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Original Article

Evaluation of the Erosive Potential of Different Amazonian Fruits on Bovine Enamel

Danielle Tupinambá Emmi[1], Carmiane Pereira Cabral[2], Max Pinto da Costa da Rocha[1]

[1]Professor, School of Dentistry, Federal University of Pará, Belém, PA, Brazil.
[2]DDS, Federal University of Pará, Belém, PA, Brazil.

Author to whom correspondence should be addressed: Danielle Tupinambá Emmi. Av. Augusto Correa, 01, Cidade Universitária José da Silveira Netto, Bairro Guamá, Belém, PA, Brazil. 66075-110. E-mail: dtemmi@yahoo.com.br.

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Abstract

Objective: To investigate the influence of the erosive potential of Amazonian fruits on the bovine enamel microhardness. **Material and Methods:** A total of 30 healthy bovine incisors were divided into three groups, according to the Amazonian fruit juice used in the erosive challenge: Group A - taperebá (*Spondias mombin*), Group B - cupuaçu (*Theobioma gandiflorum*) Group C - graviola (*Annona muricata*), with n=10. The planning of specimens polishing was performed with decreasing grain sandpaper. Specimens were submitted to microhardness initial reading carried out in microhardness meter using Knoop indenter, using load of 50g for 15 sec. Three indentations were performed on reference surfaces with a distance of at least 100 μm from each other. Specimens were stored in distilled water up to the erosive challenge, which consisted of three steps: (1) 5-minute immersion in 10 ml of acid juice; (2) washing with distilled water and mild drying with tissue paper; (3) 60-minute immersion in 10 ml of artificial saliva. Subsequently, samples were stored in distilled water for reading of the final microhardness, after erosive challenge. Data were analyzed by ANOVA with $\alpha=0.05$.. **Results:** All groups showed a statistically significant reduction in Knoop microhardness (KHN) after erosive challenge ($p<0.0001$). Group B showed the lowest average KHN (113.6) after erosive challenge, though not statistically different from Groups A and C ($p=0.1592$). **Conclusion:** Juices of fruits evaluated significantly changed the dental enamel hardness, and cupuaçu juice (*Theobioma gandiflorum*) caused the greatest surface hardness loss.

Keywords: Tooth Erosion; Fruit and Vegetable Juices; Hardness.

Introduction

In recent decades, there has been a decline in dental caries prevalence, leading to a longer life of tooth elements. This is due to the increase in the national average life expectancy, better oral hygiene habits, prevention methods and evolution of dentistry. On the other hand, increased incidence of non-carious lesions such as dental erosion has been observed, which are caused mainly by changes in lifestyle and eating habits, with high consumption of industrialized and acidic foods and beverages [1,2].

Dental erosion is the term used to characterize chronic, localized and painless loss of hard tooth tissues caused by chemical processes by acid media and chelating substances, without the involvement of bacteria [3]. It is a multifactorial disease and highly influenced by personal habits and lifestyles [4].

Acids that cause dental erosion can be intrinsic or extrinsic. Extrinsic acids include those from diet (acidic foods and drinks), drugs and environment (exposure to pool water with chlorine and industrial acids). Intrinsic factors include endogenous acids that come into contact with the tooth surface due to gastroesophageal reflux disease, anorexia nervosa, bulimia and / or diseases that cause decreased salivary flow [5].

Clinically, erosive lesion shows loss of normal anatomical contours of teeth and enamel shine, which becomes smooth, wide, shallow and without sharp angles, showing erosion of lingual and palatine faces in anterior teeth and planning of pits and fissure in posterior teeth. These characteristics can generate dentinal sensitivity and in more severe cases, pulp exposure and loss of tooth vitality, leading to functional and aesthetic problems [4].

