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Influence of Staining Solutions on Color Change and Enamel Surface Properties During At-home and In-office Dental Bleaching: An *In Situ* Study

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Clinical Relevance

The consumption of cola and coffee can affect the color change of enamel during both at-home and in-office bleaching treatment. In addition, the consumption of cola changes the microhardness, roughness, and micromorphology of the enamel surface.

SUMMARY

The purpose of this *in situ* study was to evaluate the influence of staining solutions (coffee and cola) on the color change, microhardness, roughness, and micromorphology of the enamel surface during at-home and in-office dental bleaching. One hundred and thirty-five enamel bovine blocks were prepared to perform the evaluations. Fifteen volunteers used an intraoral appliance with nine enamel blocks for 15 days. The enamel blocks were randomly assigned among the different groups according to the three treatments: in-office bleaching with high hydrogen peroxide concentration (Opalescence Boost PF 40%, Ultradent) for 40 minutes in three sessions (first, eighth, and 15th days of treatment), at-home bleaching with low carbamide peroxide concentration (Opalescence PF 10%, Ultradent) for 60 minutes daily for 15 days, and a control group (no bleaching agent applied). The enamel blocks were immersed daily in different staining solutions (coffee or cola) for 30 minutes for 15 days or were not submitted to staining (control) to obtain a factorial scheme

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(3×3) of the dental bleaching treatment and staining solution (n=15). The microhardness analyses (Knoop), roughness evaluations (Ra), surface micromorphological observations, and color measurements (using the CIELAB system and the VITA Classical scale) were made before and after the bleaching treatments to assess immersion in staining solutions. Mixed model tests showed that there was a decrease in enamel microhardness after exposure to cola compared with coffee and the control group ($p<0.0001$) for both bleaching techniques. Roughness was higher for the cola groups ($p<0.0001$), and there was no significant difference between the coffee and the control groups. Generalized linear models showed that when no staining solution was applied, lighter color scores were found for the VITA Classical scale ($p<0.0001$). Without the staining solutions, there was an increase in luminosity (ΔL) ($p=0.0444$) for in-office bleaching. Lower values of Δa ($p=0.0010$) were observed when the staining solutions were not used. The Δb ($p=0.3929$) did not vary significantly between the bleaching agents, but when cola was applied, the values were significantly higher than for the control ($p=0.0293$). Higher values of ΔE ($p=0.0089$) were observed for in-office bleaching without staining solutions, while lower values of ΔE were observed for the in-office associated with coffee immersion. Regardless of whether being submitted to bleaching, the enamel stained with cola showed a decrease in microhardness, an increase in roughness, and changes in the micromorphology. The efficacy of the bleaching agents was greater when no staining solution (cola or coffee) was used, and in-office bleaching showed greater color change than the at-home bleaching technique.

INTRODUCTION

The color of dental elements can be modified noninvasively with dental bleaching procedures using either carbamide peroxide or low-concentration hydrogen peroxide (in the at-home treatment) or else using high concentrations of hydrogen peroxides (for in-office bleaching), which leach out the chromophores present in the dental structure.[1] In dental bleaching, carbamide or hydrogen peroxides release oxygen and perhydroxyl free radicals, capable of diffusing through the dental structure, breaking down the large and pigmented molecules (chromophores) into smaller molecules, modifying the reflection of the wavelength of light, and consequently altering tooth coloration.[2]

In addition to color change, changes of a micromorphological nature may be associated with the use of bleaching agents of low or high concentration, including enamel erosion or porosity[3-6] and greater enamel surface roughness[3,6-8] and permeability.[9] These changes may be associated with decreased enamel microhardness and loss of mineral content.[4,8,10] They occur due to the composition of bleaching agents, concentration of the peroxide used, pH, application time, storage medium (water or saliva), and protocols of the bleaching techniques. In this respect, surface micromorphology changes have been observed when using agents with higher concentrations of hydrogen peroxide[9] and lower pH values.[5,11] These surface changes could lead to greater roughness and consequent higher adhesion of biofilm and pigments to the enamel,[12] thus compromising bleaching efficacy.

For this reason, patients undergoing dental bleaching have been cautioned to avoid food and drinks rich in coloring agents, such as coffee, red sauces, red wines, tea, and chocolate.[13] Although Matis and others[14] observed that the consumption of pigmented food and beverages was not related to less bleaching effectiveness, they did not evaluate a control group with patients who did not have a diet free of pigmented foods or beverages for comparative purposes. An *in vitro* study by Liporoni and others[15] found that the consumption of wine after tooth whitening with 35% hydrogen peroxide led to the staining of the enamel structure. In contrast, Attin and others[16] demonstrated that a tea solution used for 10 minutes at different time intervals during bleaching with 10% carbamide peroxide did not influence the enamel color.

Among the pigmented drinks consumed by the population, coffee has high penetration and presence in Brazilian and American households. In 2017, 1.07 tons were consumed, putting Brazil right behind the United States in terms of consumption.[17] Cola-based soft drinks, especially Coca-Cola, also have broad population penetration—worthy of note, it is one of the most well known brands in the United States and ranked first among the best beverages in 2010.[18]

Coffee and cola are listed as the leading solutions causing tooth staining.[19-22] This is because both solutions present pigments that are adsorbed or absorbed by the substrate[23] in addition to having low pH values that may potentiate dental staining: coffee

is described as having a pH of about 5.5 [24] and cola of about 2.6.[21]

Some *in vitro* studies have evaluated the staining caused by coffee or cola associated with the bleaching treatment. When performing the bleaching technique with trays, Attia and others[19] observed lower color stability of the dental element when it was exposed to coffee during tooth whitening with 16% carbamide peroxide for 28 days. Coffee also caused staining of the enamel after dental bleaching with 6% hydrogen peroxide in the study by Bazzi and others[20] De Araújo and others[21] observed enamel that was stained with cola for one hour after bleaching with 10% hydrogen peroxide for 21 days. In regard to the in-office bleaching technique, only Mori and others[22] evaluated *in situ* the effect of coffee staining after bleaching with 35% hydrogen peroxide and found no influence on color change. Briso and others[25] also evaluated *in situ* the effect of staining with coffee and grape juice during bleaching with 10% carbamide peroxide and observed no difference in the final color at the end of the treatments. In a clinical study, Rezende and others[13] showed that exposure to coffee during bleaching treatment does not seem to affect the degree of at-home bleaching. However, no *in situ* study has evaluated the effect of coffee and cola on at-home and in-office dental bleaching treatments. In an *in situ* study, which is considered a preclinical study, the constant presence of saliva may influence the surface characteristics of dental enamel bleaching and have a remineralizing effect, and saliva may also deposit pigments on the dental surface. The null hypotheses to be tested were that there are no differences in enamel's 1) microhardness, 2) roughness, 3) micromorphology, or 4) color change between at-home and in-office dental bleaching submitted to the tested staining solutions (coffee and cola) or not submitted to any solution.

METHODS AND MATERIALS

Sample-Size Calculation and Patient Selection

Fifteen participants of both sexes were selected for this *in situ* study. The participants were selected after performing a sample-size calculation, considering the experimental design as one of repeated measures, in a 3 × 3 factorial scheme (solutions and bleaching agents). The inclusion of 15 participants enabled a high degree of freedom (269) and error (112) for the factors under study of "bleaching agent," "solutions," and the interaction of "bleaching agents vs solutions." The error was also high (126) for the "time" factor and for the interactions among "time" and the other factors. The G*Power software[26] for this experimental design (n=15) indicated an effect size higher than 0.80 for a 5% level of significance and a mean effect size of 0.25.[27]

Participants were included or excluded from the study based on history taking and clinical examination. The inclusion criteria were between 18 and 30 years of age and the presence of at least 24 sound teeth. The exclusion criteria were[28] people wearing dentures or fixed/removable orthodontic appliances, pregnant or breast-feeding women, smokers, and patients using medication that could reduce their salivary flow.

