

MICROHARDNESS OF THE ENAMEL EXPOSED TO WHITENING DENTIFRICES.

ANÁLISE DA MICRODUREZA EM ESMALTE BOVINO EXPOSTO A DENTIFRÍCIOS CLAREADORES.

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ABSTRACT

Introduction: The purpose of this study is to verify the effect of three different types of dentifrices on the superficial microhardness of bovine enamel. **Methods:** Forty-eight 4x4mm dental fragments were polished and randomly divided into 4 groups: GI, conventional silica-based dentifrice; GII, hydrogen peroxide-based dentifrice; GIII, carbamide peroxide-based dentifrice; and GIV, immersion in artificial saliva. After polished, the specimens received five indentations of 25g static load, for 5 seconds. Subsequently, specimens from groups GI, GII and GIII were immersed in solution containing dentifrice and distilled water, in weight proportion of 1:2, for 15 minutes daily. After this period, fragments were rinsed in tap water and stored in artificial saliva at 37°C. This procedure was repeated for 21 days and then a new analysis of the microhardness was performed. **Results and conclusion:** The results were submitted to ANOVA and Fisher's test at 5%. It was concluded that all samples treated with dentifrices showed hardness decrease, being most pronounced in dentifrices containing peroxide.

DESCRIPTORS: Dentifrices • Dental enamel • Peroxides.

RESUMO

Introdução: O objetivo deste estudo é verificar o efeito de dentifrícios à base de peróxido de carbamida, peróxido de hidrogênio e um convencional rico em sílica, na microdureza superficial do esmalte bovino. **Métodos:** Para tanto, 48 fragmentos dentais de 4 x 4mm foram polidos, realizada então a leitura inicial da microdureza, e divididos aleatoriamente em 4 grupos: GI - dentifrício convencional; GII - dentifrício contendo peróxido de hidrogênio; GIII - dentifrício contendo peróxido de carbamida e GIV - imersão em saliva artificial. Após o polimento, os espécimes receberam 5 indentações de carga estática de 25 gramas, por 5s. Após essa fase, os espécimes dos grupos GI, GII e GIII foram imersos em suspensão contendo dentifrício e água destilada, na proporção de 1:2 em peso, durante 15 minutos diários. Decorrido este período, os fragmentos foram lavados com água corrente e armazenados em saliva artificial a 37°C. Esse tratamento foi repetido por 21 dias e, após esse período, foi realizada a análise final da microdureza. **Resultados e conclusão:** Os resultados foram submetidos aos testes estatísticos ANOVA e teste de Fisher a 5%. Concluiu-se que todas as amostras tratadas com dentifrícios sofreram diminuição na dureza, sendo esta mais pronunciada nos dentifrícios contendo peróxido.

DESCRIPTORIOS: Dentifrícios • Esmalte dentário • Peróxidos.

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INTRODUCTION

Dental aesthetics is a constant concern for both professionals and patients. For this reason, the presence of dental color changes, either extrinsic or intrinsic has motivated the development and improvement of microabrasion (Hegedus *et al.*¹, 1999, Casals *et al.*², 2007, Correa *et al.*³, 2007), as well as whitening techniques (Correa *et al.*³, 2007, Croll⁴, 1992, Gerlach *et al.*⁵, 2002, Haywood *et al.*⁶, 1994).

The aesthetic results using dental bleaching techniques allowed the development of new bleaching products, including toothpaste based on carbamide peroxide and hydrogen peroxide (Hegedus *et al.*¹, 1999, Croll⁴, 1992, Gerlach *et al.*⁷, 2002, Crews *et al.*⁸, 1997) rich in polishing agents, seeking chemical and mechanical removal of dental staining (Sasaki *et al.*⁹, 2007, Haywood¹⁰, 1992).

It is worth highlighting that although certain dentifrices promote the proposed whitening (Howard¹¹, 1992, Ernst *et al.*¹², 1996, Garcia-Godoy *et al.*¹³, 2004), they may cause high levels of abrasion, roughness and dental weariness (White¹⁴, 2001, Mankodi *et al.*¹⁵, 1999). These effects are common because whitening dentifrices are easily accessed by patients, and may be used without appropriate instructions, follow-up or awareness of the professional.

The literature reports several initial unwanted effects that highly-concentrated bleaching agents cause to the enamel structure, suggesting demineralization and hardness decrease (Casals *et al.*², 2007, Faraoni-Romano *et al.*¹⁶, 2007). Thus, the understanding of the microhardness of a dental structure exposed to any oxidizing agent is of fundamental importance to conduct even the most innocuous treatments and obtain the bleaching effect without affecting the dental integrity.

Therefore, this study analyzed the *in vitro* effect of carbamide-and hydrogen peroxide-based dentifrices and a conventional rich-in-silica dentifrice on the superficial microhardness of bovine enamel. The null hypothesis tested is that there is no hardness difference between enamels exposed to dentifrices containing carba-

mide peroxide or hydrogen peroxide.