Several studies have shown the relationship between ingestion of acidic foods and development of dental erosion, stressing that drinks with pH below critical values (5.5) have great erosive potential [2, 6-8]. Thus, any drink or food with low pH, including soft drinks, fruit juices, sports drinks, salad dressings and flavored mineral water, can cause dental erosion [9]. The erosion potential severity is related to pH, titratable acidity, type of acid, adherence to dental surface, chelating properties and calcium, phosphate and fluoride concentration, which are related to acidic drinks, as well as the quality and quantity of saliva and its acid buffering capacity [4,10].

Chemically, in dental erosion, hydrogen ions derived from weak and strong acids that come into contact with the teeth bind to carbonate and phosphate ions of hydroxyapatite crystals, removing them. This fact causes an increase in the enamel porosity and diffusion of acids in the dental substrate, starting a demineralization process [11]. Saliva plays an important role in protecting teeth from acid erosion because it has ability to dilute and eliminate potentially demineralizing agents and ability to neutralize extrinsic or intrinsic acids. Furthermore, saliva has calcium ions, phosphate and fluoride, which act in remineralization and salivary glycoproteins, which form film on teeth, protecting their surface against dental demineralization [12].

Brazil produces a large variety of fruits consumed and appreciated worldwide. Many of these fruits belong to Amazonian biodiversity and have high levels of acids [13]. However, the hot and humid Amazon climate favors the high consumption of drinks and regional fruit juices, both natural and industrialized, which appears as a refreshing and tasty option. Thus, more detailed studies on the problems that the high consumption of juices in this region can cause should be conducted, which would also help dentists to warn and prevent the population for future problems.

Thus, the aim of the research was to investigate the erosive potential of Amazonian fruits on the bovine enamel microhardness and establish a ranking of the erosive potential of the most used Amazonian fruits: taperebá (*Spondias mombin*), cupuaçu (*Theobroma grandiflorum*) and graviola (*Annona muricata*).

Material and Methods

This study was conducted after approval by the Ethics Committee for Research with Experimentation Animals of the Federal University of Pará, under protocol No. 15/2012.

For the development of this *in vitro* study, 60 bovine incisors, free of cracks, chips, pigmentations and other imperfections were used.

Disinfection was carried out with 0.1% thymol solution for one week [14]. After this period, teeth were cleaned with Gracey 3-4 and 7-8 curettes (Trinity, Trinity Ind. e Com., São Paulo, SP, Brazil) for removal of adhering residual tissues and then submitted to prophylaxis with Robinson straight brushes (Microdont, Microdont Ind. e Com., São Paulo, SP, Brazil) at low speed (Dabi Atlante, Ribeirão Preto, SP, Brazil), with pumice, being then washed with air / water jet [14]. Once clean, teeth were kept in a glass with distilled water at 4°C [15].

The coronal portion of tooth roots was separated at the amelocemental junction with the aid of a metallographic cutter (Isomet Low Speed Saw, Buhler, Illinois, USA). After cutting, the buccal surface of each tooth was worn in polisher (Aropol-E, Arotec, Cotia, SP, Brazil), at speed of 150 revolutions per minute, with sandpaper of decreasing grain size (#120, 400, 600, 800, and 1200) up to the enamel surface planning [14]. After surface wear, a marking corresponding to the delimitation of specimens cut with dimensions of 4x4x2 mm was performed with 0.5 mm graphite and with the aid of a digital caliper (Digimess, São Paulo, Brazil) [16]. Then, teeth were cut with double-sided diamond disc (#7020, KG Sorensen, Cotia, SP, Brazil) mounted on spindle and straight piece (Dabi Atlante, Ribeirão Preto, SP, Brazil) for section of enamel specimens with marked dimensions.

The enamel specimens obtained were embedded in acrylic resin (Varidur, Buheler, Illinois, USA) with the aid of PVC matrixes, so that the enamel surface is exposed to the external environment to be submitted to acidic challenges. The resin blocks obtained were then planned using Al_2O_3 abrasive discs (#500, 1200, 2400 and 4000 - Buehler, Illinois, USA) under refrigeration, followed by polishing with felt disc and polishing paste (Diamond Gloss 2, KG Sorensen, Cotia, SP, Brazil) to allow accurate reading of the enamel surface microhardness. Then, samples were rinsed and stored in distilled water to make the initial microhardness reading before the erosive challenge [16].