Four men and 11 women having a mean age of 21.7 years participated in this study. An impression of the upper arch was made for each volunteer, and a mold was obtained in dental stone on which a removable acrylic intrabuccal device for the palate was placed. It contained niches of a little over 5 × 5 × 3 mm to position nine blocks of enamel.

Preparation of Enamel Slabs

Sixty freshly extracted bovine incisors were obtained, cleaned, and stored in the freezer at -18°C until the experimental phase but no longer than two months prior. Before use, the teeth were thawed, and the roots were removed from the coronary portion with double-faced diamond discs (KG Sorensen, Barueri, Brazil). The crowns were sectioned to obtain dental blocks (5.0×5.0×3.0 mm) that were embedded in polyether resin (Maxi Rubber, Campinas, Brazil) and polished (Ecomet 250 grinder, Buehler Ltd, Lake Bluff, IL, USA) with adhesive abrasive paper (Buehler) using a granulation of 600 and then 1200. The polishing was performed with alumina suspension at 0.3 μm (Alfa Micropolish, Buehler) and a felt cloth (Buehler). The enamel blocks were cleaned in an ultrasound device with deionized water for 10 minutes to remove any residues as a result of the polishing procedure. Afterward, the enamel blocks were sterilized with ethylene oxide.[25] Two hundred enamel blocks were obtained at the end of this phase.

Microhardness Measurements

Three Knoop microhardness indents were made before applying the treatments, 150 μm from each other, on the top surface of each enamel slab using a microhardness tester (HVS-1000, Panambra, São Paulo, Brazil) under a 25*g* load applied for five seconds. The blocks selected presented microhard-

ness values closest to the mean (376.44±41.89 kgf/mm^2).

Roughness Measurements

Initial roughness measurements were performed with a profilometer (Surftest SJ-210, Mitutoyo, Suzano, Brazil) on the surface of the dental slabs. The mean roughness (Ra) was measured with a static load of 5 N and speed of 0.05 mm/s. The cutoff value was 0.25 μm in a sequential mode, and the measurement distance was 2.5 mm. Three tracings were made on each specimen at different locations, and the arithmetical mean was calculated. The values were obtained and tabulated. The mean value was obtained by selecting blocks of enamel with roughness values closest to the mean (0.04±0.01 μm). At the end of this phase, 138 enamel blocks were selected for the experiment (135 for the microhardness, roughness, and color analysis and three for the baseline micromorphological analysis).

Color Analysis

The initial color of the enamel slabs was evaluated with a spectrophotometer (Easyshade Advance, VITA, Bad Säckingen, Germany) whose tip (with a 5.0-mm diameter) was placed at the center of the enamel surface to ensure reproducibility. The evaluations were carried out in a location sheltered from outside luminosity. The measurements were taken in duplicate to improve accuracy. When the two readings were the same for the VITA Classical scale, the value obtained at the second reading was used. If the two readings did not match, another measurement was made to reach agreement between the two readings.

All the color analyses regarding the parameters of the VITA Classical scale and the Commission Internationale de L'éclairage (CIE) color coordinates were performed using a spectrophotometer, where L* represents lightness, a* represents the point on a red-green scale, and b* represents the point on a yellow-blue scale (CIELAB).

Clinical Phase: Bleaching and Staining Protocols

A week before the bleaching treatment began (run-in period) and throughout the entire treatment, the participants standardized the toothbrush (Colgate Slimsoft, Colgate-Palmolive, São Bernardo do Campo, Brazil) and dentifrice used (Colgate Maximum Anticaries Protection, 1500-ppm fluoride toothpaste, Colgate-Palmolive).

Figure 1. *Line 1: in-office bleaching (40% hydrogen peroxide); line 2: no bleaching (control); line 3: at-home bleaching (10% carbamide peroxide). Column 1: immersion in coffee; column 2: no immersion; column 3: immersion in cola solution.*

Nine enamel slabs were positioned in the appliance according to the treatments to be applied, randomly allocated in lines and columns (Figure 1). The devices were delivered to the volunteers early in the morning, together with instructions for daily use, for a total of 15 days. The volunteers were told to use the device throughout the day and night and remove it only for meals. When the device was removed from the oral cavity, it had to remain in a plastic box wrapped in damp gauze.[22] The device and the dental blocks had to be brushed twice a day with toothbrush and toothpaste, applied with 10 back-and-forth movements on top of each row.

After the first four hours of use (in which there occurred the formation of a pellicle of acquired biofilm),[29] the devices were collected by the researcher for the application of bleaching agents (Table 1). The enamel slabs on line 1 received the in-office bleaching treatment with 40% hydrogen peroxide (Opalescence Boost PF 40% [OPA40], Ultradent). The enamel slabs in line 2 received no bleaching agent (control). In line 3, the bleaching treatment was performed with 10% carbamide peroxide (Opalescence PF 10% [OPA10], Ultradent) (Figure 1). In applying the bleaching agents, 0.02 mL of each gel was applied to the surface of the dental enamel, taking care not to let the gel overflow and invade the neighboring enamel blocks. OPA40 was applied only on treatment days 1, 8, and 15; it had to remain on the surface of the enamel for 40 minutes. OPA10 was applied daily for 60 minutes, for a total of 15 days of treatment (except on weekends, when no bleaching

Table 1: *Bleaching Agents, Compositions, and Manufacturers*

Bleaching Treatment	Composition (Percentage in Weight)[a]	Mode of Use
OPA40, Opalescence Boost PF 40% (Ultradent South Jordan, UT, USA) BCRFX	Hydrogen peroxide (<40), sodium fluoride (1.1-1.5), potassium nitrate (<5), potassium hydroxide (<5)	40 min per session for three sessions with 1-wk intervals between sessions
OPA10, Opalescence PF10% (Ultradent) DO2J6	Carbamide peroxide (<20), polyacrylic acid (<10), sodium fluoride (0.25), sodium hydroxide (<5)	60 min per day for 15 d

[a] *According to the material safety data sheet of each product.*

treatment was performed). Removal of the bleaching agents was done initially with gauze, followed by washing with distilled water for 10 seconds.

After performing the bleaching treatments, the device was inserted into the oral cavity to be used by the research volunteer for two hours. After this time, the devices were again collected for immersion of the blocks into the staining solutions daily for a total of 15 days, except on weekends, where no spotting treatment was performed.

The enamel slabs from column 1 (Figure 1) were submitted to staining with coffee. The coffee was prepared with 50 mL of hot water and one teaspoon of soluble coffee (Nescafé Original, Nestlé, Araras, Brazil), according to the manufacturer's instructions. The enamel slabs were immersed into 30 mL of the coffee solution (considering a volume of 10 mL of solution per dental block) for 30 minutes daily, simulating the contact of the coffee with the dental structure for one day of consumption.[19,22] After immersion, the blocks were washed in distilled water for 10 seconds. The enamel slabs from column 2 were not submitted to any staining solution. Finally, the slabs from column 3 were submitted to the cola solution (Coca-Cola, Rio de Janeiro, Brazil) by immersing them in 30 mL of this cola (considering a volume of 10 mL of solution per dental block) for 30 minutes daily.

