Original Article

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Dentistry Section

Ex vivo Evaluation of the Erosive Effect of Acid Tea Widely Consumed in Brazil

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ABSTRACT

Introduction: Dental erosion is defined as the pathological, irreversible, chronic, and localised loss of dental enamel by prolonged acid action and often without bacterial involvement.

Aim: To evaluate ex vivo, the erosive potential of widely consumed teas in Brazil by measuring the pH, titratable acidity of beverages and the roughness and surface microhardness of dental enamel before and after erosive challenges.

Materials and Methods: Thirty human maxillary third molars, extracted for clinical reasons and obtained from Human Teeth Bank of the Dental School from 8th May 2017 to 18th May 2017 (Federal University of Juiz de Fora), were used to perform erosive cycles, which were exposed to *Morinda citrifolia*, *Uncaria tomentosa*, *Caesalpinia ferrea*, *Schizolobium amazonicum*, *Schinus aroeira* teas and 1% citric acid. The samples were immersed in the solutions for two minute, five times a day, at intervals of two hours for four days. Between the erosive cycles, the specimens remained in artificial saliva. Statistical analyses were performed using the IBM SPSS statistics version 22.0 software. The accepted significance was set has p-value <0.05.

Results: The mean pH of the five samples ranged from 3.56 to 5.56 and all the herbal teas tested showed significant buffering capacity. The results suggested that all acid teas in the study have an erosive potential. The microhardness ranged from 34.12 to 46.98, and no significant difference was observed between groups in relation to the control group (1% citric acid). The roughness results for *M. citrifolia* and *U. tomentosa* teas were similar to those of the control group.

Conclusion: The analysis of the data allows for the conclusion that all the teas present with acid pH and presence of ions capable of interacting with the dental surface and causing loss of dental tissue, reducing the microhardness of the enamel. Thus, there is a need to be careful in consuming such beverages.

Keywords: Acidity, Dental enamel, Tooth erosion, Tooth remineralisation

INTRODUCTION

Dental erosion is defined as the pathologic, irreversible, chronic, and localised loss of dental hard tissue (enamel and dentine), resulting primarily from non-bacterial chemical attack, by prolonged and frequent acid action (extrinsic and intrinsic) [1]. The prevalence of tooth erosion is high and continuously growing within populations [2]. Important parameters for erosive potential are pH value and Titratable Acidity (TA) [3]. The pH value refers to the equilibrium measure of the hydrogen ion concentration, however, it does not indicate the overall acidic content of the drink or food. Titratable acidity, in turn, gives a measure of all free hydrogen ions available to cause dental erosion [4].

In addition to the chemical aspects of erosion, to evaluate the loss of tissue of enamel surface, physical characteristics were analysed due to exposure to acid drinks with low pH values, by measurement of roughness parameters by contact stylus surface profilometry [5], as well the surface microhardness, which are directly related to alterations in the mineral content of dental hard tissues [6].

In the previous decade, the eating behaviour of the Brazilian population have undergone significant changes, particularly, in concerns with replacement of aliments that are homemade and natural instead for industrialised food [7]. Tea and its constituents are one of the significant components used in the domain of diet-based therapies to maintain the health and reduce the risk of various malignancies, being consumed worldwide, besides water [8]. Many people hold the belief that tea is a healthier option, so, consume it as a replacement for other industrialised beverages that are considered harmful. Following this change in the eating behaviour, an increase in prevalence of tooth erosion cases among children and teenagers has been observed [7].

Considering the above problems and the scarcity of literature on the erosive effect of teas consumed by the Brazilian population. The objective of this study was to evaluate ex vivo the erosive potential of five different widely consumed teas in Brazil.

MATERIALS AND METHODS

It was an experimental study conducted at the Nucleus of Identification and Analytical Quantification (NIQUA) of the Pharmacy School (Federal University of Juiz de Fora), with a duration of 10 days (from 8th May 2017 to 18th May 2017).

This study was developed in accordance with the Ethics Committee of the Federal University of Juiz de Fora (nº 1.345.640). Thirty intact human third molars extracted for clinical reasons and obtained from Human Teeth Bank of the Dental School (Federal University of Juiz de Fora) were selected for this study. The sample size was based on the methodology established by Lussi A et al., and Sener Y et al., [3,9]. Teeth were disinfected at room temperature with sodium hypochlorite for 24 hours [9].

