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# Effect of Hydrogen Peroxide at 35% on the Morphology of Enamel and Interference in the De-remineralization Process: An *In Situ* Study

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### Clinical Relevance

The 35% hydrogen peroxide bleaching gel potentiated the structural and histomorphological changes induced by the accumulation of biofilm on the enamel surface.

## SUMMARY

**This study evaluated the microhardness and histomorphology of bovine enamel when 35% hydrogen peroxide is used. A total of 44 specimens were adapted to removable devices used by 11 individuals subjected to dental caries challenge. A decrease in microhardness was observed for all groups after the cariogenic challenge. Microscopic analysis revealed that fragments subjected to cariogenic challenge associated with bleaching had more intense superficial histologic changes, but the depth of the lesions remained unchanged. It was concluded that 35% hydrogen peroxide enhanced the reduction in hardness and histomorphologic changes in the enamel surface exposed to cariogenic challenge.**

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## INTRODUCTION

Achieving a beautiful and healthy smile is a constant concern of dental professionals and patients. Therefore, cosmetic dental treatments can be decisive in

raising self-esteem and are increasingly requested by patients and stimulated by the media, which has given emphasis to health being associated with beauty.[1] In this context, the presence of dental discoloration, whether intrinsic or extrinsic, has stimulated the development and improvement of bleaching techniques.[2,3]

Among the most common products for tooth bleaching are those based on hydrogen peroxide at 35%, which should be applied in dental offices under the supervision of dental professionals.[4]

Regardless of the technique or product used, the mechanism of action of bleaching agents is based on a complex oxidation process with release of reactive forms of oxygen, which penetrate through the pores of enamel rods and reach the dentin, causing the breakdown of organic molecules and resulting in lighter, smaller, and clearer compounds.[5]

Despite the good clinical results of bleaching techniques, their usage has been associated with decreased wear resistance of enamel and dentin, an increase in surface roughness, a decrease in microhardness, and major histomorphologic changes.[2,4] However, the wide variation in dosages used in bleaching treatments, along with the fact that most studies are carried out *in vitro,* makes it difficult to compare results and to extrapolate clinical conditions in which the teeth are continuously subjected to demineralization and remineralization cycles.

*In situ* studies represent an intermediate stage between laboratory experiments and clinical trials.[6] These are attempts to reproduce the process to be studied under the influence of biological factors, such as the protective effect of saliva.[6] Therefore, intraoral models allow some clinical reality, while preserving the sensitivity and accuracy of laboratory models, because the analysis can be performed outside the oral cavity.

Little is known about the effects of bleaching materials on early caries lesions because lesion diagnosis is difficult or uninterpretable by most dentists. Moreover, in an era when esthetics is of great value for people, many professional bleaching centers in malls may practice bleaching techniques without ensuring the adequacy of the oral environment. So, there is a need to know the effects of hydrogen peroxide in high concentrations on the microhardness and histomorphology of demineralized enamel.

Thus, because of the limited number of studies on the effects of bleaching agents on incipient lesions, it is important to evaluate the structural and morphologic aspects of enamel after the application of 35% hydrogen peroxide to demineralized enamel by introducing an intense cariogenic challenge. The null hypothesis tested was that 35% hydrogen peroxide would not affect the structural features and histomorphology of the enamel surface when subjected to cariogenic challenge.

## MATERIALS AND METHODS

### Preparation and Selection of Samples

This study was approved by the Ethics in Research Board (P. 2008-01237). Eleven volunteers between 19 and 26 years of age, who were aware of the experiment through the "letter informing the volunteer" and who signed a "term of consent," were selected After receiving all relevant information concerning the research, volunteers answered the health history questionnaire and were clinically examined for criteria of exclusion (Table 1).

Once selected, volunteers received a kit containing a toothbrush (Indicator Plus, Oral B, São Paulo, Brazil), a fluoridated toothpaste (Pro-Health, Oral B), dental floss (Essential Floss, Oral B), a vial with 20% sucrose, a case to store the unit during meals, and a list of guidelines.

Experimental units were obtained from the teeth of slaughtered cattle aged approximately 30 months.