The pH for each bleaching agent and solution was obtained by using a pH meter (MS Tecnopon Special Equipment Ltd, Piracicaba, Brazil) and in triplicate. The corresponding pH values obtained were as follows: 6.64 for the at-home bleaching agent, 7.24 for the in-office bleaching agent, 2.51 for the cola, and 4.78 for the coffee.

At the end of 15 days, the intraoral device was collected on the morning following the last day of treatment. A representative figure comparing the *in situ* device before and after 15 days of use is presented in Figure 2. The enamel blocks were

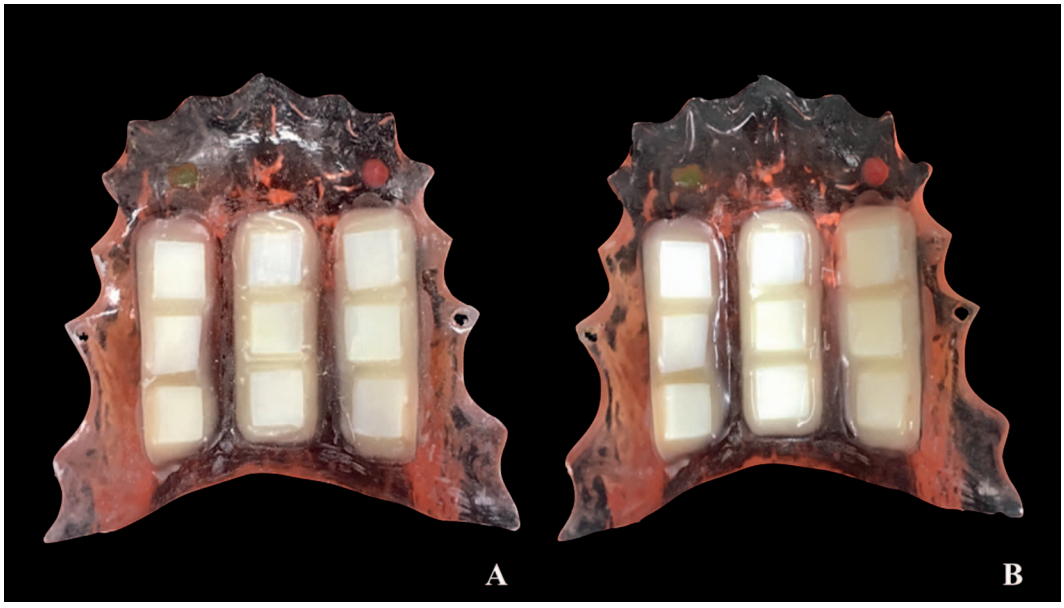

Figure 2. *Representative images of the* in situ *device before and after 15 days of use. (A):* In situ *device before insertion into the oral cavity. (B):* In situ *device after 15 days of use.*

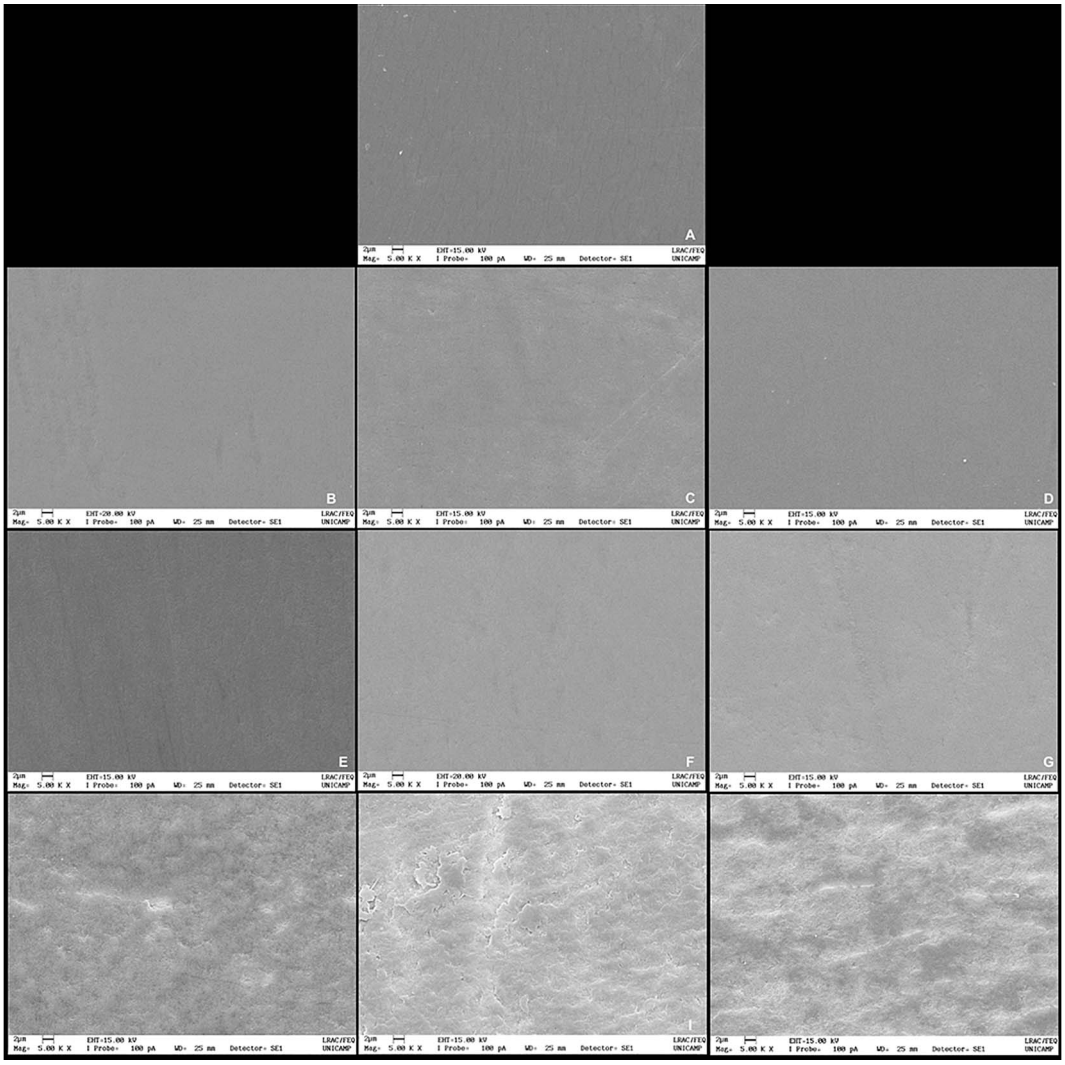

Figure 3. *Representative enamel surface micromorphological features. (A): Baseline. (B): Coffee + at-home bleaching. (C): Coffee + in-office bleaching. (D): Coffee + no bleaching. (E): No solution + at-home bleaching. (F): No solution + in-office bleaching. (G): No solution + no bleaching. (H): Cola solution + at-home bleaching. (I): Cola solution + in-office bleaching. (J): Cola solution + no bleaching.*

removed from the intraoral device and stored in Eppendorf tubes left in relative humidity for final analysis of microhardness, roughness, and color.

