

The effect of milk ingestion after an orange juice in the superficial microhardness of human enamel: An in vitro study

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ABSTRACT

Patients' diet and habits are important factors that can determine the occurrence of dental erosion. The objective of this study was to evaluate the ability of milk to reduce the erosion caused by orange juice in enamel. Specimens of human teeth were embedded in acrylic resin, polished and submitted to the initial microhardness measurement (SMH) using a Knoop indenter with 25 g load for 10 seconds. The specimens with similar values of SMH were randomly divided into 4 experimental groups (n = 10) according to the following treatments: C (Control): 30 minutes of immersion in artificial saliva; OJ: 1 minute of immersion in orange juice, followed by 30 minutes in artificial saliva; M: 1 minute of immersion in cow's milk followed by 30 minutes in artificial saliva; OJ + M: 1 minute of immersion in orange juice followed by 1 minute in milk and 30 minutes of artificial saliva. These treatments were repeated for four times. The final SMH was determined and means of SMH values were calculated. Data were analyzed using ANOVA and Tukey's Test ($p < 0.05$). The immersion of the specimens in milk after orange juice was able to reduce the surface softening caused by this acidic drink. It was concluded that milk after an erosive challenge may be an alternative to reduce enamel surface softening.

DESCRIPTORS

Dental enamel. Hardness. Tooth demineralization. Tooth erosion. Tooth remineralization.

INTRODUCTION

Dental erosion is defined as an irreversible loss of dental hard tissues caused by acids, in a process that does not include bacteria¹¹. According to Lussi¹², the etiology and pathogenesis of dental erosion involve chemical, biological and behavioral factors. Chemical factors are some properties of the acid source that make it erosive to the teeth. Biological factors are related to saliva and its characteristics, such as the acquired dental pellicle, tooth structure and surrounding soft tissues¹⁰. The behavioral factors act to modify the extent of the erosive wear and are related to individual habits, such as healthier lifestyles that may involve frequent consumption of acidic fruit and drink^{16,29}.

The consumption of acid food and drink has increased over the last few decades and many studies have related this fact to the increasing prevalence of dental erosion^{17,19}. Many fruit and their respective juices have organic acids, such as citric, maleic and tartaric acid, which can cause different degrees of erosion. Citric acid is present in one of the most consumed juices in the world — orange juice²⁵. In addition to citric acid, orange juice also has citrate, a substance capable of chelating the calcium from the saliva and the teeth¹⁵ and therefore, it is considered a drink with high erosive potential^{27,28}.

The prevention and treatment of dental erosion include guidance, advising patients to avoid the consumption of acid food and drink¹⁴. Nevertheless, results depend on patient's cooperation; thus, efforts are being made in order to minimize the erosive potential of some

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popular acid drinks²⁴. The most common method described in literature is by adding chemical substances, usually containing calcium or phosphate^{3,9,10,26}, to acidic drinks, which decrease the driving force for dissolution by the common ion effect⁴. However, despite the fact that some additives have promoted a positive effect on reducing the erosive potential of an acid solution, most of them cause important side effects on the taste and stability of the drink⁶.

Some food and drinks, such as milk and its derivatives, are naturally rich in calcium, phosphate and other components that may play an important preventive role in the erosion process. According to Grenby *et al.*⁷, milk has some proteins, such as casein, which has a recognized cariostatic and anticariogenic effect. Regarding dental erosion, Barbour *et al.*⁴ added casein to a citric acid solution in a range of pH values similar to those typical for soft drinks and found a reduction of 50–60% in hydroxyapatite demineralization. Corroborating these observations, Aidi *et al.*¹ investigated the association between a broad collection of biological and behavioral factors and the incidence and progression of dental erosion among adolescents. They found that erosive lesions were less likely to progress in subjects who consumed milk and yoghurt products.

From the exposed above, it was hypothesized that drinking milk after the ingestion of orange juice could reduce the loss of tooth tissues, acting as an important and positive behavioral factor of erosion. Thus, the objective of this study was to evaluate the ability of milk to reduce the enamel erosion caused by orange juice.

METHOD AND MATERIALS

This study was approved by the Research Ethics Committee of the School of Dentistry of Universidade de São Paulo (Protocol # 184/2008).

