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EFEITOS DE PROTOCOLOS CLAREADORES COM RADIAÇÃO VIOLETA ASSOCIADA OU NÃO A PERÓXIDOS EM ALTA CONCENTRAÇÃO NA ALTERAÇÃO DE COR E CONTEÚDO MINERAL DO ESMALTE DENTAL BOVINO PIGMENTADO

EFFECTS OF DENTAL BLEACHING PROTOCOLS MEDIATED BY VIOLET RADIATION COMBINED OR NOT WITH HIGH-CONCENTRATED PEROXIDES ON THE COLOR AND MINERAL CONTENT OF STAINED BOVINE ENAMEL

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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Cirurgião Dentista.

Undergraduate final work presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Dental Surgeon.

Orientador: Prof. Dr. Vanessa Cavalli Gobbo

Co-Orientador: Ms. Matheus Kury Rodrigues

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DO TRABALHO DE CONCLUSÃO DE CURSO APRESENTADO PELA ALUNA ROBERTA SOARES KOBAYASHI E ORIENTADA PELA PROF. DR. VANESSA CAVALLI GOBBO

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RESUMO

Este trabalho avaliou a alteração de cor e o conteúdo mineral do esmalte dental bovino artificialmente pigmentado após clareamento dental com radiação violeta (VR) associado ou não ao peróxido de carbamida 37% (CP) ou de hidrogênio 35% (HP). Duzentos blocos dentais foram pigmentados (n=50) com café (CF), fumaça de cigarro (TS) ou vinho tinto (RW), ou permaneceram sem pigmentação (NS). Os espécimes de cada tipo de pigmentação foram distribuídos aleatoriamente (n=10) em grupos, conforme o protocolo: VR, CP, VR/CP, HP e VR/HP. Avaliação colorimétrica (ΔE_{00}, ΔWI_D, ΔL, Δa, Δb) com espectrofotômetro digital foi realizada após pigmentação (T_1) e após 7 dias dos protocolos clareadores (T_2). A porcentagem dos pesos atômicos (%wt) de cálcio (Ca), fósforo (P) e a proporção Ca/P foi mensurada em T_2 utilizando espectroscopia de Raio-X por energia dispersiva (EDS). Os dados obtidos foram submetidos $\langle \alpha$=5%) à ANOVA de dois fatores e Teste de Tukey ou Kruskal-Wallis e Teste de Dunn (Δa). VR sozinho causou maior alterações colorimétricas mediante pigmentação com CF, TS e RW em comparação a NS ($p<0.05$). VR/CP exibiu maiores alterações colorimétricas que CP em esmalte pigmentado com CF ou NS. VR/CP, HP ou VR/HP não apresentaram diferenças nas alterações ($p>0.05$). Nenhum grupo apresentou diferença significativa na %wt de Ca, P ou Ca/P, independentemente do protocolo pigmentante ou clareador. Dessa maneira, LED violeta foi mais eficaz no clareamento de dentes pigmentados. Ativação de 37%CP com VR foi tão eficaz quanto o protocolo com HP. Não foram observados efeitos adversos no conteúdo mineral no esmalte.

Palavras-chave: Dentes - Clareamento. Peróxido de hidrogênio. Peróxido de Carbamida.

ABSTRACT

This study evaluated the color change and mineral content of stained bovine dental enamel after tooth bleaching with violet radiation (VR) combined or not with 37% carbamide (CP) or 35% hydrogen peroxide (HP). Two hundred dental blocks were stained (n=50) with coffee (CF), cigarette smoke (TS) or red wine (RW), or remained without stained (NS). The specimens of each type of staining were randomly distributed (n = 10) in groups, according to the protocol: VR, CP, VR/CP, HP and VR/HP. Colorimetric evaluation (ΔE_{00}, ΔWI_D, ΔL, Δa, Δb) with digital spectrophotometer was performed after stained (T_1) and after 7 days of the bleaching protocols (T_2). The percentage of atomic weights (% wt) of calcium (Ca), phosphorus (P) and the Ca/P were measured at T_2 using energy-dispersive X-ray spectroscopy (EDS). Data obtained were subjected (α=5%) to two-way ANOVA and Tukey's test or Kruskal-Wallis and Dunn's test (Δa). VR alone caused higher colorimetric changes in CF, TS and RW groups compared to NS ($p<0.05$). VR/CP exhibited higher colorimetric changes than CP in stained enamel with CF or NS. VR/CP, HP or VR/HP showed no differences in changes ($p>0.05$). No group showed a significant difference in the% wt of Ca, P or Ca/P, regardless of the stained or bleaching protocol. Therefore, violet LED was more effective in bleaching teeth artificially stained. Activation of CP 37% with VR was as effective as the protocol with HP. No adverse effects were observed on the mineral content of the enamel.

Key words: Teeth - Bleaching. Hydrogen peroxide. Carbamide peroxide.

SUMÁRIO

1 INTRODUÇÃO

A busca constante por dentes mais brancos por meio do clareamento dental vem crescendo a cada dia, uma vez que a estética do sorriso apresenta grande impacto psicossocial na vida dos pacientes (Pavicic et al., 2020) e a satisfação com coloração dental pode gerar um impacto positivo na qualidade de vida dos pacientes (Silva et al., 2018). Neste contexto, o clareamento dental supervisionado pelo cirurgião-dentista desponta como um dos procedimentos estéticos mais realizados em consultório, tanto na modalidade caseira utilizando agentes clareadores (peróxido de carbamida [PC] ou de hidrogênio [PH]) em baixas concentrações, quanto em consultório, onde são empregados PC e PH em altas concentrações (até 40%) (Basting et al., 2012). A diferença entre os agentes utilizados (PC e PH) baseia-se na presença de uréia em associação ao peróxido de carbamida, que se dissociam em amônia e peróxido de hidrogênio em contato com a estrutura dental. Sendo assim, a taxa de liberação de peróxidos e consequente dissociação em radicais livres nos géis de PC são mais lentas, e a concentração final de peróxido de hidrogênio corresponde a 1/3 presente nos géis de PH (Kwon e Wertz, 2015).

