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Effect of Mouth Rinse Treatments on Bleached Enamel Properties, Surface Morphology, and Tooth Color

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Clinical Relevance

Mouth rinses are popular agents used in combination with esthetic treatments. Some mouth rinses containing different active ingredients can affect bleached enamel properties.

SUMMARY

Objective: To evaluate, *in vitro*, the effect of mouth rinse exposure on bleached enamel.

Methods: Enamel/dentin bovine blocks (4×4×2 mm) were bleached with 35% hydrogen peroxide (HP) and were submitted to immersion twice daily for 14 days with different rinses (n=10), including those involving: distilled water (C [control]), 225-ppm NaF (FM, Colgate Plax Classic), essential oil (EM, Listerine Tartar Control), 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening), and 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate® Luminous White). The specimens were stored in a remineralizing solution during all experiments. Analyses of color (ΔE, L*, a*, b*) and roughness (Ra) were performed at the baseline, after HP, and after exposure to the rinse. The cross-sectional microhardness (CSMH) and images by scanning electron microscopy (SEM) were assessed at the end. The data were subjected to analysis of variance (ANOVA) (ΔE), repeated measures ANOVA (Ra), and split-plot ANOVA (CSMH), followed by the Tukey test. The L*, a*, and b* values were analyzed by generalized linear models (α=0.05).

Results: Color changes were not statistically different in the groups. Ra increased in all groups after bleaching; however, it was reestablished in C, FM, and HPM+F and increased in EM after 14 days of the rinse. EM and HPM reduced the CSMH values differing from C and promoted alterations on the enamel surface visualized by SEM.

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Conclusion: The mouth rinses did not affect the whitening efficacy or promote benefits on bleached enamel properties. Moreover, the 1.5% hydrogen peroxide– or essential oil–based mouth rinses affected the bleached enamel properties, promoting an alteration in morphologic surface and mineral loss in depth.

INTRODUCTION

Since the demand for esthetic dentistry has extensively increased recently, treatment protocols for discolored teeth have been developed, with tooth bleaching becoming a common treatment that promotes improvement in the appearance of the smile.[1] Categorically, there are different methods and approaches to whitening treatment:[2] 1) dentist-supervised or at-home bleaching using low-concentration bleaching agents in a high-frequency regimen, 2) in-office or power bleaching, generally applying relatively high-concentration agents on the dental substrate, and 3) over-the-counter (OTC) whitening agents represented by mass market bleaching products containing low concentrations of whitening agents that are self-applied to the teeth via mouthwash, toothpaste, or strips. In general, the action mechanism most likely involved in tooth bleaching is related to the properties of hydrogen peroxide (HP) as an oxidizing agent that can break down the chromogen and remove the pigments from the structure of enamel or dentin through diffusion, indirectly promoting the reduction of light absorption.[3] The reduction in light absorption produces a significant reduction in the yellowness of dentin and an increase in the whiteness of the tooth.[4]

Tooth bleaching has been widely indicated since this procedure is considered esthetic, relatively safe, and effective.[1,2] Scientific evidence[5-9] shows that changes in the morphology and properties of dental tissues can happen, especially related to an unspecified oxidative effect of HP that could act in the inorganic and organic composition of the tooth.[9] In order to eliminate the side effects of dental bleaching, different products and vehicles, such as fluoride in gel or toothpaste, have been suggested for use before or after treatment.[10-12] There are no investigations regarding the effects of compounds incorporated in different commercial mouth rinses on bleached enamel with 35% HP.

Mouth rinses are very popular oral hygiene agents, and combinations of different preventive and therapeutic agents are commercially present. Considering the OTC products, whitening mouth rinses appeared on the market as an alternative to treatment for tooth discoloration, with a lower cost than traditional guided approaches. The whitening effectiveness of these agents is rarely discussed and is controversial in the literature, especially due to the lack of clinical studies that validate the effectiveness and safety of these products. Previous *in vitro* studies described no efficacy,[13] slight bleaching effectiveness before 45 days of use,[14] or similar color alteration compared with 14 days of at-home bleaching therapy when used for 12 weeks.[15] Moreover, a recent study[16] demonstrated that the whitening efficacy of some OTC mouth rinses may increase the longevity of at-home whitening outcomes over time. There are few studies regarding the use of OTC whitening associated with dental bleaching or evaluating the effects of use of commercial mouth rinses containing different active principles on bleached enamel, mainly in relation to enamel properties, effectiveness of bleaching, and color stability of treatment.

