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Erosive softening and erosive loss of enamel: hardness and profilometry analysis

Erosão e desgaste erosivo do esmalte: análises por microdureza e perfilometria

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ABSTRACT

Objective: This study aimed to determine and differentiate erosive softening and enamel erosive loss induced by citric and hydrochloric acids. **Material and Methods:** Forty enamel specimens were divided into 2 groups: 1) 0.05 M citric acid (pH 2.5) simulating extrinsic erosion and 2) 0.01 M hydrochloric acid (pH 2.2) simulating intrinsic erosion. The enamel specimens were submitted to erosive challenges. Surface microhardness (softening) or contact profilometry (loss) was done after 30 s, after each 60 s up to 10 min, after each 5 min up to 30 min and after 60, 90 and 120 min. **Results:** Erosive softening (enamel hardness loss) was measurable up to 1 and 2 min for hydrochloric and citric acids, respectively. Erosive loss was significantly increased over time for both types of acids. After 8 min, citric acid was more aggressive than hydrochloric acid ($p < 0.001$). **Conclusion:** The progression of enamel erosion from erosive softening to erosive loss is highly dependent on the type of acid, being citric acid more aggressive in later stages. Therefore, this finding should be considered when choosing the method of analysis for laboratory studies.

KEYWORDS

Enamel; Erosive wear; Microhardness; Profilometry; Tooth erosion.

RESUMO

Objetivo: Esse trabalho tem como objetivo determinar e diferenciar a erosão e o desgaste erosivo do esmalte induzidos pelos ácidos cítrico e clorídrico. **Materiais e Métodos:** Quarenta amostras de esmalte foram divididas em 2 grupos: 1) 0,05 M de ácido cítrico (pH 2,5) simulando a erosão extrínseca e 2) 0,01 M de ácido clorídrico (pH 2,2) simulando a erosão intrínseca. Amostras de esmalte foram submetidas aos desafios erosivos. A microdureza de superfície (erosão) ou a perfilometria (desgaste erosivo) foi realizada após 30 s, depois a cada 60 s até 10 min, depois a cada 5 min até 30 min e depois de 60, 90 e 120 min. **Resultados:** A erosão (perda de dureza do esmalte) foi mensurável até 1 e 2 min de exposição aos ácidos clorídrico e cítrico, respectivamente. O desgaste erosivo aumentou significativamente ao longo do tempo para ambos os ácidos. Após 8 min, o ácido cítrico foi mais agressivo comparado ao clorídrico ($p < 0,001$). **Conclusão:** A progressão da erosão do esmalte do amolecimento ao desgaste erosivo é altamente dependente do tipo de ácido, sendo o ácido cítrico mais agressivo em estágios avançados. Portanto, este resultado deve ser considerado na escolha do método de análise para estudos laboratoriais.

PALAVRAS-CHAVE

Desgaste erosivo; Erosão de dente; Esmalte; Microdureza; Perfilometria.

INTRODUCTION

Dental erosion was firstly defined as an irreversible loss of dental structure due to a chemical process not involving bacteria [1]. In the last decade, the terms to define the effects of acids on the tooth surface were more precisely defined. Initially, the acid contact causes an erosive softening due to a demineralization of the outermost enamel layer. Continuous acid contact leads to a measureable erosive enamel loss [2,3]. Dental erosion is a multifactorial and complex condition determined by chemical (type of acid), biological (saliva, acquired pellicle) and behavior (frequency of acid exposure, brushing habits) factors [4,5]. It is most likely that in real life, dental erosion alone is not the main reason for dental hard tissue loss, but it is a mixture of a chemical-mechanical processes involving erosion, abrasion and attrition [6].

To determine the first phase of erosion, called surface softening, measurement of calcium and phosphate release or nano- and microhardness analyses alone or combined are probably the most appropriate methods, as they are able to measure initial demineralization directly or indirectly [7-9]. To analyze erosive loss (wear) other methods are more suitable, such as profilometry or transversal and longitudinal microradiography [7-9]. Microradiography is a reliable method only when the loss of dental hard tissue exceeds a certain amount (around 10 - 20 μm), while profilometry is able to measure dental hard tissue loss of less than 1 μm. Therefore, contact profilometry is the most applied method to measure tooth loss, despite some limitations, such as repositioning and potential tissue damage [9].

