Influence of Fluoride Concentration and pH Value of 35% Hydrogen Peroxide on the Hardness, Roughness and Morphology of Bovine Enamel

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ABSTRACT

Aim: The aim of this *in vitro* study was to evaluate the effects of different sodium fluoride (NaF) concentrations and pH values on the Knoop hardness (KHN), surface roughness (SR), and morphology of bovine incisors bleached with 35% hydrogen peroxide (HP).

Materials and methods: Sixty-five bovine incisors were fragmented (5 mm² × 2 mm) and distributed in 5 groups: Control (unbleached), Low NaF/Acidic (35% HP + 1.3% NaF, pH 5.5), Low NaF/Neutral (35% HP + 1.3% NaF, pH 7.0), High NaF/Acidic (35% HP + 2% NaF, pH 5.5), and High NaF/Neutral (35% HP + 2% NaF, pH 7.0). KHN analysis was performed with a microhardness tester under a load of 25 gf for 5 seconds. The average SR was obtained with a rugosimeter. KHN and SR were analyzed before and after treatments. For morphological analysis, specimens were dehydrated and gold-sputtered, and scanning electron micrographs were obtained and analyzed by 3 examiners with a double-blinded technique. KHN and SR results were analyzed by one-way ANOVA and Tukey's test ($p < 0.05$).

Results: Only the Low NaF/Acidic and Low NaF/Neutral groups showed significant differences between the initial and final KHN values. All bleached groups presented significant differences between the initial and final SR values. Among the bleached groups, the least and most morphological changes were shown by the High NaF/Neutral and the Low NaF/Acidic group, respectively.

Conclusion: Treatment with 35% HP and 2% NaF at pH 7.0 promoted the least changes in morphology, hardness and roughness among the bleached groups.

Clinical significance: In-office bleaching with high-concentration HP and 2% NaF at neutral pH promoted the least changes in enamel hardness, SR, and morphology compared to other treatments.

Keywords: Tooth whitening, pH, Fluoride, Hardness, Roughness, Hydrogen peroxide, Scanning electron microscopy.

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INTRODUCTION

Hydrogen peroxide (HP) is an effective bleaching agent for whitening teeth.¹ When applied to the teeth, HP decomposes into unstable nonspecific free radicals,² which break the large pigmented molecules in the enamel into smaller, less-pigmented molecules through a redox process.³ Studies have reported secondary clinical effects in the enamel hardness and morphology after peroxide exposure,⁴⁻⁷ at the enamel surface and subsurface levels.^{8,9} Although, high peroxide concentrations are not thought to cause macroscopic changes to the hard dental tissues, such increased porosity, depressions, or surface irregularities,¹⁰⁻¹² researchers have reported increased roughness and reduced hardness with their use.¹³ The strong oxidizing effect of HP on the organic matrix of the teeth contributes to the enamel changes observed after bleaching.

The pH value of in-office whitening gels also plays an important role in the bleaching process and enamel changes. According to Pinto et al¹² and Hegedüs et al¹⁴ changes to the dental structure are increased by a low pH, which causes subsequent modifications to the mineral composition. Sun et al¹⁵ verified that although neutral and acidic 30% HP solutions had the same bleaching capacity, the acidic solution was more damaging to enamel. Demineralization of the enamel surface may be caused mainly by low pH and not by the action of the peroxide itself.¹⁶ Commercial bleaching agents have different pH values. Bleaching agents with high HP concentrations generally have an acidic pH, favoring HP stability and facilitating the whitening process.¹⁷ Cadenaro et

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al¹⁸ reported that whitening agents with a low pH can cause softening of the hard dental tissue.

Some studies have shown that compared to nonfluoridated gels, fluoridated whitening gels tend to reduce the enamel's susceptibility to erosion/caries and to increase the whitened enamel's mineralization.¹⁹⁻²² Bistey et al⁶ observed a significant reduction in the fluoride content of enamel after HP bleaching. Adding fluoride to bleaching gels has been suggested to reduce the changes that occur in the enamel after whitening and to preserve the maximum resistance of the enamel.¹⁹ However, the optimal fluoride concentration to add to bleaching agents remains to be clarified.