Hipotetiza-se que o mecanismo de ação dos agentes clareadores ocorra após a geração de espécies reativas de oxigênio (ERO), por meio de uma reação de oxidação entre o peróxido de hidrogênio e os pigmentos, que são compostos orgânicos presentes no substrato dental (Joiner et al., 2006). Esta reação ocorre devido à capacidade das ERO de se difundirem através do substrato, em direção à interface esmalte-dentina e oxidar as ligações duplas de carbono presentes nos cromóforos, resultando no clareamento dental (Kwon e Wertz, 2015). Sendo assim, o grau de alteração pode depender da frequência de exposição, bem como da concentração do peróxido (Florez et al., 2009).

Apesar de ser uma abordagem ultraconservadora e minimamente invasiva, com ótimos resultados estéticos (Basting et al., 2012), a maioria dos géis de clareamento em consultório apresentam altas concentrações de peróxido para permitir um resultado mais rápido e eficaz. Entretanto, evidências reportam que estes agentes clareadores podem apresentar efeitos adversos à estrutura dental, como aumento da rugosidade do esmalte (Berger et al., 2008; Liporoni et al., 2010), diminuição da resistência intrínseca e microdureza de superfície (Cavalli et al., 2004; da Silva et al., 2005; Zantner et al., 2007) e hipersensibilidade dentinária (Basting et al., 2012; Soares et al., 2013). Além disso, de acordo com Berger et al., (2010), o esmalte após clareamento com géis de alta concentração, apresentou uma diminuição significativa no conteúdo mineral do esmalte. Acredita-se que a redução no conteúdo mineral do esmalte após o clareamento seja resultante da interação do gel clareador, que possui atividade oxidativa, e ainda pode ter seu pH reduzido ao longo da aplicação (Cavalli et al., 2019). Entretanto, um estudo revelou que as alterações *in vitro* na superfície do esmalte após exposição a gel clareador em alta concentração com baixo pH observadas, não foram detectadas em um desenho *in situ*, onde a saliva humana pode haver protegido o substrato (Sa et al., 2013). Ainda, estudos revelaram que os efeitos adversos de géis clareadores utilizados em consultório não foram exacerbados pela ativação luminosa com diferentes fontes de luz (Kury et al., 2020a; Berger et al., 2010;).

A fim de oferecer aos pacientes um protocolo clareador que diminua as alterações na morfologia, na composição mineral do esmalte e a sensibilidade dental causada pelo clareamento dental em consultório, o uso do LED violeta (≅405nm) com ou sem gel clareador vem sendo clinicamente empregado (Lago et al., 2017; Rastelli et al., 2018; Gallinari et al, 2019a; Kury et al., 2019). Estudos recentes indicam que o LED sozinho promoveu alteração de cor perceptível e não influenciou a morfologia de superfície do esmalte (Gallinari et al., 2019b; Kury et al., 2020a). Além disso, a combinação do LED violeta com peróxido de carbamida em alta concentração (37%) alcançou eficácia similar ao peróxido de hidrogênio 35%, com redução significativa do risco de sensibilidade dental e manutenção do conteúdo mineral do esmalte dental (Kury et al., 2020b,c).

Os efeitos positivos do LED violeta em protocolos combinados com géis de peróxido tem sido discutidos e creditado a um possível aumento térmico causado pela radiação violeta no gel clareador, o que aumentaria a geração de ERO (Joiner et al., 2006; Kury et al., 2020a,b). Entretanto, discute-se que o LED violeta, quando utilizado sozinho, apresentaria um mecanismo de ação fotolítico, no qual o seu comprimento de onda (≅405nm) coincidiria com o pico de absorção dos cromóforos presentes apenas na superfície do esmalte, quebrando as moléculas de pigmentos em moléculas menores deixando os dentes com aspectos mais claros (Zanin, 2016; Rastelli et al., 2018).

Enquanto a pigmentação intrínseca está presente no interior dos tecidos dentais, estando relacionadas com envelhecimento dental, desordens metabólicas, tratamentos com antibióticos à base de tetraciclina, as descolorações extrínsecas são resultados do contato com bebidas como chá e café, manchamento por tabaco, acúmulo de biofilme (Martínez, 2019). Até o presente momento, evidências reportam que o LED violeta sozinho foi capaz de gerar um efeito clareador *in vitro* perceptível em dentes pigmentados com chá preto (Gallinari et al., 2019b; Kury et al., 2020a). Entretanto, o LED apresentou eficácia significativamente inferior aos protocolos com géis de peróxido em ambos estudos. Neste contexto, e levando em consideração que os pacientes possuem hábitos alimentares variáveis, o tipo de manchamento dental extrínseco deve ser levado em consideração, uma vez que poderia influenciar os mecanismos de ação do clareamento com LED violeta (tanto fotolítico quando aplicado sozinho, quanto químico quando combinado com géis). Além disso, a interação de protocolos clareadores com LED violeta em superfícies pigmentadas extrinsecamente com soluções apresentando diferentes características ou pH poderiam influenciar no conteúdo mineral do substrato.

Diante do exposto, o objetivo desse trabalho, foi avaliar in vitro a eficácia dos protocolos clareadores que consistem na associação do LED violeta com géis de peróxido de carbamida 37% e hidrogênio 35% e seus efeitos sobre a composição química do esmalte bovino pigmentado com diferentes tipos de manchamento extrínsecos.