The duration of erosive challenges differs widely between different studies, which makes a direct comparison very difficult [3,7,9-13]. Furthermore, the progression of tooth erosion development from erosive softening to erosive loss phase has never been evaluated in detail, considering two different acid sources. Therefore, this in vitro study applied a model to evaluate the progression of erosive softening to erosive loss provoked by the most important extrinsic and intrinsic acids applied in in vitro studies, which were compared using surface microhardness (for erosive softening) and contact profilometry (for erosive loss).

MATERIAL AND METHODS

Specimen Preparation

Forty crowns of permanent bovine incisors (4-5 years old cattle, Mondelli Frigorífico, Bauru, São Paulo, Brazil) were embedded in auto-polymerized acrylic resin JET using a silicone mold (Biopdi, São Carlos, São Paulo, Brazil) and ground flat and polished with water-cooled silicon carbide discs (320, 600 and 1200 grades of Al_2O_3 papers for 2, 4 and 4 min, respectively, Buehler, Lake Bluff, Illinois, USA) [14]. This procedure removed around of 200-300 μm of enamel. Baseline hardness indentations and profile scans were obtained as described below. Prior to the erosive challenges, the reference areas were protected with red nail varnish, leaving only the central area of enamel exposed to the acid (5 mm^2). The specimens were divided into 2 groups (n = 20) and stored in deionized water until being used for the experiment (total time of 10 days).

Erosive Challenges

The specimens were exposed to 0.05 M citric acid solution (pH 2.5, n = 20) simulating an extrinsic erosive challenge [11,13] or to 0.01 M hydrochloric acid solution (pH 2.2, n = 20) simulating an intrinsic erosive challenge [10,12] at 25º C under stirring conditions (60 rpm). Specimens were removed after 30 s, each 60 s up to 10 min, each 5 min up to 30 min and after 60, 80 and 120 min of erosive challenge. At each experimental time (30 s, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 60, 90, and 120 min), the acid solution was renewed and the specimens were washed using deionized water for 5 s. The enamel erosion was then analyzed using microhardness or/and profilometry. For periods of exposition equal or longer than 10 min (for ex. between 30 and 60 min), the acid solution was changed every 5 min.

Microhardness and Profilometry

Surface knoop microhardness was measured (5 indentations, 200 μm apart from each other, under 25 g per 10 s), using a microhardness tester (Buehler Micromet 5114, Lake Bluff, Illinois, USA) at baseline and after each experimental period. The parameters to define the "erosive softening" phase were: 1) the presence of a baseline hardness indentation at each measurement and 2) the progressive decrease of the mean hardness values. The progression to "erosive loss" was defined when: 1) the mean hardness values slightly increased or remained unchanged compared to the last measurement and/or 2) the baseline indentations completely disappeared. The parameters were reproducible in most of the specimens [15,16].

"Erosive loss" was quantified using contact profilometer with a roughness tip (90o conical shape with a radius of 2 microns, applying approximately 1 mN of force, MarSurf GD 25, Göttingen, Lower Saxony, Germany). Five equidistant surface scans of each specimen were performed (5 mm of reading, 250 μm apart, area: 5 mm^2) at the same area in the baseline and after each experimental period (after removal of the nail varnish) [14]. To ensure exact repositioning, the specimens were placed in a device during the profilometric measurement, where it was possible to standardize the position at the x-, y- and z-axes. Furthermore, the specimens received a mark on the control area using a drill, to facilitate the localization of the first reading. Baseline and final profiles were compared. For that, the scans were superposed and the average depth of the under curve area was quantified (μm) using the software MahrSurf CXR20 (Marh, Göttingen, Lower Saxony, Germany). The mean of 5 readings was calculated per specimen and experimental period.

Statistical Analysis

Data were statistically analyzed using the GraphPad Instat and GraphPad Prism for Windows (GraphPad Software, San Diego, California, USA). The data were tested for normality (Kolmogorov-Smirnov test) and homogeneity (Bartlett test). Thereafter, two-way repeated measures ANOVA followed by the Bonferroni test were applied, considering the types of acid and the experimental periods as factors for microhardness and profilometry, separately. The level of significance was set at 5%.