Specimens Preparation

Thirteen recently extracted and caries free human molars were used in this study. They were cleaned with a scalpel to remove any debris, submitted to prophylaxis with pumice and Robinson's brush and stored in 0.1% thymol solution at 4°C until required. One week before the beginning of the experimental procedures, teeth were stored in artificial saliva at 4°C (CaCl₂*2H₂O, KH₂PO₄, KCl, NaCl, Tris buffer and H₂O). After this week, 52 enamel slabs were obtained using an IsoMet Low Speed Saw (Labcut 1010, Extec) with a diamond disk (Excel Technologies Inc). The slabs were embedded

in acrylic resin, ground flat and polished using water-cooled abrasive discs (400 – and 600 – and 1200-grit Al₂O₃ papers; Buehler, Lake Bluff, IL, USA) and felt paper with diamond spray (1 µm; Buehler). On the polished surface, a testing area of 3 x 3 mm was delimited with enamel varnish. This area was divided into two: the left side was designated to the initial surface microhardness (SMH) measurements, and the right side to the final.

The initial SMH was determined by performing three indentations using a Knoop diamond indenter, with 25 g load for 10 seconds (HMV-2000; Shimadzu Corp., Tokyo, Japan). The indentations had at least 100 µm distance between them¹⁸.

The specimens SMH average was calculated and one-way ANOVA test was carried out for comparisons amongst groups and ensured that there was no statistical difference between initial SMH of the specimens. Forty specimens were randomly allocated into four experimental groups (n = 10).

Experimental groups and procedures

Control (C) Group: individual immersion of the specimens in 30 mL of artificial saliva (0.213 g/L of CaCl₂.H₂O; 0.738 g/L of KH₂PO₄; 1.114 g/L of KCl; 0.381 g/L of NaCl; 12 g/L of Tris buffer and 2.2 g/L of porcine gastric mucin; pH = 7,0)²⁰ for 1 minute, at room temperature;

Orange Juice (OJ) Group: individual immersion of the specimens in 30 mL of orange juice, pH = 3.86 (Sucos Del Valle do Brasil Ltda), for 1 minute, at room temperature;

Milk (M) Group: individual immersion of the specimens in 30 mL of cow's milk, pH = 7.02 (Parmalat Brasil S.A. Indústria de Alimentos), for 1 minute, at room temperature;

Orange Juice and Milk (OJ + M) Group: individual immersion of the specimens in 30 mL of orange juice (1 minute) followed by immersion in 30 mL of cow's milk (1 minute), at room temperature;

After treatments, the specimens were stored in artificial saliva for 30 minutes. This cycle was repeated for four times in accordance to results obtained in the pilot tests.

In the end of the cycles, the final SMH measurement was performed.

The pH of each solution was assessed in pH meter (Quimis Aparelhos Científicos Ltda, Diadema, SP,

Brasil), evaluating 20 mL of each solution under gentle agitation with magnetic stirrer.

In addition, three samples from each group were randomly selected to be prepared for scanning electron micrograph (SEM) analysis. The samples were air dried, fixed in aluminium stubs and sputter — coated with gold — palladium for 120 seconds. The surface morphology of enamel was then examined by Scanning Electron Microscope (XL30, Philips Eletronics, Eindhoven, The Netherlands). Representative areas were photographed in each group.

Statistical analysis

From the SMH data, the final SMH was calculated and the results were submitted to statistical analysis using the software Minitab® 15.13. The assumptions of equality of variances and normal distribution of errors were checked and ANOVA and Tukey tests were carried out for statistical comparisons amongst groups. Statistical significance was set at $p \leq 0.05$.

RESULTS

Graphic 1 shows the means (SD) of final SMH measurements of 4 experimental groups. There was a significant increase in the SMH after milk immersion ($p \leq 0.05$) in comparison with all the other groups. Immersion in milk followed by orange juice promoted significantly less SMH changes than immersion in orange juice only. SMH changes for this group (OJ + M) were also not significantly different from the group held in saliva. The orange juice group presented the greatest SMH changes.

Analyses of Scanning Electron Micrograph (Figure 1) showed that the OJ Group presented a much rougher and irregular surface in comparison with all the other groups. The M Group seems to be covered for a thin film. The surface of the OJ + M Group showed irregularities, but they were less rough than those presented by the orange juice group.

