em geral e especialmente, nos jovens. **Objetivo:** Verificar o pH e a titulação ácida de BE e a influência de uma marca de BE na microdureza superficial do esmalte. **Material e método:** Dez amostras de BE de diferentes marcas comerciais foram selecionadas. O pH de dois lotes de cada BE foi analisado, com e sem gás. A titulação ácida foi realizada com a adição de alíquotas de NaOH, até atingir pH 7,0. Dezoito amostras de esmalte dental humano foram distribuídas aleatoriamente em três grupos (n = 6), Red Bull (RB), Red Light Bull (RBL) e água destilada (C), submetidas a um desafio ácido com a BE, seis vezes consecutivas, com intervalos de 12 horas, durante três dias. A microdureza Knoop foi medida antes e depois do desafio ácido. **Resultado:** Todas as marcas de BE testadas apresentaram baixos níveis de pH, variando de 2,1 a 3,2. Em relação à titulação ácida, verificou-se que a quantidade de base necessária para promover a neutralização das soluções variou de 1200 µL a 3750 µL. Amostras de esmalte humano nos grupos RB e RBL submetidos ao desafio ácido apresentaram diminuição significativa da microdureza Knoop. **Conclusão:** Todas as BE examinadas apresentaram baixo pH e alta titulação ácida e, portanto, potencial para promover perda mineral. As BE analisadas promoveram perdas minerais significativas na superfície do esmalte dental.

Descritores: Erosão dentária; alimentos para praticantes de atividade física; esmalte dentário; desmineralização do dente.

Abstract

Introduction: Energy drinks (ED) possess low pH and citric acid in their composition, making them potentially erosive beverages that can contribute to the high dental erosion rates found currently in the general population and also in young people. **Objective:** To evaluate the mean pH and titratable acidity of commercial ED and the influence of a brand of ED on the superficial microhardness of human enamel. **Material and method:** Ten commercial ED were selected and the pH of two lots of each ED with and without gas was obtained. Acid titration was conducted with the addition of NaOH aliquots until the pH 7 was reached. Eighteen human enamel specimens were allocated in three groups (N=6), Red Bull (RB), Red Bull Light (RBL) and distilled water (C), submitted to an acid challenge with the ED, six consecutive times, with 12 hours intervals, during three days. Knoop microhardness was measured before and after the acid challenge. **Result:** All ED brands tested presented low pH levels ranging from 2.1 to 3.2. Regarding titratable acidity, it was found that the amount of base required promoting the neutralization of the solutions ranged from 1200µL to 3750µL. Samples of human enamel in the RB and RBL groups submitted to the acid challenge presented significantly decreased Knoop microhardness when compared with the group C. **Conclusion:** All ED examined have potential to promote mineral loss due to the low pH and high titratable acidity. The ED analyzed promoted significant mineral losses on the dental enamel surface.

Descriptors: Tooth erosion; energy drinks; dental enamel; tooth demineralization.

INTRODUCTION

The fact that energy drinks (ED) can improve physical and mental performance, as well as the mood¹, has led this type of beverage to be widely consumed, especially among young people². The consumption of ED has been increasing globally every year,

with the leading ED brand (Red Bull - with 43% of the international market) selling more than 5 billion cans worldwide alone³.

ED it is the kind of beverage that could be included in the erosive drinks category. Dental erosion is a concerning issue as it represents



an irreversible mineral loss caused by the acid dissolution of the dental structure, with no bacterial involvement. Dental erosion may be caused by the exposition to acids of extrinsic (acidic beverages) and intrinsic (gastric acid) origin⁴. Present feeding habits include the large consumption of acid food and drinks⁵. The evidence emerging from *in vitro* and *in situ* studies show that fruit juices, wine and soft drinks can potentially cause dental erosion due to low pH and the presence of acids in their composition⁶. However, to the best of our knowledge there have been no studies that verified the enamel softening with ED.

