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RESEARCH ARTICLE

Assessment of the Erosive Potential of Mineral Waters in Bovine Dental Enamel

Gabriela Monteiro Barbosa Xavier[1], Aila Silva De Almeida[1], Alexandra Gabrielly de Souza Bentes[1], Issae Sousa Sano[1], Cecy Martins Silva[1] and Jesuína Lamartine Nogueira Araújo[1,*]

[1]*Department of Conservative Dentistry, School of Dentistry, Health Science Center, Federal University of Pará, Belém, Pará, Brazil*

Abstract:

Background:

High intake of acidic foods and beverages has been often associated with the onset of dental erosive wear.

Objetive:

This study *in vitro* assessed the pH of different mineral waters marketed in Brazil and their effects on the properties and surface of dental enamel.

Methods:

Forty-eight bovine incisor specimens were divided into four groups (n=12): CG-control group, PeG-Perrier, PrG-Prata, and SLG-São Lourenço. The immersion cycles were performed after analysis of the pH of the waters, for 5 days (5 minutes in mineral water and 60 minutes in artificial saliva). Knoop micro-hardness was assessed by means of three indentations with a load of 50kgf for 15 seconds, and surface roughness with a cut off of 0.25mm. The data were analysed using Student's *t*-test, ANOVA, and Tukey test, with a significance level of 5%.

Results:

The groups of waters with lower pH (Perrier® and São Lourenço®) exhibited a reduction in Knoop micro-hardness ($p<0.0001$) and an increase in surface roughness ($p=0.04$ and $p=0.004$, respectively). The Prata water group did not exhibit significant changes in Knoop micro-hardness ($p=0.07$) and surface roughness ($p=0.26$).

Conclusion:

Mineral waters with a pH below the critical value can lead to a reduction in surface hardness and roughness in the bovine enamel.

Keywords: Erosion, Mineral water, Tooth enamel, Acidic drinks, Micro-hardness, Surface roughness.

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1. INTRODUCTION

Dental erosion is a process of irreversible dental hard tissue loss caused by acids without bacterial involvement [1]. With a prevalence rate ranging from 20 to 50% in the world population [2], it is a condition that, when uncontrolled, can progress and bring functional and aesthetic damage [3]. The surface of the eroded tooth becomes highly susceptible to abrasive wear and mechanical impacts, which can easily remove superficially demineralised tooth tissue [4, 5].

The etiology of dental erosion is related to intrinsic factors associated with gastric juice and extrinsic factors that includediet (acidic foods and drinks), environmental factors (exposure to acidic products), and chronic drug use [1, 6 - 8]. Among these, excessive and frequent consumption of acidic foods and beverages is one of the factors most commonly associated with this condition in several countries, including Brazil [2, 9, 10].

The pH and titratable acidity are is relevant parameters for determining the erosive potential of these products and the possible rate of dissolution of dental tissue [7 - 10]. In that regard, saliva plays an important role in maintaining the intra-oral pH at a physiologically healthy level, *i.e.*, around 7.4 [5, 11]. When salivary pH increases, acid buffering occurs effectively and promotes tooth enamel remineralisation [11, 12]; however, its protective action is limited when there is an

* Address correspondence to this author at the Department of Conservative Dentistry, School of Dentistry, Federal University of Pará, 01 Augusto Correa Avenue, Campus of Guamá, Belém, PA 66075-110, Brazil;
Tel: +55 91 99144-2849; E-mail: jesuinalamartine@hotmail.com.

excessive frequency of beverages and acidic food consumption [13].

Drinks such as soft drinks, energy drinks, and fruit juices are commonly mentioned in some studies because they influence the erosive tooth wear process [6, 14]. In this context, some mineral waters can also have acidic pH with the potential to damage the tooth structure nevertheless studies that ress this issue are scarce and little addressed [15]. Bottled mineral waters are valid options to satisfy the water needs of the body. As these waters are beneficial to health, they are routinely consumed [16]. Therefore, it is important to assess the possible damage to tooth enamel caused by drinking these beverages, both because of the frequency of ingestion and the low pH present in some brands [15, 17].

In addition, it is necessary that professionals become aware of the erosive potential of the various brands of bottled water found in the market. This way, they will be able to guide patients, especially those whose teeth already exhibit signs of erosion, in order to prevent further deterioration and demineralization of the tooth structures. Most patients and professionals incorrectly assume that bottled water is innocuous to health [18]. Thus, clarification about the pH of mineral waters and their implications is essential for the prevention and progression of injuries in the population.

