Influence of toothpastes containing tricalcium phosphate on dental enamel microhardness, color, and topography

Influência de dentifrícios contendo tricálcio fosfato na microdureza, cor e topografia do esmalte dental

Influencia de los dentífricos que contienen fosfato tricálcico en la microdureza, el color y la topografía del esmalte dental

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Abstract
The aim of this study was to evaluate the effect of using dentifrices containing tricalcium phosphate (β-TCP) on microhardness, enamel color, and topography before bleaching treatment with 35% hydrogen peroxide (HP). Fifty bovine incisor enamel/dentin blocks (4 × 4 × 2 mm) were randomized into five groups (n=10): (**C**) negative control (no treatment), positive control (35% HP), Colgate Total 12 then 35% HP (CT 12 + 35% HP), Elmex Sensitive then 35% HP (ES + 35% HP), and Bianco Pro Clinical then 35% HP (BPC + 35% HP). Knoop microhardness (KHN) and scanning electron microscopy (SEM) were performed. The color was evaluated using CIELAB (ΔE_{ab}^*) and CIEDE 2000 (ΔE_{00}) in 3 times: initial time (T_0), 24 hours after exposure to dentifrice (T_1), and 24 hours after bleaching treatment (T_2). Data for microhardness, ΔE_{ab}^*, and ΔE_{00} data were analyzed using generalized linear models, considering the model's group effect. The ΔL, Δa, and Δb data were analyzed using Kruskal–Wallis and Dunn's nonparametric tests. A significance of 5% was used. KHN was significantly higher in the BPC + 35% HP and ES + 35% HP groups than in the C and 35% HP groups (p=0.0295). ΔL was greater in the groups that received 35% HP than in the C group 24 hours after bleaching (*p*=0.0001). There was no significant difference between groups in ΔE_{ab}^* (*p*=0.0679) and ΔE_{00} (*p*=0.1463) 24 hours after bleaching. Using dentifrices containing β-TCP before bleaching treatment with 35% HP increases enamel microhardness but does not significantly alter tooth color. Clinical Significance: Dentifrices containing β-TCP in their formulation increase enamel microhardness without interfering with substrate color when used with previous in-office tooth bleaching.
Keywords: Tooth bleaching; Hydrogen Peroxide; Carbamide Peroxide; Dental enamel; Dentifrices.

Resumo
O objetivo deste estudo foi avaliar o efeito do uso de dentifrícios contendo tricálcio fosfato (β-TCP) na microdureza, cor e topografia do esmalte antes do tratamento clareador com peróxido de hidrogênio 35% (HP). Cinquenta blocos de

esmalte/dentina bovinos (4 × 4 × 2 mm) foram randomizados em cinco grupos (n=10): **(C)** controle negativo (sem tratamento), controle positivo (35% HP), Colgate Total 12 e 35% HP (CT 12 + 35% HP), Elmex Sensitive e 35% HP (ES + 35% HP) e Bianco Pro Clinical e 35% HP (BPC + 35% HP). Foram realizadas microdureza knoop (KHN) e microscopia eletrônica de varredura (SEM). A cor foi avaliada por meio do CIELAB (ΔE_{ab}^{*}) e CIEDE 2000 (ΔE_{00}) em 3 tempos: inicial (T_0), 24 após exposição ao dentifrício (T_1), e 24 horas após o tratamento clareador (T_2). Os dados de microdureza, ΔE_{ab}^{*}e ΔE_{00} foram analisados utilizando modelos lineares generalizados, considerando o efeito de grupo do modelo. Os dados ΔL, Δa e Δb foram analisados utilizando-se os testes não paramétricos de Kruskal-Wallis e Dunn. Utilizou-se significância estatística de 5%. KHN foi significativamente maior nos grupos BPC + 35% HP e ES + 35% HP do que nos grupos C e 35% HP (p=0,0295). ΔL foi maior nos grupos que receberam 35% de HP do que no grupo C (24 horas após o clareamento) (p=0,0001). Não houve diferença significativa entre os grupos em ΔE_{ab}^{*} (p=0,0679) e ΔE_{00} (p=0,1463) 24 horas após o clareamento. O uso de dentifrícios contendo β-TCP antes do tratamento clareador com 35% HP aumenta a microdureza do esmalte, mas não altera significativamente a cor do dente. Relevância Clínica: Dentifrícios contendo β-TCP em sua formulação aumentam a microdureza do esmalte sem interferir na cor do substrato quando usado previamente ao clareamento de consultório com 35% HP.
Palavras-chave: Clareamento dental; Peróxido de Hidrogênio; Peróxido de Carbamida; Esmalte dentário; Dentifrícios.