In addition to OTC products, other agents are frequently incorporated into mouth rinses in order to decrease or prevent biofilm-associated oral diseases as an adjunct to mechanical oral hygiene measures. Essential oil mouth rinses are very popular agents used in a combination with thymol, menthol, eucalyptol, and hydroalcoholic vehicles. In particular, blue-colored alcohol and essential oil–containing mouth rinses have been shown to be capable of causing color change of enamel.[17] No investigations have evaluated the effects of these compounds on bleached enamel, which is necessary since it has been suggested that the low pH of rinses can promote some enamel erosion.[18,19]

Further investigation is warranted considering the absence of studies investigating the effects of different commercial mouth rinses on bleached enamel, which may have its mineral content altered.[8] The aim of this study was to investigate the effects on enamel of mouth rinses with different active agents used after in-office dental bleaching, using analysis of color, cross-sectional microhardness, and surface roughness in order to evaluate the properties and morphology of bleached enamel. The null hypotheses tested were that 1) in-office dental bleaching would not affect the enamel properties of the surface and subsurface, 2) the use of a mouth rinse after in-office dental bleaching would not affect the whitening efficacy or promote the incorporation of pigments, and 3) the use of a mouth rinse after dental bleaching would not affect the bleached enamel properties of the surface and subsurface.

Table 1: *Components of Products Used According to the Manufacturer's Information*

Product	Manufacturer	Composition	Color	pH[a]
Whiteness HP (bleaching agent)	FGM (Santa Catarina, Brazil)	35% hydrogen peroxide, carbopol, glycol, and water	Initial = red; after 15 min = colorless	Initial = 5.64; after 15 min = 4.87
Colgate Plax Classic (FM)	Colgate-Palmolive (São Paulo, Brazil)	225-ppm NaF (sodium fluoride), triclosan 0.03%, PVM/MA copolymer, 0.20% gantrez, alcohol, water, sorbitol, glycerin, sodium lauryl sulfate, disodium phosphate, sodium hydroxide, sodium saccharin, CL 16035	Red	6.11
Listerine Tartar Control (EM)	Johnson & Johnson (São Paulo, Brazil)	21.6% alcohol, 0.064% thymol, 0.092% eucalyptol, 0.06% methyl salicylate, water, n-propranolol, sorbitol, polaxamer 407, peppermint flavoring, benzoic acid, sodium benzoate, sodium saccharin, zinc chloride, blue dye FD&C	Blue	4.30
Colgate Plax Whitening (HPM)	Colgate-Palmolive	1.5% hydrogen peroxide, water, sorbitol, ethyl alcohol, poloxamer 338, polissorlato 20, methyl salicylate, menthol, saccharin sodium, CI 42090	Light blue	4.07
Colgate Luminous White (HPM+P)	Colgate-Palmolive	2% hydrogen peroxide, tetrapotassium pyrophosphate, tetrasodium pyrophosphate, 225-ppm NaF, zinc citrate, glycerine, propylene glycol, phosphoric acid, saccharin sodium, sucralose, flavor	Light blue	6.92

[a] *The pH was determined in triplicate using a pH meter (Procyon, São Paulo, Brazil).*

METHODS AND MATERIALS

Sample Preparation

Enamel/dentin blocks ($4 \times 4 \times 2$ mm), with 1 mm of enamel and 1 mm of dentin, were obtained from the middle third of the buccal surface of sound bovine incisor teeth that were stored in a 0.01% thymol solution at 4°C for 30 days until use. The sections were obtained using a low-speed water-cooled diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA). The samples were subsequently serially ground with 600-, 1000-, and 2000-grit SiC papers (Buehler) and polished with cloths and diamond spray (1, 0.5, and 0.25 μm, – Buehler). The samples were placed in an ultrasonic machine for 10 minutes (Marconi, Piracicaba, Brazil) to remove residues in order to obtain a standardized enamel surface. All surfaces of the blocks, except the enamel surface, were protected with acid-resistant varnish. During the experiment, all prepared samples were stored in a remineralizing solution containing 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 μg F/mL, and 0.1 M Tris buffer at pH 7.0[20] that was renewed every day of the study. The initial L* values of each sample were used to stratify and allocate specimens into all groups, aiming to reduce the initial variability among the treatments. The evaluation method of the L* coordinate is described in the "Color Measurements" section.