MATERIAL AND METHODS

Forty-eight recently-extracted healthy bovine incisors were used. After removal, the teeth were cleaned with periodontal cures (Duflex Ltda. Rio de Janeiro, Rio de Janeiro, Brazil), polished with pumice and water using a Robinson bristle brush (KG Sorensen Ind. e Com. Ltda, Barueri, SP, Brazil). The teeth were then stored in a 0.1% timol solution until use.

The roots and dental pulp remnants were removed using a metallographic cutter (Isomet 2000 - Buehler, Lake Bluff, Illinois, USA). Subsequently, the crowns were analyzed in stereomicroscope (Carl Zeiss Company - DSM-940 A, Oberkochen, Baden- Württemberg, Germany) and only that the ones showed no cracks or other structural defects were selected. Dental fragments measuring 4x4mm were embedded in an acrylic resin base using an embedding machine (PRE-30S, Arotec S.A. Indústria e Comercio Ltda, Cotia, São Paulo, Brazil). The specimens were flattened and polished using a rotation machine (Aropol E, Arotec S.A. Industria e Comércio Ltda, Cotia, São Paulo, Brazil) and sandpaper #600, #800 and #1200 granulation, for 2 minutes each. The finishing was done using felt disc and 1µm polishing diamond paste (Arotec S.A. Indústria e Comercio Ltda, Cotia, São Paulo, Brazil), for 5 minutes.

Subsequently, the specimens were randomly divided into four study groups (n=12) and received the treatments presented in Table 1.

All samples were initially cleaned with distilled water in an ultrasonic cleaner (BRANSON 2210, Danbury, Connecticut, USA), submitted to initial microhardness measurements by means of a microhardness tester (HMV-2000 SHIMADZU, Columbia, Maryland, USA), and a Knoop indenter under a static load of 25 g applied for 5 seconds. Each specimen received 5 indentations each time period: baseline (before immersion in dentifrice slurry) and final (after treatment).

According to the values obtained, each group was exposed to a different type of

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Table 1. List of experimental groups according to the characteristics of each product, brand names, manufacturers and ingredients.

Groups	Dentifrice type	Dentifrice brand name	Manufacturer	Ingredients
I	Silica-based dentifrice	Colgate	Colgate-Palmolive Company	Calcium carbonate, Water, Sorbitol, Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Flavour, Cellulose Gum, Tetrasodium Pyrophosphate, Methylparaben and Sodium Silicate
II	Hydrogen peroxide-based dentifrice	Colgate Simply White Toothpaste	Colgate-Palmolive Co.	Glycerin, hydrated Silica, PEG-12, Sodium Lauryl Sulfate, Flavor, Hydrogen Peroxide, Sodium Hydroxide, Cellulose, Sodium Saccharin, EDTA, Propylparaben, Tetrasodium Pyrophosphate, Titanium Dioxide.
III	Carbamide peroxide-based dentifrice	Rembrandt Plus™	Gillette Company, Boston, MA, USA	Sodium Monofluorophosphate, Glycerin, Silicon, Carbamide Peroxide, Alumina, Acidulated Amylopectin, Flavor, Sodium Citrate, Papain, Sodium Saccharin, Sodium Lauryl Sulfate and EDTA
IV	Artificial saliva	—	Aphoticario Manipulation Pharmacy, SP, Brazil	1.5 mmol/l Ca 50 mmol/l KCl 0.9 mmol/l PO ₄ 20 mmol/l TRIS (tri-hydroxymethyl-aminomethane) buffer, pH 7.0

treatment. Dentifrices were applied in the form of oral suspension, obtained by mixing them with distilled water in the weight proportion of 1:2, using 18 ml distilled water to 9g dentifrice (da Silva *et al.*¹⁷, 2007). After treatment accomplishment, the pH of toothpaste samples were measured using the pHmeter Analyser (2A14-KA, São Paulo, SP, Brazil). For this purpose, suspension samples were obtained in a proportion of 1:2 and three measurements in each dentifrice sample were performed in a 10-minute interval between them, so that the pH was confirmed.

The fragments were in contact with the suspension for 15 minutes daily, simulating the time spent in 3 brushings. Following, the fragments were washed in tap water and stored in artificial saliva at 37 oC. This procedure was repeated for 21 days and during this period of time, group IV specimens remained in artificial saliva.

Next, specimens were cleaned with distilled water in an ultrasonic cleaner (BRANSON 2210, Danbury Connecticut, USA) for 10 minutes, dried and submitted to a second knoop microhardness analysis.

The results obtained were submitted to Kolmogorov-Smirnov normality test and analysis of variance (ANOVA) followed by Fisher's PLSD (Protected least significance difference) post test at 5% significance level.