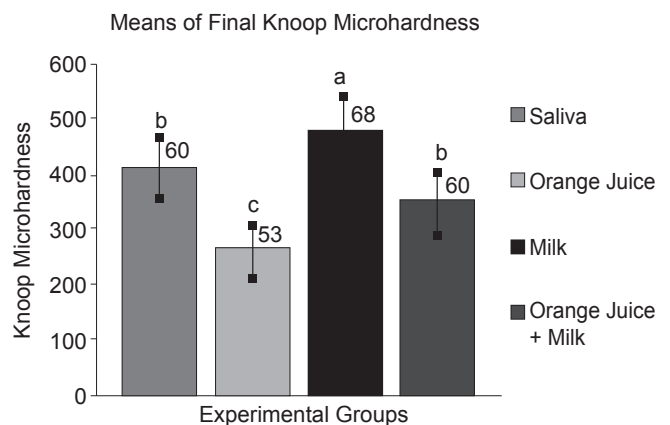
DISCUSSION

The hypothesis of this study, that milk can reduce the erosion caused by an acid drink, was confirmed. The SMH changes for the specimens immersed in milk after orange juice were significantly lower than those found for the group immersed in orange juice only, which means that milk was able to reduce the surface softening caused by orange juice.

While there is no doubt that a high consumption of acid food and drink can cause dental erosion, it is also clear that the clinical manifestation is modified by behavioral and biological factors^{8,13,29}. This fact might be the key to understand why some individuals, whose diet includes high consumption of acid drinks; show few or absent clinical signs of erosion, while others with similar acid intake present erosive lesions. The possibility that diet variations could minimize the erosive effects of acid drinks must be considered, and for this matter, milk and its derivatives could play a very important role in the development of non carious lesions.

The methodology used in this study is in accordance with the current recommendation: that to assess enamel softening the duration of an erosive challenge did not last more than a few minutes that simulate clinical conditions. To assess the enamel softening the most useful method is to measure microhardness²¹.

Milk is a complex colloidal mixture of proteins, fat, lactose, minerals and other constituents⁷. Despite the fact that milk is an excellent protein food that provides essential amino acids and organic nitrogen for humans and animals of all ages², milk and dairy products also have been identified as having important protective effects against dental caries^{5,7,25} and erosion²³. Recently, a study showed that adolescents who consumed milk and yogurt products were less likely to progress erosive wear and the mean consumption of milk and yogurt products was significantly smaller in the group who had erosion progression process than in the non-progression group¹. The findings of our study help to explain the results found by Aidi *et al.*¹ clinically.



Graphic 1 - Means (SD) of the final SMH of the experimental groups. Different letters means statistical difference between groups ($p \leq 0.05$).