Micromorphological Analysis of the Enamel Surface

Three enamel blocks from each group (before and after each combination of bleaching treatments and staining solutions) were separated for analysis by scanning electronic microscope (SEM) (LEO Electron Microscopy, Oxford, England) to obtain representative images. They were submitted to gold sputtering. Images were taken at 5000× magnification. A single operator evaluated the enamel surface for the presence of erosion, irregularities, and depressions. The examiner was blinded during the SEM evaluation.

Statistical Analysis

The color of the tooth was obtained according to the VITA Classical scale and was converted into numbers according to numerical values previously established in the literature:[13] B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, and C4. Note that the smaller the numerical value, the brighter the tooth appears. The values of ΔL^*, Δa^*, and Δb^* were calculated by comparing the baseline to the after-treatment time period. After obtaining the values of ΔL^*, Δa^*, and Δb^* for each treatment, ΔE^* was calculated using the following mathematical formula: $\Delta E^* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$, where ΔE^* = the color change, ΔL^* = L*final – L*initial, Δa^* = a*final – a*initial, and Δb^* = b*final – b*initial. The color data in the VITA Classical scale and CIELAB

Table 2: *Microhardness Means (Standard Deviations) in kgf/mm² According to Staining Solution, Bleaching Agent, and Time Period*[a]

Time Period	Solution	Bleaching Agent		
		No Bleaching	At-Home	In-Office
Baseline	Coffee	370.56 (40.84) Aa	377.53 (43.73) Aa	395.13 (49.24) Aa
	Cola	396.00 (37.40) Aa	365.09 (36.94) Aa	375.93 (43.70) Aa
	No solution	367.40 (36.59) Aa	370.22 (48.73) Aa	370.13 (35.62) Aa
Final	Coffee	353.16 (98.86) Aa	364.69 (106.85) Aa	304.90 (85.90) Aa
	Cola	121.44 (78.10) Ab[b]	130.76 (77.71) Ab[b]	116.76 (90.31) Ab[b]
	No solution	398.98 (116.51) Aa	339.77 (91.74) Aa	358.80 (99.47) Aa

[a] *Means followed by different letters (uppercase horizontally and lowercase vertically, comparing solutions at each time period) differ among the time periods (p≤0.05).*
[b] *Differ significantly from the baseline in the same conditions of bleaching agent and solution (p≤0.05).*

system did not meet the assumptions of a parametric analysis and were analyzed by generalized linear models considering the study design.

The microhardness data met the assumptions of a parametric analysis. Roughness was determined by performing logarithmic transformation. Mixed-model methodology was applied for repeated measurements in a 3×3 (PROC mixed) factorial scheme for microhardness and roughness. All analyses were carried out using SAS software (version 9.2, SAS Institute Inc, Cary, NC, USA) considering a 5% level of significance.

RESULTS

There was no significant interaction among the factors of type of bleaching treatment, staining solution, and time period influencing microhardness ($p=0.1556$), but there was a significant effect resulting from the interaction between staining solution and time period ($p<0.0001$). Microhardness averages were not significantly different among the treatments at the baseline time. At the final time period (after the last bleaching session), the microhardness of the enamel submitted to staining with cola was significantly lower than that of the other groups ($p<0.0001$), highlighting a significant decrease in microhardness ($p<0.0001$) in relation to the initial time period regardless of whether bleaching was performed (Table 2).

There was no significant interaction among the factors of type of bleaching, staining solution, and time period influencing roughness values ($p=0.9112$), but there was a significant effect of these factors on the interaction between staining solution and time period ($p<0.0001$). At the beginning, there was no significant difference among the treatments. However, at the final time period, the roughness was greater for the enamel submitted to cola ($p<0.0001$) and increased significantly by the final time period regardless of whether bleaching was performed ($p<0.0001$) (Table 3).

There was a significant effect between staining solution and time period for the VITA Classical shade ($p<0.0001$) but no significant effect of the interaction among the factors of bleaching treatment, staining solution, and time period ($p=0.5684$). In the groups where no staining solution was used, a smaller color score was observed at the final vs the

Table 3: *Roughness Means (Standard Deviations) in μm According to Staining Solution, Bleaching Agent, and Time*[a]

Time Period	Solution	Bleaching Agent			
		No Bleaching	At-Home	In-Office	
Baseline	Coffee	0.04 (0.01)	0.04 (0.01)	0.03 (0.01)	a
	Cola	0.04 (0.01)	0.03 (0.01)	0.05 (0.02)	a
	No solution	0.04 (0.02)	0.04 (0.01)	0.03 (0.01)	a
		A	A	A	
Final	Coffee	0.05 (0.06)	0.04 (0.02)	0.04 (0.02)	b
	Cola	0.20 (0.05)[b]	0.18 (0.05)[b]	0.21 (0.06)[b]	a
	No solution	0.04 (0.01)	0.04 (0.01)	0.03 (0.01)	b
		A	A	A	

[a] *Means followed by different letters (uppercase horizontally and lowercase vertically, comparing solutions at each time period) differ among each time period (p≤0.05).*
[b] *Differ significantly from the baseline in the same conditions of bleaching agent and solution (p≤0.05).*

Table 4: *Means (Standard Deviations) of the VITA Classical Scores According to Staining Solution, Bleaching Agent, and Time Period*[a]

Time Period	Solution	Bleaching Agent			
		No Bleaching	At-Home	In-Office	
Baseline	Coffee	11.13 (1.55)	10.73 (2.66)	10.67 (2.61)	a
	Cola	10.33 (2.55)	10.20 (3.23)	10.33 (2.38)	a
	No solution	10.36 (2.59)	11.27 (1.71)	10.67 (3.58)	a
		A	A	A	
Final	Coffee	10.60 (3.20)	10.47 (2.77)	8.93 (4.20)	a
	Cola	10.79 (2.58)	11.07 (3.01)	9.47 (3.48)	a
	No solution	8.27 (3.03)[b]	8.93 (3.41)[b]	5.67 (3.85)[b]	b
		A	A	B	

[a] *Means followed by different letters (uppercase horizontally and lowercase vertically, comparing solutions for each time period) differ among each time period ($p \leq 0.05$).*
[b] *Differ significantly from the baseline in the same conditions of bleaching agent and solution ($p \leq 0.05$).*

initial time periods ($p<0.0001$). The color scores for OPA40 were significantly lower than those of the other treatments at the final time period ($p=0.0034$). The enamel color scores were lower at the final time period, when no staining solution was used ($p=0.0154$) (Table 4).

When in-office bleaching was performed and no staining solution was used, a greater increase in the ΔL value was observed than for at-home bleaching ($p=0.0444$). There was no significant difference between the bleaching agents when they were associated with the staining solutions. There was also no significant difference between the bleaching agents in regard to Δa ($p=0.8270$), and lower values were observed when no staining solution was used ($p=0.0010$). The Δb value also did not vary significantly between the bleaching agents ($p=0.3929$) but was significantly higher when cola was used ($p=0.0293$). In contrast, higher ΔE values were observed when cola was associated with the bleaching treatments ($p=0.0089$). Even when no staining solution was used, lower values were observed for the control and the at-home bleaching groups. Higher ΔE values were observed for in-office bleaching when no staining solution was used (Table 5), while lower values of ΔE were observed for the in-office associated with coffee immersion.

The SEM images (Figure 3) at baseline show that the enamel surface had a smooth and polished appearance (A). The groups submitted to the coffee solution and those not submitted to any solution (control) had the same characteristics of no erosion or porosity regardless of application or nonapplication of the bleaching agents (B to G). However, when the enamel was immersed in cola (H to J), the surface had a rough appearance, irregularities, and cracks regardless of whether the bleaching agent was applied.

