EFFECTS OF XYLITOL ON ENAMEL EROSION CONTROL
Efeitos do xilitol no controle da erosão dentária

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ABSTRACT - In this study was verified the in vitro influence of xylitol in the process of teeth demineralization, evaluating its preventive and/or corrective performance, in comparison with the action of fluoride, the standard drug used in cases of dental erosion. It were used 60 bovine teeth, divided equally into 10 groups, which were exposed to lemon juice, xylitol (10%, 25% and 40%) and fluoride (500 ppm) in different ways. We evaluated the microscopic characteristics of the enamel by scanning electron microscopy (SEM). Xylitol (10% and 25%) was not enough to prevent the microstructural changes of the dental enamel and, consequently, to prevent the process of dental erosion both in the prevention and correction studies. However, 40% xylitol showed protective and correction effect on the erosive process, similar to that one adopted as standard, 500 ppm of fluoride. This study highlights the importance of knowledge of substances that can prevent the progression or treating the erosive process, and the 40% xylitol may be an appropriate replacement substance of fluoride.

DESCRIPTORS - Xylitol; tooth erosion; demineralization; prevention and control.

INTRODUCTION
The surface of dental enamel can be attacked by acids generating mineral loss at the microscopic and macroscopic levels, leading to a process called dental erosion, the type of non-curious cervical lesion, which occurs by the loss of tooth structure caused by chemical action, without bacterial involvement.¹-³ This phenomenon may occur due to the action of acids from extrinsic (diet, acidic beverages, medicines) or intrinsic (gastric reflux, anorexia, vomiting, regurgitation among others) sources or from unknown sources.³,⁴

The most common clinical feature of dental erosion is the loss of brightness of the enamel, taking a concave form, developing an usually large and shallow injury, without sharp angles. The cervical area of the vestibular face of anterior teeth is the region most affected. When the lesion reaches the dentin, it arouses sensitivity to cold, heat and osmotic pressure.¹ Currently, in order to reduce the erosive potential associated with the diet, calcium, phosphate and fluoride ions have been added in the composition of various beverages.⁵,⁶ However, these compounds may have negative effect upon the taste and reduce the expiry date, which industry may become impracticable and, clinically, significant reductions are not yet demonstrated in the erosive potential of drinks with these changes.⁷

Xylitol is a five-carbon polyalcohol which appears in the form of a white crystalline powder, having the same sweetening power as sucrose.⁸,⁹ Among the methods of chemical control of dental plaque, xylitol is assuming an important role, since there is no fermentation characteristic of the majority of bacteria present in the oral cavity. It is believed that xylitol has the ability to move and to form complexes with calcium ions, and therefore it is able to go through the tooth enamel. Xylitol renders the dental plaque less adherent and easily removed by brushing, and, this
Xylitol was used with a purity level greater than 98% (Fluka BioChemika, Switzerland).

**Experimental design**
The samples were divided into 8 experimental groups (A,B,C,D,E,F,G and H) and 2 control groups (I,J) (n = 6 teeth/group). In order to test the preventive effect of xylitol against enamel erosion, the teeth of A,B,C and D groups were immersed and mixed on vortex for 5 minutes in 60 mL of a solution of lemon juice containing 10%, 25% or 40% xylitol, respectively, and 500 ppm of fluoride (group D). In order to test the corrective effect of xylitol against enamel erosion, the teeth of E, F, G and H groups were first immersed for 5 minutes in 60 mL of lemon juice and then exposed for 5 minutes to 60 mL of solutions 10%, 25% or 40% xylitol, respectively, and 500 ppm of fluoride (group H). Each group was exposed to 60 mL of the final solution for 4 times daily (immersions for 5 minutes) for 7 days. The positive control (group I) was exposed only to the lemon juice and the negative control (group J) was stored in artificial saliva.

**Artificial saliva and solutions**
All 10 experimental groups were stored in artificial saliva at intervals of immersions. The composition of the artificial saliva was: 0.12% KCl; 0.089% NaCl; 0.005% MgCl$_2$; 0.146% Ca$_2$(C$_6$H$_5$O$_7$)$_2$; 0.002% nipagim; 0.013% nipazol; 1.0% carboxymethylcellulose (CMC); 3.0% sorbitol; distilled water quantity sufficient for 1 mL. The solutions were stored 4°C and the pH of lemon juice (optimum pH range 1.5 to 2.0) and pH of artificial saliva (optimum pH 7.0) were checked daily to avoid significant changes from one day to another.

**Scanning Electron Microscopy (SEM)**
In order to observe microstructural features of the enamel after the treatments using microscopic analysis (scanning electron microscopy - SEM), the treated teeth were analyzed. The SEM images were used to quantify the surface changes and to evaluate the preventive and/or corrective performance of xylitol and fluoride.