Resumen
El objetivo de este estudio fue evaluar el efecto del uso de dentífricos que contienen fosfato tricálcico (β-TCP) sobre la microdureza, el color del esmalte y la topografía antes del tratamiento de blanqueo con peróxido de hidrógeno (HP) al 35%. Cincuenta bloques incisivos bovinos de esmalte/dentina (4 × 4 × 2 mm) fueron aleatorizados en cinco grupos (n=10): control negativo (C) (ningún tratamiento), control positivo (35% HP), Colgate Total 12 luego 35% HP (CT 12 + 35% HP), Elmex Sensitive luego 35% HP (ES + 35% HP) y Bianco Pro Clinical luego 35% HP (BPC + 35% HP). Se realizaron microdureza Knoop (KHN) y microscopía electrónica de barrido (SEM). El color fue evaluado utilizando CIELAB (ΔE_{ab}^{*}) y CIEDE 2000 (ΔE_{00}) en 3 tiempos: inicial (T_0), 24 después de la exposición a la pasta de dientes (T_1) y 24 horas después del tratamiento de blanqueamiento (T_2). Los datos de microdureza, ΔE_{ab}^{*} y ΔE_{00} se analizaron utilizando modelos lineales generalizados, considerando el efecto de grupo del modelo. Los datos ΔL, Δa y Δb se analizaron utilizando las pruebas no paramétricas de Kruskal-Wallis y Dunn. Se utilizó una significación del 5%. La KHN fue significativamente mayor en los grupos BPC + 35% HP y ES + 35% HP que en los grupos C y 35% HP (p = 0,0295). ΔL fue mayor en los grupos que recibieron 35% de HP que en el grupo C 24 horas después del blanqueamiento (p=0,0001). No hubo diferencia significativa entre los grupos en ΔE_{ab}^{*} (p=0,0679) y ΔE_{00} (p=0,1463) 24 horas después del blanqueamiento. El uso de dentífricos que contienen β-TCP antes del tratamiento de blanqueamiento con 35% de HP aumenta la microdureza del esmalte, pero no altera significativamente el color del diente. Importancia Clínica: Los dentífricos que contienen β-TCP en su formulación aumentan la microdureza del esmalte sin interferir con el color del sustrato cuando se usan con blanqueamiento dental previo en el consultorio.
Palabras clave: Blanqueamiento de dientes; Peróxido de Hidrógeno, Peróxido de Carbamida; Esmalte dental; Dentífricos.

1. Introduction

Dental bleaching is essential for obtaining a harmoniously pleasant smile (Pecho et al. 2019) and is considered a conservative treatment (Corcodel et al. 2017). Many methods for vital teeth bleaching have been described using different oxidative chemical agents, such as hydrogen peroxide (HP) or carbamide peroxide (CP), at different concentrations, application times, and product formulas (Côrtes et al. 2013).

HP is a low molecular weight oxidizing agent that penetrates mineralized tissues by diffusion (Eimar et al. 2012). The free radical derived from HP reacts with organic chromogens, the molecules responsible for color change (Ontiveros et al. 2009), promoting their breakdown by an oxygen reduction reaction, decreasing light absorption and allowing their removal from the dental structure, resulting in whiter teeth (Sulieman, 2004). However, studies have shown adverse effects (Haywood, 2005), such as mineral calcium and phosphate loss (Vieira-Júnior et al. 2018), resulting in changes in the enamel's physical (Torres et al. 2019) and topographic properties (D'Amario et al. 2012).

Toothpastes have been used in association with bleaching treatments to minimize these adverse effects (Vieira-Júnior et al, 2016; Vieira-Júnior et al. 2018). In addition, different active ingredients have been investigated due to their remineralizing potential in the context of erosive challenges (João-Souza et al. 2019; Korner et al. 2020; Tomaz et al.2020;

Ionta et al. 2014). Tricalcium phosphate (β-TCP) is one such remineralizing agent (Viana et al.2020; Bekes et al. 2017) that dissociates into Ca^{2+} and PO_4^{3-} ions, making them available in the form of ions and ion clusters (Jin et al. 2013) Additionally, it has reduced solubility compared to other calcium salts and minerals, allowing it to act as a source of mineral components (Scaramucci et al. 2013), such as calcium and phosphate (Coceska et al. 2016; Bae et al 2015). HP's deleterious effects can be reversed through remineralizing agents (Sulieman, 2004). The importance of evaluating the remineralizing potential of toothpastes containing β- TCP is evident since no studies have evaluated the effects of these toothpastes when associated with in-office bleaching treatments.

