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Original Article

Effect of the Mixture of Coffee or Chocolate to Milk in the Progression of Des-Remineralization of Tooth Enamel - An in Vitro Study

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Abstract

Objective: To evaluate if there is any interference when adding coffee or chocolate to cow milk in the enamel des-remineralization process (orange juice/saliva). **Material and Methods:** 48 specimens of human enamel (4x4 mm) were included in acrylic resin, ground flat, polished and randomly divided into the following experimental groups (n = 8): G1-saliva, G2-orange juice, G3 orange juice / milk; G4-Orange Juice / Chocolate, G5- Orange Juice / Coffee + milk and G6- milk. Each group was immersed for 60 seconds on each solution proposed and then immersed for 30 minutes in saliva. This cycle was repeated 4 times. Prior to these cycles, the Knoop microhardness average of each specimen was obtained. After the challenges proposed, the final microhardness average was calculated. The values obtained from the difference between the initial and final microhardness were subjected to ANOVA followed by Tukey test ($p < 0,05$). **Results:** The orange juice had the highest change in microhardness and statistically different from all other groups. The microhardness change was statistically similar in the groups submitted to orange juice followed by immersion in milk, in chocolate and in the mixture milk + coffee. The pure milk and saliva caused no change in surface hardness of enamel. **Conclusion:** Milk or the addition of chocolate and coffee to milk was able to produce a protective effect of the enamel surface against an erosive challenge.

Keywords: Tooth erosion; Dental enamel; Tooth wear.

Introduction

Dental erosion is characterized by the loss of dental hard tissue structure, which is dissolved by the action of nonbacterial acids [1,2]. These acids, in general have an extrinsic origin, when derived from the diet, medications, environmental factors or intrinsic origin, when gastric acid from the stomach reaches the oral cavity in episodes of vomiting, regurgitation and acid reflux [1].

In the initial stage of erosion, there is a softening of the tooth surface, and gradually, the tooth structure shall be dissolved, layer by layer, from the surface, featuring a progressive loss of tissue. [3]. Whereas the lost tissue is not regenerated, the process of structure loss has great clinical significance [4].

The population's life style has been chaging over the years, including dieteray habits. The frequency of consumption of acidic foods and drinks has also changed and at present, a higher consumption of soft drinks and fruit juices by the population has been noted [5,6]. At the same time, it has been observed an increase in the prevalence of dental erosion in all age groups [5].

To prevent progression of the lesion caused by dental erosion, it is important that the diagnosis is concluded early. It is essential to identify the risk factors that lead to the appearance of the erosion process so that preventive measures can be applied [5].

Strategies for treatment and prevention of these lesions consist of changes in the lifestyle and dietary counseling, involving the orientation in relation to decrease in the frequency and consumption of acidic foods and drinks or even the replacement of these foods. A recent clinical study demonstrated [7] that there is an association between dental wear and dietary habits, showing that patients who ingest fewer amounts of low acid foods develop reduced loss of tooth structure.

One attempt to mitigate the effects of an acidic diet on tooth surface is that patients are instructed to use some remineralizing agent immediately after eating acidic food or drink [8]. These agents could be fluoride mouthwash, or ingestion of milk to promote a faster remineralization of softened tooth surface or prevent demineralization. Milk has a protective capacity on the tooth against dental erosion, especially when ingested after an acid (8) challenge. Milk can act as a remineralizing agent, acting as a donor of calcium and phosphate, acting together with saliva to remineralize, but it can also promote a deposit of mineral and organic material on the enamel surface, forming a protective film [9]. This film is related to the adsorption of this casein in the enamel surface [10], thereby reducing the rate of dissolution of hydroxyapatite crystals and their stabilization by inhibiting the release of ions [11].

The literature has demonstrated the protective effect of milk against dental erosion, but there is little acceptance of individuals regarding the consumption of pure milk. It is common the intake of milk mixed with other foods such as chocolate or coffee. It is assumed that the addition of these ingredients could influence this beneficial protective effect of milk against dental erosion, but little is reported on this subject. Therefore, the aim of this study was to evaluate the effects of pure milk and if the mixing with coffee or chocolate interferes with the ability to reduce the demineralization of tooth enamel caused by orange juice.