RESULTS

Surface microhardness

Erosive softening was measurable up to 1 min and 2 min for hydrochloric and citric acids, respectively. Despite a significant loss of surface microhardness was seen for citric acid between 2-3 min, the baseline indentations were not longer visible. In case of hydrochloric acid, there was an increase of hardness values after 1 min. Figure 1 shows a significant decrease in the mean of surface microhardness from 0 to 1 min for both acids ($p < 0.001$), which did not differ from each other ($p > 0.05$). At 2 and 3 min, surface softening differed significantly between both acids, but this difference should be carefully interpreted due to the feasible surface loss.

Profilometry

Erosive loss was increased overtime for both types of acids as shown in Figure 2. For citric acid, erosive loss was significantly increased after 4 min (compared to 2 min) and thereafter after each third minute up to 10 min. From 10 to 120 min, there was a significant increase of tooth loss among the experimental periods. In case of hydrochloric acid, erosive loss was significantly increased after 8 min (compared to 2 min) and then after 15 and 25 min. From 30 to 120 min,

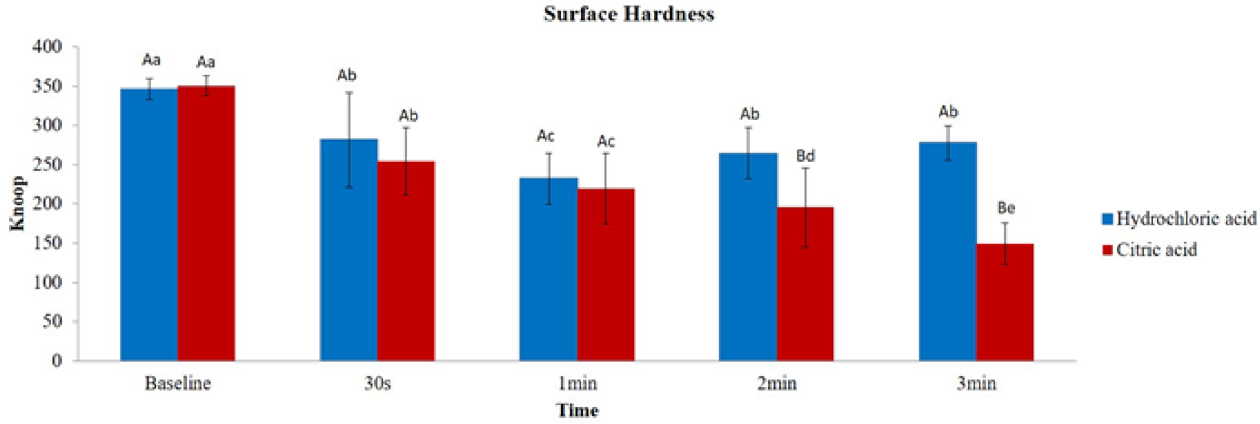

Figure 1 - Surface microhardness (mean ± standard deviation) of eroded enamel specimens (n = 20). Different capital letters indicate significant differences between the acids. Different lowercase letters indicate significant differences among the experimental periods for the same acid.