The goal of the present study was to assess the pH of different mineral waters usually consumed by the Brazilian population and their effects on the surface of bovine dental enamel by means of Knoop micro-hardness (KHN), and surface roughness. The null hypothesis stated that the pH of the waters tested would have no influence on the hardness and roughness of the bovine enamel after erosion.

2. MATERIALS AND METHODS

The present *in vitro* study was approved by the Ethics Research Committee on Experimental Animals of the Federal University of Pará, State of Pará, Brazil (Protocol No. 4027190520). Forty-eight healthy bovine incisors of animals with an average age of 24 months were selected, sanitised, and kept in 0.1% thymol. The teeth were evaluated under a 10x magnifying glass and those with cracks or enamel defects were excluded. Four experimental groups were assessed in terms of exposure to water with different pH, according to information provided by the manufacturers (Table **1**). Distilled water was used as the control group (CG).

Table 1. PH value provided by the manufacturers for the products, according to their respective groups.

Groups/water	pH	Manufacturer/ Batch no.
CG - Distilled water	7.0	Asfer Indústria Química Ltda (SP, Brazil)/2683
PeG - Perrier® mineral water	5.5	ASB Bebidas E Alimentos Ltda (Vergèze, France)/ L0254181628
PrG - Prata® mineral water	6.7	Águas Prata Ltda (SP, Brazil)/13:55L250821
SLG - São Lourenço® mineral water	5.3	Nestlé S.A. (MG, Brazil)/ L20A0094

2.1. Sample Preparation

The teeth were sectioned, initially separating the root from the crown with a cutting machine (Labcut 1010, Extec, Enfield, CT, USA) and diamond discs (Extec, Enfield, CT, USA), until vestibular enamel samples were obtained (4 x 4 x 2 mm). These samples were included in blocks of chemically activated acrylic resin (Jet Classic, São Paulo, SP, Brazil) and polished with silicon carbide sandpaper (320, 600, and 1,200 - 3M, SP, Brazil) in a polishing tool (AROTEC-multiple polishing device, Cotia, SP, Brazil). After changing the sandpaper and at the end of polishing procedure, performed with felt discs and diamond paste (Diamond Excel FGM, SC, Brazil), the samples were washed with ultrasound and deionised water (Ultrasonic-T14, L&R Ultrasonic, USA) for two minutes. After preparation, the specimens were randomly divided into 4 experimental groups (n=12) (Table **1**).

2.2. Assessment of pH

The pH values of the waters selected for the study were measured before the immersions using a ph meter (Kasvi K39-1410A, Paraná, Brazil). This procedure was performed in triplicate, with 50 ml of each mineral water at room temperature.

2.3. Erosive Challenges

The samples were subjected to alternate erosive cycles (demineralisation and remineralisation). Each cycle was composed of five-minute immersion in 10 ml of demineralising solution (mineral water), washing with deionised water for 10 seconds, light drying with absorbent paper, and a 60-minute immersion in 10 ml of remineralising solution (artificial saliva, pH = 7.0). This artificial saliva had the following composition: potassium chloride (11182, 50 mg/l); calcium nitrate (60.12 mg/l); sodium fluoride (0.066 mg/l); monobasic sodium phosphate (160.19 mg/l); 2-Amino-2-hydroxymethyl-propane-1, 3-diol (12114.00 mg/l); and deionised water (1,000 ml).

The demineralisation and remineralisation (DES-RE) cycles were repeated six times a day for five days, totalling two hours and thirty minutes of immersion in demineralising solution. At night, between cycles, the samples were stored in artificial saliva. The mineral waters were renewed at each erosive challenge, as well as the artificial saliva that was replaced once a day, before the first cycle. All solutions were used at room temperature (29 °C). After the last immersion cycle, the samples were submitted for analysis.

2.4. Knoop Micro-hardness

The Knoop micro-hardness method was performed using a micro-hardness tester (FM 700, Future Tech, Japan), before the first and after the last the exposure cycle. Three indentations were performed, spaced 500 µm apart, with a load of 50 g for 15 seconds. The average of the indentations was calculated.

2.5. Surface Roughness

The assessment of surface roughness was performed by rugosimeter (SJ - 301, Mitutoyo, Los Angeles, CA, USA), through three readings taken for all samples performed in two different moments (before the first and after the last exposure cycle). The mean roughness (Ra) was adopted as a parameter, corresponding to the arithmetic mean of the absolute values of the roughness profile ordinates (peaks and valleys) concerning the midline, within the measurement run. At each reading, the rugosimeter needle crossed a 5 mm long area on the surface with a cutoff sampling of 0.25 mm.