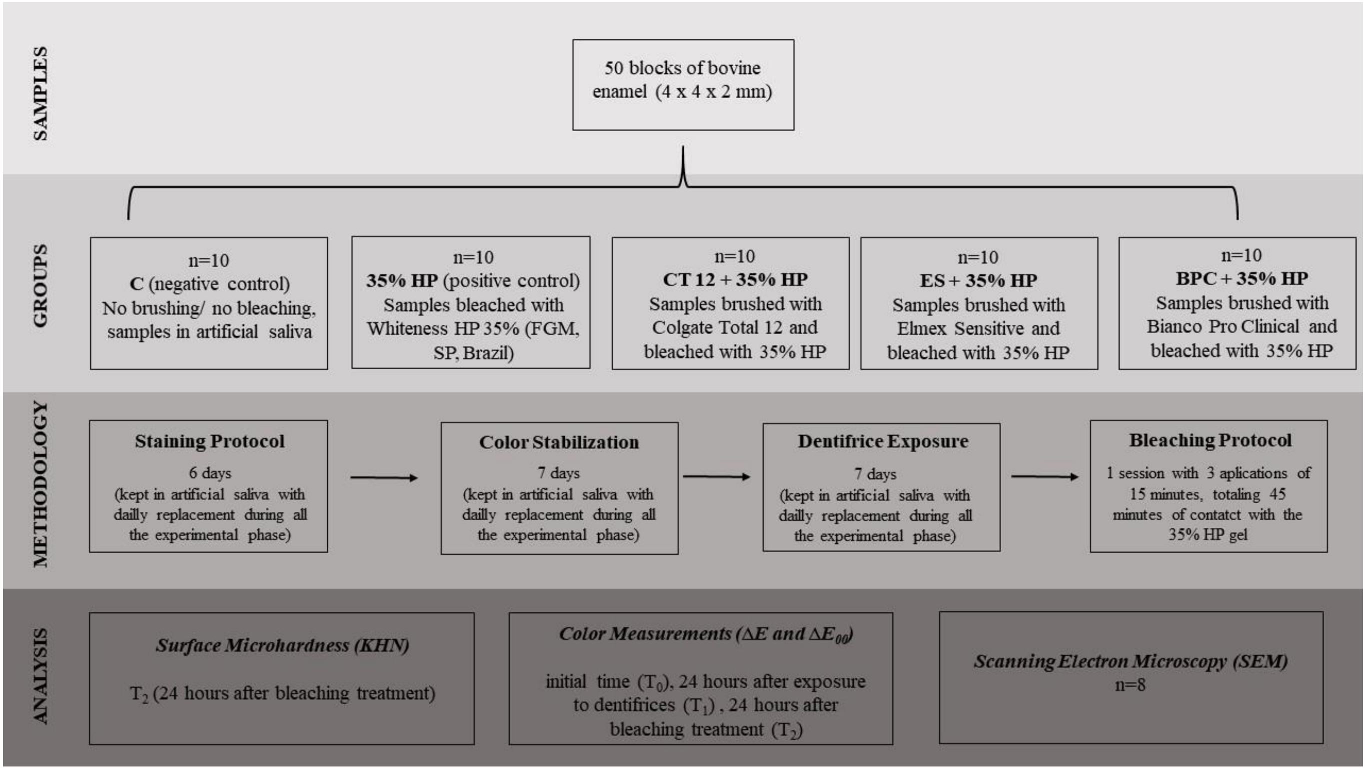
Therefore, this study evaluates the effect of using dentifrices containing β-TCP prior to in-office bleaching with 35% HP on dental enamel microhardness, color, and topography compared to bleaching without prior brushing. The null hypotheses tested were: (1) Toothpastes containing β-TCP in its formulation will not affect dental enamel microhardness after brushing and bleaching with 35% HP; (2) Toothpastes containing β-TCP will not affect dental enamel color after brushing and bleaching with 35% HP; (3) Toothpastes containing β-TCP will not affect dental enamel topography after brushing and bleaching with 35% HP.

2. Methodology

Experimental design

Sample calculation (n=10) was performed using G*Power program, large effect size (f=0.38). with power of at least 80% (β=0.20) for the main effects: group and time, as well as for the interaction between them (group x time), with significance level of 5% (α=0.05). The flowchart of the experimental design is shown in Figure 1.

Figure 1 - The experimental design.