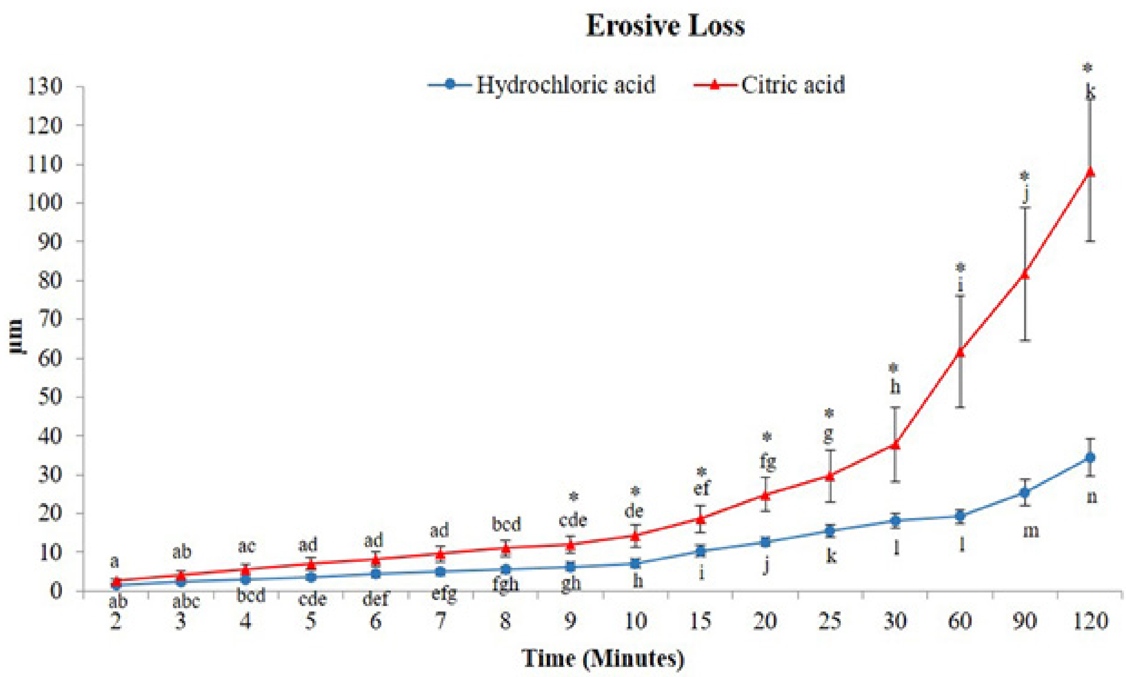

Figure 2 - Loss (mean ± standard deviation) of eroded enamel specimens (n = 20). Asterisks (*) indicate differences between the acids. Different lowercase letters indicate significant differences among the experimental periods for the same acid.

there was also a significant increase of tooth loss among the experimental periods.

No significant difference was found between the acids from 2 to 8 min. However, after 8 minutes citric acid was more aggressive (2-fold from 8 to 30 min and 3-fold from 60 to 120 min) than hydrochloric acid ($p < 0.001$).

DISCUSSION

There is a lack of concise information about the progression of enamel softening to erosive loss by the effect of different acids. Thus, this in vitro study monitored the progression of enamel erosion induced by an extrinsic and an intrinsic acid from 30 s to 120 min of exposure.

The times of erosive challenges were chosen to simulate conditions from a short to a long exposure to acids (e.g. baby bottle-feeding during the night) [5]. However, in the clinical situation short erosive challenges are more common, e.g. vomiting or a short consumption of an erosive drink. Hydrochloric acid and citric acid were chosen, at specific concentration and pH, to simulate gastric juice and the main acid source in erosive drinks, respectively, as applied in several previous in vitro studies [3,7,9-13]. As these acids were used at different pH values, a direct comparison of their erosive effects is difficult, but it was not aimed in the present study.

To measure erosive softening and erosive loss we have applied the most useful methods, surface microhardness and contact profilometry, respectively, as recommended by Shellis et al. [3]. It is a challenge to choose an appropriate method as response variable. The decision must be based on the acid exposure time, in which there is only softening without any detectable tooth loss, and from which moment enamel loss is measurable. Attin [7] discussed that erosive challenge times below 10 min cause only erosive softening, and large attacks, preferable longer than 30 min, are able to provoke erosive loss. No information was given for periods between 10-30 min of erosion. Oppositely, some studies in the literature applied microhardness for exposure times higher than 10 min [17,18], and profilometry for erosive challenges bellow 30 min [6,19]. We should also bear in mind that the progression of erosion might be dependent on variables as type of acid, pH values, temperature and stirring, among others.

In our study, erosive softening could be measured by microhardness in the first minutes. Our results showed that the times for the progression from "erosive softening" to "erosive loss" are shorter than those suggested by Attin [7], but comparable to other studies that were able to quantify erosive loss earlier than 10 min. Gracia et al. [20] showed 4.46±0.40 μm of enamel erosive loss after 5 min in 0.05 M citric acid (pH 3.8) while Aykut-Yetkiner et al. [6] showed 6.8±0.6 μm of enamel erosive loss after 10 min in 0.01 M citric acid (pH 2.5).

The erosive attack provokes a progressive softening of enamel until the moment that the first layer is outworn. When this happens, a new layer (the underlying enamel) is exposed, which may be less softened than the former layer as shown for hydrochloric acid. In case of citric acid, the hardness was still decreasing after 2 min. However, the baseline indentation was not more visible, which means that the first layer was also removed. The indentations have a length around 30-35 μm, which is equivalent to 1.00-1.15 μm penetration [8]. Considering that the enamel loss mean was 2.7 μm for citric acid after 2 min of erosion, the removal of the indentation is expected.