Table 1: *Exclusion Criteria Applied to Select Volunteers for This Research*

| EXCLUSION CRITERIA |
|---|
| Pregnant or nursing volunteers |
| Smoking volunteers |
| Volunteers with fixed or removable prostheses |
| Volunteers with braces |
| Volunteers with caries activities |
| Volunteers with periodontal disease |
| Take drugs that affect salivary flow (Antidepressants, narcotics and diuretics) |
| Presence of digestive disorders |
| Unable to attend pre-booked appointments for the laboratory procedures |

The teeth were cleaned and cut into sections, resulting in 100 pieces measuring 4 × 4 × 2 mm. The fragments were ground flat and polished on a polishing unit (Aropol and Arotec SA Industria e Comercio Ltda, Cotia, Sao Paulo, Brazil) with the use of aluminum oxide sandpaper in grits of 600, 800, and 1200, at low speed under water cooling. Final polishing was performed with felt disks soaked in 1 μm diamond paste (Arotec SA Industria e Comercio Ltda) for five minutes. Between changes of sandpaper, the fragments were cleaned in an ultrasonic tank with distilled water (Branson 2200® **ultrasonic** cleaner, Shelton, CT, USA) to remove debris left by the sandpaper on the enamel surface.

The 100 polished fragments, none of which were found to have cracks, were subjected to initial readings of microhardness and received three indentations in the central region of each fragment with a Knoop-type indenter (HMV-2000, Shimadzu, Columbia, MD, USA) with a static load of 25 g for five seconds. Microhardness values were used to select 44 enamel blocks. For this, the general average was calculated for the 100 blocks (246), and extreme values (above and below this average) were excluded.

### Making of Intraoral Palatal Devices

After preparation and selection of enamel blocks, the manufacture of intraoral palatal devices (IPDs) was started by molding the upper arch of each volunteer with alginate (Avagel, Dentsply Industria e Comercio Ltda, Petropolis, Rio de Janeiro, Brazil) and casting in dental stone (Durone IV, Dentsply Indústria e Comércio Ltda). Later, the palatal devices were made of acrylic resin containing four 4 × 4 × 3 mm niches, two on each side, which served to fix the blocks of bovine enamel.

Specimens were arranged in the device to avoid interference from site-specific factors. Thus, two blocks were placed in the anterior region of the device: one exposed to hydrogen peroxide at 35%, and one, a control, the same in the posterior region. Specimens were fixed on the intraoral device with sticky wax (Kota Industrial e Comercio Ltda, Sao Paulo, Brazil), positioned 1 mm below the surface of the resin. To enhance the accumulation of biofilm on the enamel samples, a polyethylene screen was fixed on the acrylic covering the specimens.

### Treatment of Samples

Volunteers used the IPD for seven days for initial formation of the biofilm; subsequently they were instructed to apply a drop of 20% sucrose solution on the enamel, eight times a day, every two hours. After the dripping, the device remained at rest for five minutes before returning to the oral cavity, allowing the sucrose to spread on the biofilm. The first daily exposure to sucrose was at 8 AM and the last at 10 PM. A new sucrose solution was prepared every two days.

After seven days, samples were carefully removed from the IPD and were submitted to 35% hydrogen peroxide bleaching gel (Whiteness HP Maxx, FGM Produtos Odontológicos Ltda, Santa Catarina, Brazil). The product was handled according to the manufacturer's specifications (three drops of peroxide for each drop of thickener), and 0.06 ml of bleach was applied to each fragment, remaining in contact with the enamel for 15 minutes. This procedure was repeated three times in each session. Then, the samples were washed with distilled-deionized water to waste from the gel, carefully dried with absorbent paper, and placed back into their niches. Three bleaching sessions were held with seven days between sessions. Afterward, the samples were removed and were kept in deionized water with daily changes for seven days.

### Knoop Microhardness

After the experimental procedures had been performed, the samples were subjected to a final enamel surface microhardness test. Three new indentations were made in the center of the sample, 100 μm away from the site of the initial microhardness reading. The average of the three indentations was used for statistical analysis.

The Knoop microhardness values were transformed into percentage of loss of hardness (% LH) obtained by the following formula:

$$\%LH = 1 - KHN(I) - KHN(F)/KHN(I) \times 100$$

where KHN(I) is the average of initial microhardness of the group, and KHN(F) is the average of final microhardness. The results obtained were submitted to analysis of variance at 5% (repeated measures ANOVA).

### Scanning Electron Microscopy (SEM)

Twenty-two samples (11 in the control group and 11 in the bleached group) were fixed with double-sided carbon tape (Electron Microscopy Sciences 19034, Washington, DC, USA) on stubs and were sputter-coated with gold, using the vacuum camera (Balzers SCD 050 Sputter Coater, Balzers Aktiengesellschaft Union, Fürstentum, Liechtenstein, Germany), at a current of 45 mA for 160 seconds. Evaluation was

performed using a scanning electron microscope (JSM 5600, Joel Inc, Peabody, MA, USA) with magnifications of 1000× and 3000×, taking into account the enamel surface morphology. Representative areas were photographed for benchmarking.