The aim of this *in vitro* study was to evaluate the effects of different sodium fluoride (NaF) concentrations and pH values on the Knoop hardness (KHN), surface roughness (SR), and morphology of bovine incisors bleached with 35% HP. We used bovine enamel for this study because of its similarity to human enamel in terms of hardness and composition.^{23,24} The null hypothesis was that the concentration of NaF and the pH of high-concentration HP would not promote changes in the hardness, roughness, or morphology of the enamel.

MATERIALS AND METHODS

Specimen Preparation

The study was approved by the Research Ethics Committee for Animal Experimentation of the Federal University of Pará (CEPAE-UFPA) as case N° 110-13. Sixty-five bovine incisors (Cooperativa da Indústria Pecuária do Pará LTDA, Maguari, Belém, Pará, Brazil) without cracks, stains, or any other defect to the enamel were used for this study. Each tooth was sectioned transversally on the crown/root edge across the amelocemental junction. The crowns were separated, and the roots were discarded. The vestibular surfaces were cut out with a double-sided steel disk (KG Sorensen, model 18002.7020, Cotia, Brazil) attached to a low-speed micromotor, under cooled water, to form fragments with an area of 25 mm² and thickness of 2 mm. The fragment dimensions were standardized and measured with a digital pachymeter. The cut-out portion always corresponded to the central area of the vestibular surface of the dental crown, so as to obtain enamel prisms with the same angles.

Surfaces of the specimens were planed with a polisher (APL-4 AROTEC Ltda, São Paulo, Brazil) by using wet sandpaper with granulations of 600, 1200 and 2000 (3M Brazil, Sumaré, Brazil). Samples used for KHN and SR analyses were planed and polished with a felt disk (Diamond Flex, FGM, Joinville, Brazil) attached to a micromotor, together with diamond polishing paste (Diamond Excel,

FGM, Joinville, Brazil). Specimens used for scanning electron microscopy (SEM) analyses were not planed; their surfaces were maintained whole.

Experimental Groups

Specimens were divided into groups according to the NaF concentration and pH value of the 35% HP used (Table 1). Each group contained 13 fragments, including 10 with planed and 3 with unplaned surfaces. The 10 planed fragments were used to analyze SR, with 5 of these also being used for the KHN test. The remaining 3 unplaned fragments from each group were used for SEM.

Bleaching Procedure

Bleaching agents were obtained from the A Fórmula pharmacy (Belém, Pará, Brazil) (Table 1). Three applications of bleaching agent were applied to the enamel for 15 minutes each, with an interval of 7 days between applications, over a 3-week period. After each bleaching session, specimens were washed in running water. The surfaces were polished with a felt disk and diamond polish attached to a low-speed hand piece. The specimens were stored in artificial saliva (219 mg sodium bicarbonate, 12.5 mg magnesium chloride, 82 mg potassium chloride, 10 mg nipasol, 0.8 mg carboxymethyl, 127 mg potassium phosphate, 44.1 mg calcium chloride, 0.45 mg NaF, 2.4 mg sorbitol and 100 ml distilled water), which was replaced daily, in a biological incubator at 37 °C.

Knoop Hardness

Five specimens from each group were tested for KHN. Five indentations, separated by 100 µm, were made on each specimen by using a load of 25 gf for 5 seconds in a microdurometer (FM-700, Future Tech Corp., Tokyo, Japan). Indentations were placed so that it was possible to map the whole area of the specimens (the left lateral, right, top, bottom, and central extremities). KHN was read before (initial) and immediately after (final) the bleaching treatment. The percentage of change in KHN (%KHN) was calculated as: %KHN = [(Final KHN – Initial KHN)/Initial KHN] × 100%.

Table 1: Chemical components, concentration of sodium fluoride (%NaF), and pH of the bleaching agent in each group

Groups (n = 13)	Chemical components	% NaF	pH
G1	Controle (sem tratamento)		
G2	HP 35%; carbopol 2.5%;	1.3	5.5
G3	methylparaben 0.2%;	1.3	7.0
G4	propylene glycol 0.15%;	2	5.5
G5	EDTA 0.1%; distilled water qsp 100%	2	7.0

Surface Roughness

Ten specimens were used for the SR reading. A rugosimeter (Surftest Mitutoyo Sul Americana Ltda, São Paulo, Brazil) was used to measure the SR before and after the whitening procedures. The point of the rugosimeter touched the specimen and explored the central 4 mm, performing 3 diametrically opposite measurements. The average roughness superficial (average RS) was obtained, which had been previously established and calibrated using the machine's own program. The program compensated for (planar or convex) changes in the surface anatomy of the piece, without affecting the roughness results. The average RS is an arithmetic measurement of the roughness deviation profile that is obtained through the arithmetic average of the sum of the absolute values of the roughness deviation profile from the central line along the evaluated path. As a complementary analysis, the percentage change in SR (%ARS) was calculated for each group, as shown in Graph 1 and according to the following formula: $\%ARS = [(Final\ SR - Initial\ SR) / Initial\ SR] \times 100\%$.