Bleaching Procedure

The bleaching treatment was performed using an agent of high HP concentration (35% HP, Whiteness HP, FGM, Joinville, Brazil), according to the manufacturer's instructions. Information on the product and its pH are shown in Table 1. All prepared samples were stored in a remineralizing solution for 24 hours prior to the bleaching procedure. The bleaching agent was applied three times for 15 minutes on the enamel surface. After the procedure, the samples were washed with distilled water, and a daily exposure regime with mouth rinses was initiated.

Mouth Rinse Application Protocol

The samples were positioned with a sticky wax on metal fins coupled with conical centrifuge tubes

(Falcon, Fisher Scientific, Loughborough, UK) and were submitted to daily simulated rinses. Enamel blocks were exposed to 5 mL of mouth rinses or distilled water twice daily for 14 days under agitation (100 rpm) at room temperature. The exposure time and frequency were performed in accordance with the manufacturer's recommendations. Considering the different mouth rinses used and their commercial presentations, the exposure regimen of 14 days was used as the total consumption time of a bottle of commercial mouth rinse. Information about the groups and mouth rinses used, including manufacturers, pH, and components, are detailed in Table 1. After the stratification of L* values, the samples were randomly divided into five groups (n=10) according to the following treatments:

1. Bleaching with 35% HP and immersion in distilled water for one minute, twice daily for 14 days (C [control])
2. Bleaching with 35% HP and immersion in 225-ppm NaF mouth rinse for one minute, twice daily for 14 days (FM, Colgate Plax Classic)
3. Bleaching with 35% HP and immersion in essential oil–based mouth rinse for 30 seconds, twice daily for 14 days (EM, Listerine Tartar Control)
4. Bleaching with 35% HP and immersion in 1.5% HP-based mouth rinse for two minutes, twice daily for 14 days (HPM, Colgate Plax Whitening)
5. Bleaching with 35% HP and immersion in a mouth rinse containing 2% HP, pyrophosphates, and 225-ppm NaF for one minute, twice daily for 14 days (HPM+P, Colgate Luminous White)

After each rinse treatment, the samples were washed with distilled water for 10 seconds and stored in a remineralizing solution until the next cycle.

Color Measurements

Color reading was performed in an ambient light condition (GTI MiniMatcher MM 1, GTI Graphic Technology Inc, New York, NY, USA) in standardized daylight. The spectral distribution was measured using a reflectance spectrophotometer (CM 700d, Minolta, Osaka, Japan) based on the CIE L*a*b* system. The L* coordinate represents the luminosity (white–black) axis, a* represents the green–red axis, and b* represents the blue–yellow axis. Before the measurements, the spectrophotometer was calibrated using white and black reflectance standards. The analysis was performed at initial time (baseline), 24 hours after dental bleaching, and after use of mouth rinses for 14 days. The color change was calculated using the following equation: $\Delta E = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$.

Surface Roughness

The enamel roughness (Ra) was analyzed using a profilometer tester (Mitutoyo Surfitest 211, São Paulo, Brazil) at three time frames: initial time (baseline), 24 hours after dental bleaching, and after mouth rinse exposure. Three different equidistant scans of 1.25 mm each were measured on the surface of each sample, with a cutoff of 0.25 mm, a load of 5 N, and a velocity of 0.1 mm/s.

Cross-Sectional Microhardness Analysis

The enamel cross-sectional microhardness (CSMH) was analyzed at the end using a Future-Tech FM-ARS microhardness tester (Future-Tech Corp, Tokyo, Japan) with a Knoop diamond under a 50-g load for five seconds. For the CSMH tests, the samples were longitudinally sectioned through the center and embedded in acrylic resin. The exposed area was gradually polished, as previously described. Three rows of five indentations were made in the central area of the slab, the measurements occurring at 10, 25, 50, 75, and 100 μm from the enamel surface. The mean values at all three measuring points at each distance were then determined. Due to the destructive characteristic of the analysis and impossibility of evaluating the baseline values, an unbleached enamel group (n=10) was standardized in order to compare the values found in the experimental groups with intact enamel under the same conditions as those established by the study design.

Scanning Electron Microscopy (SEM)

Three representative samples of groups were randomly selected and subjected to vacuum in a sputter coater (SCD 050 Sputter Coater, Balzers, Liechtenstein) to deposit a thin layer of gold, in order to increase the surface reflectance. Then images of representative areas of the specimens were obtained at 4000× using a scanning electron microscope (JSM 5600LV, JEOL, Tokyo, Japan).