Similarly to other soft drinks, the majority of ED also have low pH and contain citric acid in its formulation⁷. Previous *in vitro* studies making use of profilometry and electron microscopy have demonstrated changes in the enamel and dentin caused by ED⁸⁻¹¹. Decreased enamel hardness is the first sign of tooth erosion¹², which is an important evidence of the action of erosive drinks in short exposures.

Therefore, the aim of this study was to verify the mean pH and titratable acidity of commercial ED and the influence of a well-known brand of ED on the superficial microhardness of human enamel after a short period of acid challenge.

MATERIAL AND METHOD

Study Design

This is an *in vitro* experimental study (Figure 1). All procedures described herein were approved by the Human Experimentation Ethics Committee of the São Paulo University (protocol CEP - FOU SP 181-05).

Potential of hydrogen (pH) analysis and acid titration

Ten commercially available ED were selected for pH analysis and the titratable acidity test. In order to establish the mean pH of each ED, samples from two different lots were acquired, and measured. An electrode connected to a potentiometer (pH-Meter E520, Switzerland) was used for pH analysis. The electrodes were calibrated at pH 7 and pH 4 with a standard solution. The tested beverages were maintained in ambient temperature (~ 25°C), and the measurements were performed in two different conditions,

immediately after opening the can and after the drink was placed in a magnetic agitator to remove the CO₂.

The titratable acidity test was conducted using an electrode connected to the drinks (30 ml). Aliquots (50 µl) of NaOH 1N were added in order to check the amount of base required to reach pH 7.

Selection and preparation of specimens

The ED brands Red Bull® and Red Bull Light®, widely consumed for sports and recreational purposes, were selected for the Knoop microhardness test.

Eighteen sound premolars were obtained from the Tooth Bank at the Dentistry College, University of São Paulo, Brazil. These teeth were examined under a light microscope (2X), to ascertain that no gaps, cracks, caries, erosion and/or stains were present. The selected teeth were sectioned with a diamond disc in a sectioning machine (Labcut 1010). First the crown was separated from the root, and then the teeth were cut in the mesial distal direction near the central sulcus, and a fragment from buccal face was obtained and used in the study.

The dental specimens were submitted to a superficial enamel planning to obtain homogenous surfaces. Polishing was performed on a rotating polishing machine (Ecomet 4, Buehler, Lake Bluff, USA), with decreasing size abrasives and final polishing with felt discs and diamond paste (Arotec, São Paulo, Brasil). Each specimen was placed in an acrylic tube (10mm × 10mm) that was then filled with autopolymerizing acrylic resin. Test specimens were ultrasonically cleaned (Thoron) during 10 minutes, to remove any residues.

The specimens were randomly allocated (drawing lots) to three experimental groups with six specimens in each group: CG – Control Group (distilled water); RB – Red Bull® Group; RBL – Red Bull Light® Group.

An area of 2 × 2 mm of the enamel surface was delimited and covered with parafilm, while the remaining surrounding areas received two layers of an acid-resistant varnish (Colorama®). After the varnish had completely dried the parafilm was removed, exhibiting the test area. Specimens were then kept in distilled water until use.