DISCUSSION

The effectiveness of bleaching agents containing hydrogen peroxide and carbamide peroxide in high and low concentrations is influenced by the physical, mechanical, and micromorphological properties of the enamel subjected to beverages rich in pigments consumed during the bleaching technique. Even though laboratory studies have been carried out to evaluate this influence,[16,19-21] intraoral conditions cannot be reliably reproduced and must be analyzed with studies using *in situ* methodology.

When evaluating the microhardness of dental enamel not submitted to staining solutions, no significant changes were observed between the evaluation time periods with or without the bleaching agents. Both bleaching agents used have fluorides in their composition. This may have contributed to slowing the demineralization process[30] and enhancing enamel remineralization.[31,32] In addition, the manufacturer of the bleaching gels does not specify what thickening agent is used. Since carbopol (polycarboxylic acid)—present in the composition of many bleaching agents as a thickening agent—is known to cause enamel demineralization,[33,34] it may be suggested that both gels presented another component as the thickener. The pH values of the bleaching agents are also close to neutral (6.64 for the at-home agent and 7.24 for the in-office agent), thus contributing to balancing the demineralization and remineralization processes. Furthermore, the permanent presence of saliva—because this was a *in situ* study—has an enamel remineralizing action because it is supersaturated in minerals.[35-37] *In vitro*

Table 5: *Means (Standard Deviations) of the CIELAB Color Change According to Staining Solution, Bleaching Agent, and Time Period*[a]

Variable	Solution	Bleaching Agent		
		No Bleaching	At-Home	In-Office
ΔL[b]	Coffee	0.39 (6.40) Aab	3.91 (6.69) Aa	2.33 (4.03) Ab
	Cola	−0.20 (4.03) Bb	0.67 (6.47) Aa	2.74 (5.44) Aab
	No solution	5.27 (5.56) ABa	3.47 (7.15) Ba	7.68 (5.20) Aa
Δa[c]	Coffee	1.70 (2.28) Aa	1.54 (1.83) Aa	0.34 (1.89) Aa
	Cola	1.30 (2.01) Aa	1.63 (3.04) Aa	1.19 (2.52) Aa
	No solution	0.14 (0.91) Ab	−0.41 (2.23) Ab	−1.50 (2.60) Ab
Δb[d]	Coffee	−0.27 (5.97) Aab	1.42 (5.49) Aab	−3.27 (6.41) Aab
	Cola	3.69 (4.44) Aa	2.52 (7.49) Aa	−0.33 (8.67) Aa
	No solution	−2.47 (3.48) Ab	−3.00 (3.78) Ab	−5.75 (6.25) Ab
ΔE[e]	Coffee	7.77 (4.53) Aa	8.07 (5.44) Aa	7.83 (3.56) Ab
	Cola	6.40 (3.53) Ba	9.12 (5.32) Aa	9.89 (3.98) Aab
	No solution	7.44 (4.55) Ba	6.83 (6.56) Ba	11.47 (5.74) Aa

[a] *Means followed by different letters (uppercase horizontally and lowercase vertically, comparing solutions at each time period) differ among each time period ($p \leq 0.05$).*
[b] *p(bleaching)=0.0444; p(solution)=0.0886; p(bleaching vs solution)=0.0530.*
[c] *p(bleaching)=0.8270; p(solution)=0.0010; p(bleaching vs solution)=0.7010.*
[d] *p(bleaching)=0.3929; p(solution)=0.0293; p(bleaching vs solution)=0.2950.*
[e] *p(bleaching)=0.0089; p(solution)=0.7486; p(bleaching vs solution)=0.0718.*

studies have shown that even when artificial saliva solutions are used, bleaching gels with more acidic pH have been found to decrease enamel microhardness[35] and can negatively influence hardness values during bleaching.[38]

Even in the groups not submitted to any bleaching agent, the application of coffee did not promote any changes in enamel microhardness. However, the association of cola caused a decrease in microhardness in all the bleaching groups. Thus, the first null hypothesis was rejected, namely, that there are no differences in enamel microhardness between at-home and in-office dental bleaching submitted to staining solutions (coffee and cola) or not submitted to any solution. It is known that enamel exposed to solutions with a low pH for a certain period of time can present demineralization[37] or erosion.[40] Although the coffee solution presented a pH of 4.78, it was unable to cause demineralization in contact with the enamel for 30 minutes a day. Therefore, it can be suggested that, even with an acidic pH and with the same immersion time for coffee and cola, the demineralizing or erosive potential of coffee was not as harmful to the enamel as the effects caused by cola, which had a pH of 2.51. Among the soft-drink beverages, cola has the highest erosive potential[41,42] and requires more time for saliva to neutralize the acid medium.[42] Cola is also different from other soft drinks because of the phosphoric acid in its composition, which gives the beverage its acidity. Although there are a number of studies that show that cola causes a reduction in microhardness,[21,42] no studies were found that evaluated the influence of coffee on enamel microhardness. In the present study, even the constant presence of saliva and fluoride (from the fluoride toothpaste used for brushing the enamel blocks) was not enough to maintain or recover the initial microhardness values observed prior to immersion in cola.

The reduction in enamel microhardness when using cola was accompanied by increased roughness and by micromorphological changes similar to depressions and enamel erosion with or without use of the bleaching agents. Likewise, the second and the third null hypotheses were also rejected since differences were in fact observed in the enamel roughness and micromorphology between at-home and in-office dental bleaching submitted to staining solutions (coffee and cola) or not submitted to any solution. Other studies also reported that cola promotes greater enamel roughness[44,45] and leads to dental erosion.[41,46] These effects are duly explained by the acidic and erosive characteristics of the beverage, which raise roughness values to a threshold of about 0.2 μm.[47] These higher values may increase plaque accumulation. However, when neither coffee nor cola nor any bleaching agent was used, the roughness and micromorphology of the enamel were not altered, and the enamel surface looked smooth and polished, just like the bleached enamel surfaces observed in the *in situ* study by Zeczkowshi and others.[48] Although the literature

has reported that both at-home bleaching using 10% carbamide peroxide[7,49] and in-office bleaching[50] can change tooth roughness and promote erosion, the products currently available for dental bleaching have fewer detrimental effects due to the modernizing changes made in their composition over time. For example, the bleaching agents used today are less acidic than those available in the 1990s and in 2000, which had a pH from 4.3 to 5.2 for at-home use[51] and from 4.3 to 5.5 for in-office use.[5,10,32] Today's bleaching process has a shorter time protocol for using the agents in trays[52,53] and a modified thickener.[32,33] Although manufacturers do not declare the composition of bleaching agents in their entirety, it is known that the ingredients have been modified over time in response to the constant innovations and solutions that the research community has offered to manufacturers over time.

When no staining solution was used, the enamel had lower color scores for the VITA Classical scale, thus confirming that the bleaching treatments were effective in promoting the desired result. In regard to the bleaching agent used for at-home use, the color changed about three shades on the scale (from 11.27 before bleaching to 8.93 afterward), whereas the color change in the in-office treatment was five shades on average (from 10.67 to 5.67). Although these results may be related to the differences in the concentrations of the hydrogen peroxide agents used (10% carbamide peroxide for the at-home bleaching and 40% hydrogen peroxide for in-office) and the application time of only one hour per day for the at-home bleaching agent[52] (instead of overnight as recommended by the manufacturer), other studies have also shown that bleaching with carbamide peroxide in low concentrations[54,55] and hydrogen peroxide in high concentrations[56,57] has had satisfactory results. Furthermore, similar effectiveness between the techniques was reported in a systematic review,[58] but it did not take into consideration the variations in protocols (daily use time, number of bleaching sessions, and product concentration) of the bleaching techniques in the studies included.