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Staining Protocol

The specimens were submerged daily in a fresh black tea solution (Dr. Oetker LTDA, São Paulo, SP, Brazil) for 5 minutes for six days. Then, the specimens were stored in artificial saliva (AS; pH 7.0; composition: Ca 1.5 mmol/L; P 0.9 mmol/L; KCl mmol/L; 0.1mol/L tris buffer) at 37 ± 1ºC[11] for seven days. During this period, AS was changed daily for color stabilization (Serra & Cury, 1992; Sulieman, 2004; Lima et al. 2008; Zeczkowski et al. 2015). Before analysis, prophylaxis was performed on enamel and dentin surfaces with a rubber cup and a 2:1 mixture of pumice and water (Lima et al. 2008). A final polishing was used with a #4000 silicon carbide sandpaper (SiC), with constant water irrigation to obtain a smooth and polished surface.

Dentifrice Exposure

The specimens were randomly divided into five groups with differing dentifrices application and bleaching protocol (n=10): (**C**) negative control (no brushing or bleaching, with specimens kept in AS replaced daily; (**HP 35%)** positive control (in-office bleaching with 35% HP; (**CT12 + 35% HP**) exposed to Colgate Total 12 (CT12) dentifrice then 35% HP; (**ES + 35% HP**) exposed to Elmex Sensitive (ES) dentifrice then 35% HP; (**BPC + 35% HP**) exposed to Bianco Pro Clinical (BPC) dentifrice then 35% HP. The technical profile of dentifrices is presented in Table 1. Specimens were subjected to contact with the dentifrice, as occurs during the daily oral hygiene habits, using a 1:3 solution of dentifrice and AS (Lima et al. 2008) which formed a "slurry" solution (Lima et al. 2008).

Table 1 - Technical profile of the toothpaste.

Product	Composition*	Active Ingredient	Manufacturer	Batch Number/Expiration
Colgate Total 12	Sodium fluoride 0.32% (1450 ppm), Triclosan 0.3%, Water, Sorbitol, hydrated silica, Sodium lurylsulfate, PVM/MA copolymer, Carrageenan flavor, Sodium hydroxide Triclosan, Sodium saccharin, Titanium dioxin (CI 77891) and, dipenteno.	Sodium Fluoride (NaF) 1450 ppm	Colgate-Palmolive Comercial Ltda, São Paulo -SP, Brazil	0205BR122B 01/2022
Bianco Pro Clinical	Water, Sorbitol, Glycerin, Cellulose Gum, Sodium Benzoate, Silica, Sodium lurylsulfate, Mica, Titanium Dioxide, Aroma, Menthol.	1450 ppm F- (Sodium Fluoride), 3% Tricalcium phosphate (β-TCP)	Bianco® Oral Care, Raymounds Eireli Ind. São Paulo -SP, Brazil	05/18 05/2021
Elmex Sensitive	Water, Calcium Carbonate, Sorbitol, Arginine Bicarbonate, Hydrated Silica, Sodium lurylsulfate, Aroma, Cellulose Gum, Sodium Bicarbonate, Sodium Saccharin, Benzyl Alcohol, Xanthan Gum, CI 77891, Limonene.	1450 ppm F- (Sodium Monofluorophosphate), Pro-Argin and CalSeal Technologies, β-TCP and Tetrasodium Pyrophosphate	Colgate-Palmolive Comercial Ltda, São Paulo -SP, Brazil	0046PL1134 01/2023

Source: According to the manufacturer´s information.

Specimens were brushed with an electric toothbrush (Oral-B Professional Care 3000; Oral-B, Schwalbach am Taunus, Germany) (Schlueter, et al.2014). A custom dense silicone (Express™ XT Denso - 3M) device was made to standardize the toothbrush position, enabling the toothbrush's head to be parallel to the enamel surface (Moron et al. 2013). The pressure exerted by the toothbrush was 2.5 N, indicated by a light alert. The toothbrush was triggered for 15 seconds on each specimen surface (Comar et al. 2012). The specimens were kept in the "slurry" solution for 2 minutes. Then, they were thoroughly washed with distilled water and stored in AS until the next cycle. At the end of the last brushing cycle, the specimens were stored in AS for 24 hours. The brushing protocol was performed daily for 7 days (Vieira-Júnior et al, 2016; Vieira-Júnior et al. 2018).

Bleaching Protocol

The bleaching treatment was performed with 35% HP (Whiteness HP, FGM, Joinville – Santa Catarina/ Brazil) simulating a single in-office bleaching session (Vieira-Júnior et al, 2016). The gel was applied three times to the sample's surface for 15 minutes, totaling 45 minutes of exposure, according to the manufacturer´s recommendations. During all experiment stages, the specimens were kept in AS solution with daily exchanges, simulating an oral condition (Zeczkowski et al. 2015).