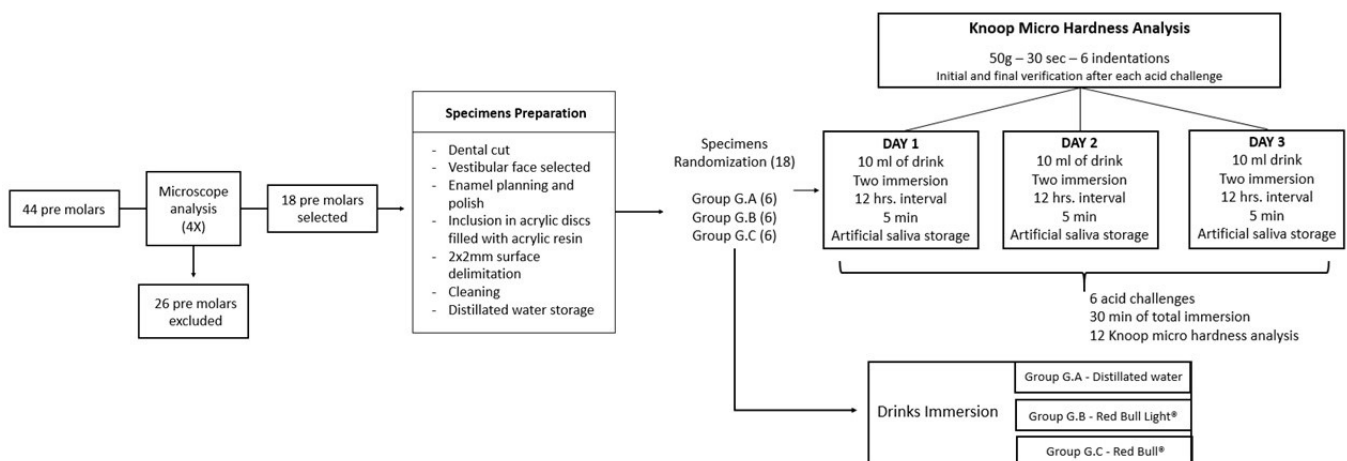


Figure 1. Study design.

Cycle immersion in the ED

Specimens were removed from the distilled water, blown dry, and individually conditioned in 10 ml recipients. ED were conditioned in a clean dry glass container. A magnetic stirrer was used during 3 minutes to eliminate the CO₂. After that, 10 ml of the ED was poured into the individual recipients containing the dental specimens and kept for 5 min (Motorola® chronometer). Then, the specimens were removed from the container and thoroughly washed in distilled water.

Two acid challenges, with intervals of 12 hours, were performed per day, during three consecutive days. Between the acid challenges, specimens were kept totally immersed in artificial saliva. To simulate the natural oral remineralization conditions, the artificial saliva was replaced after each experimental day. At the end of the experiments, specimens were stored in a recipient with distilled water.

Knoop microhardness analysis

The superficial alterations on the enamel were verified with a microdurometer (HMV - Microhardness Tester, Shimadzu Corporation – Japan) using a pyramidal diamond-type Knoop indenter, with a load of 50 gf for 30 sec. Six indentations were made at a distance of 100 µm between them before (baseline measure) and after (final measure) the acid challenge. Knoop microhardness values were automatically calculated by the equipment software.

Statistical Analysis

The pH and acid titration analysis of the ED tested were described in terms of means and standard deviation. The Kolmogorov-Smirnov test at a significance level of 0.05 was used to determine the normal distribution of Knoop microhardness values.

RESULT

Mean values for pH of the samples analyzed are displayed in Table 1. The pH values of all samples demonstrated no differences with or without gas, varying from pH 2.1 (Flying Horse Light®) to pH 3.25 (Pepsi® Energy).

Red Bull Light was the ED to require the greatest amount of base solution to neutralize acidity (~ 3750 µl). The standard Red Bull version was the second to require more NaOH aliquots (~ 3500 µl) to reach basic pH (Figure 2). Some ED, like Pepsi Energy, suffered a slight pH drop, that is attributable to the hydrolysis of the weak base, which comes from the salt formed.

The Knoop microhardness values of each specimen obtained before and after the acid challenge are presented in the Table 2. Baseline microhardness values, before the acid challenge of specimens, were similar among groups, with no statistical differences being observed. The comparative assessment of baseline and final microhardness values revealed significant statistical differences for the RB and RBL groups, while the control group displayed no statistically significant differences.