Statistical Analysis

After exploratory analysis using the SAS software (Release 9.1, 2003, SAS Institute Inc, Cary, NC, USA), the data were submitted to one-way analysis of variance (ANOVA) (ΔE), repeated measures ANOVA (Ra), and split-plot ANOVA (CSMH), followed by the Tukey test, at a 5% level of significance.

Table 2: *Mean (SD) for Results of L*, a*, and b* Coordinates Based on Treatment Groups (n=10)*[a]

	Baseline	After Dental Bleaching	After Mouth Rinse
L*			
Control	82.87 (1.80) Ba	85.62 (0.76) Aa	85.38 (0.89) Aa
FM	83.23 (1.62) Ba	86.99 (0.83) Aa	84.44 (1.78) Aa
EM	82.17 (2.45) Ba	85.34 (2.85) Aa	85.60 (0.91) Aa
HPM	83.93 (2.13) Ba	86.86 (1.23) Aa	86.46 (0.65) Aa
HPM+P	81.30 (1.17) Ba	85.93 (1.17) Aa	86.03 (1.03) Aa
a*			
Control	−0.30 (0.50) Aa	−0.71 (0.26) Ba	−0.65 (0.39) Ba
FM	−0.51 (0.38) Aa	−0.60 (0.24) Aa	−0.19 (0.76) Aa
EM	−0.34 (0.37) Aa	−0.49 (0.37) ABa	−0.75 (0.33) Ba
HPM	−0.18 (0.38) Aa	−0.55 (0.31) Ba	−0.96 (0.19) Ca
HPM+P	−0.39 (0.31) Aa	−0.52 (0.27) Ba	−0.97 (0.18) Ca
b*			
Control	10.72 (1.87) Aa	6.20 (1.93) Ba	5.78 (1.80) Ba
FM	9.77 (2.21) Aa	5.08 (1.90) Ba	7.26 (3.70) Ba
EM	9.58 (2.69) Aa	5.26 (1.14) Ba	5.53 (1.16) Ba
HPM	8.70 (3.12) Aa	5.23 (1.59) Ba	3.85 (0.82) Ba
HPM+P	8.83 (2.57) Aa	4.95 (1.38) Ba	5.37 (1.66) Ba

Abbreviations: Rinses based on distilled water (control); 225-ppm NaF (FM, Colgate Plax Classic); essential oil (EM, Listerine Tartar Control); 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening); 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White).
[a] Means followed by different letters (upper in horizontal and lower in vertical) are different (p<0.05).

The L*, a*, and b* data were analyzed by generalized linear models (Proc Genmod model) for repeated measures data (α=0.05). Considering the ΔE values, a power calculation was performed with the following parameters: a power value of 0.80, significance level of 0.05, standard deviation = 1.00, and the values proposed by Alghazali and others[21] for the relevant difference between the groups, being 1.9 ΔE units for assessment of perceptibility and 4.2 ΔE units for clinical acceptability of color changes. In this condition, the statistical analysis showed that a minimum sample size of seven would be necessary considering the 1.9 ΔE units or four considering the 4.2 ΔE units. Thus, with a margin of safety, the experiment was conducted with n = 10.

RESULTS

The L*, a*, and b* results are presented in Table 2. For the L* results, the statistical analysis demonstrated an effect of the time factor ($p<0.001$) and an absence of effect of the mouth rinse factor ($p=0.2345$) or interaction of the factors ($p=0.1209$). For the b* values, an effect of the time factor was found ($p<0.001$) and no effect of the mouth rinse factor ($p=0.1363$) or interaction of the factors ($p=0.2427$). For a* data, although no effect was demonstrated for the mouth rinse factor ($p=0.42$), an effect of the time factor ($p<0.001$) and interaction with the mouth rise factor ($p=0.0127$) was statistically significant. Concerning Table 2, for baseline data, there was no statistical difference among the groups. After dental bleaching, the L*, a*, and b* values differed statistically from the baseline values, with increasing L* values and decreasing b* values. Moreover, the groups did not differ statistically for any coordinate studied after the dental bleaching. For the a* values, the EM group did not differ statistically between the times, and the L* and b* values were not different from the other bleached groups. For L* and b* results after mouth rinse exposure, there was no statistical difference among the rinse groups and the control, nor was there a difference compared to the previous time. For the a* results, although no difference among groups was found, a decrease in a* values was statically demonstrated in groups exposed to HP-based mouth rinses (HPM and HPM+P).