### Polarized Light Microscopy (PLM)

The remaining 22 samples were analyzed by PLM. They were longitudinally sectioned with double-faced diamond disks (7020 KG Sorensen Ind e Com Ltda, Barueri, SP, Brazil) to obtain two samples of each block of enamel. The samples were polished with aluminum oxide sandpaper in grits of 600 and 1200 and at low speed and were water cooled to obtain slices that were approximately 100 μm thick.

The specimens were placed on glass slides, immersed in distilled water, and covered with a coverslip. Analysis was performed using a polarized light microscope (Axiophot Zeiss DSM-940 A, Oberkochen, Germany) at magnifications of 25× and 100×. The images, taken by a digital camera attached to the microscope, were analyzed as to the shape and depth of lesions using AxioVision 4 software (Zeiss, Oberkochen, Germany).

## RESULTS

Mean and standard deviation values of microhardness before and after bleaching are shown in Table 2. Results show that specimens of the control group and those of the bleaching treatment group had similar microhardness values in the initial period ($p$=0.7871). However, at the end of the experiment, a general decrease was seen in microhardness values, which was especially more significant in the bleached group ($p$=0.0069).

Statistical analyses of the data showed that the control group had a reduction of 19.2% for microhardness (submitted to cariogenic challenge), and the bleached group (submitted to cariogenic challenge and bleaching) had a reduction of 24.9% for microhardness.

PLM analysis showed that both the bleached group (Figures 1 and 2) and the control group

Table 2: *Mean (Standard Deviation) of Knoop Hardness (KHN) Before and After Bleaching Treatment According to Experimental Groups**

| Group | Mean (SD) | |
|---|---|---|
| | Initial | Final |
| Control | 237.3 (17.9)$^{Aa}$ | 191.7 (15.8)$^{Ba}$ |
| Bleached | 238.9 (21.3)$^{Aa}$ | 179.4 (12.5)$^{Bb}$ |

* *Capital letters compare columns; lowercase letters compare lines.*

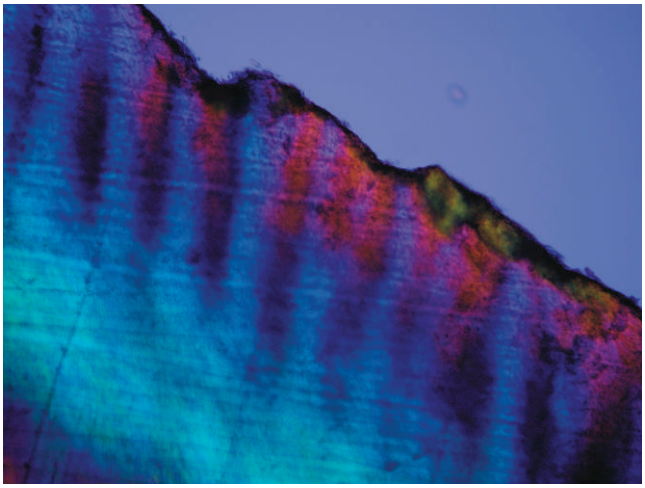

Figure 1. *Image from polarized light microscopy of the bleached group in a cut where the cavitated region represented by a more brownish region in the image can be clinically seen (100×).*