Lighter tooth staining was also found in the present study when no gel was applied and no pigmented solution was used. Although all procedures were performed with utmost care to avoid contamination of the gel with blocks belonging to another group positioned alongside, it is believed that there may have been diffusion of the residual gel into the oral cavity, influencing the magnitude of ΔL^* and ΔE^*, which were high for this group despite meticulous removal of the gel. Because the devices were positioned on the palate, contact of the tongue with the surface of the enamel was unavoidable, and a small amount of gel residue could have diffused to the other blocks due to the low molecular weight of the free radicals released by the degradation of the peroxides. This is because peroxides can move freely between the structures of the substrates,[59] leading to the color changes observed in the nonbleached group caused by the proximity between the blocks. Although this approximation may have influenced the results for the no-bleaching and no-staining group, its effect cannot be avoided in the at-home and in-office bleaching groups submitted to the staining solutions together with other factors that may also explain the changes observed in the negative control. For example, although all care was taken to measure the colors correctly, the spectrophotometer may have been positioned incorrectly on the specimens, or the size of the enamel blocks may have been too small to accommodate the tip of the spectrophotometer, issues that will have to be dealt with more knowingly in future studies.

When analyzing the staining with cola vs coffee solutions, no differences were found based on the color analysis by the VITA Classical scale between before and after bleaching or between these solutions in the group that did not undergo bleaching. Thus, it may be suggested that, although the bleaching treatment was effective (as observed in the nonstained groups), the staining solutions used may have interfered in the final result of the treatment. Hence, the fourth null hypothesis was rejected since there were no differences in the enamel color change between at-home and in-office dental bleaching submitted to staining solutions (coffee and cola) or not submitted to any solution. In this respect, it is known that extrinsic staining is associated with the adsorption of pigments on the enamel surface, which can be removed efficiently with professional prophylaxis.[60] It has been reported that coffee and cola can stain teeth due to their dark coloration and acidic pH.[21,61] Low pH values may cause increased enamel permeability,[9] which may facilitate the penetration of the pigments during bleaching, thus interfering in the color results.[13,21] However, it has been found that the compounds that cause staining are constituted by chains of macromolecules, which barely permeate the enamel,[62] causing the pigments to be adsorbed on the surface of the enamel.

When analyzing the change in brightness (ΔL), the groups not subjected to bleaching or staining presented higher values than the group submitted

to staining with cola, which showed a reduction in luminosity. A more pronounced change in luminosity was found for teeth submitted to in-office bleaching without staining (greater luminosity) than for at-home bleaching without staining. This result could be related to the higher concentration of hydrogen peroxide contained in the bleach used in the in-office versus at-home treatments. In the latter, the bleach may require more time in contact with the teeth to produce a result with higher luminosity, as obtained by Briso and others[25] and Attin and others,[16] who associated different staining solutions (coffee, grape juice, and black tea) with 10% carbamide peroxide and observed higher values of brightness variation in the absence of staining. The present study also showed that the cola reduced the brightness of the enamel when no bleaching treatment was used, corroborating the findings by Pirolo and others,[63] who demonstrated the potential of cola to stain.

An analysis of the color change in the Δa variable, in the groups submitted to staining, indicated that more reddish pigments,[25] such as those present in coffee (brown pigment) and coca (caramel pigment), may not have actually been removed by different bleaching techniques but rather may have been adsorbed into the surface of the enamel. However, the most important color spectrum for whitening studies is the Δb variable since it is related to the color change of positive values, corresponding to colors from the yellow color band to the blue range, which presents negative values.[64] It was found that teeth became more yellow when the enamel blocks were subjected to cola solutions and showed intermediate yellowing values when submitted to coffee. When submitted to cola, the enamel blocks presented positive values, corresponding to the yellow color band regardless of having been submitted to a lightening process. When tooth enamel is exposed to an acidic substance, surface demineralization may occur, leaving the enamel more susceptible to pigment deposition, thereby explaining the results found.

Regarding color change (ΔE), values above 3.3 present clinically perceptible color changes and prove the effectiveness of the bleaching procedures.[65] ΔE values above 8 cause extremely perceptible changes.[66] In the group where the enamel blocks were not exposed to staining but were submitted to office bleaching, the value of ΔE was 11.47. This group was significantly different from the other nonstained groups, resulting in a desirable clinical response by the patients who completed the treatment. Even though all the other groups presented ΔE values above 3.3, indicating that all the groups presented clinically perceptible color changes after treatment, it still cannot be concluded that all the groups were affected by the bleach when considering each group (L*, a*, and b*) individually. This is because there is a "competitive" effect between the bleaching agents and the staining solutions that promotes bleaching (by degrading pigmented molecules) and darkening (by depositing dark pigments). Because ΔE values are the result of the interaction of all the parameters, they should not be evaluated separately; otherwise, there may be misinterpretation. Thus, it can be observed that the groups submitted to staining with cola had the following characteristics: 1) less alteration in luminosity than the bleached groups; 2) the teeth presented more red pigments than those not submitted to staining, whether or not they received the bleaching treatment; and 3) the teeth were more yellowish than those not submitted to staining, whether or not they received the bleaching treatment. In relation to staining with coffee, the lesser change in luminosity and the greater presence of reddish pigment may have influenced the ΔE values when associated with in-office bleaching. This can be explained by the daily immersion in coffee, whereas the in-office bleaching session was performed only once a week. Hence, this daily immersion influenced the color change, whereas the less frequent in-office dental bleaching decreased staining effectiveness, although a clinically perceptible color change was in fact achieved with in-office bleaching (ΔE value higher than 3.3).

Thus, considering the results for the VITA Classical scale and the L*, a*, and b* parameters, it can be concluded that the association of staining solutions to the bleaching treatment influences the color of the teeth during both at-home and in-office bleaching treatments and results in a less effective color change. In addition, cola also led to significant changes in microhardness, roughness, and micromorphology of the enamel. This may indicate that its consumption in everyday situations may also result in damage to the enamel surface.

CONCLUSIONS

Regardless of the bleaching treatment used (10% carbamide peroxide for at-home use and 40% hydrogen peroxide for in-office bleaching), there was a decrease in microhardness, an increase in roughness, and changes in the micromorphology of the cola-stained enamel. The effectiveness of the bleaching agents was higher in the absence of the

staining solutions (coffee or cola), and the in-office bleaching option presented a greater color change than at-home bleaching.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the São Leopoldo Mandic Research Institute Ethical Committee. The approval code for this study is 59850416.0.0000.5374.