Table 3: *Mean (SD) for ΔE Values Based on Treatment Groups (n=10)*[a]

	Baseline vs Dental Bleaching	Dental Bleaching vs Mouth Rinse	Baseline vs Mouth Rinse
Control	5.54 (1.15) a	0.88 (0.40) a	5.79 (0.32) ab
FM	5.59 (0.60) a	3.33 (2.58) a	4.18 (1.55) b
EM	6.27 (1.49) a	1.34 (1.94) a	5.85 (0.58) ab
HPM	5.06 (1.97) a	1.92 (0.98) a	6.28 (1.54) ab
HPM+P	6.56 (1.94) a	1.08 (0.32) a	6.63 (0.96) a

Abbreviations: Rinses based on distilled water (control); 225-ppm NaF (FM, Colgate Plax Classic); essential oil (EM, Listerine Tartar Control); 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening); 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White).
[a] Means followed by different letters in vertical are different (p<0.05).

Considering the ΔE values (Table 3) when comparing baseline vs dental bleaching or dental bleaching vs mouth rinse treatment, no difference was shown for all groups, and the groups were not statistically different compared to the control or among them ($p=0.1983$). In assessment of baseline vs mouth rinse exposure, the groups were not different compared to the control ($p=0.0846$); however, FM differed statistically from HPM+P ($p=0.0181$).

Based on the roughness values (Table 4), the initial results of groups were not statistically different. After dental bleaching, a slight increase of Ra values was found in all groups compared to

Table 4: *Mean (SD) for Ra Values (Roughness) Based on Treatment Groups (n=10)*[a]

	Baseline	After Dental Bleaching	After Mouth Rinse
Control	0.13 (0.01) Ba	0.18 (0.02) Aa	0.13 (0.01) Bc
FM	0.13 (0.01) Ba	0.18 (0.02) Aa	0.15 (0.01) Bbc
EM	0.14 (0.01) Ca	0.18 (0.02) Ba	0.21 (0.02) Aa
HPM	0.13 (0.01) Ba	0.18 (0.02) Aa	0.16 (0.02) Ab
HPM+P	0.13 (0.01) Ba	0.18 (0.02) Aa	0.13 (0.01) Bc

Abbreviations: Rinses based on distilled water (control); 225-ppm NaF (FM, Colgate Plax Classic); essential oil (EM, Listerine Tartar Control); 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening); 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White).
[a] Means followed by different letters (upper in horizontal and lower in vertical) are different (p<0.05).

baseline values ($p<0.001$), with no difference between groups. In terms of effects on roughness after the mouth rinse application protocol, the control, FM, and HPM+P groups reestablished the baseline values of Ra, which statistically differed from the means of the previous time ($p<0.001$), referring to the values found 24 hours after dental bleaching. Distinct events were observed in the groups exposed to HP mouth rinses: HPM+P showed lower Ra values, statistically differing from the HPM group ($p<0.001$). HPM presented Ra values similar to the previous time and statistically different from the baseline values ($p<0.001$). The blocks treated with EM had the highest increase of Ra, which statistically differed from all other groups ($p<0.01$) or the Ra values in other frames ($p<0.001$).

With regard to cross-sectional microhardness (Table 5), the results showed that the bleached control did not demonstrate a statistical difference from unbleached enamel at 10 and 25 μm. Therefore, the reestablishment of CSMH values was not enabled at all depths after 14 days, being statistically different at 50, 75, and 100 μm from reference values of unbleached enamel ($p<0.001$). There were distinct effects between the rinse groups and the control groups at all depths. The HPM+P group did not differ statistically from the unbleached enamel at depths of 10 and 25 μm, and the FM group did not differ statistically at 25 μm. The HPM+P group did not differ statistically from the bleached control at all depths, and comparing the values of the FM group to the bleached control revealed a statistically significant difference only at 10 μm ($p<0.001$), similar to the results found at depths of 25, 50, 75, and 100 μm. The EM and HPM groups were statistically different from the bleached or unbleached control at all depths ($p<0.001$). In particular, HPM presented lower values of CSMH at all depths. Finally, no statistical differences were found in the evaluation of the CSMH values at the different depths within the group itself.