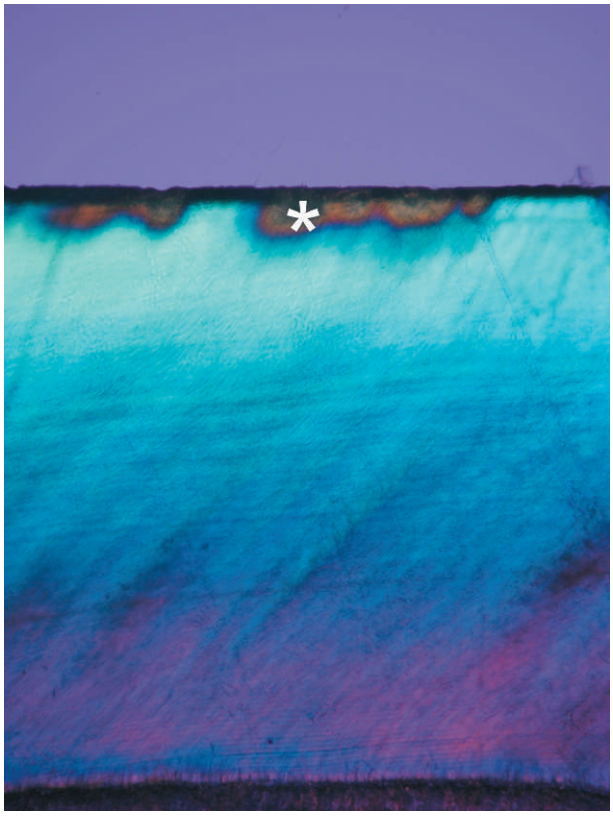


Figure 2. *Image from polarized light microscopy of the bleached group, showing an intense birefringence difference in enamel. The asterisk (*) shows areas of enamel demineralization (100×).*

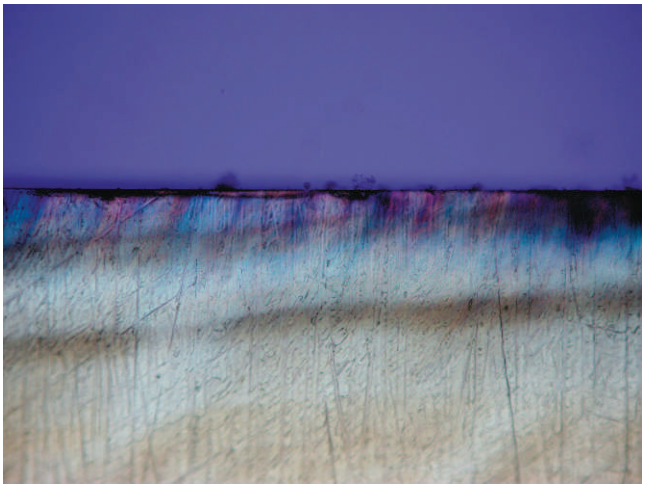

Figure 3. *Image from polarized light microscopy of a cut in the control group, with areas of subsurface demineralization (darkened region) (100×).*

(Figures 3 and 4) had areas of demineralization in all specimens, represented by regions of negative birefringence. This proves the effectiveness of the cariogenic challenge. However, it is worthwhile to

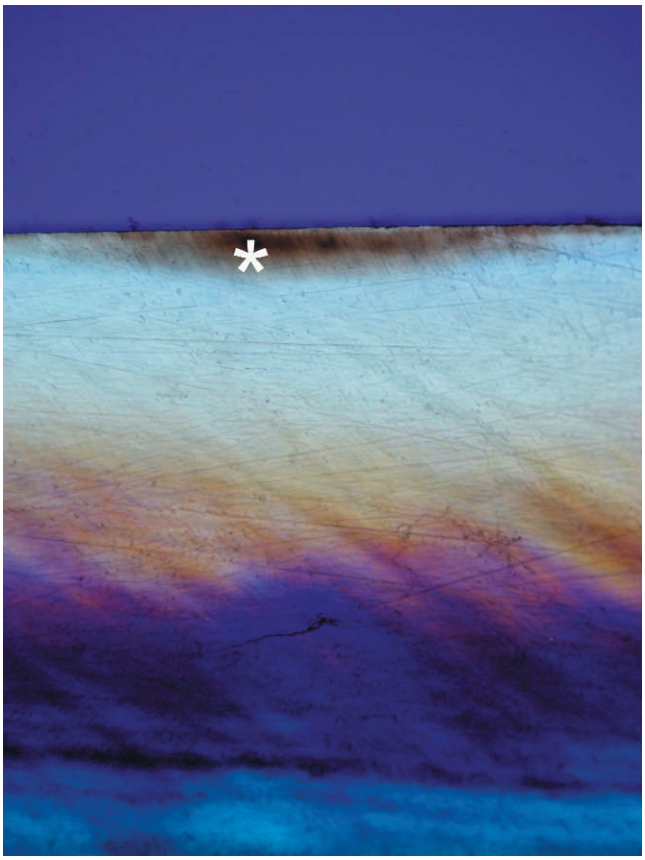


Figure 4. *Image from polarized light microscopy of a specimen of the control group, showing isolated areas of demineralization (darkened region). The asterisk (*) shows area of enamel demineralization (100×).*