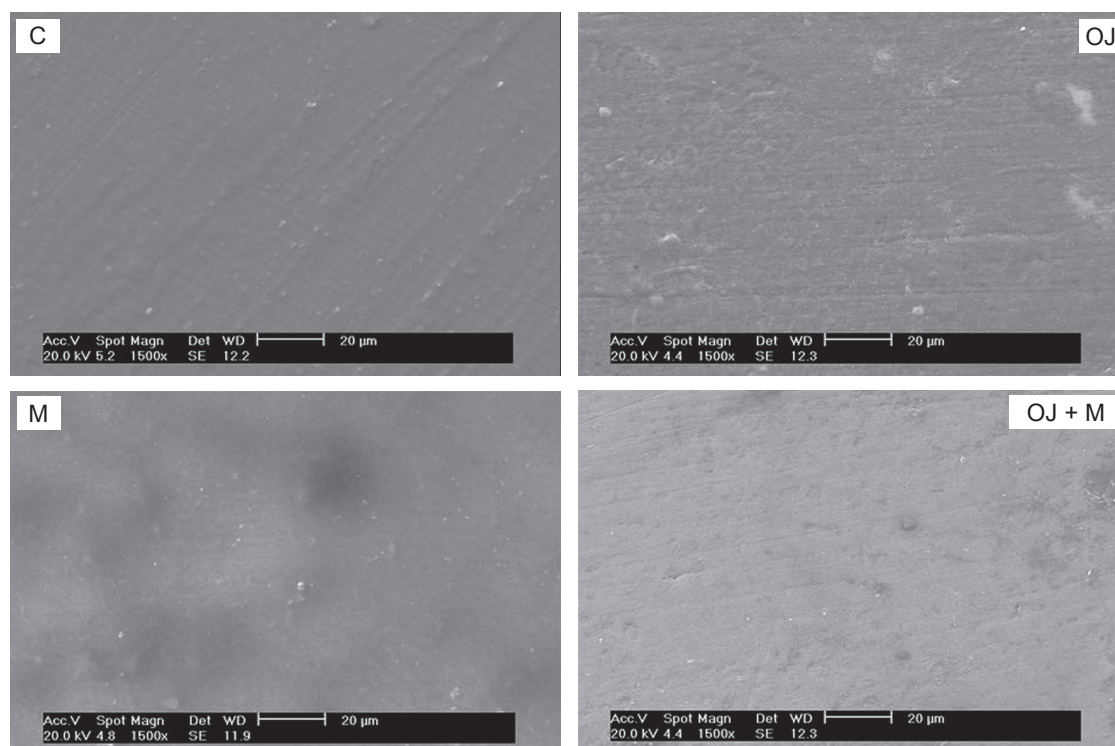


Figure 1 - Scanning Electron Micrograph illustrations of each experimental group. The Control Group C shows the effects of saliva in the enamel surface. In the OJ Group, a rough surface that suggests demineralization can be observed. In the M Group, the surface seems to be covered by a regular mist. In the OJ+M Group, a regular surface with spare irregular areas can be noted.

This protective effect is not only related to the large amount of calcium and phosphates in its composition, but also because it has some phosphoproteins, such as casein. The role of casein in demineralization was hypothesized by Barbour *et al.*⁴. According to these authors, casein can bind to the hydroxyapatite surface and stay there as a thin layer, which acts as a dissolution barrier, restricting the H⁺ access to the crystal surface and preventing diffusion of ions away from hydroxyapatite. Another alternative theory also proposed by these authors says that casein molecules may bind to sites of high energy on the crystal surface, preventing ion detachment from these sites.

This study highlights the importance of the presence of milk and its derivatives in the daily diet. They will not only counteract the harmful effects of a high cariogenic or erosive diet, but also avoid other general health problems, such as those associated to calcium

deficiency¹². In view of this fact, clinicians should encourage their patients, especially those suffering from dental erosion, to include dairy milk or its derivative products in their diet.

It is worth noting that the present study was conducted in enamel specimens and in terms of future directions, further investigations must be performed to verify whether milk exerts the same protective effect on dentin.

CONCLUSION

The ingestion of milk after orange juice may be an alternative to reduce enamel loss by erosion.

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RESUMO

O efeito da ingestão do leite após um suco de laranja na microdureza superficial do esmalte humano: Um estudo *in vitro*

A dieta e os hábitos dos pacientes são fatores importantes que podem determinar a ocorrência de erosão dental. O objetivo deste estudo foi avaliar a capacidade do leite em reduzir a erosão causada pelo suco de laranja

no esmalte. Espécimes de dentes humanos foram inseridos em resina acrílica, polidos e as microdurezas iniciais (MDS) foram mensuradas, utilizando-se um penetrador Knoop com carga de 25 g por 10 segundos. Os espécimes com valores similares de SMH foram divididos aleatoriamente em 4 grupos experimentais ($n = 10$), de acordo com os seguintes tratamentos: C (controle): 30 minutos de imersão em saliva artificial; OJ: 1 minuto de imersão em suco de laranja, seguido por 30 minutos em saliva artificial; M: 1 minuto de imersão em leite de vaca, seguido por 30 minutos em saliva artificial; OJ + M: 1 minuto de imersão em suco de laranja, seguido de 1 minuto no leite e 30 minutos de saliva artificial. Esses tratamentos foram repetidos por quatro vezes. As MDS finais foram determinadas e as médias dos valores de MDS calculadas. Os dados foram analisados por ANOVA e teste de Tukey ($p < 0,05$). A imersão dos espécimes de esmalte no leite foi capaz de reduzir o amolecimento da superfície causado pela bebida ácida. Concluiu-se que o leite após um desafio erosivo pode ser uma alternativa para reduzir o amolecimento superficial do esmalte.

DESCRIPTORES

Esmalte dentário. Dureza. Desmineralização do dente. Erosão dentária. Remineralização dentária.

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