The SEM images collected are presented in Figure 1. The unbleached enamel (Figure 1A) demonstrated a smooth, regular, and uniform surface. After 24 hours of bleaching (Figure 1B), the enamel surface presented evidence of a slight demineralization process associated with mineral loss, demonstrating a loss of interprismatic substance and an increase in porosity. However, after 14 days in the remineralizing solution, the enamel (Figure 1C) showed mineral recovery and a surface very similar to that verified in the unbleached enamel (Figure 1A). The FM (Figure 1D) and HPM+F (Figure 1G) mouth rinses promoted an enamel surface very similar to unbleached control (Figure 1A) or enamel after 14 days of whitening (Figure 1C) despite rare signs of the demineralizing event that were found in the FM group, as shown in Figure 1D. In fact, distinct severity of such events could be observed throughout the enamel surface in mouth rinse groups as the morphologic changes became much more pronounced in the EM (Figure 1E) and HPM (Figure 1F) groups. The bleached enamel exposed to EM or HPM (Figure 1E,F,

Table 5: *Mean (SD) for Cross-Sectional Microhardness Values Based on Treatment Groups (n=10)*[a]

	10 μm	25 μm	50 μm	75 μm	100 μm
Unbleached enamel	359.2 (28.6) Aa	356.1 (30.0) Aa	372.9 (28.4) Aa	374.6 (30.3) Aa	371.6 (29.6) Aa
Dental bleaching (35% HP) +					
Distilled water	324.8 (30.0) Aa	325.7 (24.9) Aa	321.4 (28.6) Ab	325.4 (31.8) Ab	315.2 (27.2) Ab
FM	258.6 (34.1) Ab	309.7 (22.6) Aa	301.8 (31.6) Ab	297.7 (30.5) Abc	299.0 (32.3) Ab
EM	241.1 (28.4) Ab	258.7 (24.7) Ab	246.9 (28.8) Ac	260.7 (29.8) Ac	248.7 (29.8) Ac
HPM	172.5 (32.4) Ac	181.1 (34.8) Ac	187.7 (32.5) Ad	180.3 (30.5) Ad	183.2 (23.9) Ad
HPM+P	313.0 (14.1) Aa	316.1 (27.0) Aa	306.4 (20.6) Ab	321.1 (11.5) Ab	321.5 (16.2) Ab

Abbreviations: Rinses based on water (control); 225-ppm NaF (FM, Colgate Plax Classic); essential oil (EM, Listerine Tartar Control); 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening); 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White).
[a] Means followed by different letters (upper in horizontal and lower in vertical) are different (p<0.05).