Scanning Electron Microscope

Three specimens from each group were dehydrated and gold-sputtered with an EMITECH K550 metallizer. Metallization in this equipment was based on the interaction between a pure-gold target and argon ions (argon gas) at a pressure of 2.10^{-1} mbar and current of 25 mA for 2 minutes and 30 seconds, resulting in the deposit of a film with an average thickness of ± 15 nm. Samples were mounted on aluminum supports of 10 mm in diameter (stubs) by using adhesive tape of carbon metalized with gold. Photomicrographs were obtained in a SEM (LEO, model 1450VP). Secondary electrons were detected at a voltage acceleration of 15 kV and digitally recorded in high resolution, in a 'tiff' format. For

the microstructural analysis, increases of 2000 and 6000 Kx were made, recorded and analyzed by 3 independent examiners using a double-blinded technique.

Statistical Analysis

To analyze the KHN and SR results, the BioEstat 5.0 statistical software was used. To analyze the statistical significance between the initial and final KHN and SR averages between the groups, one-way analysis of variance (ANOVA) and the Tukey test were used, with a 5% significance level.

RESULTS

Knoop Hardness

Table 2 shows the between- and within-group comparisons for the KHN results. Initial KHN averages were similar in all groups. Groups with 1.3% NaF (Low NaF/Acidic and Low NaF/Neutral) showed significant differences between the initial and final KHN averages. The Control group demonstrated the highest final KHN average among the groups. The final KHN average in the Low NaF/Neutral group was similar to those of all other bleached groups. The final KHN average of the Low NaF/Acidic group differed from those of the High NaF/Acidic and High NaF/Neutral groups, which were not significantly different from each other. The Control group showed a gain in %KHN. The Low NaF/Acidic group had the highest %KHN loss, followed by the Low NaF/Neutral, High NaF/Acidic, and High NaF/Neutral groups, respectively (Graph 2).

Surface Roughness

Table 3 shows the comparisons between the initial and final SR averages. All of the groups showed similar initial SR averages and significant differences between the initial and final SR averages. The final SR averages for the bleached

Graph 1: Percent change in average surface roughness after treatment

Graph 2: Percentage change in Knoop hardness (%KHN) after treatment

Table 2: Average (mean $\pm$ SD) Knoop hardness values for each group before (initial) and after (final) treatment

Groups <i>n</i> = 5	Initial	Final
G1 (control)	372.57 $\pm$ 6.44 ^{Aa}	384.21 $\pm$ 4.59 ^{Aa}
G2	358.41 $\pm$ 12.55 ^{Aa}	321.40 $\pm$ 12.93 ^{Bb}
G3	354.82 $\pm$ 9.10 ^{Aa}	334.70 $\pm$ 9.82 ^{BCb}
G4	351.75 $\pm$ 12.00 ^{Aa}	348.02 $\pm$ 7.56 ^{Ca}
G5	353.94 $\pm$ 18.28 ^{Aa}	352.17 $\pm$ 14.21 ^{Ca}

*Groups with the same letter are statistically similar, capital letters on the vertical and lowercase letters on the horizontal

groups were significantly different from the final SR average of the Control group. There were no significant differences between the Low NaF/Neutral and High NaF/Neutral groups, although they differed from the other groups.

Morphological Analysis

The enamel for the control group was smooth and whole (Fig. 1). In contrast, the bleached groups showed morphological changes, characterized by the partial removal of the aprismatic bed, depressions, porosities, and surface irregularities of different degrees of severity (Figs 2 to 5). Of the treated groups, the High NaF/Neutral group showed the fewest morphological changes (Fig. 5).