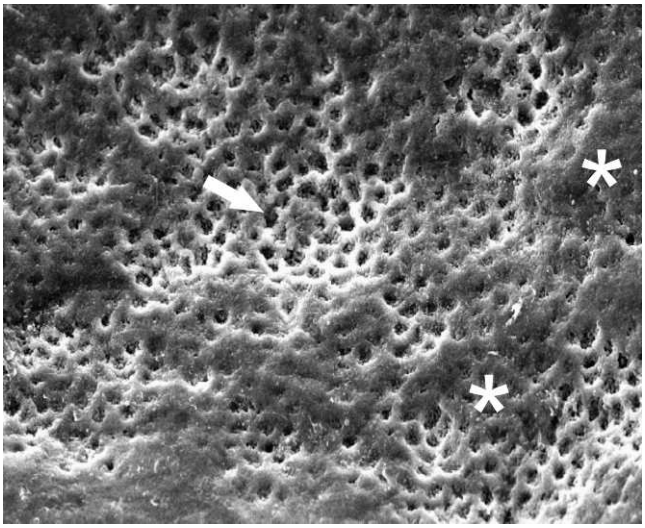


Figure 5. *Photomicrograph of a specimen of the control group showing areas of aprismatic layer removal and exposure of the prisms (→) and better conserved regions (*) (1000×).*

emphasize that histologic changes in the bleached enamel were sharper and reached most of the surface, causing cavitation in some specimens. The depth of demineralization varied from 58.85 μm to 103.52 μm in the control group (average, 74.89 μm) and from 63.71 μm to 132.06 μm in the bleached group (average, 88.13 μm). Surface analysis in scanning electron microscopy showed that the control group (Figures 5 and 6) and the bleached group (Figures 7 and 8) exhibited surface morphologic changes similar to dissolution of the central region of the prisms and increased surface porosity. Greater removal of the aprismatic layer was noted in the group exposed to hydrogen peroxide.

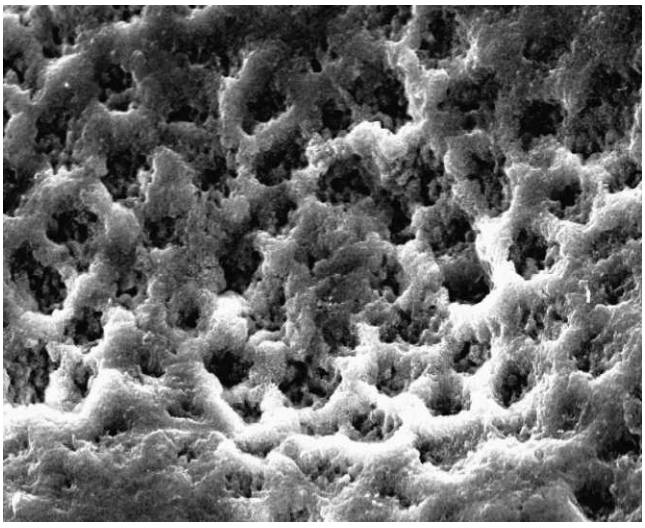

Figure 6. *Detail of morphologic changes. Removal of the aprismatic layer and removal of the center of the prisms can be seen (3000×).*

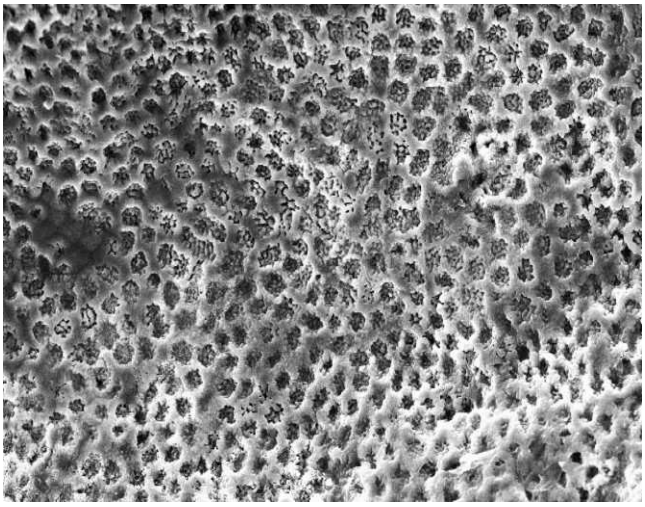

Figure 7. *Photomicrograph of a specimen of the bleached group, showing morphologic changes that are more homogeneous than in the control group and present on the whole surface (1000×).*