Microhardness tests were performed on Microdurometer (FM-700, Future Tech Corp., Tokyo, Japan), using a Knoop-type pyramid indenter with static load of 50 g applied for 15 seconds [14, 17]. Three indentations were carried out on reference surfaces at a distance of at least 100 μm from each other [18]. The average of surface indentations was calculated.

To select the specimens, samples were submitted to initial surface microhardness, selecting specimens with initial average microhardness between 253.7 KHN and 385.9 KHN, according to the enamel hardness standards [19] and hardness similarity between human and bovine enamel [20]. Blocks with limit value 20% above or below the average of all blocks were excluded to reduce standard deviation and variability of hardness among specimens. After selection, samples were randomly divided into 3 groups according to Table 1.

Table 1. Description of study groups.

Group	Juice	Samples
A	Taperebá (*Spondias mombin)*	10
B	Cupuaçu (*Theobioma gandiflorum)*	10
C	Graviola (*Annona muricata)*	10

To obtain the fruit juice for the erosive challenge, 25ml of industrialized pulp and 25 mL of natural mineral water were used. For this, specimens were submitted to alternating cycles of erosion-remineralization. A complete cycle comprised the following steps: (1) 5 minute immersion in 10ml of acidic juice (2) washing with distilled water and drying with tissue paper (3) 60 minutes immersion in 10 mL of remineralising solution (artificial saliva - Saliform, pH 7.0 - Fórmula & Ação, São Paulo, SP, Brazil). This cycle was repeated 5 times per day for 5 days, totaling 2 hours and 5 minutes of immersion in demineralizing solution. At night, between cycles, specimens remained stored in artificial saliva. All solutions were used at room temperature [16].

The pH was measured using a calibrated pH electrode (Quimis, Diadema, SP, Brazil), being performed in triplicate and with juices at room temperature. Distilled water was used as control. The initial pH value of juice was measured using 50 ml of each solution [16].

Table 2. Origin and pH of juices used in the study.

Fruit	Scientific Name	Manufacturer / Location	Initial pH
Taperebá	*Spondias mombin*	CAMTA (Companhia Agrícola de Tome-Açú)	2.75
Cupuaçu	*Theobioma gandiflorum*	CAMTA (Companhia Agrícola de Tome-Açú)	3.75
Graviola	*Annona muricata*	CAMTA (Companhia Agrícola de Tome-Açú)	4.02

After the acid challenge, specimens were submitted to a new microhardness reading using Knoop indenter in order to obtain parameters on enamel mineral loss. Thus, for the analysis of mineral loss due to erosive challenge, the arithmetic average of 3 indentations made on the surface of specimens was obtained, and after, the mean and standard deviation of the Knoop surface microhardness in each group before and after erosive challenge. The BioEstat software 5.0 for statistical analysis was used. Data normality was verified using the Shapiro-Wilk test. For analysis of differences before and after erosive challenge, the Student t-test was used and for analysis between groups, analysis of variance was used. The significance level adopted was 5%.

Results

According to Table 3, all groups showed statistically significant reduction in Knoop microhardness (KHN) after erosive challenge ($p < 0.0001$). It appears that Group B - Cupuaçu (*Theobioma gandiflorum*) was the one with the lowest average KHN (113.6) after erosive challenge, though not statistically different from Groups A and C ($p = 0.1592$).

Table 3. Mean and standard deviation of Knoop microhardness (KHN) before and after erosive challenge in the different study groups.