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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REFERENCES

1. Sulieman M (2005) An overview of tooth discoloration: Extrinsic, intrinsic and internalized stains *Dental Update* **32(8)** 463-471.
2. Polydorou O, Hellwig E, & Auschill TM (2007) The effect of at-home bleaching on the microhardness of six esthetic restorative materials *Journal of the American Dental Association* **138(7)** 978-984.
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. Faraoni-Romano JJ, Turssi CP, & Serra MC (2007) Concentration-dependent effect of bleaching agents on microhardness and roughness of enamel and dentin *American Journal of Dentistry* **20(1)** 31-34.
5. Xu B, Li Q, & Wang Y (2011) Effects of pH values of hydrogen peroxide bleaching agents on enamel surface properties *Operative Dentistry* **36(5)** 554-562.
6. Dominguez JA, Bittencourt B, Michel M, Sabino N, Gomes JC, & Gomes OM (2012) Ultrastructural evaluation of enamel after dental bleaching associated with fluoride *Microscopy Research and Technique* **75(8)** 1093-1098.
7. Basting RT, Rodrigues AL, & Serra MC (2007) Micromorphology and surface roughness of sound and demineralized enamel and dentin bleaching with a 10% carbamide peroxide bleaching agent *American Journal of Dentistry* **20(2)** 97-102.
8. Sasaki RT, Catelan A, Bertoldo Edos S, Venâncio PC, Groppo FC, Ambrosano GM, Marchi GM, Lima DA, & Aguiar FH (2015) Effect of 7.5% hydrogen peroxide containing remineralizing agents on hardness, color change, roughness and micromorphology of human enamel *American Journal of Dentistry* **28(5)** 261-267.
9. Berger SB, Tabchoury CP, Ambrosano GM, & Giannini M (2013) Hydrogen peroxide penetration into the pulp chamber and dental permeability after bleaching *General Dentistry* **61(3)** e21-e25.
10. Basting RT, Antunes EV, Turssi CP, do Amaral FL, Franca FM, & Florio FM (2015) In vitro evaluation of calcium and phosphorus concentrations in enamel submitted to an in-office bleaching gel treatment containing calcium. *General Dentistry* **63(5)** 52-56.
11. Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T, & Wang Y (2013) Effects of two in-office bleaching agents with different pH on the structure of human enamel: An in situ and in vitro study *Operative Dentistry* **38(1)** 100-110.
12. Ittatirut S, Matangkasombut O, & Thanyasrisung P (2014) In-office bleaching gel with 35% hydrogen peroxide enhanced biofilm formation of early colonizing streptococci on human enamel *Journal of Dentistry* **42(11)** 1480-1486.
13. Rezende M, Loguercio AD, Reis A, & Kossatz S (2013) Clinical effects of exposure to coffee during at-home vital bleaching *Operative Dentistry* **38(6)** E229-E236.
14. Matis BA, Wang G, Matis JI, Cook NB, & Eckert GJ (2015) White diet: Is it necessary during tooth whitening? *Operative Dentistry* **40(3)** 235-240.
15. Liporoni PC, Souto CM, Pazinatto RB, Cesar IC, de Rego MA, Mathias P, & Cavalli V (2010) Enamel susceptibility to coffee and red wine staining at different intervals elapsed from bleaching: A photoreflectance spectrophotometry analysis *Photomedicine and Laser Surgery* **28(Supplement 2)** S105-S109.
16. Attin T, Manolakis A, Buchalla W, & Hannig C (2003) Influence of tea on intrinsic colour of previously bleached enamel *Journal of Oral Rehabilitation* **30(5)** 488-494.
17. Brazilian Agricultural Research Corporation–Embrapa (1972) *Brasil consome 21,5 milhoes de sacas de café em 2017*; Retrieved online July 14, 2018 from: https://www.embrapa.br/en/busca-de-noticias/-/noticia/31768082/brasil-consome-215-milhoes-de-sacas-de-cafe-em-2017
18. National Broadcasting Company (1926) Sweet! America's top ten brands of soda; Retrieved online July 27, 2018 from: http://www.nbcnews.com/id/42255151/ns/business-us_business/t/sweet-americas-top-brands-soda/#.W1X8SNJKjIV
19. Attia ML, Aguiar FH, Mathias P, Ambrosano GM, Fontes CM, & Liporoni PC (2009) The effect of coffee solution on tooth color during home bleaching applications *American Journal of Dentistry* **22(3)** 175-179.
20. Bazzi JZ, Bindo MJ, Rached RN, Mazur RF, Vieira S, & de Souza EM (2012) The effect of at-home bleaching and toothbrushing on removal of coffee and cigarette smoke stains and color stability of enamel *Journal of the American Dental Association* **143(5)** e1-e7.
21. De Araújo LS, dos Santos PH, Anchieta RB, Catelan A, Fraga Briso AL, Fraga Zaze AC, & Sundfeld RH (2013) Mineral loss and color change of enamel after bleaching and staining solutions combination *Journal of Biomedical Optics* **18(10)** 108004.

22. Mori AA, Lima FF, Benetti AR, Terada RS, Fujimaki M, & Pascotto RC (2016) Susceptibility to coffee staining during enamel remineralization following the in-office bleaching technique: An in situ assessment *Journal of Esthetic and Restorative Dentistry* **28(Supplement 1)** S23-S31.

23. Topcu FT, Sahinkesen G, Yamanel K, Erdemir U, Oktay EA, & Ersahan S (2009) Influence of different drinks on the colour stability of dental resin composites *European Journal of Dentistry* **3(1)** 50-56.

24. De Andrade IC, Basting RT, Lima-Arsati YB, do Amaral FL, Rodrigues JA, & França FM (2011) Surface roughness evaluation and shade changes of a nanofilled resin composite after bleaching and immersion in staining solutions *American Journal of Dentistry* **24(4)** 245-249.

25. Briso AL, Fagundes TC, Gallinari MO, Moreira J, de Almeida L, Rahal V, Gonçalves RS, & Santos PD (2016) An in situ study of the influence of staining beverages on color alteration of bleached teeth *Operative Dentistry* **41(6)** 627-633.

26. Faul F, Erdfelder E, Buchner A, & Lang AG (2009) Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses *Behavior Research Methods* **41(4)** 1149-1160.

27. Cohen J (1988) *Statistical Power Analysis for the Behavioral Sciences* Lawrence Erlbaum Associates, Mahwah, NJ.

28. Rodrigues JA, Marchi GM, Ambrosano GM, Heymann HO, & Pimenta LA (2005) Microhardness evaluation of in situ vital bleaching on human dental enamel using a novel study design *Dental Materials* **21(11)** 1059-1067.

29. Lee YH, Zimmerman JN, Custodio W, Xiao Y, Basiri T, Hatibovic-Kofman S, & Siqueira WL (2013) Proteomic evaluation of acquired enamel pellicle during in vivo formation *PloS One* **8(7)** e67919.

30. Attin T, Albrecht K, Becker K, Hannig C, & Wiegand A (2006) Influence of carbamide peroxide on enamel fluoride uptake. *Journal of Dentistry* **34(9)** 668-675.

31. Cavalli V, Rodrigues LK, Paes-Leme AF, Brancalion ML, Arruda MA, Berger SB, & Giannini M (2010) Effects of bleaching agents containing fluoride and calcium on human enamel *Quintessence International* **41(8)** e157-e165.

32. Magalhães JG, Marimoto AR, Torres CR, Pagani C, Teixeira SC, & Barcellos DC (2012) Microhardness change of enamel due to bleaching with in-office bleaching gels of different acidity *Acta Odontologica Scandinavica* **70(2)** 122-126.

33. Basting RT, Rodrigues AL Jr, & Serra MC (2005) The effect of 10% carbamide peroxide, carbopol and/or glycerin on enamel and dentin microhardness *Operative Dentistry* **30(5)** 608-616.