Red Bull® presented higher titratable acidity and consequently needed a greater amount of base for neutralization to occur. This means that when this solution is ingested, buffering in the oral cavity can be compromised, leading to pH maintenance under acidic conditions for a longer period of time. In our study, Pepsi Energy® was the

Table 1. Mean values for pH of the samples analyzed

Energy Drink	With Gas	Without Gas
Red Bull®	3.05	3.05
Red Bull Light®	3.00	3.02
Pepsi® Energy	3.25	3.17
Burn® Energy	2.30	2.32
Atomic Sugar Free	2.65	2.60
Rush! Energy™	2.30	2.32
Flying Horse Light®	2.10	2.10
Flying Horse Booster®	2.12	2.15
220V Energy Drink	2.55	2.57
Bad Boy Power Drink	2.67	2.65

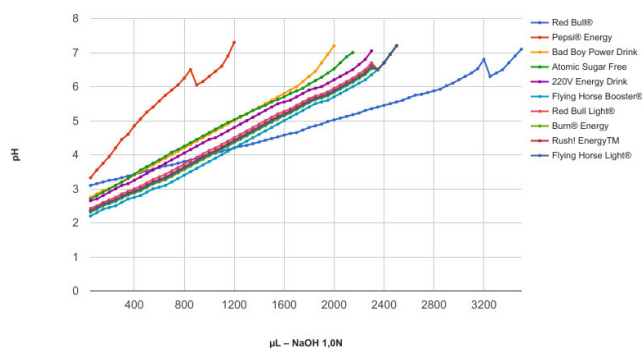


Figure 2. Mean pH values of energy drinks in NaOH 1N aliquots.

Table 2. Mean and standard deviation (SD) of Knoop microhardness values before and after the acid challenge

GROUP	BEFORE	AFTER
Control	403.5 (19.61) Aa	380.75 (32.16) Aa
Red Bull Light	394.85 (54.37) Aa	129.65 (13.28) Bb
Red Bull	434.62 (62.90) Aa	156.90 (20.89) Bb

Different uppercase letters show statistically significant differences before and after the acid challenge. Different lowercase letters show statistically significant differences between groups.

energetic that presented lower titratable acidity (1200 µl of NaOH), requiring less amount of base for its neutralization, indicating that among the evaluated ED, from the point of view of the maintenance of acidity, Pepsi Energy® was the most easily neutralized.

DISCUSSION

In this study, the erosive potential of ED was demonstrated after a rapid acid challenge based on the decreased superficial microhardness of the dental specimens tested. The pH reflects the dissociated hydrogen ion concentration, thereby we observed that the brand Flying Horse had the greater dissociated hydrogen ion concentration. The acids present in the EDs would correspond to the total acidity, the titratable acidity performed is the sum of the [H⁺] and neutralized undissociated acid molecules up to pH = 7.

Thus, it is important to indicate the presence of undissociated acid, given by its viability of real demineralization of an acidic beverage¹³.

The ED Red Bull used in the microhardness test was not the one with the lowest pH but was the most difficult to be neutralized.

The knowledge and identification of the beverages with the potential to cause dental erosion are important. Dental erosion represents an irreversible loss of tooth structure, even in populations without dental caries. Besides, erosion tends to occur early. Several surveys observed the prevalence of erosion in children and adolescents in different locations around the world¹⁴. Only few studies have investigated the action, and the possible role, of ED in dental erosion⁸⁻¹¹.

Pinto et al.¹¹, using electron microscopy, observed that ED have the potential to cause the dissolution of the smear layer and, consequently, increase human mineral dentin loss. Kitchens, Owens⁸, Ehlen et al.⁹ and Caneppele et al.¹⁰ using profilometry to assess the effects of ED on human enamel and bovine dentin, observed that ED caused damage to the samples exposed to the acid challenge, in agreement with our results. The higher the titratable acidity of the solution, the greater its erosive potential¹⁵. Owens¹⁶ observed that Red Bull® had the highest titratable acidity, indicating its high potential for erosion to the dental enamel. Red Bull® was the ED that needed a larger amount of base to reach pH 7.0 (3750 µl NaOH), followed by Red Bull Light® (3500 µl NaOH). Thus, it is the ED ones that present greater erosive potential.