Surface Microhardness

Surface Knoop microhardness (KHN) was evaluated using a microdurometer (Shimadzu HMV -2000, Tokyo, Japan). The specimens were positioned with the enamel surface parallel to the indentator. Five indentations were made in the sample's central region with the Knoop penetrator using a load of 50 grams, for 5 seconds, with 100 micrometers of the distance between them. KHN was obtained through the arithmetic mean of the five indentations. The indentations can change the topography of the enamel surface and modify the light reflectance pattern, negatively impacting color analysis.[11] Consequently, KHN was evaluated only at the end (24 hours after bleaching treatment; T_2). The KHN results were compared with the values obtained from the negative control group (Vieira-Júnior et al, 2016).

Color Measurements

The color measurement analysis was performed in triplicate using a digital spectrophotometer (EasyShade, Vita Zahnfabrik, Bad Säckingen, Germany), and the average of the three readings for each coordinate was recorded. The readings followed the CIELAB (ΔE_{ab}^* and ΔL) and CIEDE 2000 (ΔE_{00}) (Pérez et al. 2019; Ramos et al. 2019) standards, according to the Commission Internationale de l'Eclairage 1978 (CIE; International Commission on Illumination) (do Carmo Públio et al. 2019) in three-dimensional modes L*, a*, and b*, where L* represents the luminosity from 0 (black) to 100 (white), a* is the measurement along the red-green axis, and b* is the measurement along the yellow-blue axis (Pan et al. 2018; Gómez-Polo et al. 2016). The following formulas present the color variation:

- $\Delta E_{ab*} = [(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2]^{1/2}$
- $$\Delta E00 = \left[\left(\frac{\Delta L'}{KLSL}\right)^2 + \left(\frac{\Delta C'}{KCSC}\right)^2 + \left(\frac{\Delta H'}{KHSH}\right)^2 + RT\left(\frac{\Delta C'}{KCSC}\right)\left(\frac{\Delta H'}{KHSH}\right)\right]^{\frac{1}{2}}$$

Evaluations were performed three times: initial time (T_0), 24 hours after exposure to dentifrice (T_1), and 24 hours after bleaching treatment (T_2).

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Enamel Topography Analysis by Scanning Electron Microscopy

Eight samples were randomly selected for qualitatively evaluating dental enamel morphology and mineral precipitates. The samples were previously metalized (Bal-Tex SCD 050 sputter coter, Germany) with gold alloy and evaluated by scanning electron microscopy (Jeol, JSM 5600LV, Tokyo, Japan) at 15 kV under 2000× magnification.

Statistical Analysis

All statistical analyses were performed using the R statistical software program (R Core Team, 2021. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria), considering a 5% significance level. The data did not meet the assumptions for an analysis of variance (ANOVA). Therefore, microhardness, ΔE_{ab}^{*}, and ΔE_{00} data were analyzed using generalized linear models, considering the model's group effect. The ΔL, Δa, and Δb data did not fit a known distribution and were analyzed using Kruskal–Wallis and Dunn's nonparametric tests.

3. Results

Surface Microhardness (KHN)

KHN was significantly higher in the BPC + 35% HP and ES + 35% HP groups than in the C and 35% HP groups (p=0.0295; Table 2). The CT12 + 35% HP group was observed to be similar to the other four groups.

Table 2 - Knoop Microhardness (KHN) (T_2).

Group	Mean (standard deviation)
C	253,03 (62,93) b
35% HP	260,84 (68,82) b
CT 12 + 35% HP	295,12 (32,17) ab
ES + 35% HP	310,10 (30,44) a
BPC + 35% HP	318,69 (41,27) a
p-value	0,0295

C (Negative Control); 35% HP (in-office bleaching with Whiteness HP 35%); CT12 + 35% HP (exposure to Colgate Total 12 dentifrice + 35% HP); ES + 35% HP (exposure to Elmex Sensitive dentifrice + 35% HP); BPC + 35% HP (exposure to Bianco Pro Clinical dentifrice + 35% HP);
(Distinct letters indicate statistically significant differences (p≤0.05).
Source: Own Authorship.

Color Measurements

- 24 hours after dentifrices exposure

ΔL did not differ significantly between groups (p=0.7941; **Table 3**). However, there was a significantly smaller Δa difference in the BPC + 35% HP group than in the C group (p=0.0244). Δb values were significantly more negative in the BPC + 35% HP group than in the C group (p=0.0336). ΔE_{ab}^{*} (p=0.1832) and ΔE_{00} (p=0.3754) did not differ significantly between groups.