RESULTS

ANOVA test was applied to the microhardness values and significant difference was detected among treatments ($p < 0.0001$). Table 2 shows means and standard deviation of the enamel microhardness obtained after immersion in saliva (control) or in conventional dentifrice slurries, containing hydrogen peroxide (HP) or carbamide peroxide (CP). The





mean obtained for the specimens before the immersion (baseline) was 232.4 (5.1). Fisher's Test showed that the use of any of the studied dentifrices caused significant reduction in the microhardness values ($p < 0.001$). It was also verified that the groups which received carbamide or hydrogen peroxide dentifrices showed similar microhardness results ($p = 0.41$) and these groups had higher reduction of microhardness than the group treated with conventional dentifrice ($p < 0.0001$).

DISCUSSION

At present, abrasive-rich dentifrices or the ones containing bleaching agents can be easily acquired and used by patients who want to get whiter teeth at a lower cost, even knowing that these products offer less intense whitening than in-office or at-home techniques (Perdigao *et al.* 18, 1998). Although dentifrices used daily remain in contact with the teeth for a short period of time, it has been reported that their frequent use may decrease the abrasion resistance of the enamel (Perdigao *et al.*¹⁸, 1998, Kakar *et al.*¹⁹, 2004). In this study, the immersion in dentifrice slurries caused a decrease of the enamel microhardness. On the other hand, enamel samples treated with artificial saliva alone (control group) preserved their hardness, showing values similar to the baseline.

Regarding the conventional dentifrice, the hardness decrease is possibly associated with the acid pH which varied between 5.2 and 5.4, inferior to the critical pH (5.5) for dissolution of enamel (Titley *et al.* 20, 1993). This fact is indirectly minimized through in vivo remineralization by

deposition of calcium fluoride, this fact is indirectly minimized through remineralization in vivo by the deposition of crystals of calcium fluoride from the saliva, which despite being a weak link can auxiliary to reduce the commitment of the surface microhardness structure (Casals *et al.*², 2007, Dawes e Kubieniec²¹, 2004, Dodds *et al.*²², 2005).

In relation to whitening dentifrices, besides the influence of the acid pH, it is believed that the notable decrease of the enamel microhardness may be associated with the action of oxygen free radicals, present both in hydrogen and carbamide peroxides, which may react with organic structures of the dental tissues (da Silva *et al.*¹⁷, 2007, Lima *et al.*²³, 2008). It is worth highlighting that the concentration of hydrogen peroxide found in the Colgate Simply White is 1%, while the Rembrandt Plus dentifrice shows 3% of carbamide peroxide. This last peroxide, when in contact with the dental tissues, decomposes into hydrogen peroxide at approximately 1%, that is, same concentration of hydrogen peroxide as in the first dentifrice (Antonini *et al.*²⁴, 2007).

Another factor which should be taken into account in the formulation of carbamide peroxide-based dentifrices is the presence of sodium citrate, a chelating salt which may react with the calcium present in the enamel and dentin (Ong e Strahan²⁵, 1989). This reaction results in calcium-citrate complex, which causes mineral loss and microhardness decrease (da Silva *et al.*¹⁷, 2007, Perdigao *et al.*¹⁸, 1998). Moreover, the presence of urea may contribute to the denaturation of proteins existing in the spaces interprismatic, contributing to the occurrence of microstructural damage (Perdigao *et al.*¹⁸, 1998, Lima *et al.*²³, 2008).

The EDTA present in the composition of both dentifrices containing peroxide, may also be considered a potential factor in enamel microhardness loss, since this product, when present in high concentrations, acts in dental demineralization. The 17% EDTA is the one responsible for the chelation of calcium ions in enamel structure. Besides the EDTA, the papain, present in the toothpaste Rembrandt Plus™

Table 2. Mean values, standard deviation and statistical analysis (ANOVA and Fisher's test) of the microhardness measurements after immersion in slurry.

Groups	Means (SD)	Statistical decision*
Control	230.1 (9.3)	A
Conventional	125.2 (11.9)	B
HP	106.8 (12.7)	C
CP	103.2 (12.8)	C

*Similar letters represent statistical equality

composition may also have influenced the results, once it acts as a chemical debridement in the chemo mechanical methods of caries removal, while it promotes the removal of protein content by "breaking" collagen molecules (Correa *et al.*³, 2007, Piva *et al.*²⁶, 2008, Niu *et al.*²⁷, 2002).

Consequently, the null hypothesis must be rejected as dentifrices containing bleaching agents affected the enamel microhardness. Despite the reduced period of time (21 days), and the fact that the in vitro investigation limit the study, it can be stated that peroxide-based dentifrices affected the enamel microhardness, and, thus, further longitudinal studies are needed to understand the action of these agents over

time (Sasaki *et al.*⁹, 2007), as well as in situ and clinical studies to analyze the behavior of these dentifrices in the oral environment.

The present in vitro study demonstrated that carbamide- and hydrogen peroxide-based dentifrices, as well as a conventional silica-based dentifrice decreased the superficial microhardness of the bovine enamel. The most significant decrease was observed following peroxide-based dentifrices treatment.

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