**Material and Methods**
The method of comparative approach involved a procedure that takes into account the qualitative changes of dental enamel submitted to: 1) lemon juice; 2) lemon juice associated with xylitol (10%, 25% and 40%) and with 500 ppm of fluoride (used as standard against the demineralization) as a possible inhibitor of dental erosion; 3) first to lemon juice, and then submitted to solutions of xylitol (10%, 25% and 40%) and with 500 ppm of fluoride with possible corrective effect of demineralization. Fluoride (500 ppm) was used as standard because it is very effective in protecting teeth against caries and strongly reduces enamel demineralization while also enhance remineralization.

**Bovine teeth**
Sixty bovine teeth obtained from recently extracted and caries-free were used in this study. Every tooth was examined and those presenting visible enamel cracks and/or fractures were rejected. Bovine teeth were selected not only for the ease of production, but mainly because it has a similar behavior to human teeth in studies of erosion and have a greater surface area of analysis. The bovine teeth were cleaned up with a fluid mixture of pumice stone and distilled water, using a rubber cup, mounted on a contra-angle with a low engine speed, for 10 seconds and washed afterwards thoroughly with distilled water for 15 seconds.

**Xylitol**
Xylitol can act in the prevention of demineralization and on the remineralization of teeth. Thus, in this study it was verified the in vitro influence of xylitol on the process of demineralization of teeth, checking the preventive and/or corrective performance of this compound, through analysis of tooth enamel by scanning electron microscopy (SEM).
microscopy - SEM), slides were prepared from enamel blocks from teeth selected at random from each group. The laminar enamel blocks were obtained by diamond disc and drill on high speed, under constant irrigation, selecting then the middle third of the vestibular face of dental elements, which average extent was approximately 5mm x 5mm. The enamel blocks were metalized in Balzers Union FL - 9496 (Balzers, Germany) metalizer with 2 nm of gold for 2 minutes, and subsequently they were analyzed in the scanning electron microscope JSM 5310 (Jeol, Japan) in high vacuum in mode of secondary electrons. Data analysis was performed in a descriptive way, noting the qualitative changes in the morphology of the structure of dental enamel.

RESULTS
Areas of wastage were observed in lemon juice-treated teeth associated to the presence of erosion in enamel (Figure 1 B), while such structural changes were not observed in the negative control (Figure 1 A), indicating that acidic drinks cause the demineralization of dental enamel.

Fig. 1 - Photographs of SEM showing the dental enamel. The negative control (Group J - Panel A) was stored in artificial saliva while the positive control (Group I - Panel B) was exposed only to the lemon juice. It was observed that in the presence of 10% xylitol (Figure 2 A), there is no inhibition of the erosive process, because the teeth showed morphological changes comparable to positive control. In the presence of 25% xylitol (Figure 2 B), dental enamel erosions are still observed, although at lower levels than the positive control and group A (10% xylitol). The erosion inhibition by xylitol was dose-dependent and the best preventive effect was observed with 40% xylitol (Figure 2 C). In this group (group C) the inhibition of erosions in the dental enamel was similar to those found with 500 ppm of fluoride (group D) which is considered standard against demineralization (Figure 2 D). Only in the group G, with 40% xylitol (Figure 3 C) the dental enamel appears comparable to the negative control, whereas there were no morphological changes. In this case, the erosion process was fixed. Used as a standard, the 500 ppm of fluoride (group H) corrected changes in the structure of dental enamel (Figure 3 D). It was observed in the group E, with 10% xylitol (Figure 3 A), no correction of the erosive process and in the group F, with 25% xylitol (Figure 3 B), dental enamel erosions are still observed, although at lower levels than the positive control and group E (10% xylitol). The erosion correction by xylitol was dose-dependent and the best preventive effect was observed with 40% xylitol (Figure 3 C) similar to those found with the standard treatment (500 ppm of fluoride - Figure 3 D)
Fig. 2 - Photographs of SEM showing the effect of xylitol as inhibitor of enamel demineralization. The teeth in group A to C (Panel A to C) were exposed to lemon juice containing 10%, 25% and 40% of xylitol, respectively, and in group D (Panel D) to 500 ppm of fluoride.