- 24 hours after bleaching treatment

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There was greater positive ΔL variation in the 35% HP group than in the C group (p=0.0001; **Table 3**). However, ΔL did not differ significantly between groups treated with dentifrices and the 35% HP group (*p*=0.0001). Δa showed significantly greater negative variation in the 35% HP group than in the C group (*p*=0.0007). However, Δa did not differ significantly between the groups treated with dentifrices and the 35% HP group (*p*=0.0007). Δb variation was significantly more negative in the BPC + 35% HP group than in the C group (*p*=0.0212). However, Δb did not differ significantly between the groups treated with dentifrices and the 35% HP group (*p*=0.0212). ΔE_{ab}^{*} (*p*=0.0679) and ΔE_{00} (*p*=0.1463) did not differ significantly between groups.

Table 3 - Color Variation T_1 x T2.

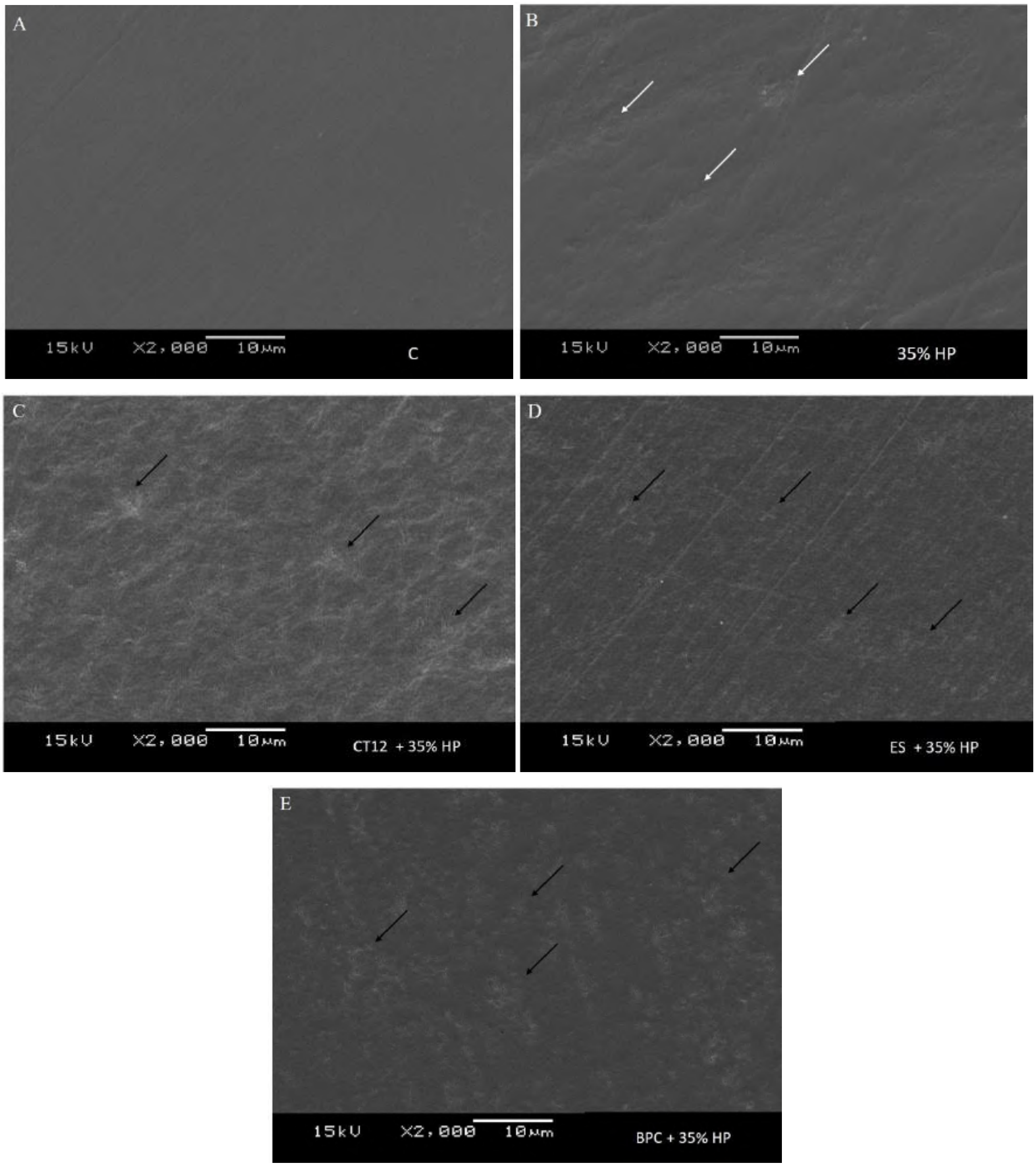
Variable	Group	Mean T1 (standard deviation)	Mean T2 (standard deviation)
	C	-3,03 (2,59) a	-3,01 (3,28) b
	35% HP	-1,71 (1,83) a	5,60 (2,89) a
ΔL	**CT12 + 35% HP**	-1,97 (3,57) a	6,83 (4,82) a
	ES + 35% HP	-1,23 (4,55) a	5,88 (5,87) a
	BPC + 35% HP	-2,83 (3,11) a	4,55 (2,75) a
p-value		0,7941	0,0001
	C	2,02 (1,14) a	1,52 (1,35) a
	35% HP	1,35 (0,48) ab	-1,84 (1,32) b
Δa	**CT12 + 35% HP**	0,83 (2,13) ab	-2,30 (2,81) b
	ES + 35% HP	0,27 (2,50) ab	-2,61 (3,84) b
	BPC + 35% HP	0,61 (1,01) b	-2,07 (1,42) b
p-value		0,0244	0,0007
	C	2,96 (3,14) a	1,36 (3,29) a
	35% HP	0,18 (1,90) ab	-2,38 (2,61) ab
Δb	**CT12 + 35% HP**	-0,55 (4,83) ab	-4,14 (5,99) ab
	ES + 35% HP	-0,82 (5,81) ab	-4,08 (5,44) ab
	BPC + 35% HP	-1,19 (2,94) b	-4,14 (4,16) b
p-value		0,0336	0,0212
	C	5,15 (3,58) a	5,07 (3,10) a
	35% HP	2,91 (1,77) a	7,02 (2,67) a
$\Delta E_{a,b}^{*}$	**CT12 + 35% HP**	5,59 (3,36) a	9,85 (6,00) a
	ES + 35% HP	5,36 (5,58) a	8,77 (7,61) a
	BPC + 35% HP	4,62 (2,54) a	7,83 (2,38) a
p-value		0,1832	0,0679
	C	3,18 (2,07) a	3,23 (2,24) a
	35% HP	2,06 (1,20) a	4,83 (2,10) a
ΔE_{00}	**CT12 + 35% HP**	3,41 (1,86) a	6,21 (3,74) a
	ES + 35% HP	3,42 (3,24) a	5,63 (5,69) a
	BPC + 35% HP	3,13 (1,97) a	4,78 (1,88) a
p-value		0,3754	0,1463