Material and Methods

Sound human teeth were used, and the experiment was initiated after the Ethics Committee of Faculty of Dentistry, University of Sao Paulo approval (114/2010). The teeth were cleaned with curettes to remove the residual adherent tissues and then subjected to prophylaxis with Robinson brushes, at low speed, with pumice and water and washed with jet air / water. Subsequently, they were examined with a stereomicroscope (SZ-PT/SZ40, Olympus, Tokyo, Japan) with 10X magnification and those endowed with cracks or structural anomalies were discarded. 24 selected teeth were stored in 0.1% thymol solution (disinfectant) until the start of preparation of specimens. Once they were prepared, were stored in deionized water.

For specimens preparation, the teeth were sectioned to separate the crown of the root in an automatic cutter (Labcut 1010, Extec, USA). Then the crowns were sectioned vertically in mesiodistally to obtain two fragments of enamel. The roots were discarded. The enamel fragments obtained were cut with dual face diamond (KG Sorensen, Barueri, SP, Brazil) under refrigeration to obtain blocks of 4x4 mm. These blocks were embedded in acrylic resin (Arazyn ® 1.0, Araçariguama, SP, Brazil), polished on a polishing machine (Buehler Automet 2000 Illinois, USA) with 600, 800, 1200 and 4000 grit abrasive discs, under refrigeration, and then with diamond paste and buff disc resulting in a flat, regular, smooth and polished surface. The initial microhardness of the enamel surface of each specimen was performed with a microhardness tester (Buhler, Illinois, USA) and the Knoop indenter, using the static load of 50 g for 15 seconds. For each specimen three indentations were made and the initial microhardness mean was obtained. The specimens were stored in deionized water humidifier containers under refrigeration. The 48 enamel samples were randomly divided into six groups (n = 8), as shown in Table 1.

Table 1. Division of experimental groups according to the immersion solution.

Groups	Immersion Solution
G1	Orange juice
G2	Artificial saliva
G3	Orange juice + Whole milk
G4	Orange juice + chocolate
G5	Oranje juice + milk with coffee
G6	Whole milk

For the erosive challenge was used: manipulated artificial saliva, industrialized orange juice (Del Vale Mais Laranja Caseira®,)whole milk (Day ®), industrialized chocolate (Milky drink Chocolate Flavor Nescau ®) and powdered coffee (Pilão Traditional Coffee ®).

Artificial saliva was manipulated with the following composition (12): Calcium chloride dihydrate (0.213 g); Dihydrogen phosphate (0.738 g); Potassium chloride (1.114 g); Sodium chloride

(0.381 g); TrisBuffer (12 grams); and deionized water. The pH was adjusted to 7.0 using calcium hydroxide solution.

The orange juice, milk and chocolate milk were used directly from the packages. The coffee was prepared dissolved in drinking water and milk mixed in a 1:1 ratio.

After measuring the pH of the solutions, in triplicate, (Quimis Scientific Instruments LTD, Diadema, SP, Brazil), 80ml of each one was used in individual recipients for the specimens cycling, which were totally submerged in room temperature (24 ± ° C):

- Group 1: orange juice, for 60 seconds;
- Group 2: artificial saliva for 30 minutes – control;
- Group 3: orange juice for 60 seconds, followed by milk for 60 seconds;
- Group 4: Orange juice for 60 seconds, followed by chocolate for 60 seconds;
- Group 5: Orange juice for 60 seconds, followed by milk with coffee for 60 seconds;
- Group 6: milk for 60 seconds.

In the groups in which the exchange of immersion solution was needed, the specimens were rinsed with deionized water for 5 seconds. After their cycling, all specimens were immersed individually in artificial saliva for 30 minutes. This cycling process was repeated four times.

At the end of the cycling, the occurred changes in hardness at the specimens enamel surface were analyzed in a similar way used to obtain the initial microhardness The indentations, both initial and final, were performed near the center of the specimen at regions with uniformity and without risks. The three indentations were made on horizontal line, 100μm distant from each other. The final three indentations were performed at a distance of 100μm below the initial ones.