Groups	Pre-erosive challenge KHN	Post-erosive challenge KHN	p-value
GA - Taperebá (*Spondias mombin)*	319.8 ± 29.4 $^{(a)}$	130.9 ± 24.5 $^{(b)}$	p< 0.0001
GB - Cupuaçu (*Theobioma gandiflorum)*	320.4 ± 25.0 $^{(a)}$	113.6 ± 19.3 $^{(b)}$	p<0.0001
GC - Graviola (*Annona muricata)*	303.9 ± 38.6 $^{(a)}$	122.0 ± 13.1 $^{(b)}$	p<0.0001

* Different letters indicate statistical difference ($\alpha = 0.05$)

To obtain the surface hardness loss, the following formula was used [18]:

$$\%SHL = \frac{\text{initial microhardness} - \text{final microhardness} \times 100}{\text{initial microhardness}}$$

According to Figure 1, Group B showed the highest average surface hardness loss (0.64), followed by Group C (0.59) and Group A (0.58); however, there was no statistical difference between groups ($p = 0.1926$).

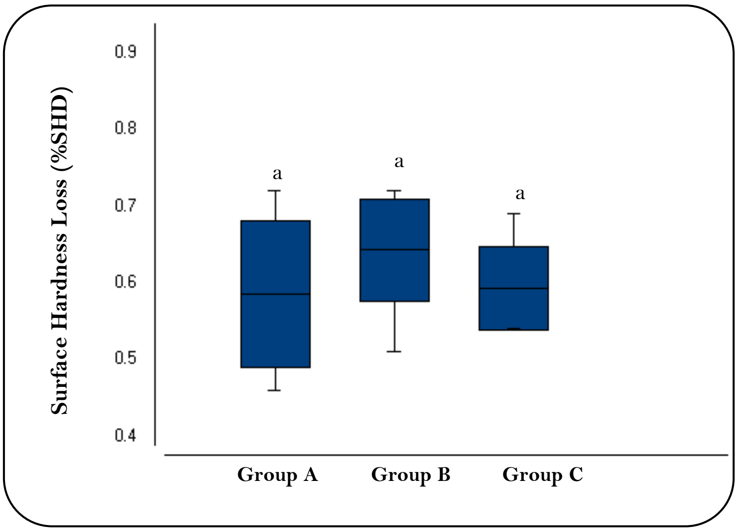

Figure 1. Mean and standard deviation of surface hardness loss (SHL) between study groups. (*Equal letters indicate statistical similarity).

Discussion

The present study examined the influence of erosive challenge using fruit juices from the Amazon region on the enamel hardness, thus confirmed the ability of these juices to cause structural changes in the dental enamel. This may be attributed to the low pH values of juices analyzed (Table 2), below critical pH (5.5) for enamel dissolution, because any solution with pH values below this limit will cause loss of tooth structure, especially if the contact with the acid is long-lasting and frequent [2,21].

The pH of beverages is an important factor in determining their erosive potential, being the parameter that best characterizes the erosive capacity of acidic drinks when individually evaluated [22]. However, the intensity of mineral loss caused by acidic beverages also depends on the interaction of physicochemical aspects related to beverages such as acid concentration and type; sugar content; calcium and fluoride content; temperature; and biological and behavioral factors such as time the beverage remains in the oral cavity and intake frequency [2,23]. In this study, the pH of juices was measured at room temperature and was the factor considered to evaluate the erosive capacity of tropical juices. In general, acidic beverages at room temperature have higher erosive potential than those consumed cold [24]. Study conducted with isotonic drinks at room temperature showed that the products analyzed showed low pH, demonstrating erosive potential to dental tissues if consumed improperly and with high frequency, and to reduce this risk, these drinks should be consumed cold [21].

In this experiment, the results show that taperebá juice (*Spondias mombin*), despite having more acid pH, was not the juice that caused the greatest surface hardness loss, probably due to other related physicochemical and sensory characteristics such as the low viscosity. Similar results were found when evaluating the pH of different beverages, including fruit juices, sports drinks, alcoholic and soft drinks, showing that although cola-based beverages present the lowest pH value, they did not cause the greatest enamel loss, suggesting that factors other than pH and titratable acidity must be analyzed to determine the erosive potential of beverages [25].