C (Negative Control); 35% HP (in-office bleaching with Whiteness HP 35%); CT12 + 35% HP (exposure to Colgate Total 12 dentifrice + 35% HP); ES + 35% HP (exposure to Elmex Sensitive dentifrice + 35% HP); BPC + 35% HP (exposure to Bianco Pro Clinical dentifrice + 35% HP);
Distinct letters indicate statistically significant differences between groups (p≤0.05).
Source: Own Authorship.

Enamel topography analysis by SEM

The qualitative evaluation of the enamel surface showed a smooth and healthy aspect (Figure 2A) with a rougher enamel surface with areas of depression (Figure 2B, white arrowheads) and distinctive demineralization caused by HP action. The presence of whitish areas in interprismatic regions, characteristic of mineral precipitation, was evident (Figure 2C-E, black arrowheads). A rougher surface was evident in Figure 2C than in Figures 2D and 2E.

Figure 2 - A- C (negative control) - It can be observed a smooth aspect; **B- 35% HP** (positive control), white arrowheads indicate the presence of demineralization (whitish patterns); **C- CT 12 + 35% HP** (Colgate Total 12 + 35% HP), black arrowheads indicate demineralization patterns; **D- ES + 35% HP** (Elmex Sensitive + 35% HP), black arrowheads indicate the presence of mineral deposition; **E- BPC + 35% HP** (Bianco Pro Clinical + 35% HP), black arrowheads indicate the presence of mineral deposition.



Source: Own Authorship.

4. Discussion

First null hypothesis was rejected once there was increased enamel surface microhardness when toothpastes containing β-TCP were used. While dental bleaching is considered a safe and effective treatment when properly indicated (Corcodel et al. 2017), physical and topographic changes in enamel structure can occur (Zeczkowski et al. 2015; D'Amario et al. 2012; Vieira-Júnior et al. 2016). Changes in biomechanical and morphological properties of mineralized structures may be similar to an erosive process (Coceska et al. 2016) and are more associated with using oxidative agents at high concentrations (Jin et al. 2013). Consequently, research is moving towards finding approaches to minimize and reverse deleterious effects on the enamel surface (Coceska et al. 2016). Toothpastes containing fluoride have been proposed since they have obliterating action on dentin tubules, and their use can be recommended during or after bleaching treatment (João-Souza et al. 2019). Active ingredients with remineralizing potentials, such as potassium nitrate (João-Souza et al. 2019) (desensitizing agent with neural action), bioactive glass, arginine, (Vieira-Júnior et al. 2016), β- TCP (Jin et al. 2013) and calcium carbonate (Bekes et al. 2017) have also been introduced.