For statistical analysis, the difference in initial and final means (Δ) of each specimen microhardness was performed. Data were subjected to ANOVA test followed by Tukey test to detect differences between groups. The level of significance was 5% ($p < 0.05$).

Results

The pH of the immersion solutions used in cycling can be seen in Table 2.

Table 2. pH obtained in immersion solutions.

Immersion Solution	pH
Artificial Saliva	7.0
Orange juice	3.7
Milk	6.8
Chocolate	6.9
Coffee with milk	6.7

The orange juice had the lowest pH value while other solutions showed pH between 6.7 and 7.0. Differences in initial – final microhardness means (Δ) and standard deviation for each experimental group are presented in Figure 1.

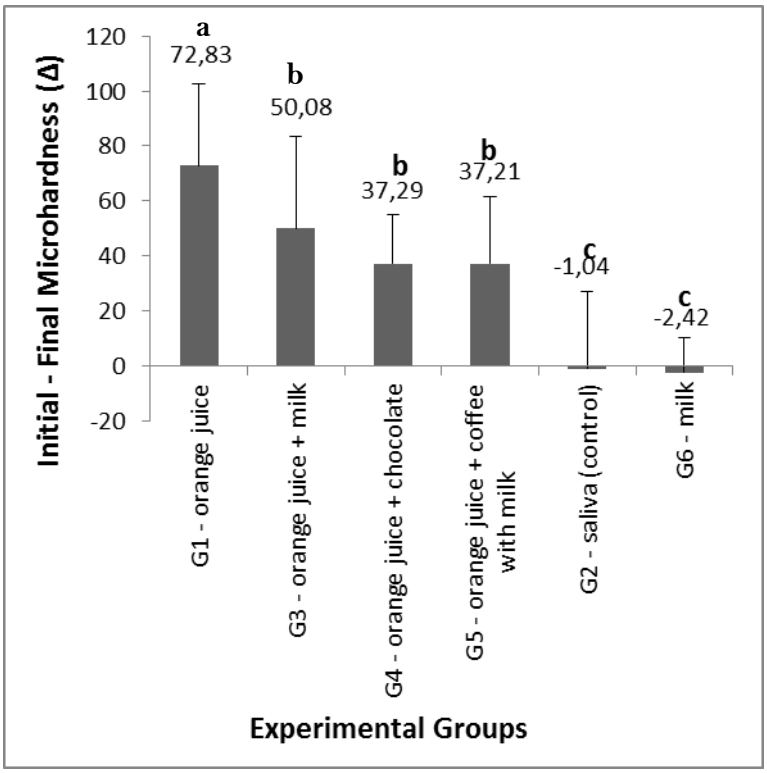

Figure 1. Microhardness mean (Δ) and standard deviation of the experimental groups. Different letters indicate significant differences according to ANOVA and Tukey test (p <0,05).

G1, which was subjected to orange juice cycling, showed the highest value of Δ, resulting in greater mean reduction in microhardness of the enamel surface. This group was found to be statistically different from the other groups.

G3 (juice + milk), G4 (juice + chocolate) and G5 (juice + coffee with milk) groups showed similar microhardness change. There was a minor variation of surface hardness in these 3 groups and it presented statistically significant difference when compared to the orange juice group.

G2 (control-saliva) and G6 groups (milk + saliva) showed negative values of Δ, suggesting an increase in initial surface microhardness. These groups showed a statistically significant difference when compared to the other groups.

Discussion

Tooth enamel is a highly mineralized structure and may be subject to the action of acids, whether intrinsic or extrinsic, in this case it can be mentioned drinks and acidic foods. Processed

beverages such as sodas and fruit juices play an important role in this process, since they are often consumed due to the pleasant taste, convenience and constant advertising [13]. Some of these products may be classified as acidic because they have a low pH and may contain acidulants substances such as citric acid, phosphoric acid, malic acid, sodium citrate and other preservatives and flavorings in addition to the acid found on fruit juice, which is naturally present [14].