2 ARTIGO: EFFECTS OF DENTAL BLEACHING PROTOCOLS WITH VIOLET RADIATION ON THE COLOR AND CHEMICAL COMPOSITION OF STAINED BOVINE ENAMEL

Submetido no periódico *Photodiagnosis and Photodynamic Therapy* (Anexo 3)

ABSTRACT

Objectives: To evaluate the bleaching efficacy of a violet radiation (VR) combined or not with bleaching gels on the color and mineral content of stained teeth. **Material and Methods:** Enamel/dentin blocks were obtained and stained (n=50) with coffee, red wine, tobacco smoke, or were left non-stained. The stained or not-stained bocks (n=10) were distributed into five bleaching groups (n=10): VR, CP (37% carbamide peroxide), VR/CP, HP (35% hydrogen peroxide), and VR/HP. Color (ΔE_{00}, ΔL, Δa, and Δb) and whiteness index (ΔWI_D) changes were evaluated after staining and after bleaching using a spectrophotometer. Calcium (Ca), phosphorous (P), and Ca/P contents (in wt%) were measured after bleaching using energy-dispersive X-ray spectroscopy (EDS). Data was statistically analyzed (〈=0.05) using two-way ANOVA and Tukey tests (ΔE_{00}, ΔWI_D, ΔL, Δb, wt%) or Kruskal-Wallis and Dunn tests (Δa). **Results:** VR alone caused higher colorimetric changes on coffee, tobacco and red wine-stained groups compared to non-stained enamel ($p < 0.05$). VR/CP exhibited higher colorimetric changes compared to CP in coffee and non-stained groups. The VR/CP, HP and VR/HP groups exhibited no change differences ($p > 0.05$). None of the bleaching protocols decreased the enamel mineral content.

Conclusions: The violet radiation was more effective in bleaching stained rather than non-stained teeth. VR combined with 37% carbamide peroxide was as effective as the HP agent. Besides, no adverse effects were observed in the enamel mineral content, regardless of the bleaching protocol tested.

Keywords: Violet radiation, dental bleaching, hydrogen peroxide, carbamide peroxide, minimally invasive, ultra-conservative

1. Introduction:

Dental discolorations can be treated using minimally invasive and ultraconservative approaches including at-home and in-office dental bleaching procedures. In-office power bleaching techniques (IPB) [1] are very popular amongst patients and clinicians because the removal of hard tissues is not necessary, and esthetic outcomes may be perceived after one clinical session. The mechanism of action of the IPB revolves around the generation of reactive oxygen species (ROS) [2-4]. These short-lived molecules must migrate through the gel-enamel interface, diffuse through the enamel and reach deep areas within the tooth structure (enamel-dentin interface), [5] prior to the oxidation of the carbon-to-carbon conjugated double bonds present in chromophores (high molecular weight molecules, organic origin).

Therefore, the efficacy of dental bleaching procedures is strongly dependent on the exposure time and peroxide concentration (either carbamide [CP] or hydrogen peroxide [HP]), [6] wherein higher concentrations and longer exposure times are typically translated into more intense and durable esthetic outcomes. In this context, the majority of bleaching gels currently available for IPB are often associated with high peroxide concentrations [7] (30-44%, v/v), allowing immediate perceivable results and decreasing the exposure of mineralized tissues to highly caustic and oxidative agents. Even though dental bleaching procedures are considered safe and effective, numerous reports [8-14] have indicated the occurrence of several adverse effects including gingival irritation, dentin hypersensitivity, and irreversible modifications on the morphology (increased roughness), chemical makeup (decreased calcium, phosphorous, and fluoride), and on the mechanical properties (decreased strength and hardness) of the treated enamel. In addition, HP (10%, 35% and 50%) was capable of significantly increasing (up to 150%) enamel's permeability in a concentration-dependent manner (10%< 35%<50%) [15].

Other studies [16,17] have shown that HP diffusion becomes exacerbated in regions of gingival recession, abfraction, erosion, or in the presence of polymer-based adhesive restorations. According to Markowitz, [5] pain during dental bleaching procedures precipitates from oxidizing agents' abilities to stimulate nociceptive afferents leading to both pain and neurogenic inflammation. Soares et al., [18] investigated the effects of HP (concentration [35% and 17.5%] and application protocols [45 min, 15 min or 5 min]) on the viability of pulp cells, and demonstrated that

HP is cytotoxic in a time- and concentration-dependent manner. It has also been suggested that lower HP concentrations (17.5%) produced appropriate esthetic outcomes and reduced cytotoxicity to pulp cells, independently of the investigated application protocol.

Metaloxide nanoparticles, non-thermal atmospheric plasma and violet radiation (VR) are being currently investigated in terms of their potential to overcome the limitations associated with traditional IPB techniques. The peroxide-free bleaching of teeth using VR (405 ± 15 nm) [19-21] involves a photophysical process (photolysis) [22,23] based on the intense absorption of highly energetic photons by the chromophores, which are capable of breaking the conjugated double bonds. Clinically, the photonic bleaching technique changed color of treated teeth (from A3 to A1) after 3 clinical sessions (30 min/each; 7 days apart) without the development of trans- or post-operatory dentin hypersensitivity [24]. Previous in vitro studies [22,23] suggested, based on well-known concepts of light-matter interaction (e.g., absorption, penetration, scattering, and transmission), that VR may preferentially excite and degrade extrinsic chromophores [25] into smaller compounds, thereby indicating that photonic bleaching procedures might be associated with limited efficacy [1,22,26,27] and short-term esthetic outcomes.

The utilization of HP or CP combined with VR has been recently proposed [23,26,28] to allow the attainment of appropriate esthetic outcomes (immediate and long-term) because VR has been shown to optimize the chemical dissociation of peroxides, thereby improving the overall efficacy of these novel bleaching modalities [26,27]. Despite these promising results, limited information is available regarding the impact of bleaching techniques based on peroxide-containing gels and VR on the chemical, mechanical and optical properties of bovine enamel artificially stained by coffee, tobacco smoke, or red wine. Besides, no information is available regarding the impact of different types of pigments on the efficacy of these novel bleaching procedures.

Therefore, and based on the critical context exposed, the objective of the present study was to determine the efficacy of bleaching protocols with VR with or without peroxide-containing gels (HP and CP). In addition, this study evaluated the potential impacts on the chemical makeup of bovine enamel as a secondary outcome. The null hypotheses tested were: (i) the efficacy of photonic bleaching (peroxide-free) does not depend on the presence or type of pigments in the tooth structure and (ii) dental bleaching protocols investigated do not change the mineral composition of treated enamel.