Acid food and drinks are considered the main cause of cervical lesions in teeth, a condition commonly associated with hypersensitivity due to the exposure of dentin tubules¹⁷. Class V restorations are still a challenge in many regards such as access to the lesion, field control, material placement and handling, marginal finishing and marginal infiltration¹⁸. Thus, knowledge about the possible causes or agents of cervical lesions is essential to draw attention to initial alterations, even if they are not still clinically apparent.

In vivo analyzes of the direct influence of the consumption of ED are almost impossible to be performed. Erosion is a multifactorial condition associated to many variables difficult to control over time. *In situ* analyzes represent the next logical step to verify the erosive potential of ED in conditions closer to reality, similarly to previous studies that investigated the effect of several other beverages¹⁹. The methodologies described in the literature and used for the immersion cycles in refreshments and juices are not applicable to the ED, since the maintenance of the dental fragments immersed in the solution by the proposed time has caused excessive mineral loss. So, it was necessary to establish a new strategy with a shorter exposure time to avoid the previously obtained failure. The methodological sequence used in this experiment recommends the exposure time of 5 minutes in each challenge, totaling 10 minutes daily. At the end of the cycles, each test specimen was exposed to the energetic for a total time of 30 minutes and by subjecting them to the microdurometer a significant decrease in the hardness values obtained was verified. The methodology was adapted from the work of Amaechiet al.²⁰ and Van Eygen et al.²¹.

The pH and the titratable acidity of acidic beverages are important to the investigation of potentially erosive drinks²². In the mouth, during the consumption of a drink, the amount of saliva initially secreted is small. Thus, at first, pH has a substantial role in the erosive capacity of a drink²². Prior to performing the experiments,

it is common to remove the gaseous portion by means of stirring. This measure was used in this study to minimize volume variation during the measurement process. As we also could see in this study, there is no significant change in the pH of carbonate solutions before or after the removal of the gas, however this can happen, there is the importance of measure. Thus, the pH with or without stirring is similar, it can be attributed that the pH of these beverages is defined by the acidic components constituting the beverage and not by the partial pressure or concentration of the CO₂ present therein.

After the drink is ingested, the titratable acidity represents the amount of salivary buffer necessary to neutralize the acid environment caused by the drink and return to the usual pH²². During the ingestion of beverages, a continuous sipping process over a period of a few minutes, the influence of pH, the titratable acidity on the salivary buffering capacity, and the time that the pH remains low may differ among people²³. This is an aspect that still requires investigation with ED. Nevertheless, the titratable acidity of a drink is considered to play a more significant role than the initial pH²³.

Consumption of ED among young people is high and tends to increase in the future. Thus, the knowledge about the possible damages that a type of beverage can cause seem to be essential to prevent irreversible injuries. Moreover, recent studies have also focused on additive compounds to prevent erosion²⁴, or on the influence of external agents, such as toothpastes and its components, to recover the demineralized dental tissue²⁵.

This *in vitro* study verified the rapid influence of commercially available ED on the early demineralization of human enamel, demonstrating that ED can be potentially erosive even after a rapid acid challenge. Warning about the possible causes of future dental erosion is essential to prevent injuries that can cause pain and are difficult to treat. To further elucidate the processes involved in the consumption of ED and their implications on tooth wear, *in situ* studies should be performed. Moreover, analyses of the effects of ED intraorally, regarding their consumption patterns, their titratable acidity and the time oral pH remains low should also be performed. The addition of compounds that could diminish the erosive potential of ED, already observed with others acid drinks, also deserve to be tested. Studies on tissue remineralization with the application of fluoride (toothpastes or topical flour) on the dental surface damaged by ED are also required.

CONCLUSION

In summary, all ED examined have the potential to promote mineral loss and consequently dental erosion due to the low pH and high titratable acidity. The EDs analyzed promoted significant mineral losses on the dental enamel surface.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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