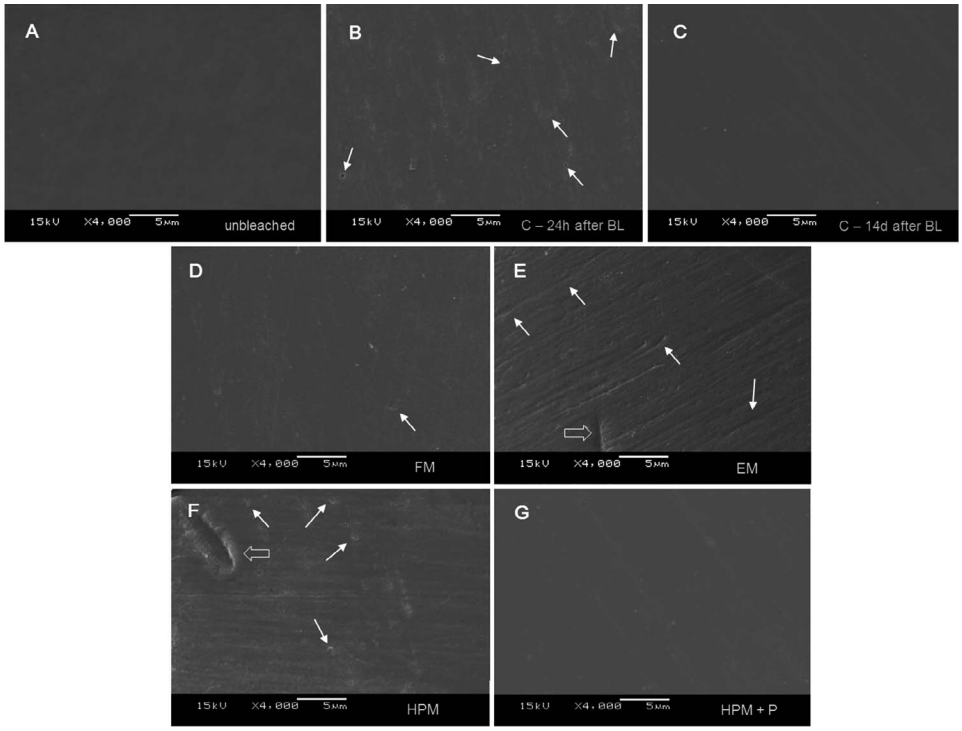

Figure 1. *Representative scanning electron microscopic images (4000×) of the samples. (A): Unbleached enamel. (B) Bleached enamel after 24 hours. (C): Bleached enamel after 14 days and exposed to distilled water (control). Bleached enamel with 35% hydrogen peroxide and submitted to mouth rinses.: (D): 225-ppm NaF (FM, Colgate Plax Classic). (E): Essential oil (EM, Listerine Tartar Control). (F): 1.5% hydrogen peroxide (HPM, Colgate Plax Whitening). (G): 2% hydrogen peroxide, pyrophosphates, and 225-ppm NaF (HPM+P, Colgate Luminous White). The morphological alterations found on the enamel surface of groups are represented in the images in B, D, E, and F; the fine arrows represent areas with pores or surface irregularities, and thick arrows indicate depressions of enamel.*

respectively) presented pores or superficial irregularities with intermittent depressions. Linear markings associated with SiC polishing can be visualized in the images (Figure 1A-G); however, only the results associated with morphological changes, such as loss of interprismatic substance, depressions, and porosities, were described, and possible linear markings were not considered or associated with the treatments studied.

DISCUSSION

In the present study, null hypotheses 1 and 3 were rejected because the in-office dental bleaching affected the physical properties of enamel, and the mouth rinse exposure promoted different effects on enamel, modifying the physical properties and mineral recovery of the tooth. However, null hypothesis 2 was accepted because the exposure to mouth rinse after the in-office dental bleaching did not affect the whitening effectiveness of treatment. Dental enamel is the hardest mineralized biological tissue, containing approximately 96% mineral, 3% water, and 1% organic matter by weight. The enamel blocks were obtained from bovine incisors, as they present physical-chemical properties resembling that of human enamel[22] and are considered a practical model for bleaching studies.[23] All groups in this investigation indicated significant change in L* and b* values following dental bleaching. The whitening effectiveness of treatment was evidenced due to the fact that the mean L* values increased while the mean b* values decreased, which represents a lighter and less yellowish color for the tooth. The mean values of total color change (ΔE) after in-office dental bleaching were greater than 4.2 units[21] or 3.3 units,[24,25] the standard values suggested for clinical acceptability of color differences.

On the other hand, the use of mouth rinses did not act directly on the L*, b*, and ΔE values. The HP mouth rinses (HPM and HPM+P), commercially available as whitening mouthwashes (OTC), were not able to promote an improvement of the bleaching effect. These mouth rinses are composed with a low concentration of HP that could diffuse through the dental structure and produce free radicals that lead to successful bleaching;[3,26] however, the effect found in the present study may have been low due to the fact that they stay in contact with the enamel for a short period of time compared with those offered by dentist-guided treatments[27] in addition to a lower concentration of active ingredient.

In relation to colorful mouth rinses, essential oil–containing mouth rinses (EM) are made available as a blue-colored alcohol solution and have been associated with enamel pigmentation after prolonged contact exposure.[17] Despite the evidence that bleached enamel may be more susceptible to staining,[28,29] the EM group did not promote color change

or disrupt the color stability of treatment, possibly because the exposure was performed daily under more real conditions. The FM group, commercially available as a red solution, presented $\Delta E = 3.33$, which could even indicate visually unacceptable discoloration according to the previous investigations;[24,25] however, the color results showed an absence of statistical differences, demonstrating the necessity of more studies to investigate the effects on color of enamel exposed to FM mouth rinse for a longer time. Overall, the use of a colorful mouth rinse after in-office dental bleaching did not affect the efficacy of whitening treatment, corroborating a previous review[30] that concluded that the use or ingestion of products with dyes does not limit the effect of tooth whitening.