2. Materials and Methods:

2.1. Experimental Design

The experimental units were submitted to four different *types of staining (n=50)*:

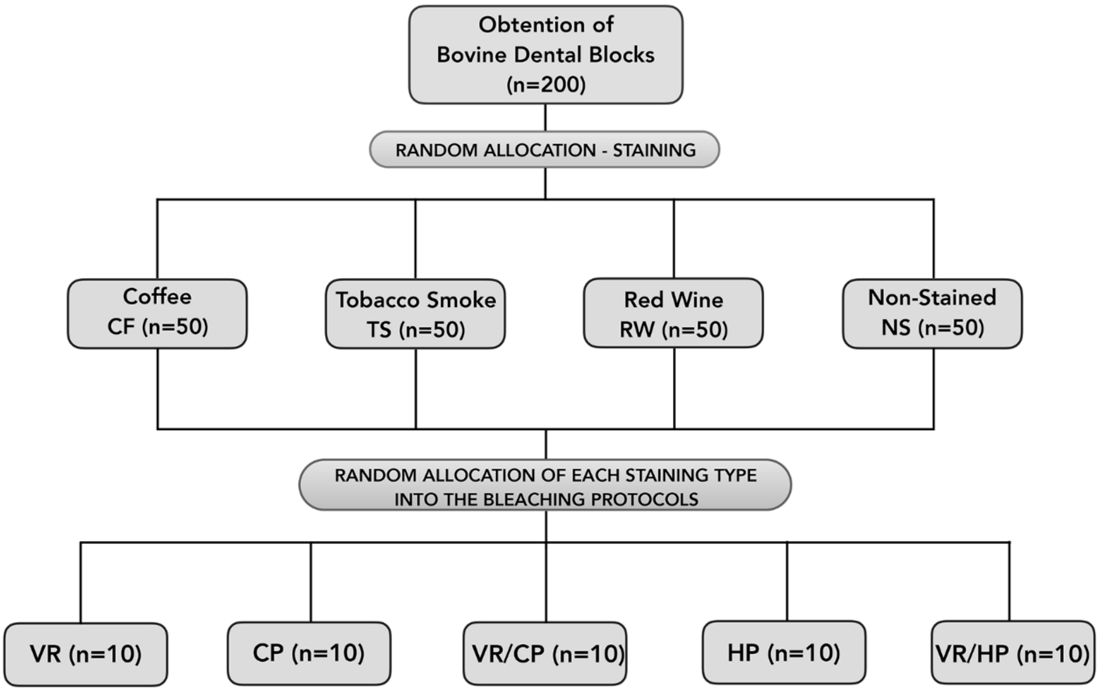
- Coffee (CF)
- Tobacco Smoke (TS)
- Red Wine (RW)
- Non-stained (NS)

Afterward, the specimens were randomly distributed into groups according to the dental *bleaching protocols (n=10):*

- Violet Radiation (VR)
- 37% Carbamide Peroxide (CP)
- VR combined with CP (VR/CP)
- 35% Hydrogen Peroxide (HP)
- VR combined with HP (VR/HP)

Objective color analysis (ΔE_{00}, ΔWI_D, ΔL, Δa and Δb) was performed after staining (T_1) and seven days elapsed from the last bleaching application (T_2), using a digital spectrophotometer. Also, the mineral content was evaluated at T_2 using energy-dispersive X-ray spectroscopy (EDS). Figure 1 exhibits the flow chart of the experimental design.

Figure 1. Flow chart of the experimental design of the present study.



2.2. Specimen fabrication

Bovine central incisors (n=200) were extracted and subjected to traditional prophylaxis procedures before being stored in a thymol solution (0.1%, 40°C, 60 days). Teeth exhibiting surface defects, i.e. cracks or abrasion, were discarded at this stage of the research. The roots were coronally sectioned 2.0 mm from the enamel-cementum interface using a low-speed metallographic diamond saw (Isomet, Buehler; Lake Bluff, IL, EUA) under copious water irrigation. Square-shaped blocks (Area= 6.0 mm^2, thickness= 3.0mm) were fabricated (1 block/tooth) from the central region of bovine crowns. Only the dentin surface was flattened, using a rotary polisher (Arotec, São Paulo, Brazil) and abrasive discs (SiC, 600 grit) under refrigeration, in order to enable the colorimetric evaluation. Transparent nail polish (Impala, Guarulhos, SP, Brazil) was manually and carefully applied onto all dentin surfaces exposed in preparation for experimental staining protocols investigated.

2.3. Staining of specimens

Prepared specimens were then stained (n=50) by immersion either in a coffee solution (powder= 16.0 g [Nescafé Tradition, Nestlé], water= 200 mL; 3· day, 15 min/each, 37ºC for 14 days) [29] or in red wine (200 mL [Casillero del Diablo Malbec, Santiago del Chile, Chile], 48 hours, 37ºC) [30]. Specimens stained with tobacco were exposed to the smoke of 20 cigarettes [Marlboro, Phillip Morris] per day (5 days, 25ºC) in a laboratory inhalation device (developed by the Department of Restorative Dentistry, Operative Dentistry Area, Piracicaba Dental School – University of Campinas - 2011 (registered under # 01810012043 INPI - National Institute of Industrial Property), following a previously published protocol [31]. Non-stained specimens (NS) were stored in artificial saliva at 37ºC during the execution of the present study [32]. The specimens were stored in artificial saliva (3.125mL for each mm^2 of exposed enamel) at the intervals of the staining exposures. After the completion of staining protocols, specimens in all groups were subjected to cleaning procedures using a bristle brush and pumice slurry before being stored (at 37ºC) in artificial saliva (24 hours).

2.4. Dental bleaching procedures

Specimens (n=10/staining condition) were then randomly distributed into five experimental groups, as follows: VR (10 sessions [4 days apart], 20 irradiations [1 min/each, 30 s apart]/session), CP (3 sessions [7 days apart, 30 min/session]), VR/CP (3 sessions [7 days apart], 20 irradiations [1 min/each; 30 s apart]) + CP (30 min/session), HP (3 sessions [7 days apart], 30 min/session) and VR/HP (3 sessions [7 days apart], 20 irradiations [1 min/each; 30 s apart]) + HP (30 min/session) [29]. Violet radiation was provided by an LED light source (Bright Max Whitening, MMO, São Carlos, Brazil) composed of four diodes (405 ± 15 nm, 1.2 W/cm^2, optical window area= 10.7 cm^2) fixed 8.0 mm apart from the specimens' surfaces [1,22,28]. The application of the commercial bleaching gels followed the instructions of the LED device's manufacturer, which recommends a 30-min exposure of HP and CP without refreshments [22,27].Table 1 describes information about the LED source, 35% HP (Whiteness HP, FGM, Joinville, SC, Brazil) and 37% CP (Whiteness Super Endo, FGM) gels. Before the bleaching procedures, one-way ANOVA detected no significant differences ($p < 0.05$) among groups belonging to the same staining type in regard to L*, a* and b* coordinates. Specimens were stored in artificial saliva (37ºC) for either 4 or 7 days between clinical sessions, as described in a previously published protocol [32].