2.6. Statistical Analysis

The data obtained were submitted to the Shapiro-Wilk test of normality, Student's *t*-test for intra-group assessment (before and after immersions), and analysis of variance (ANOVA) with Tukey's post-hoc test, for evaluation between the experimental groups after the immersion cycles. A significance level of 5% was used in the Biostat 5.0 software (Instituto Mamirauá, Amazonas, Brazil).

3. RESULTS

The assessments of pH indicated mean values of 6.95 ±0.05 for the CG (distilled water), 5.52 ±0.04 for the PrG (Prata group), 5.27 ±0.03 for the PeG (Perrier group), and 4.73 ±0.02 for the SLG (São Lourenço group). The results obtained for Knoop micro-hardness in the intra-group comparison did not show statistically significant differences in the CG (p = 0.63) and PrG (p = 0.07), after a five-day immersion in mineral water. However, the results also indicated a significant reduction in the values of enamel hardness in the PeG (p <0.0001) and the SLG (p <0.0001). The comparison of the groups at the end of the exposure cycles indicated statistically significant differences, i.e., the CG in comparison to the PeG (p <0.05) and the SLG (p <0.01), and between the PrG and the SLG (p <0.01) (Table **2**).

Table 2. Mean and standard deviation according to Knoop micro-hardness test.

Groups	KHN	KHN
	Before immersions Average (±SD)	**Five days after immersion Average (±SD)**
CG - Distilled water	326.33 (±3.7) aA	325.20 (±7.8) aA
PrG - Prata	325.21 (±2.7) aA	321.98 (±3.7) aAB
PeG - Perrier	332.68 (±12.7) aA	316.78 (±8.0) bBC
SLG - São Lourenço	329.12 (±4.53) aA	310.78 (±4.2) bC

Note. KHN = Knoop micro-hardness; SD = standard deviation. Distinct letters represent statistically significant difference (Student's *t*-test and ANOVA with Tukey's post-hoc; p ≤0.05). Lowercase letters compare intra-group differences, and uppercase letters compare inter-group differences.

With respect to surface roughness, there was a statistically significant difference only in the PeG (p = 0.04) and the SLG (p = 0.004) after the five-day immersion cycle. The CG (p = 0.39) and the PrG (p = 0.26) did not show major changes over the analysed period. Regarding the comparison between the groups, no significant differences were observed between the final averages (p = 0.92) (Table **3**).

4. DISCUSSION

Acidic beverages are one of the main agents that cause dental erosion due to the high volume of ingestion and the increasingly frequent consumption [2, 3, 19]. In the early stages, this condition leads to changes in the physical and chemical properties of the teeth [4, 20]. In Brazil, there is a wide variety of bottled mineral water brands available and in the present study, mineral waters with acidic pH caused damage to bovine dental enamel after erosive cycles. Therefore, the null hypothesis was partially rejected.

Table 3. Mean and standard deviation according to the surface roughness test.

Groups	SR	SR
	Before immersions Average (±SD)	**Five days after immersion Average (±SD)**
CG - Distilled water	0.234 (±0.02) aA	0.233 (±0.2) aA
PrG - Prata	0.236 (±0.03) aA	0.239 (±0.03) aA
PeG - Perrier	0.235 (±0.01) aA	0.242 (±0.01) bA
SLG - São Lourenço	0.233 (±0.01) aA	0.247 (±0.01) bA

Note. SR = surface roughness; SD = standard deviation. Different letters represent statistically significant difference (Student's *t*-test and ANOVA; p ≤0.05). Lowercase letters compare intra-group differences, and uppercase letters compare inter-group differences.

The groups represented by Perrier® and São Lourenço® mineral waters exhibited statistically significant changes in Knoop micro-hardness and surface roughness in dental enamel specimens, which may be related to the pH found below the critical level [21, 22]. Moreover, these two brands of mineral water are naturally carbonated (reinforced with carbon dioxide from the source itself), as informed by the manufacturers. In previous studies [23, 24], carbonated waters showed greater erosive potential than those without gas. Ryu *et al*. [23], observed that the higher the level of carbonation, the greater the tendency for enamel erosion.