In sequence, all teeth were submitted to horizontal sections at the cemento enamel junction, separating coronary and root pieces, with a metallographic cutter (Isomet® 1000 Precision Saw, Buehler, United States) and a diamond disc (15 LC, Diamond Wafering Blade, Buehler, USA). Then, dental coronal sections were made, at the middle third, so that only one dental block for each tooth was obtained and the cuts were made in the flat area under cooling with distilled water to avoid cracks in the enamel, obtaining enamel blocks with dimensions of 4 mm×4 mm×2 mm

[10,11]. The fragments were embedded in acrylic resin block with the buccal surface projected to the external environment, ground flat and polished [10,11]. The buccal surfaces were abraded using a belt sander (Politriz PL02, Teclago, Brazil) with grit sandpaper 600, 1000, 2000 and 4000, under finger pressure. Felt discs and diamond pastes (Diamond AC I and II) were used to promote the final polishing [10]. At the end, the enamel blocks were washed with distilled water (Direct-Q®, Millipore, France) in an ultrasonic cleaning bath (USC 1400, Unique, Brazil) for five minutes and stored in plastic containers with absorbent paper, moistened with distilled water and stored in a refrigerator at 4°C.

The experimental material (stem bark) was herbal tea bags of *Caesalpinia ferrea* (Martius), *Schinus aroeira* (Vell), *Uncaria tomentosa* (Willd) DC., *Morinda citrifolia* L., and *Schizolobium amazonicum* F Smith available in the retail trade (Moinho Central Indústria Comércio, Belém, Pará, Brazil). Teas were prepared with water, according to the manufacturer's instructions, kept at room temperature and used on the same day. Samples of ready to drink tea were collected directly from the product package immediately after its opening.

pH Measurements

The measurements of the beverages pH values were made at 25°C using an electronic pH meter (B474, Micronal, São Paulo, Brazil) connected to a glass electrode. Prior to the measurement, the equipment was calibrated using standard solutions with known pH 7.0 and 4.0 (Indústria Comércio eletro-eletrônica Gehaka Ltda, São Paulo, Brazil). Afterwards, three readings of the freshly prepared drinks were obtained in order to give a mean pH measurement for each sample.

Titratable Acidity Assessment

To determine titratable acidity (buffering capacity), 50 mL of each beverage was combined with 0.1 mL increments of 0.1 mol/L sodium hydroxide (NaOH). The amount of NaOH required to reach pH level of 7.0 was recorded. The titrations for each beverage were also repeated two times to obtain a mean value.

Erosion Remineralisation Cycling Model

The study was conducted in an erosion remineralisation cycling model, in order to simulate the conditions in the human oral cavity. The specimens were immersed in artificial saliva {(0.12% KCl, 0.089% NaCl, 0.005% $MgCl_2$, 0.146% $Ca_3(C_6H_5O_7)_2$, preservatives (0.002% nipagin and 0.013% nipasol), 1.0% Carboxymethyl Cellulose (CMC), 3.0% sorbitol, distilled water (quantity sufficient for 100 mL)}, according Silva AF et al., at a volume of 2.5 mL/mm^2 of the enamel surface and were maintained under soft agitation for 30 minutes before the first erosive challenge and then washed in running deionised water [12].

The enamel blocks (n=5 specimens) were then randomly assigned into six groups and immersed in the respective treatment groups (teas): A) *M. citrifolia*; B) *U. tomentosa*; C) *C. ferrea*; D) *S. amazonicum*; E) *S. aroeira*; F) positive control (1% citric acid) and remained still, without stirring for two minutes at room temperature, five times a day with intervals of two hours between the five daily erosive challenges, lasted for five days. During the interval between erosive challenges, the samples remained in artificial saliva and then were washed with deionised water and lightly dried with absorbent paper. The assay was performed with five specimens and in triplicate.

Surface Hardness and Roughness of Samples

Before the beginning of the erosion cycle and the sequence cycles, samples were analysed for roughness with a portable surface roughness tester (SURFTEST SJ-301, Mitutoyo, Japan). Each group was subjected to surface microhardness measurement to obtain a baseline value. The hardness value (0.05 kg/mm^2) of each specimen was determined using a microhardness test (HMV-2, Shimadzu, Kyoto, Japan). The average of these values (n = 15) was used to calculate the percentage of surface hardness loss, using the following formula:

$$\%PDS = \frac{\text{Initial microhardness} - \text{Final microhardness}}{\text{Initial microhardness}} \times 100$$

In relation to the roughness of the surface of the samples, the difference between the final roughness and the initial roughness was analysed. Both assays were performed with five specimens and in triplicate.