Table 1. Bleaching gels, composition and manufacturer's specification.

2.5. Objective colorimetric analysis

Bleaching agent and Manufacturer	Specification/Composition	Manufacturer instructions
Hydrogen Peroxide (HP) Whiteness HP (FGM, Joinville, SC, Brazil)	35% hydrogen peroxide, glycol, deionized water, dyes, inert filler, thickener, pH = 7.0. Intense carmine coloration at the beginning of the reaction, but it becomes colorless during the first 5 min.	The gel should be applied to vital teeth and should be changed every 15 minutes. An interval of 7 days between sessions is required and treatment should be repeated up to four times.
Carbamide Peroxide (**CP**) Whiteness HP (FGM, Joinville, SC, Brazil)	37% carbamide peroxide, glycol, deionized water, inert filler, neutralized carbopol, pH = 7.0 Colorless.	The gel should be applied to the pulp chamber of non-vital teeth, using the ‘‘walking bleaching technique”. If necessary, the gel could be changed in three or four days, up to eight times.
Violet radiation (VR) Bright Max Whitening – BMW (MMOptics, São Carlos, SP, Brasil)	Four light emitting diode lamps (401.82 nm = violet wavelength). Illumination area of the curved acrylic tip = 10.7 cm2. Total power = 1.2 W. Irradiance at the position corresponding tooth right upper incisor = 8.0 mW/cm2.	Twenty 1-minute irradiations of violet LED with consecutive 30-s intervals without application of gel should be applied on patients with previous intense tooth sensitivity. This cycling can also be combined with bleaching gel's application in patients with minor (37% CP) or no tooth sensitivity (35% HP). Four to ten sessions at 4-day intervals are indicated when only the light is used. The combination with the bleaching gel (VR/CP and VR/HP) limits the protocols to 3 sessions at 7-day intervals. The gels should be applied on the enamel surface during the entire irradiation cycling (30 min), without refreshments.

Objective color measurements (n=5/specimen) were performed after obtention of the specimens (T_0), immediately after staining procedures (T_1) and seven days after the completion of experimental bleaching protocols investigated (T_2) by means of a digital spectrophotometer (Vita Easyshade Advance, VITA Zahnfabrik H. Rauter GmbH & Co. KG, Bad Sackingen, Germany). The colorimetric evaluation was determined in terms of ΔE_{00} (color change - CIEDE2000), ΔWI_D (Whitening Index for Dentistry change), ΔL (luminosity difference), Δa (red*green difference) and Δb (yellow*blue difference) using calculated mean values of “L”, “a”, “b”, “C” and “h” [33].

2.6. Mineral content assessment

Specimens (n=3/group) were dry-incubated (37ºC, 24 hours) and carbon-coated in preparation for energy-dispersive X-ray spectroscopy EDS by means of a Vantage microanalysis system (Noran Instruments, Middleton, U.S.A.) in conjunction with a scanning electron microscopy (SEM-JSM 5600 LV; JEOL, Tokyo,Japan). The images (3000x of magnification) were acquired (with 15 kVp, 20 mm of distance, and spot sized 45) at three-patterned areas in random locations. The semi-quantitative analyses of these areas were obtained in terms of chemical elemental composition of calcium (Ca), phosphorous (P) and Ca/P ratio (atomic weight percentage) of treated enamel.

2.7. Statistical analysis

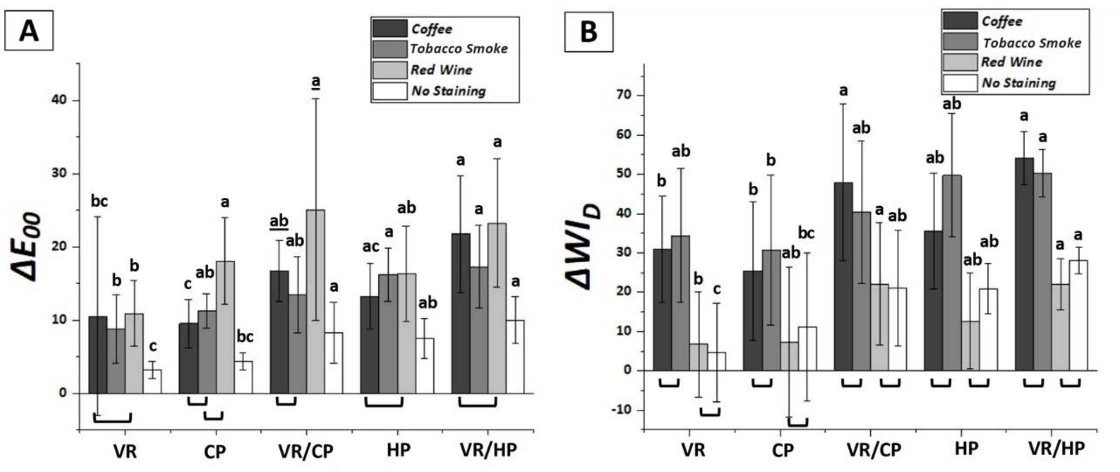
Experimental data obtained were subjected to normality and homoscedasticity analyses using the Shapiro-Wilk and Levene tests. ΔE_{00} and ΔWI_D were considered normally distributed after being transformed using Log_{10} and Sqrt, respectively. Transformed and normally distributed data (Ca, P and Ca/P) were then parametrically analyzed using two-way ANOVA and Tukey post hoc tests. Values of Δa did not meet the assumptions for normality, and therefore, were analyzed using Kruskal-Wallis and Dunn non-parametric tests. All statistical analyses in the present study were performed using the SigmaStat (Version 3.0, Informer Technologies, San Jose, U.S.A.) at a 5% significance level.