34. Rodrigues JA, Oliveira GP, & Amaral CM (2007) Effect of thickener agents on dental enamel microhardness submitted to at-home bleaching *Brazilian Oral Research* **21(2)** 170-175.

35. Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T, & Wang Y (2013) Effects of two in-office bleaching agents with different pH on the structure of human enamel: An in situ and in vitro study *Operative Dentistry* **38(1)**100-110.

36. Borges AB, Guimaräes CA, Bresciani E, Ramos CJ, Borges AL, & Rocha Gomes Torres C (2014) Effect of incorporation of remineralizing agents into bleaching gels on the microhardness of bovine enamel in situ *Journal of Contemporary Dental Practice* **15(2)** 195-201.

37. Henn-Donassollo S, Fabris C, Gagiolla M, Kerber Í, Caetano V, Carboni V, Salas MM, Donassollo TA, & Demarco FF (2016) In situ and in vitro effects of two bleaching treatments on human enamel hardness *Brazilian Dental Journal* **27(1)** 56-95.

38. Zantner C, Beheim-Schwarzbach N, Neumann K, & Kielbassa AM (2007) Surface microhardness of enamel after different home bleaching procedures *Dental Materials* **23(2)** 243-250.

39. Driessens FC, Theuns HM, Borggreven JM, & van Dijk JW (1986) Solubility behavior of whole human enamel *Caries Research* **20(2)** 103-110.

40. Hughes JA, West NX, Parker DM, van den Braak MH, & Addy M (2000) Effects of pH and concentration of citric, malic and lactic acids on enamel, in vitro *Journal of Dentistry* **28(2)** 147-152.

41. Jensdottir T, Holbrook P, Nauntofte B, Buchwald C, & Bardow A (2006) Immediate erosive potential of cola drinks and orange juices *Journal of Dental Research* **85(3)** 226-230.

42. Torres CP, Chinelatti MA, Gomes-Silva JM, Rizóli FA, Oliveira MA, Palma-Dibb RG, & Borsatto MC (2010) Surface and subsurface erosion of primary enamel by acid beverages over time *Brazilian Dental Journal* **21(4)** 337-345.

43. Lussi A, Jaeggi T, & Zero D (2004) The role of diet in the aetiology of dental erosion *Caries Research* **38(Supplement 1)** 34-44.

44. Barac R, Gasic J, Trutic N, Sunaric S, Popovic J, Djekic P, Radenkovic G, & Mitic A (2015) Erosive effect of different soft drinks on enamel surface in vitro: Application of stylus profilometry *Medical Principles and Practice* **24(5)** 451-457.

45. Karda B, Jindal R, Mahajan S, Sandhu S, Sharma S, & Kaur R (2016) To analyse the erosive potential of commercially available drinks on dental enamel and various tooth coloured restorative materials—An in-vitro study *Journal of Clinical and Diagnostic Research* **10(5)** ZC117-ZC121.

46. Colombo M, Mirando M, Rattalino D, Beltrami R, Chiesa M, & Poggio C (2017) Remineralizing effect of a zinc-hydroxyapatite toothpaste on enamel erosion caused by soft drinks: Ultrastructural analysis *Journal of Clinical and Experimental Dentistry* **9(7)** e861-e868.

47. Bollen CM, Lambrechts P, & Quirynen M (1997) Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: A review of the literature *Dental Materials* **13(4)** 258-269.

48. Zeczkowski M, Tenuta LMA, Ambrosano GMB, Aguiar FHB, & Lima DANL (2015) Effect of different storage conditions on the physical properties of bleached enamel:

An in vitro vs. in situ study *Journal of Dentistry* **43(9)**1154-1161.

49. Cvikl B, Lussi A, Moritz A, & Flury S (2016) Enamel surface changes after exposure to bleaching gels containing carbamide peroxide or hydrogen peroxide *Operative Dentistry* **41(1)** E39-E47.
50. Trentino AC, Soares AF, Duarte MA, Ishikiriama SK, & Mondelli RF (2015) Evaluation of pH levels and surface roughness after bleaching and abrasion tests of eight commercial products *Photomedicine and Laser Surgery* **33(7)** 372-377.
51. Shannon H, Spencer P, Gross K, & Tira D (1993) Characterization of enamel exposed to 10% carbamide peroxide bleaching agents *Quintessence International* **24(1)** 39-44.
52. Cardoso PC, Reis A, Loguercio A, Vieira LC, & Baratieri LN (2010) Clinical effectiveness and tooth sensitivity associated with different bleaching times for a 10 percent carbamide peroxide gel *Journal of the American Dental Association* **141(10)** 1213-1220.
53. Alonso de la Peña V & López Ratón M. (2014) Randomized clinical trial on the efficacy and safety of four professional at-home tooth whitening gels *Operative Dentistry* **39(2)** 136-143.
54. Bernardon JK, Sartori N, Ballarin A, Perdigao J, Lopes GC, & Baratieri LN (2010) Clinical performance of vital bleaching techniques *Operative Dentistry* **35(1)** 3-10.
55. López Darriba I, Novoa L, & de la Peña VA (2017) Efficacy of different protocols for at-home bleaching: A randomized clinical trial *American Journal of Dentistry* **30(6)** 329-334.
56. Reis A, Kossatz S, Martins GC, & Loguercio AD (2013) Efficacy of and effect on tooth sensitivity of in-office bleaching gel concentrations: A randomized clinical trial *Operative Dentistry* **38(4)** 386-393.
57. de Paula EA, Nava JA, Rosso C, Benazzi CM, Fernandes KT, Kossatz S, Loguercio AD, & Reis A (2015) In-office bleaching with a two- and seven-day intervals between clinical sessions: A randomized clinical trial on tooth sensitivity *Journal of Dentistry* **43(4)** 424-429.
58. de Geus JL, Wambier LM, Kossatz S, Loguercio AD, & Reis A (2016) At-home vs in-office bleaching: A systematic review and meta-analysis *Operative Dentistry* **41(4)** 341-356.
59. Kwon SR & Wertz PW (2015) Review of the mechanism of tooth whitening *Journal of Esthetic and Restorative Dentistry* **27(5)** 240-257.
60. Joiner A (2004) Tooth colour: A review of the literature *Journal of Dentistry* **32(Supplement 2)** 3-12.
61. Azer SS, Hague AL, & Johnston WM (2010) Effect of pH on tooth discoloration from food colorant in vitro *Journal of Dentistry* **38 (Supplement 2)** e106-e109.
62. Nicholson JW (1996) Biologic considerations In: Schwartz RS, Summitt JB, Robbins JW, Santos J (eds) *Fundamentals of Operative Dentistry: A Contemporary Approach* Quintessence Publishing, Chicago, IL 1-25.
63. Pirolo R, Mondelli RF, Correr GM, Gonzaga CC, & Furuse AY (2014) Effect of coffee and a cola-based soft drink on the color stability of bleached bovine incisors considering the time elapsed after bleaching *Journal of Applied Oral Science* **22(6)** 534-540.
64. Gerlach RW, Gibb RD, & Sagel PA (2002) Initial color change and color retention with a hydrogen peroxide bleaching strip *American Journal of Dentistry* **15(1)** 3-7.
65. Ruyter IE, Nilner K, & Moller (1987) Color stability of dental composite resin materials for crown and bridge veneers *Dental Materials* **3(5)** 246-251.
66. Guler AU, Kurt S, & Kulunk T (2005) Effects of various finishing procedures on the staining of provisional restorative materials *Journal of Prosthetic Dentistry* **93(5)** 453-458.