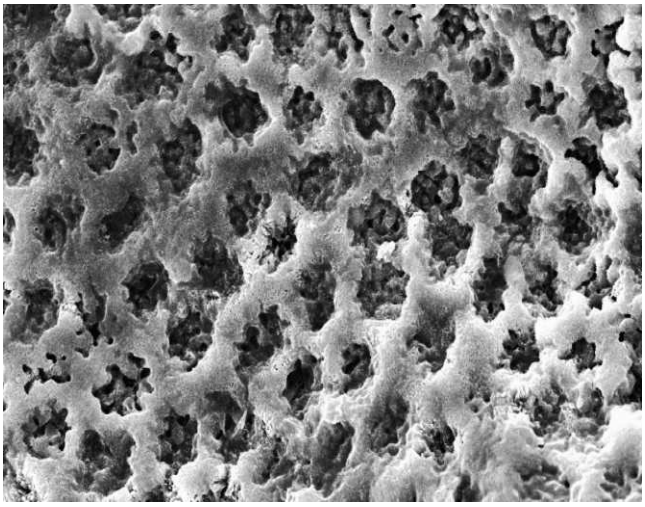

Figure 8. *Detail of morphologic changes observed in the bleached group. Despite the greater presence of demineralized areas, the pattern of changes was similar to that in the control group (3000×).*

## DISCUSSION

*In situ* models provide conditions of the oral cavity and allow all factors related to the development of carious lesions to be present in the experiment. Thus, the dental substrate, the formation of plaque with cariogenic potential, the presence of carbohydrates, and the time are properly valued in the results.[6,7]

The accumulation of biofilm was achieved by fixing the specimens 1 mm below the level of the site, and by covering the sites with a plastic screen that allowed the formation of a uniform and thick bacterial plaque possessing great demineralization ability, consistent with regions of high accumulation of biofilm. This was associated with frequent dripping of 20% sucrose solution, which has been effective for the formation of carious lesions.[6,8–10]

Current results show that bleached and control groups experienced a significant decrease in microhardness, which was most pronounced in the bleached group. It is believed that in the control group, frequent exposure to sucrose caused a change in the microbial composition of the plaque, selecting for acidogenic and aciduria microorganisms; this brought the pH below the critical level, causing demineralization and consequently a reduction in microhardness.[11] Aires and others[8] showed that *Streptococcus mutans,* in the presence of sucrose, produce large quantities of extracellular polysaccharides, which are responsible for the increase in cariogenicity of the plaque.

SEM analysis showed that specimens from the control group had surface features similar to those observed in the bleached group, which were the surface changes described by Silverstone,[12] who reported the type 1 conditioning pattern with dissolution of the center of enamel prisms. This suggests that biofilm formed in the presence of 20% sucrose produces micromorphologic damage as great as, or more significant than, that caused by hydrogen peroxide. However, it is noteworthy that changes in the control group reached a smaller surface area, and that this most likely occurred as a result of the noninfluence of products from degradation of hydrogen peroxide.[10,11,13–15]

PLM analysis targeted primarily the qualitative evaluation of mineral loss or gain, allowing the ability to analyze the specimen in depth and to identify incipient enamel lesions.[9,16] Specimens from the control group had isolated areas of superficial demineralization that were less intense, but had a depth of lesion similar to that of bleached specimens. This emphasizes the effectiveness of the cariogenic challenge and the surface demineralizing action of peroxide. It is believed that the presence of highly reactive oxygen free radicals and prolonged contact with the bleaching agent of high concentration and low pH[17] are responsible for the increased sensitivity of the substrate to cariogenic challenge.

Because of close contact of the bleaching agent with the tooth structure, microscopic changes in the enamel surface have been reported. The acidic pH of bleaching agents, the time of application, and the composition of products are some of the factors discussed in the literature as being responsible for these superficial changes.[4,18,19]

In this context, Marson and others[20] found that Whiteness HP Maxx ranges in pH from 6 to 5 over time. This fact makes the enamel prone to decalcification, decreasing its microhardness.[21] It is known that peroxides can affect not only the surface but also the interprismatic and intraprismatic portions of the enamel, promoting mild deproteinization. Thus, any mineral element associated with enamel proteins is also removed, which would explain the loss of calcium and phosphorus in these areas, thus contributing to the occurrence of microstructural damage and possibly even to changes in microhardness.[22,23]

The most frequent alterations observed through SEM in the bleached group were removal of the aprismatic layer of enamel, exposure of prisms, and partial dissolution of this structure. These changes are potentially related to the cariogenic challenge and the great reactivity of oxygen.[13] In analysis through polarized light microscopy, surface desmineralization, subsurface carious lesions, and cavitated regions were observed in the bleached group, confirming the potentiated action that hydrogen peroxide exerts on surfaces with an accumulation of cariogenic biofilm.