Studies report that the progression of dental erosion is directly related to the frequency of intake of drinks with low pH (1.15). In countries with a tropical climate, the frequency of liquid intake is usually higher, and often the water is replaced by some other kind of drink. In the present study, the acid challenge was performed with the industrialized orange juice which, admittedly, is an acid food (pH = 3.7) [16] and has a large consumption by the population. Moreover, it presents significant demineralization capacity and this action alone produces loss of tooth structure [17], which can be proven by the reduced time of cycling performed in this study. There was a clear demineralization of tooth structure despite having been employed short-time exposure to orange juice, even through having been submitted to remineralization intervals in saliva.

Some attempts have been suggested to reduce the erosive potential of certain beverages, for example, modifying the composition by adding calcium, or through the addition of milk or its components as casein, to the acid products consumed frequently [10,18]. Milk, besides calcium, has in its composition phosphate, minerals which are considered inhibitors of demineralization, thereby reducing the erosive potential of such beverages. Thus the consumption of milk immediately after ingestion of a potentially erosive beverage could work as a strategy for limiting the progression of dental erosion [8,9]. In this study it was demonstrated that pure milk and saliva have no demineralizing effect on tooth enamel and this effect was similar for these two solutions, confirming the results found in the literature [18] that milk does not exercise demineralizing enamel effect. However, it must be considered that fermented milks available and widely consumed by children and adolescents, have acidic pH and can contribute to the erosive potential of the diet [19].

It is considered that many individuals consume pure milk, but it is undeniable that a large part of the population consumes the milk mixed with coffee or chocolate. Studies have demonstrated that chocolate powder when mixed with milk or water, was unable to decrease the pH at a level sufficient to cause demineralization and it can be consumed without restriction, when we mention dental erosion for not confering a risk to dental structure [13,21]. On the other hand, the population must be careful because chocolate, which contains a high amount of sucrose in the formulation, can lead to the development of caries as a result of the sucrose metabolize by bacteria. The chemical composition of chocolate was evaluated [21] and demonstrated that the pH of such beverage depends on the degree of alkalization that the cocoa used for this purpose has, which is around 7.1, and the amount and acidity of the milk whey used, which can vary between 6.5 and 6.7, which means a pH nearby neutrality. The chocolate milk is an industry packaged product and because of that could present acidic pH, once acidulants for conservation are incorporated. In this study it was

proven that this composition does not change its ability to protect tooth enamel against acid demineralization.

Coffee has in its composition chlorogenic acid [22], which leads to assume that this drink could result in an acid pH. Also, the effects of some drinks on the surface roughness of the dental enamel, including coffee, was studied [23] and demonstrated that despite containing ascorbic acid in its composition, it was not able to cause significant changes in enamel roughness , suggesting that there is no erosive potential.

Therefore, in groups in which there was a mixture of chocolate and coffee to the milk, a smaller reduction in microhardness (compared to orange juice), with values that did not differ from milk was found, suggesting that the addition of these components to milk does not interfere in its protective capacity and confirming the results found in the literature in relation to chocolate milk [13] and compared to pure coffee [23].

Within the limitations of this in vitro study, the findings have clinical importance. Other methods of erosion control such as application of fluoride varnish and fluoride gel would depend on the cooperation of the patient going the dental office every period of time for the application to be made, implying a cost to this type of treatment. The prevention of erosion lesions can be achieved more easily and can be more widespread by giving instructions and encouraging patients to the milk intake, whether or not with any additive such as chocolate or coffee after the consumption of soft drinks, juices of acidic fruits or just fruits. It is a type of food that is part of the routine of the population and which has been incorporated into the dietary habits and it can help to stabilize the progression of loss of tooth structure. However, more studies proving the efficacy of milk intake should be conducted, with some in situ or in vivo model, so that it is possible to simulate the real oral conditions, such as saliva and brushing.

Conclusion

Within the limitations of this in vitro study, it can be concluded that milk has a protective effect of enamel and that the addition of chocolate or coffee to milk, does not interfere with its protective effect against an erosive challenge.