This study used toothpastes containing fluorinated compounds, ES and BPC, and β-TCP, but only ES contained arginine and calcium carbonate in its composition. The mechanism of action of these components consists of dentin tubule obliteration with salivary glycoproteins containing calcium and phosphate (Bae, Kim & Myung, 2015) which, when associated with β-TCP, can possibly cause a more significant increase in microhardness. The dentifrices containing β-TCP provided similar enamel microhardness results with higher values when compared to CT12.

Therefore, this study confirms that using toothpastes containing β-TCP prior to bleaching treatment with 35% HP has remineralizing potential. The mineral precipitate formation may be responsible for the increased dental enamel microhardness (Viana et al. 2020). In addition, the favorable result of these dentifrices may be due to the association of fluorinated compounds with β-TCP once its remineralizing action (Ionta et al. 2014; Viana et al. 2020) forms mineral precipitates on the dentin surface (Viana et al. 2020).

Second null hypothesis was accepted because using dentifrices did not contribute to bleaching efficacy. Performing one session of in-office dental bleaching with 35% HP (three 15-minute applications) did not change dental enamel color. Changes in the tooth shade must be associated with more than one perceptual color attribute, such as luminosity, chroma, and hue (Pérez et al. 2019; Ramos et al. 2019; Pan & Westland, 2018). The CIELAB and CIEDE 2000 systems were used to measure the color variation with the L*, a*, and b* parameters (Gómez-Polo et al. 2016) and limits of 50%:50% perceptibility (1.22 ΔE_{ab}^* and 0.81 ΔE_{00}) and acceptability (2.66 ΔE_{ab}^* and 1.77 ΔE_{00}) (Gómez-Polo et al. 2016; Pecho et al. 2019; Pérez et al. 2019).

The CIELAB system is related to color perception in all three-color space dimensions (Gómez-Polo et al. 2016). The CIEDE 2000 system is more complex, including differences in color parameters in its calculations (Gómez-Polo et al. 2016). The L* coordinate represents luminosity, while a* and b* represent chromaticity (Gómez-Polo et al. 2016). Therefore, correlated changes occur in the coordinates L*, a*, and b* as a consequence of whitening (usually, the values for L* increase while the values for a*, b*, and C* decrease) (Pan & Westland, 2018). Consequently, the overall color variation was not significantly modified. These findings agree with a previous study (Vieira-Júnior et al. 2016) and support a safe and effective indication for dentifrices, particularly those containing desensitizing agents (Haywood, 2005), which can minimize adverse effects such as dentine hypersensitivity.

Third null hypothesis was rejected since mineral precipitates were observed on the enamel surface. The SEM images indicated the occurrence of demineralization (Figure 2B) and mineral deposition (Figures 2C, 2D, and 2E) in groups treated with 35% HP and CT12, ES, and BPC dentifrices, respectively. The mineral precipitates (whitish spots, indicated by the black arrowheads in Figure 2) are distinctive of mineral deposition caused by β-TCP particles (Vieira-Junior et al. 2018). Furthermore, mineral precipitation on the dentin surface and inside the dentinal tubules should be accounted for when they occur by the remineralizing agent action found in toothpastes.

Therefore, the formation of these mineral precipitates may interfere with, HP diffusion, and the action of remineralizing agents (β-TCP and fluorinated compounds) may have contributed to findings related to dental enamel microhardness, color variation, and topography.

It is possible for dentifrices containing β-TCP to be used without adversely affecting dental enamel microhardness and general color variation. However, clinical studies are recommended to confirm the results of this *in vitro* study.

5. Conclusion

Considering the limitations of this in vitro study, it can be concluded that:

1. Using dentifrices containing β-TCP prior to bleaching treatment with 35% HP increases enamel microhardness.
2. One bleaching session with 35% HP performed after brushing with dentifrices containing β-TCP was insufficient to cause the color change of the dental substrate, and more sessions are required to achieve a bleaching effect.
3. The use of dentifrices containing β-TCP prior to bleaching treatment with 35% HP alters enamel topography, cooccurring with mineral deposition.

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