3. Results:

3.1. Objective colorimetric analysis

Figure 2 (A and B) shows mean and standard deviation values of color change (ΔE_{00}) and whiteness index change (ΔWI_D) results. Bleaching protocols presented clinically perceivable color changes (ΔE_{00}>0.8), regardless of the presence (non-stained or stained) or type of staining (CF, TS or RW). Bleaching with VR caused higher color changes (ΔE_{00}) in the stained groups in comparison to the non-stained ones ($p < 0.05$). VR alone exhibited higher ΔWI_D ($p < 0.05$) in groups stained with CF and TS in comparison to RW and NS.

Figure 2. Mean and standard deviation values of ΔE_{00} (A) and ΔWI_D (B), according to bleaching protocols and types of staining.



Means followed by distinct letters or absence of connecting bars differ statistically at 5%, according to two-way ANOVA and Tukey test. The distinct letters indicate statistical differences between the different bleaching protocols within the same type of staining (defined by the color of the bars). Connecting bars or underlined letters indicate no significant differences between the different types of staining within the same bleaching protocol.

(p type of staining < 0.001)

(p bleaching protocol < 0.001)

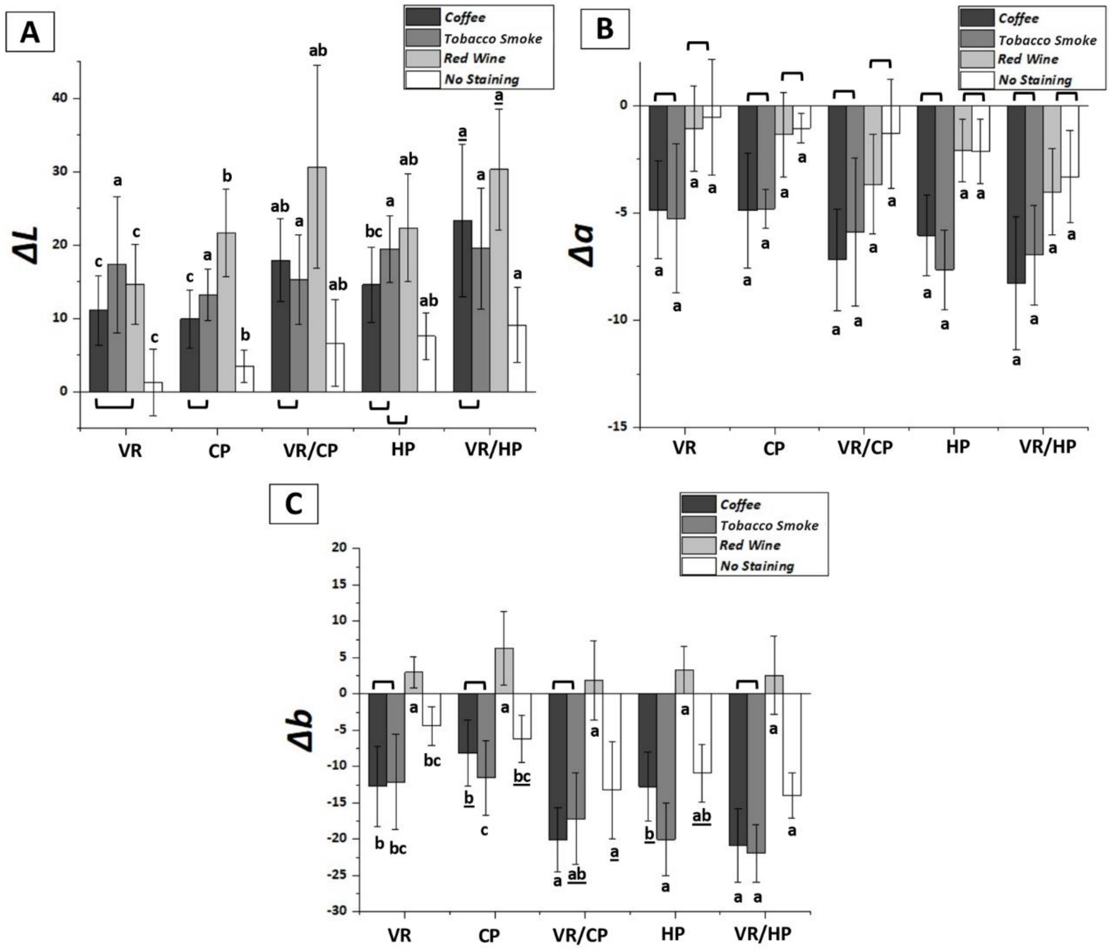
*(p type of staining * bleaching protocol = 0.121 ΔE_{00}; 0.290 ΔWI_D)*

VR/CP presented significant higher changes in the groups CF (ΔE_{00} and ΔWI_D) and NS (ΔE_{00}) compared to CP, but these bleaching protocols showed no differences in color (ΔE_{00} and ΔWI_D) in TS and RW-stained groups. Besides, HP and VR/HP or VR/CP and HP exhibited similar ΔE_{00} and ΔWI_D ($p > 0.05$). Regardless of the type of the bleaching procedure, staining with CF and TS caused a higher ΔWI_D than RW and NS ($p < 0.05$).

Figure 3 (A-C) illustrates the mean and standard deviation values according to the CIELAB coordinates (ΔL, Δa and Δb). The variation in the specimens luminosities (ΔL, Figure 3A) was significant lower in non-stained groups (NS). VR/CP and VR/HP resulted in higher ΔL than CP and HP alone, respectively, in the presence of CF staining. Figure 3B demonstrates that statistically significant differences were not present ($p > 0.05$) for mean values of Δa (variation in red*green) between the bleaching procedures. Absolute values of Δa in CF and TS groups were statistically higher in comparison to those in RW and NS, which were similar among each other ($p > 0.05$). Figure 3C illustrates the values of Δb, where it can be noticed that specimens VR/CP led to higher Δb than CP protocol in CF, TS and NS groups (p <

0.05). No differences were detected between the bleaching procedures for RW stained ($p > 0.05$), whose b* values remained positive (b* - yellow), differing from the other groups which migrated to negative b* values (b* - blue).