Fig. 3 - Photographs of SEM showing the influence of xylitol on the correction of the enamel demineralization. The teeth in group E to G (Panel A to C) were first exposed to lemon juice and then the solutions containing 10%, 25% and 40% of xylitol, respectively, and group H (Panel D) to 500 ppm of fluoride.
DISCUSSION
The comparison of the results obtained in different groups of this study allowed evaluating the influence of xylitol and fluoride in regard the process of demineralization of dental enamel. It was possible to observe that only 40% xylitol promoted the protection and correction of the teeth erosion caused by exposure to lemon juice (acid drink). It was evaluated the influence of acid fluid diet in the development of dental erosion and found that lemon juices, which have values below the critical pH for dental demineralization (pH = 5.5), are potentially erosive.\textsuperscript{1,3,7}

The period of 5 min of exposure of teeth to the lemon juice was determined in view of the short interval of time that the liquid diet is under normal conditions in the oral cavity, and the time that the saliva exerts its buffer capacity, and this time of exposure to acidic drinks was established to have an effect on dental enamel.\textsuperscript{5}

Amaechi et al.\textsuperscript{5} studied in vitro demineralization of bovine incisor teeth, and observed that after exposure to pure orange juice, the teeth treated with 25% xylitol + 0.5 ppm of fluoride had lower mineral loss than teeth treated with 0.5 ppm of fluoride, 25% of xylitol, and only orange juice, in that order. It was concluded that fluoride and xylitol have an additive effect in reducing dental erosion in vitro. Chunmuang et al.\textsuperscript{13} conducted a study with human teeth (3rd molar) which were exposed to orange juice and various protective agents (40% xylitol, 227 ppm of fluoride, and 40% xylitol + 227 ppm of fluoride). The results showed that post-treatment with 40% xylitol alone, after being exposed to an acidic drink, was unable to reduce tooth surface and mineral loss. The addition of xylitol, fluoride and a xylitol/flouride combination to the acidic drink or post-treatment with a solution containing either fluoride or a xylitol/flouride combination after the acid challenge could reduce enamel dissolution, in vitro. However, the strongest anti-erosive treatment was to add xylitol or xylitol/flouride combined to the drink itself. These studies\textsuperscript{5,13} become stronger the results obtained in this work. That way, it can lead us to consider how powerful xylitol and fluoride is to reduce enamel erosion. Probably, 40% xylitol can move and a form complex with calcium ions, in the oral cavity, and it is able to go through the tooth enamel. This suggested how xylitol can act in the remineralization of teeth. Most likely xylitol inhibits acid dissolution of enamel by interfering with the transport of dissolved enamel from the lesion to the bulk solution (by lowering the diffusion coefficients of calcium and phosphate ions).\textsuperscript{14}

Despite the limitations of the in vitro study, regarding to the reproduction of the natural conditions of the mouth, this study allowed estimating the erosive potential of the utilized juice, representing the whole range of acidic beverages, as well as, it enabled to examine microstructural changes on the surface of the enamel when it is subjected the action of this type of drink. The process of enamel dissolution by erosion involves softening of the enamel and slight subsurface mineral loss and 40% xylitol, as well as 500 ppm of fluoride, may be inhibiting this dental enamel softening.

CONCLUSION
The results showed that 40% xylitol promoted a corrective and protective effect on the process of demineralization, similar to that achieved with the use of fluoride (500 ppm). This fact highlights the important role of xylitol as an adjunct agent in the alternative procedures to prevent the development and progression of the erosive process. It may be of practical interest for the use of xylitol in toothpastes or chewing gums.

RESUMO – Neste trabalho foi verificada, in vitro, a influência do xilitol no processo de desmineralização dos dentes, avaliando sua atuação preventiva e/ou corretiva, em comparação com a ação do fluoreto, substância padrão usada em casos de erosão dentária. Foram utilizados 60 dentes bovinos divididos igualmente em 10 grupos, que foram expostos a suco de limão, xilitol (10%, 25%...
e 40%) e fluoreto (500 ppm) de maneiras diferentes. Foram avaliadas as características microscópicas do esmalte, através da microscopia eletrônica de varredura (MEV). O xilitol, nas concentrações de 10% e 25%, não foi suficiente para impedir as alterações microestruturais do esmalte e, consequentemente, o processo de erosão dentária, tanto no estudo de prevenção quanto de correção da desmineralização. Contudo, 40% de xilitol exerceram efeito protetor e/ou corretivo do processo erosivo, de forma similar àquele adotado como padrão, 500 ppm de fluoreto. Este estudo destaca a importância do conhecimento de substâncias que possam prevenir a progressão ou tratar o processo erosivo, sendo que o xilitol a 40% pode ser um substituto adequado do fluoreto.

**DESCRITORES** - Xilitol, Erosão Dentária, Desmineralização, Prevenção e Controle.

**ACKNOWLEDGMENTS** - The authors would like to thank Noêmia Rodrigues, Frederico Aiex, Lilian Azevedo and Urias P. Vaz for technical assistance. They would also like to thank CAPES for the scholarship.

**REFERENCES**

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