As citric acid has shown a higher erosive potential than hydrochloric acid, it is likely that this underlying layer now exposed after 2 min was quickly demineralized by additional 1 min of erosive challenge only (3 min data). This might explain the different behavior of enamel exposed to citric acid compared to hydrochloric acid in the early stages of enamel erosion. This result highlights the importance of checking the response variables according to the erosive challenge before planning any study on this field. We tried to correlate the hardness and profile data at 2 and 3 min, but not significant relationship was found (data not shown). Therefore, when enamel loss exists, hardness is not more apropriate response variable to be considered.

With respect to erosive loss, the sensitivity of the profilometry is highly dependent on the system and software applied [7]. Our profilometry system has the sensitivity to measure tooth loss values above 0.5 μm. Therefore, we could measure erosive loss after 1 min of erosive challenge for both acids.

A clear difference between the acids was mainly seen in the advanced phase of erosive loss, which can be explained by the characteristics of the two acids. Hydrochloric acid is a strong acid that completely dissociate at all pH values [21]. Strong acids have a high grade of dissociation, thus their effects are restricted to the beginning of the erosive challenge [22]. On the other hand, citric acid is a weak acid; its dissociation progresses with increasing pH (pKa values 3.15, 4.77 and 6.40) [21]. Weak acids, with a high amount of titratable acid and a low level of dissociation, are able to release additional protons if the acid is consumed over time [22]. Additionally, citrate may act as calcium chelating agent, but this is relevant at pH values around 6.0, but not for highly erosive conditions as happened in the present study [21]. Our results are in accordance with West et al. [23]. However, Hannig et al. [22] showed that even at short erosive time (5 min) hydrochloric acid was least erosive when compared with citric acid, both at pH 2.3 and 3.0, by analyzing phosphate release. However, two points should be taken in mind when we compare our study with the work of Hannig et al. [22]: 1) the authors compared the acids at the same pH. In our case, the acids were compared at different pH values [3,7,9-13]; 2) measurement of phosphate release might be more sensitive to detect differences between the acids than microhardness/profilometry.

In further studies, it would be interesting to include surface roughness analysis to check if enamel alterations could be measurable earlier and if this measurement could add some important information to the other response variables [24]. According to Schlueter et al. [9], there is a roughness increase in the initial stage of tooth erosion, which could be potentially used as a tool to identify different erosion stages or the transition between them.

CONCLUSION

This in vitro study was able to evaluate the progression of tooth erosion from erosive softening to erosive loss provoked by the most important extrinsic and intrinsic acids, whose results can contribute for the design of future in vitro studies. The progression of enamel erosion is highly dependent on the type of acid. Therefore, this result should be considered when choosing the method of analysis.

The authors declare no conflict of interest.