Figure 3. Mean and standard deviation values of ΔL (A), Δa (B) and Δb (C), according to bleaching protocols and types of staining.



Means followed by distinct letters or absence of connecting bars differ statistically at 5%, according to two-way ANOVA and Tukey test or Kruskal-Wallis and Dunn test (only for Δa). The distinct letters indicate statistical differences between the different bleaching protocols within the same type of staining (defined by the color of the bars). Connecting bars or underlined letters indicate no significant differences between the different types of staining within the same bleaching protocol.

(p type of staining <.001 ΔL e Δb)

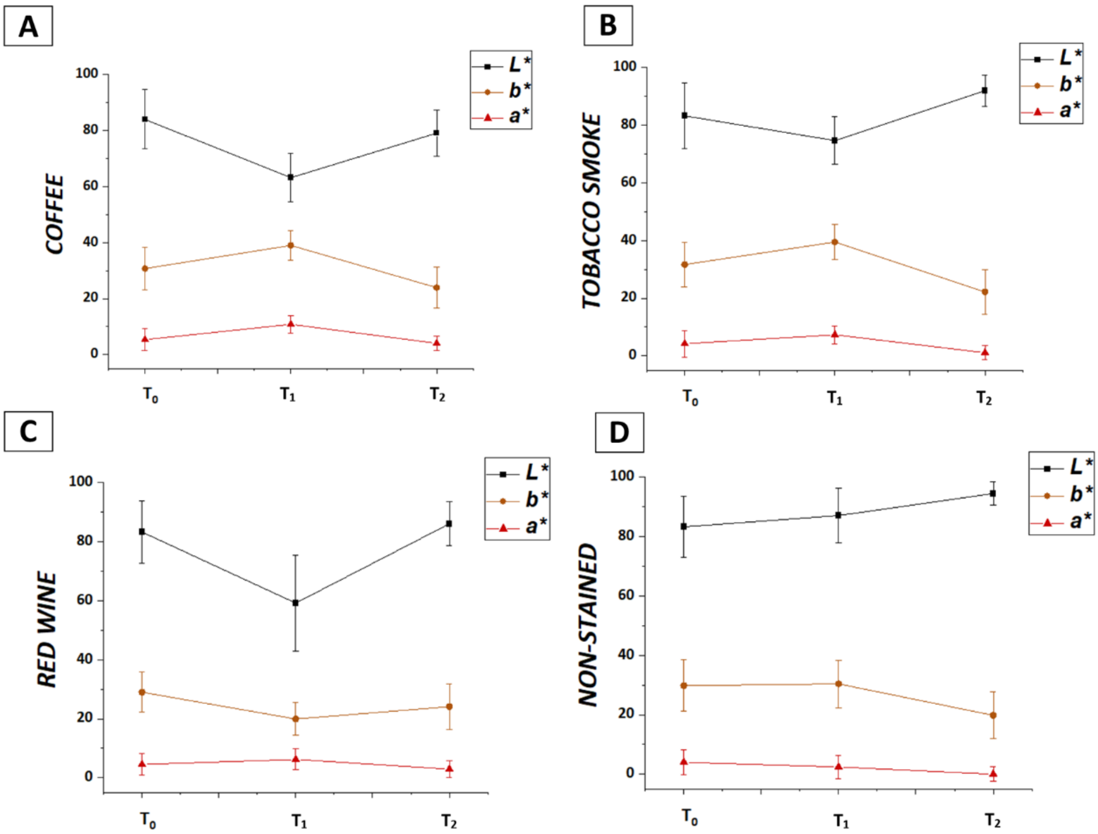
(p bleaching protocol <.001 ΔL e Δb)

*(p type of staining * bleaching protocol o = .117 ΔL; .043 Δb)*

(p <.001 Δa)

Figure 4 (A-D) displays the average L*, a* and b* values of enamel within each staining type at T_0, T_1 and T_2. While the luminosity (L*) decreased, the red (a*) and yellow appearance (b*) increased (CF, TS and NS) after staining (T_1). These coordinate values were overall recovered after bleaching (T_2), with the exception of b* values in RW groups. The coordinates of non-stained specimens were not changed between T_0 and T_1, but bleaching increased L* and decreased a* and b* values (T_2).

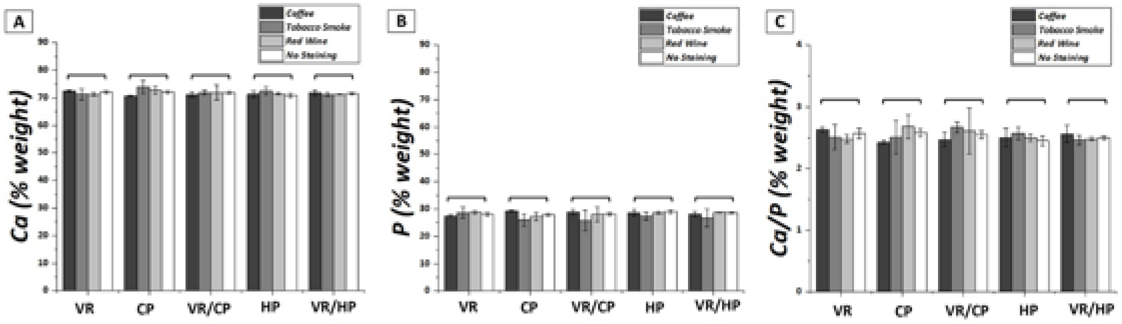
Figure 4. Line graphs presenting the average L*, a* and b* values of enamel stained with CF (A), TS (B), RW (C) and AS (D) over time (T_0 – baseline; T_1 – after staining; T_2: after bleaching).