STATISTICAL ANALYSIS

Statistical analyses were performed using the IBM SPSS statistics 22.0 software. The results were expressed as mean±standard error of the mean and were analysed using analysis of variance (ANOVA) followed by Tukey's post-hoc test. The accepted significance was set at p-value <0.05.

RESULTS

The 1% citric acid had a pH of 2.14±0.01 and the pH of the teas ranged from 3.56 to 5.56 [Table/Fig-1]. Although, the teas were not statistically similar to the positive control group (1% citric acid), they showed values close to the critical pH for the demineralisation of dental enamel.

Group	Mean±SE
1% Citric acid	2.14±0.02*
M. citrifolia	3.56±0.02*
U. tomentosa	5.56±0.18*
C. ferrea	4.83±0.26*
S. amazonicum	5.08±0.04*
S. aroeira	5.02±0.10*

[Table/Fig-1]: Average pH values of tested substances. Data are presented as mean±SE of 16 samples/group.
*p<0.05 vs. the control group (one-way ANOVA followed by Tukey's post-hoc test)

In reference of titratable acidity, the amount of NaOH needed to reach neutrality (pH 7.0) decreased with the following order: *M. citrifolia*, *S. aroeira*, *C. ferrea*, *S. amazonicum*, and *U. tomentosa* [Table/Fig-2].

Group	Mean±SE
1% Citric acid	7.77±0.03*
M. citrifolia	3.08±0.13*
U. tomentosa	0.11±0.01*
C. ferrea	0.28±0.05*
S. amazonicum	0.16±0.02*
S. aroeira	0.33±0.03*

[Table/Fig-2]: Mean titratable acidity values of tested substances. Data are presented as mean±SE of 16 samples/group.
p-value <0.05 vs. the control group (one-way ANOVA followed by Tukey's post-hoc test)

All of the tested teas promoted significant loss of superficial enamel hardness (34.12 to 46.98) and there was no statistical significant difference between the results and the positive control group (1% citric acid) [Table/Fig-3]. In relation to the surface roughness of the samples, there was a statistically significant difference between 1% citric acid and *S. amazonicum*, *S. aroeira*, and *C. ferrea* (p-value <0.01) [Table/Fig-4].

Group	Mean±SE
1% Citric acid	45.09±5.24
M. citrifolia	46.98±2.12
U. tomentosa	44.28±3.26
C. ferrea	37.03±3.68
S. amazonicum	34.55±3.62
S. aroeira	34.12±3.18

[Table/Fig-3]: Difference between superficial microhardness of the samples before and after acid challenge. Data are presented as mean±SE of 15 samples/group.
*p-value <0.05 vs. the control group (one-way ANOVA followed by Tukey's post-hoc test)

Group	Mean±SE
1% citric acid	0.41±0.05
M. citrifolia	0.29±0.02
U. tomentosa	0.33±0.05
C. ferrea	0.09±0.31*
S. amazonicum	0.16±0.02*
S. aroeira	0.15±0.03*

[Table/Fig-4]: Difference between superficial roughness of the samples before and after acid challenge. Data are presented as mean±SEM of 15 samples/group.
*p-value <0.05 vs. the control group (one-way ANOVA followed by Tukey's post-hoc test)

DISCUSSION

According to Jaâfoura S et al., there are few studies that explore the relationship between tea consumption and dental erosion [13]. Consumption of teas and damage to teeth are often associated with discolouration or with the remineralisation process, but little is known about their involvement in the pathological process of dental erosion [7,13].

Several characteristics and properties of saliva play an important role in dental erosion. Erosion makes this modulation through its buffering, dilution of acids, demineralisation and training acquired film [14]. Artificial saliva was used in this study with the intention to simulate the oral environment, offsetting the limitations of ex vivo study. After soaking in teas, there was no evidence of the remineralisation of tooth enamel, which highlights the importance of knowledge of the erosive potential of consumed teas.

The enamel begins to demineralise naturally below pH 5.5, so it is necessary to avoid reaching that lower pH or, alternatively, to make hydroxyappatite more resistant to acid dissolution [13,15,16]. Once erosion has begun, there is no method to enable the regeneration of tooth structure; among other reasons, this is because the ameloblasts secreting tooth enamel disappear before teeth sprout [15]. All evaluated teas presented a pH≤5.5, which is considered critical for tooth enamel demineralisation and demonstrates erosive potential of the teas.