Although Prata® water (without gas) sample had a pH of 5.5, considered borderline [13, 25], no differences were observed in the Knoop micro-hardness and surface roughness averages before and after the demineralisation and remineralisation cycles. These mineral waters did not differ statistically with respect to the control group, in this group, distilled water was used, which has a characteristic pH neutral of around 7.4 [14, 21]. In a study conducted by Enam *et al*. [26], bottled waters did not show erosive potential; however, according to the authors, that fact may have been related to the neutral pH of the analysed brands, which were unlikely to promote the dissolution of tooth structures. In the present study, borderline pH also does not seem to promote major changes in the properties analyzed.

The large concentration of hydrogen ions (H) present in acidic beverages allows them to become available and promote the replacement of dental enamel minerals (*e.g*., calcium), inducing the degradation of the dental structure [7, 8, 26]. In this context, high levels of calcium and phosphate in beverages can reduce the release of calcium ions from the enamel surface [27, 28]. However, bottled mineral waters generally do not have considerable amounts of these substances [26], so this was not evaluated in this study.

As the level of enamel erosion caused by acidic foods and beverages is associated with factors such as titratable acidity, exposure time, temperature, solution concentration, and pH, some studies have included these variables in their designs [28 - 31] However, in shorter challenges, such as the one of the present study (5 min), the erosive capacity is mainly determined by the acidic type and pH, and not by the concentration or amount of titratable acid [32, 33]. Furthermore, the concentration of these ions is the probable cause of mineral dissolution and consequent enamel surface softening, since other chemical and physical factors do not influence this loss when related to acidic waters [34].

The progression of erosive lesions is also related to the failure of the protective properties of saliva performed by proteins and by the buffering system, which neutralises acid attacks resulting from food and other extrinsic means [13, 35, 36] Artificial saliva compared to human saliva has differences in its composition that can cause the appearance of an eroded surface more easily [37]. However, Baumann *et al*. [38] evaluated different formulations of artificial saliva and natural human saliva concluding that the efficacy between them is equivalent, in terms of protective activity, and is closely related to the proportions of components present.

Formulations that do not have mucin as a remineralising agent can favour the onset of enamel lesions, as it is an important component of the salivary pellicle and acts to reduce erosive demineralization [36, 39]. Several studies have indicated the effects of artificial saliva in erosion models [19, 36 - 38, 40]. One of the precursors in the use of artificial saliva demonstrated its effect after the erosive challenge, as well as experiments that did not use mucin in its composition, pointing out that this component did not interfere with enamel mineral loss [41 - 43]. However, further studies assessing artificial salivary compositions are needed to reach the closest to the natural conditions of human saliva.

It is worth emphasising that there are considerable differences between *in vitro* erosive cycle models and natural clinical conditions, which can be pointed out as a limitation of the present study. Thus, the pH cycling model cannot completely and accurately simulate the conditions in which the pH fluctuates in the oral cavity, as the levels reached depend on factors inherent to the individuals, such as eating habits, oral hygiene, use of fluoride, and the composition and quality of saliva and biofilm [44].

Although the consumption of acidic beverages has been determined by the literature as a significant agent in the onset of erosive tooth wear [30, 31], few studies have assessed the implication of consuming mineral waters with acidic pH. Despite the statistical difference found in the present study for the microhardness and surface roughness analyses, the values observed in the baseline and after exposure samples lead us to assume that the consumption of mineral waters with low pH can be more harmful when the teeth are already compromised. In this way, an eroded enamel, when frequently exposed to mineral waters with acidic pH, can have its erosion process intensified [2, 7, 45].

Furthermore, despite the important role of saliva in protecting the progression of dental erosion, studies suggest a more efficient protective effect of the salivary pellicle in healthy patients when compared to those with dental erosion [46 - 48]. Thus, the loss of mineral components promoted by the use of water with acidic pH, observed in this study, may provide possible damage to this population in question.

As in Brazil, the usual presence of mineral waters with low pH available on the market may also be common in other countries and regions. Therefore, it is worth considering the importance of conducting further studies to assess enamel mineral loss and the increase in the progression of erosive processes resulting from the use of that product, since mineral water is a universal drink with no consumption restrictions [17, 45, 48].

CONCLUSION

According to the results obtained in the present study, it can be concluded that the pH below the critical value of bottled water marketed in Brazil can cause a reduction in surface hardness and roughness in the bovine dental enamel.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study was approved by the Ethics Research Committee on Experimental Animals of the Federal University of Pará, State of Pará, Brazil (Protocol No. 4027190520).

HUMAN AND ANIMAL RIGHTS

No human were used that are the basis of this study. all the animal procedures were followed in accordance with the standards established by The US National Research Council's “Guide for the Care and Use of Laboratory Animals”.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author, [J.L.N.A.], on reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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