The pH of industrialized juices of different flavors found in the market of Campina Grande, Paraiba, Brazil, ranged from 3.53 to 3.93, highlighting the capacity of these juices to solubilize apatite of permanent enamel [6]. In another study, eighteen tea brands marketed in the city of Porto Alegre, Rio Grande do Sul, Brazil showed low pH values, suggesting erosive potential for ready-to-drink teas [26]. These results corroborate the findings of this study, which found that tropical juices with low pH are potentially erosive. These results are pioneer for these juices, since no studies relate to this issue have been previously found in literature.

The structural and hardness similarity of bovine and human enamel [20], and the diversity of studies with both substrates regarding the erosive action of beverages regularly consumed by the population allows comparing the findings of the present study with several others found in literature with both human and bovine enamel. Several studies have shown the reduction in the enamel hardness after erosive challenge in these substrates and with different beverages [6, 10, 14, 17, 27, 28].

The average surface hardness loss (SHL) caused by Amazonian juices evaluated in this study showed no statistical difference between them (Figure 1). However, Group B - cupuaçu juice (*Theobioma gandiflorum*) was the group that presented the greatest surface hardness loss (0.64) (Figure 1) and the lowest average KHN (113.6) after erosive challenge (Table 3), which can be explained by the high viscosity of cupuaçu juice, remaining longer in contact with the surface of specimens, thereby providing continuous dissolution of hydroxyapatite crystals. In a study with bovine enamel submitted to the action of honey-based herbal syrup, it was observed that the product presented erosive potential, possibly due to its high viscosity, high acidity and low pH [29], findings that corroborate the results of this study. Different adherence patterns of acidic beverages to tooth structure may increase the risks of development of erosive lesions, since the greater the ability of a liquid to adhere to the tooth surface, the stronger its erosive effect and its cariogenic potential [30]. This is due to the greater difficulty of saliva in removing the film formed by the beverage on the tooth structure than the drink to remove the acquired film [23].

The quantity and quality of saliva are essential for the prevention of dental erosion. [4] Thus, searching for results closer to what occurs in the oral cavity results; this experiment used *in vitro* methodology, artificial saliva, to simulate the protective and remineralizing action provided by saliva against the potential erosion caused by Amazonian juices. However, when acids are present in the oral cavity, salivary flow volume increases, as well as its buffering capacity, and within a few minutes, the acid is neutralized and removed from the mouth, causing the pH to return to normal values, stimulating remineralization [12]. In the *in vitro* condition, the increase in salivary flow volume is a limitation that could favor the erosive wear due to further delay in pH neutralization and remineralizing action.

The daily consumption of acidic beverages such as soft drinks, sports drinks, artificial powder and natural and processed juices have become increasingly frequent. In addition, concern for a healthier diet promotes increased consumption of fruits and juices. However, consumption of some juices, both natural and industrial, has shown to be associated with high risk of dental erosion because these beverages present extremely acidic pH [8,14]. Accordingly, the modification of acidic beverages by incorporating calcium ions, phosphate and / or fluoride would minimize the structural loss of the tooth by erosion [23]. In the case of Amazonian fruit juices widely consumed, especially in a region where the hot and humid climate favors the frequent consumption of these drinks, it is important to know the physicochemical characteristics of these juices to determine possible erosion damage that the use of these drinks can cause the tooth enamel, suggesting safer protocols and standards for the consumption of these beverages.

Conclusion

The three fruit juices assessed in this study significantly reduced the dental enamel microhardness, and cupuaçu juice (*Theobroma grandiflorum*) showed the highest final hardness loss, followed by graviola (*Annona muricata*) and taperebá (*Spondias mombin*).

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