If the softened layer of enamel is not rehardened and the acid impact continues, progressive softening and dissolution of the consecutive layers of enamel lead to permanent loss of volume, with a softened layer on top of the remaining tissue [17]. This softened layer on the enamel surface is highly susceptible to wear from, for example, an abrasive toothbrush insult [18]. In severe long-term erosive wear, when there is frequent substantial erosive attack on the enamel surface, the volume of hard tissue permanently lost is clearly visible clinically as a defect in the tooth structure [19].

Assessing the erosive potential of beverages, Lunkes LB and Hashizume LN elucidated the variables that may be considered influential in this process, such as calcium, fluoride, and phosphorus concentrations and the type and concentration of acids present; however, the main factor in the erosion analysis is pH [7]. Measurement of the TA has also been used to evaluate the erosive potential of beverages. This method considers the type and concentration of the acid present. According to these authors, the majority of erosive beverages have weak acids and the concentration of these acids determines not only the pH value but also the buffering properties. Their analyses used corroborate with the present study, which also opted to evaluate pH and TA, but the concentrations of these ions were not measured.

Soares AK et al., emphasises that the TA of a beverage influences salivary pH more than the pH of the beverage [20]. As a consequence of the high TA, there is an increase in the time for saliva to neutralise the acid, causing differences in the erosive potential of a beverage even within the same pH range. The TA reflects the amount of a base that must be added to a beverage to raise the pH to 7.0 (neutral), and represents the erosive potential of the beverage [21]. Thus, a beverage with low TA is readily neutralised by salivary buffers, preventing prolonged dropping of buccal pH and, consequently, causing less mineral loss in dental structure [21].

According to Amoras DR et al., chemical composition of an acidic beverage clearly has an important effect on the hardness properties of enamel [22]. Jameel RA et al., reported that dental erosion induces demineralisation, thus affecting the hardness of dental tissue, which may be related to the degree of remineralisation [16]. This explains the loss of hardness due to erosion of dental enamel in the present study, in which all the analysed teas were statistically similar to 1% citric acid (45.09%, p-value >0.05). According to De Moraes MD et al., they agree that hardness analysis is a sensitive method for detecting changes in mineral density of eroded surfaces/abrasive lesions on a substrate enamel, but not on dentin [23].

According to Pereira CA et al., most beverages available on the market have high acidity and are able to demineralise enamel and dentin [24]. These demineralised dental structures are more susceptible to wear and abrasion by brushing, thereby accelerating the loss of dental surface structure, which can be assessed indirectly through surface roughness. The surface is considered rough if it is characterised by the presence of peaks and valleys of high amplitude and reduced ripple. The surface roughness value considered critical for the retention and adhesion of microorganisms is 0.2 µm. This finding corroborated that of the present study, in which all the teas were statistically similar to citric acid (increase in surface roughness after erosive cycles), except *S. amazonicum*, *S. aroeira*, and *C. ferrea* teas. Further, roughness directly interferes with the aesthetic properties of the tooth and restorative materials, such as gloss and surface smoothness, colour change and staining, dental biofilm accumulation and ultimately secondary caries lesion formation. Therefore, researches that measure the effect of acidic beverages on the structure of dental enamel are of great value. According to Barac R et al., apart from the chemical aspects of erosion, physical characteristics of the enamel surface were evaluated to indicate the loss of tissue due to exposure to soft drinks with low pH values [5]. These characteristics can be evaluated by measurement of roughness parameters along a line by contact stylus surface profilometry.

LIMITATION

The technical difficulty of standardising the sample cuts and the absence of other factors that could influence dental erosion. In spite of this, it is suggested that it provides valuable insight into the erosive effects of tested teas, which are widely consumed in Brazil.

CONCLUSION

The combined analysis of the results allows us to conclude that all evaluated teas presented a pH ≤5.5, considered critical for demineralisation of tooth enamel and the presence of ions capable of interacting with the dental surface and causing loss of dental tissue. The mean roughness values of *M. citrifolia* and *U. tomentosa* teas were similar to those of citric acid. As many of the teas tested were at least as erosive as 1% citric acid the potential for harm is obvious. Thus, there is a need to be careful regarding consuming such beverages.