References

1. Imfeld T. Dental erosion. Definition, classification and links. Eur J Oral Sci 1996; 104:151-5.
2. Bartlett D. Etiology and prevention of acid erosion. Compend Contin Educ Dent 2009; 30:616-20.
3. Lussi A, Megert B, Peter Shellis R, Wang X.Analysis of the erosive effect of different dietary substances and medications. Br J Nutr. 2011; 30:1-11.
4. Ganss C, Young A, Lussi A.Tooth wear and erosion: methodological issues in epidemiological and public health research and the future research agenda.Community Dent Health. 2011; 28(3):191-5.
5. Lussi A, Jaeggi T. Erosion – diagnosis and risk factors. Clin Oral Investig 2008; 12:S5-13.
6. Lussi A. Erosive tooth wear - a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 2006; 20:1-8.
7. Bartlett DW, Fares J, Shirodaria S, Chiu K, Ahmad N, Sherriff M.The association of tooth wear, diet and dietary habits in adults aged 18-30 years old. J Dent 2011; 39(12):811-6.

8. .Amaechi BT, Higham SM. Dental erosion: possible approaches to prevention and control. J Dent 2005; 33:243-52.
9. Gedalia I, Dakuar A, Shapira L, Lewinstein I, Goultschin J, Rahamim E. Enamel softening with Coca-Cola and rehardening with milk or saliva. Am J Dent 1991; 4:120-2.
10. Pearce EI, Bibby BG. Protein adsorption on bovine enamel. Arch Oral Biol 1966; 11(3):329-36.
11. Barbour ME, Shellis RP, Parker DM, Allen GC, Addy M. Inhibition of hydroxyapatite dissolution by whole casein:the effects of pH, protein concentration, calcium, and ionic strength. Eur J Oral Sci 2008; 116(5):473-8.
12. Scaramucci T, Hara AT, Zero DT, Ferreira SS, Aoki IV, Sobral MAP. In vitro evaluation of the erosive potential of Orange juice modified by food additives in enamel and dentine. J Dent 2011; 39:841-8.
13. Ferreira FV, Pozzobon RT. Processed dairy beverages pH evaluation: consequences of temperature variation. J Clin Pediat Dent 2009; 33(4):319-23.
14. Prati C, Montebugnoli L, Suppa P, Valde G, Mongiorgi R. Permeability and Morphology of dentin after erosion induced by acidic drinks. J Periodontol 2003; 74:428-436.
15. Lussi A, Jaeggi T. Erosion – diagnosis and risk factors. Clin Oral Investig 2008; 12:S5-13.
16. Sobral MAP, Luz MAAC, Gama-Teixeira A, Garone Netto N. Influência da dieta líquida ácida no desenvolvimento de erosão dental. Pesqui Odontol Bras 2000; 14(4):406-10.
17. West NX, Hughes JA, Addy M. Erosion of dentine and enamel in vitro by dietary acids: the effect of temperature, acid character, concentration and exposure time. J Oral Rehabil 2000; 27:875-80.
18. Syed J, Chadwick RG. A laboratory investigation of consumer addition of UHT milk to lessen the erosive potential of fizzy drinks. Br Dent J 2009; 206(3):153-6.
19. Farias MMAG, Ozelame SB, Schmitt BHE, Capristano DF, Silveira EG. Avaliação da acidez de diversas marcas de leite fermentado disponíveis comercialmente. Pesq Bras Odontoped Clin Integr 2012; 12(4):451-5.
20. Cavalcanti AL, Oliveira KF, Paiva OS. Determinação dos sólidos solúveis totais (Brix) e pH em bebidas lácteas e sucos de frutas industrializados. Pesq Bras Odontoped Clin Integ 2006; 6:57-64.
21. Eduardo MF, Lannes SCS. Achocolatados: análise química. Rev Bras Cienc Farmaceuticas 2004; 40(3):405-12.
22. Ferruzzi MG. The influence of beverage composition on delivery of phenolic compounds from coffee and tea. Physiology & Behavior 2010; 100(1):33-41.
23. Kitchens M, Owens BM. Effect of carbonated beverages, coffee, sports and high energy drinks, and bottled water on the in vitro erosion characteristics of dental enamel. J Clin Pediatr Dent 2007; 31(3):153-9.