According to the results of this present study, the dental bleaching with 35% HP promoted an increase in enamel surface roughness, a slight change in topography visualized by SEM, and a decrease in the cross-sectional microhardness, as previously described.[6,12,31,32] During dental bleaching, a mineral dissolution[8,33,34] could occur, explaining the alterations on enamel properties. These deleterious effects can be attributed to the oxidation of the organic and inorganic components of the tooth by free radicals[3,26] as well as to the acidic pH of the bleaching agent used,[35] which was 4.87 in the current study (Table 1). However, after 14 days, the bleached enamel showed Ra values and enamel surface (SEM) similar to the unbleached enamel as well as no difference from unbleached enamel at 10 and 25 μm in the CSMH analysis. The remineralizing solution was used like artificial saliva to simulate the inorganic composition of human saliva, and this storage produced an environment rich in calcium, phosphorus, and fluoride. This environment promoted enamel remineralization, enabling the mineral recovery of dental substrates and almost completely reversing the demineralization caused by the bleaching agent, with an exception at the depths of 50, 75, and 100 μm.

Saliva[36] and other active agents[10-12] play an essential role in promoting remineralization or decreasing demineralization of teeth submitted to bleaching treatment. The rinses studied did not present additional or beneficial effects to bleaching therapy, and in some cases were able to potentiate an injury to the dental structure. After mouth rinse cycling, the EM (Listerine Tartar Control) induced enamel demineralization, verified through changes on the surface, the highest increase of Ra, and a decrease of microhardness values in depth. These alterations may have occurred due to the low pH of the product associated with the absence of remineralizing agents in an alcoholic vehicle.[18,19,37] Although the impact of this increased roughness promoted by EM in clinical practice is undesired, these results indicate a change in surface topography promoted by a demineralizing event with possible dissolution of hydroxyapatite crystals with slight structural alterations, as shown in Figure 1E.

In addition, the OTC whitening products could cause undesirable local effects, such as sensitivity, oral mucosa irritation, alterations of physical properties of restorative materials, and slight erosion in the tooth structure.[9] The potential abusive use of these self-agents, especially in young patients, could promote potential harmful results.[38] The OTC agents evaluated in the present study exhibited different results in relation to enamel properties; while HPM was damaging to dental structure for all variables studied, the HPM+P presented a surface and subsurface similar to the unbleached enamel. This could be explained by the different pH of these products (Table 1) and the fact that the HPM (Colgate Plax Whitening) does not have any remineralizing agent in its composition and remained in greater contact with the enamel, which happened for two minutes, as the manufacturer indicated for a prebrushing rinse. Furthermore, pyrophosphates and fluoride are incorporated into HPM+P (Colgate Luminous White).

Pyrophosphates are agents with high affinity for hydroxyapatite crystal, interacting with calcium.[39] During the chemical reactivity with enamel, the pyrophosphates reduce the binding capacity of proteins or chromogens, being considered an anticalculus or antistaining agent.[39] Additionally, HPM+P and FM include added fluoride (225-ppm NaF), which is currently used as an agent that promotes remineralization of dental hard tissues and decreases the effects of demineralization.[40] The HPM+P and FM presented neutral pH; however, the presence of low-concentration fluoride during the demineralizing event in rinse solutions or bleaching gel[41] appears to be more important than its use after tooth whitening to remineralize because no evidence of remineralization was found in the FM group, commercially available as a neutral fluoride rinse solution (pH=6.11).

This study was designed to evaluate the effects on enamel of mouth rinse exposure after in-office tooth bleaching. The impact of active agents incorporated into mouth rinses on enamel is very relevant because oral home care products are purchased

and sold cosmetically and, unfortunately, often used without supervision by a dentist. The consumption of oral home care products has spread around the world, and HP- or alcohol-containing mouth rinses need to be extensively investigated, especially with regard to the rational use and safety of these products. However, it is important to note that the *in vitro* studies of solutions at low pH have been shown to exaggerate the erosive/demineralization effect. In the mouth, the mineral dissolution could be lower because these effects on mineral content are decreased by the protective effect of the acquired pellicle and the buffering capacity of human saliva. Nevertheless, controlled *in vitro* studies are necessary and important precursors of *in vivo* studies. Further research is needed to investigate the performance of mouth rinses in *in situ* and *in vivo* models regarding the combination of compounds, wear time, and the association of different oral care products with the dentist-supervised bleaching, whether using at-home or in-office techniques.

CONCLUSIONS

The use of a mouth rinse after in-office dental bleaching did not affect the efficacy of whitening treatment or enhance tooth staining. Additionally, the mouth rinses did not promote additional benefits to treatment, and the 1.5% HP- or essential oil–based rinses impaired mineral reestablishment of enamel, promoting a decrease in bleached enamel properties.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the University of Campinas.

Conflict of Interest

The authors of this article 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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