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REFERENCES

[1] Derceli JD, Faraoni JJ, Pereira-da-Silva MA, Palma-Dibb RG. Analysis of the early stages and evolution of dental enamel erosion. Braz Dent J. 2016;27(3):313-17.
[2] Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monogr Oral Sci. 2006;20:44-65.
[3] Lussi A, Megert B, Shellis RP, Wang X. Analysis of the erosive effect of different dietary substances and medications. Brit J Nutr. 2012;107(2):252-62.
[4] Benjakul P, Chuenarrom C. Association of dental enamel loss with the pH and titratable acidity of beverages. J Dent Sci. 2011;6(3):129-33.
[5] Barac R, Gasic J, Trutic N, Sunaric S, Popovic J, Djekic P, et al., Erosive effect of different soft drinks on enamel surface in vitro: Application of stylus profilometry. Med Princ and Pract. 2015;24(5):451-57.
[6] Torres CP, Chinelatti MA, Gomes-Silva JM, Rizóli FA, Oliveira MA, Palma-Dibb RG, et al., Surface and subsurface erosion of primary enamel by acid beverages over time. Braz Dent J. 2010;21(4):337-45.
[7] Lunkes LB, Hashizume LN. Evaluation of the pH and titratable acidity of teas commercially available in Brazilian market. Rev Gaúcha de Odontol. 2014;62(1):59-64.
[8] Hayat K, Iqbal H, Malik, U, Bilal U, Mushtaq S. Tea and its consumption: benefits and risks. Crit Rev Food Sci Nutr. 2015;55(7):939-54.
[9] Sener Y, Botsali MS, Kucukyilmaz E, Tosun G, Altunsoy M. Influence of soft drinks on dental enamel: an in vitro study. J Pediatr Dent. 2013;1(2):42-45.
[10] Gonçalves GK, Guglielmi CD, Corrêa FN, Raggio DP, Corrêa MS. Erosive potential of different types of grape juices. Braz Oral Res. 2012;26(5):457-63.
[11] Medeiros IC, Brasil VL, Carlo HL, Santos RL, De Lima BA, De Carvalho FG. In vitro effect of calcium nanophosphate and high concentrated fluoride agents on enamel erosion: an AFM study. Int J of Paediatr Dent. 2014;24(3):168-74.
[12] Silva AF, Devito KL, Silva SS, Raposo NR. Effects of xylitol on enamel erosion control. Arquivo Brasileiro de Odontologia. 2010;6(1):03-09.
[13] Jaâfoura S, Khemiss F, Kammoun D, Chebbi R, Baccouche C, Ghoul-Mazgar S. Dental erosion and tea: a systematic review. Int J Sci Res. 2014;3(11):2436-2439.
[14] Karda B, Jindal R, Mahajan S, Sandhu S, Sharma S, Kaur R. To analyse the erosive potential of commercially available drinks on dental enamel and various tooth coloured restorative materials-an in-vitro study. J Clin Diagn Res. 2016;10(5):117-21.
[15] Patiño-Herrera R, González-Alatorre G, Estrada-Baltazar A, Escoto-Chavéz SE, Pérez E. Hydrophobic coatings for prevention of dental enamel erosion. Surf Coat Technol. 2015;275:148-54.
[16] Jameel RA, Khan SS, Abdul RZ, Bakri MM, Siddiqui S. Analysis of dental erosion induced by different beverages and validity of equipment for identifying early dental erosion, in vitro study. J Pak Med Assoc. 2016;66(7):843-48.
[17] Lussi A, Schueter N, Rakhmatullina E, Ganss C. Dental erosion-An overview with emphasis on chemical and histopathological aspects. Caries Res. 2011;45(Suppl 1):02-12.
[18] Lussi L, Carvalho TS. Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci. 2014;25:01-15.
[19] Seong J, Virani A, Parkinson C, Claydon N, Hellin N, Newcombe RG, et al. Clinical enamel surface changes following an intra-oral acidic challenge. J Dent. 2015;43(8):1013-20.
[20] Soares AK, Bonvini B, Farias MM. Evaluation of erosive and cariogenic potential of artificial soft drinks. Rev Odontol Univ Cid São Paulo. 2014;26(3):197-203.
[21] Silva TA, Sampaio CS, Furtado JE, Abílio GM, Xavier AF, Cavalcanti AL. Evaluation of erosive potential of soy-based drinks. Revista Brasileira de Ciências da Saúde. 2010;14(1):109-14.
[22] Amoras DR, Corona SA, Rodrigues AL, Serra MC. Effect of beverages on bovine dental enamel subjected to erosive challenge with hydrochloric acid. Braz Dent J. 2012;23(4):367-72.
[23] De Moraes MD, Carneiro JR, Passos VF, Santiago SL. Effect of green tea as a protective measure against dental erosion in coronary dentine. Braz Oral Res. 2016;30(13):01-06.
[24] Pereira CA, Wanderley RB, Andrade AK, Duarte RM. Diet children's influence in net roughness of cattle teeth enamel. Arq Odontol. 2016;52(3):154-59.

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