DISCUSSION

Nonbleached control specimens stored in artificial saliva demonstrated an increased average KHN and decreased SR over time, consistent with the remineralizing effect of artificial saliva reported by Borges et al.²⁵ All of the bleached groups showed reduced KHN and increased SR values of the enamel compared to the Control group, consistent with previous studies.^{8,12,14,26} Groups in which 2% NaF was added to the bleaching agent had lower %KHN, independent of their pH values. Therefore, we rejected the null hypothesis that the NaF concentration and pH value of high-concentration

Table 3: Average (mean $\pm$ SD) surface roughness before (initial) and after (final) treatment for each group

Groups	Initial	Final
G1	0.17 $\pm$ 0.01 ^{Aa}	0.17 $\pm$ 0.01 ^{Aa}
G2	0.18 $\pm$ 0.01 ^{Aa}	0.31 $\pm$ 0.03 ^{Bb}
G3	0.17 $\pm$ 0.01 ^{Aa}	0.24 $\pm$ 0.01 ^{Bc}
G4	0.17 $\pm$ 0.01 ^{Aa}	0.27 $\pm$ 0.01 ^{Bd}
G5	0.18 $\pm$ 0.01 ^{Aa}	0.23 $\pm$ 0.01 ^{Bc}

*Groups with the same letter are statistically similar, capital letters on the vertical and lowercase letters on the horizontal

HP would not promote changes to the enamel hardness, roughness and morphology.

Previous studies have demonstrated that fluoride can promote remineralization in dental lesions, increase resistance to acid attacks, inhibit demineralization, and promote an increase in enamel hardness.²⁶⁻³¹ One potential mechanism for these effects is that fluoride may contribute to the repair of microstructural defects through the absorption and precipitation of calcium and phosphate in saliva.³² In this study, the use of 2% NaF with the bleaching agent minimized the decrease in KHN compared to 1.3% NaF, corroborating the findings of previous studies.^{26,31}

Adding 2% NaF to the whitening agent did not prevent the increase in SR in the High NaF/Acidic group. This result may have been due to the low pH (= 5.5) of this group. Groups with neutral pH (= 7.0) had less of an increase in SR compared to the control group, whereas the group with pH 5.5 and 1.3% NaF demonstrated the greatest increase in SR. Borges et al.²⁵ reported that use of an acidic 35% HP solution reduced the microhardness of the enamel compared to bleaching with 35% HP with a neutral pH, corroborating our results. Cadenaro et al.¹⁸ performed an *in situ* study in which they evaluated the roughness caused by 2 in-office whitening agents, 38% HP and 35% carbamide peroxide (CP). The use of 35% CP did not change the SR of the enamel, presumably due to the neutral pH (= 6.5) of CP.

Fig. 1: Photomicrographs of untreated enamel, amplified 2000 and 4000 K $\times$

Fig. 2: Photomicrographs of enamel bleached with 35% HP + 1.3% NaF at 7.0"

Fig. 3: Photomicrographs of enamel bleached with 35% HP + 1.3% NaF at pH 7.0

Fig. 4: Photomicrographs of enamel bleached with 35% HP + 2% NaF at pH 5.5

Previous studies have correlated the changes caused by bleaching with the pH of the bleaching agent.^{25,33-36} Price et al³⁷ reported that higher concentrations of peroxide are associated with more acidic whitening products. Azrak et al³⁸ showed that greater acidity of the bleaching agent was associated with greater damage to the hard dental tissue.

Among the bleached groups, we observed the least morphological changes in the group treated with 2% NaF at pH 7.0. The greatest changes were displayed by the group with 1.3% NaF and pH 5.5, with partial removal of the aprismatic enamel bed, prism exposure and many porosities. The SEM results showed that the NaF concentration and pH of the HP

Fig. 5: Photomicrographs of enamel bleached with 35% HP + 2% NaF at pH 7.0"

influenced the morphological pattern of the enamel, with a higher concentration of NaF and a neutral pH preventing demineralization and bleaching-related changes.

CONCLUSION

Based on the method used in this study, we can conclude that the use of 35% HP with 2% NaF at pH 7.0 promoted the least change to the morphology and to the percentages of KHN and SR of the bovine enamel.

CLINICAL SIGNIFICANCE

In-office whitening with high-concentration HP with added 2% NaF and a neutral pH promoted fewer changes to the hardness, roughness and morphology of bovine enamel.

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