The noncavitated carious lesions observed through PLM showed a characteristic that is already well documented in the literature[16] in which two distinct zones are quite evident: the surface area represented by a relatively intact layer and the body of the lesion with intense demineralization observed as darkened regions.[12]

Throughout the present study, the specimens remained in the oral cavity in contact with saliva, which was not able to inhibit demineralization. Many studies suggest that the action of saliva may reverse mineral loss,[24,25] because stimulation of the flow of saliva increases the constituents, such as carbonic acid, hydrogen carbonate, hydrogen phosphate, calcium, and fluoride, which are associated with increased buffer capacity and maintenance of the balance between demineralization and remineralization phenomena.[26] However, frequent and substantial accumulation of bacterial plaque prevented direct contact between these constituents and the enamel surface.[27,28]

In this current study, bleaching gel was applied to the enamel before it was submitted to cariogenic challenge. After bleaching treatment on these carious lesions, a statistically significant loss of enamel microhardness occurred. The carious enamel is more susceptible to the demineralization effect of the bleaching gel because it becomes more porous (Figures 5 and 7). The current study simulated a dynamic process of demineralization/remineralization, hypothesizing that saliva would be able to mitigate the effects caused by 35% hydrogen peroxide; however, this effect was not observed. The interval of seven days may not have been sufficient to reverse the effects of the bleaching treatment.

After the bleaching cycle, the samples were removed from the palatal devices and were stored in deionized water for seven days. The hypothesis was that if the samples had remained in the oral environment for this same period of time, demineralization of the bleached samples could have been minimized.

PLM and SEM analyses showed that histomorphologic changes caused by frequent exposure to sucrose were possibly caused by changes in the microbial composition of the plaque and allowed an acid environment that led to demineralization, increased by the unspecific action of oxygen radicals released by the bleaching agent, and also by the low pH that the product presents. Given these findings, the influence of hydrogen peroxide and its action on ionic imbalance with mineral loss of the dental substrate are evident.[17,29]

As a consequence of the current results, the null hypothesis should be rejected because 35% hydrogen peroxide bleaching gel potentiated the structural and histomorphologic changes induced by the accumulation of biofilm on the enamel surface. However, additional studies should be conducted, including the use of other materials and bleaching techniques, to minimize doubts and conclusions on the effects of bleaching healthy or demineralized dental tissue. Only then will application of bleaching agents be proven safe and to have satisfactory cosmetic and biological results.

It is concluded that application of 35% hydrogen peroxide bleaching gel intensified changes in the bovine enamel surface that had been subjected to an *in situ* cariogenic challenge.

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#### Conflict of Interest Declaration

The authors of this manuscript certify that they have no proprietary, financial or other personal interest of any nature or kind in any product, service and/or company that is presented in this article.