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REFERENCES

1. Imfeld T. Dental erosion. Definition, classification and links. Eur J Oral Sci. 1996 Apr;104(2(Pt2)):151-5.
2. Huysmans MC, Chew HP, Ellwood RP. Clinical studies of dental erosion and erosive wear. Caries Res. 2011 May;45(Suppl 1):60-8.
3. Shellis RP, Ganss C, Ren Y, Zero DT, Lussi A. Methodology and models in erosion research: Discussion and conclusions. Caries Res. 2011 May;45(Suppl 1):69-77.
4. Lussi A, Carvalho TS. Erosive tooth wear: A multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci. 2014;25:1-15. doi: 10.1159/000360380.
5. Zero DT, Lussi A. Behavioral factors. Monogr Oral Sci. 2006;20:100-5.
6. Aykut-Yetkiner A, Wiegand A, Bollhalder A, Becker K, Attin T. Effect of acidic solution viscosity on enamel erosion. J Dent Res. 2013 Mar;92(3):289-94.
7. Attin T. Methods for assessment of dental erosion. Monogr Oral Sci. 2006 May;20:152-72.
8. Attin T, Wegehaupt FJ. Methods for assessment of dental erosion. Monogr Oral Sci. 2014 Jun;25:123-42.
9. Schlueter N, Hara A, Shellis RP, Ganss C. Methods for the measurement and characterization of erosion in enamel and dentine. Caries Res. 2011 May;45(Suppl 1):13-23.
10. Austin RS, Stenhagen KS, Hove LH, Dunne S, Moazzez R, Bartlett DW, et al. A qualitative and quantitative investigation into the effect of fluoride formulations on enamel erosion and erosion-abrasion in vitro. J Dent. 2011 Oct;39(10):648-55. doi: 10.1016/j.jdent.2011.07.006.
11. Ganss C, Lussi A, Grunau O, Klimek J, Schlueter N. Conventional and anti-erosion fluoride toothpastes: Effect on enamel erosion and erosion-abrasion. Caries Res. 2011;45(6):581-9. doi: 10.1159/000334318.
12. Hove LH, Holme B, Stenhagen KR, Tveit AB. Protective effect of TiF(4) solutions with different concentrations and pH on development of erosion-like lesions. Caries Res. 2011;45(1):64-8. doi: 10.1159/000324155.
13. Wiegand A, Hiestand B, Sener B, Magalhães AC, Roos M, Attin T. Effect of TiF4, ZrF4, HfF4 and AmF on erosion and erosion/abrasion of enamel and dentin in situ. Arch Oral Biol. 2010 Mar;55(3):223-8. doi: 10.1016/j.archoralbio.2009.11.007.
14. Magalhães AC, Kato MT, Rios D, Wiegand A, Attin T, Buzalaf MAR. The effect of an experimental 4% TiF4 varnish compared to NaF

varnishes and 4% TiF4 solution on dental erosion in vitro. Caries Res. 2008;42(4):269-74. doi: 10.1159/000135672.

15. Brito JS, Santos Neto A, Silva L, Menezes R, Araújo N, Carneiro V, et. al. Analysis of dental enamel surface submitted to fruit juice plus soymilk by micro x-ray fluorescence: In Vitro study. ScientificWorldJournal. 2016;2016:8123769. doi: 10.1155/2016/8123769.

16. Mesquita-Guimarães KS, Scatena C, Borsatto MC, Rodrigues-Júnior AL, Serra MC. Effect of foods and drinks on primary tooth enamel after erosive challenge with hydrochloric acid. Braz Oral Res. 2015;29. pii: S1806-83242015000100291. doi: 10.1590/1807-3107BOR-2015.vol29.0096.

17. Carvalho FG, Brasil VL, Silva Filho TJ, Carlo HL, Santos RL, Lima BA. Protective effect of calcium nanophosphate and CPP-ACP agents on enamel erosion. Braz Oral Res. 2013 Nov-Dec;27(6):463-70. doi: 10.1590/S1806-83242013000600004.

18. Khamverdi Z, Vahedi M, Abdollahzadeh S, Ghambari MH. Effect of a common diet and regular beverage on enamel erosion in various temperatures: An in-vitro study. J Dent (Tehran). 2013 Sep;10(5):411-6.

19. Moazzez RV, Austin RS, Rojas-Serrano M, Carpenter G, Cotroneo E, Proctor G, et al. Comparison of the possible protective effect of the salivary pellicle of individuals with and without erosion. Caries Res. 2014;48(1):57-62. doi: 10.1159/000352042.

20. Gracia LH, Rees GD, Brown A, Fowler CE. An in vitro evaluation of a novel high fluoride daily mouthrinse using a combination of microindentation, 3D profilometry and DSIMS. J Dent. 2010 Nov;38 Suppl 3:S12-20. doi: 10.1016/S0300-5712(11)70004-5.

21. Shellis RP, Featherstone JD, Lussi A. Understanding the chemistry of dental erosion. Monogr Oral Sci. 2014;25:163-79. doi: 10.1159/000359943.

22. Hannig C, Hamkens A, Becker K, Attin R, Attin T. Erosive effects of different acids on bovine enamel: Release of calcium and phosphate in vitro. Arch Oral Biol. 2005 Jun;50(6):541-52.

23. West NX, Hughes JA, Addy M. The effect of pH on the erosion of dentine and enamel by dietary acids in vitro. J Oral Rehabil. 2001 Sep;28(9):860-4.

24. Field J, German M, Waterhouse P. Using bearing area parameters to quantify early erosive tooth surface changes in enamel: A pilot study. J Dent. 2013 Nov;41(11):1060-7. doi: 10.1016/j.jdent.2013.08.015.

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