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## REFERENCES


1. Alves EA, Alves FK, Campos E, & Mathias P (2007) Susceptibility to carieslike lesion after dental bleaching with different techniques *Quintessence International* **38(7)** 404-409.
2. Basting RT, Rodrigues AL, & Serra MC (2007) Micromorphology and surface roughness of sound and desmineralized enamel and dentin bleaching with a 10% carbamine peroxide bleaching agent *American Journal of Dentistry* **20(2)** 97-102.
3. Cavalli V, Arrais CA, Giannini M, & Ambrosano GM (2004) High-concentrated carbamide peroxide bleaching agents effects on enamel surface *Journal of Oral Rehabilitation* **31(2)** 155-159.
4. Ernst CP, Briseño M, & Zönnchen BM (1996) Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel *Quintessence International* **27(1)** 53-56.
5. Tredwin CJ, Naik S, Lewis NJ, & Scully C (2006) Hydrogen peroxide tooth-whitening (bleaching) products: Review of adverse effects and safety issues *British Dental Journal* **200(7)** 371-376.
6. Zero DT, & Lussi A (1995) In situ caries models *Advance in Dental Research* **9(3)** 214-230.
7. Featherstoni JDB, & Zero DT (1992) In situ model for simultaneous assessment of inhibition of demineralization and enhancement of remineralization *Journal of Dental Research* **71(4)** 804-810.
8. Aires CP, Tabchoury CPM, Del Bel Cury AA, Koo H, & Cury JA (2006) Effect of sucrose concentration on dental biofilm formed in situ and on enamel desmineralization *Caries Research* **40(1)** 28-32.
9. Paes Leme A, Dalcico R, Tabchoury CPM, Del Bel Cury A, Rosalen P, & Cury JA (2004) In situ effect of frequent sucrose exposure on enamel desmineralization and on plaque composition after APF application and F dentifrice use *Journal of Dental Research* **83(1)** 71-75.
10. Cury JA, Marques AS, Tabchoury CPM, & Del Bel Cury A (2003) Composition of dental plaque formed in the presence of sucrose and after its interruption *Brazilian Dental Journal* **14(3)** 147-52.
11. Gao XJ, Fron Y, Kent RL Jr, Van Houte J, & Margolis HC (2001) Association of caries activity with the composition of dental plaque fluid *Journal of Dental Research* **80(9)** 1834-1839.
12. Silverstone LM (1975) Variation in the pattern of acid etching of human dental enamel examined by scanning microscopy *Caries Research* **9(5)** 373-387.
13. Fu B, Hoth-Hanning W, & Hannig M (2007) Effects of dental bleaching on micro and nano-morphological alterations of the enamel surface *American Journal of Dentistry* **20(1)** 35-40.
14. Pinto CF, Oliveira R, Cavalli V, & Gianinni M (2004) Peroxide bleaching agent effect on enamel surface microhardness of human enamel *Brazilian Dental Journal* **18(4)** 306-311.
15. Spalding M, Taveira LAA, & Assis GF (2003) Scanning electron microscopy study of dental enamel surface exposed to 35% hydrogen peroxide: Alone, with saliva, and with 10% carbamide peroxide *Journal of Esthetic and Restorative Dentistry* **15(3)** 154-165.
16. Arnold WH, Sonkol T, Zoellner A, & Gaengler P (2007) Comparative study of in vitro caries-like lesion and natural caries lesion at crown margins *Journal Prosthodontics* **16(6)** 445-451.
17. Robinson C, Shore RC, Brookes SJ, Strafford S, Wood SR, & Kirkham J (2000) The chemistry of enamel caries *Critical Reviews in Oral Biology and Medicine* **11(4)** 481-495.
18. Smidt A, Weller D, & Roman I (1998) Effect of bleaching agent on microhardness and surface morphology of tooth enamel *American Journal of Dentistry* **11(2)** 83-85.
19. Ben-Amar A, Liberman R, Gorfil C, & Bernstein Y (1995) Effect of mouthguard bleaching on enamel surface *American Journal of Dentistry* **8(1)** 29-32.
20. Marson FC, Sensi LG, & Reis R (2008) Novo conceito de clareação dentária pela técnica no consultório *Revista Dental Press Estética Maringa* **5(3)** 55-66.
21. Lee KH, Kim HI, Kim KH, & Kwon YH (2006) Mineral loss from bovine enamel by a 30% hydrogen peroxide solution *Journal of Oral Rehabilitation* **33(3)** 229-233.
22. Justino LM, Tames DR, & Demarco FF (2004) In situ and in vitro effects of bleaching with carbamide peroxide on human enamel *Operative Dentistry* **29(2)** 219-225.
23. Park HJ, Kwon TY, Nam SH, Kim HJ, Kim KH, & Kim YJ (2004) Change in bovine after treatment with a 30% hydrogen peroxide bleaching agent *Dental Materials Journal* **23(4)** 517-521.
24. De Oliveira RO, Basting RT, Rodrigues JA, Rodrigues AL Jr, & Serra MC (2003) Effect of a carbamide peroxide agent and desensitizing dentifrices on enamel microhardness *American Journal of Dentistry* **16(1)** 42-46.
25. Akal N (2001) Effect of carbamide peroxide containing bleaching agents on the morphology and subsurface hardness of enamel *The Journal of Clinical Pediatric Dentistry* **25(4)** 293-296.
26. Dods MW, Johnson DA, & Yeh CK (2005) Health benefits of saliva: A review. *Journal of Dentistry* **33(3)** 223-233.
27. Dawes C, & Kubieniec K (2004) The effect of prolonged gum chewing on salivary flow rate and composition *Archives of Oral Biology* **49(8)** 665-669.
28. Bardow A, Madsen J, & Nountofte B (2000) The bicarbonate concentration in human saliva does not exceed the plasma level under normal physiological conditions *Clinical Oral Investigations* **4(4)** 306-312.
29. Bollet-Quivogne FRG, Anderson P, Dowker SEP, & Elliot JC (2007) Desmineralization of permeable with alternative water and acidic buffer: Scanning microradiographic study of effect of switching period *Caries Research* **41(2)** 152-160.