3.2. Mineral content assessment

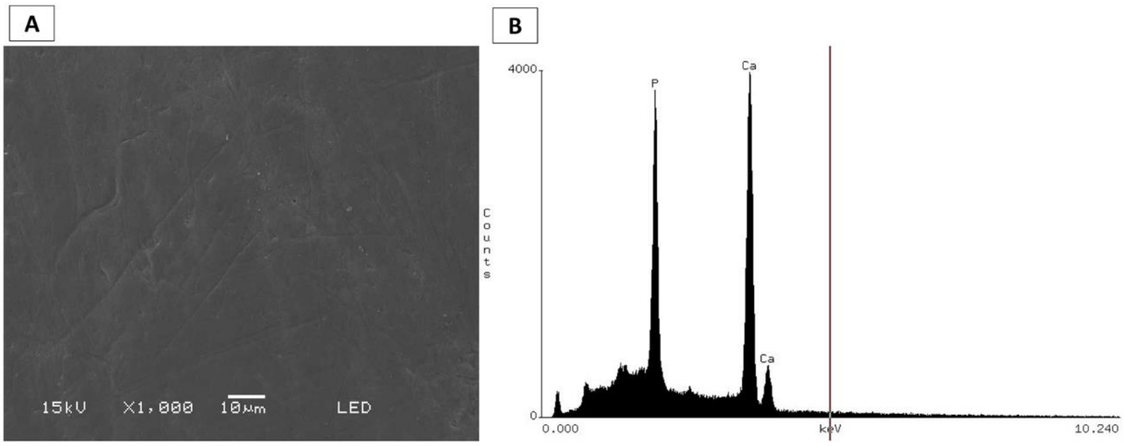
Figure 5 (A-C) exhibits the mean and standard deviation values (in terms of atomic percentage) for the contents of calcium (Ca, 5A), phosphorous (P, 5B) and calcium to phosphorous ratio (Ca/P, 5C), as determined by EDS at T_2. Specimens showed no decrease in mineral contents independently of the bleaching protocol investigated and the presence and type of staining. Mean values of atomic weight percentage were not statistically different ($p > 0.05$) within Ca, P or Ca/P evaluations. A representative SEM image of a specimen with its corresponding EDS mapping is illustrated in Figure 6 (A-B).

Figure 5. Mean and standard deviation values of the Ca (A), P (B) and Ca/P (C) - atomic weight (%), according to bleaching protocols and types of staining.



Connecting bars not differ statistically at 5%, according to two-way ANOVA and Tukey test. The absence of letters comparing the groups indicates that there were no statistical differences.

Figure 6. Representative image (A) observed under scanning electron microscopy, to find the spot to be assessed in terms of elemental composition. The EDS mapping (B) exhibits the peaks corresponding to calcium (Ca) and phosphorous (P) detected.



4. Discussion:

The color change evaluated in the present study was based on spectrophotometric analysis and the utilization of CIEDE2000 [33]. The use of ΔE_{00} allows for color assessments that are more accurate than those provided by CIELAB system, [34] because ΔE_{00} has adequately resolved the perceptual uniformity issue by adding five corrections including a hue rotation term (R_t) and compensation for neutral colors (L*C*h), lightness (S_L), chroma (S_C) and hue (S_H). In addition, the utilization of whiteness index (ΔWI_D), which is based on CIELAB, allows for bleaching efficacy determination where higher values of ΔWI_D correlate to more efficient bleaching protocols [35]. Both ΔE_{00} and ΔWI_D were taken into consideration to determine the efficacy of the present bleaching protocols, since previous multi-center studies already addressed the individual ΔE_{00} and ΔWI_D values compatible with bleaching efficacy [34,35].

Intergroup comparisons reported in the present study demonstrated that VR (405 ± 15 nm, 1.2 W/cm^2, optical window area= 10.7 cm^2, height= 8.0 mm) was capable of significantly ($p < 0.05$) changing the color (ΔE_{00}) of specimens (NS, CF, TS, RW). Bleaching protocols based solely on VR exhibited higher efficacy against CF/TS (ΔE_{00} and ΔWI_D) and RW (ΔWI_D). Based on these results, the first null hypothesis tested that the efficacy of photonic bleaching protocols (peroxide-free) does not depend on the presence or type of pigments was not accepted. The range of mean ΔE_{00} values (from 3.19 ± 1.16 [NS] to 10.91 ± 4.49 [RW]) achieved using photonic bleaching protocols are above the clinically perceivable threshold ($\Delta E_{00} > 0.8$), previously reported by Paravina et al [34]. The findings reported in the present study corroborates further the hypothesis that photonic bleaching protocols could be used to efficiently resolve discolorations precipitating from the surface accumulation of extrinsic pigments [19,23]. According to Rastelli et al., [23], organic pigments' optical absorption profiles facilitate the photolysis process when using near-UV wavelengths. It is important to note that the overall higher changes detected for stained specimens could be explained by the decrease in luminosity (L*) and increase in red (a*) and yellow (b*) appearance caused by the pigments at T_1 (Figure 4A-C). Since NS specimens (Figure 4D) presented stabilization of the coordinate values at T_1, due to the absence of the artificial staining, the average chromatic changes in NS groups were less pronounced between T_1 and T_2.

However, the results of the present study indicated that the investigated photonic protocols were also effective in changing the color of NS-bovine enamel above the clinically perceivable threshold ($\Delta E_{00} > 0.8$) [34]. In fact, color changes caused by VR and CP protocols in NS specimens were comparable (3.19 ± 1.16 and 4.43 ± 1.17, respectively) and significant differences between groups could not be detected ($p > 0.8$). As a result, these data suggest that photonic bleaching protocols can be efficiently used in clinical situations where teeth are not severely discolored. These findings corroborate the subjective color evaluation (visual shade guide) results from a recent randomized clinical trial [1]. However, the findings reported [1] have demonstrated that even though photonic bleaching protocols were able to yield clinically perceivable color changes ($\Delta E_{00} > 0.8$), the objective esthetic outcomes attained were significantly less pronounced when compared to the outcomes produced by either CP or HP. It is important to mention that the authors of the previous randomized clinical trial on the efficacy of VR [1] discussed that VR alone protocol also presents the limitation of a longer treatment (up to 10 sessions).

ΔE_{00} and ΔWI_D results indicated that VR was not able to improve the efficacy of HP independently of the considered type of staining (either CF, TS or RW) and displayed efficacy levels that were similar to those attained with the use of HP alone (VR + HP = HP). These results are similar to previous studies [22,27], possibly explained by hydrogen peroxide's typical optical absorption profile that preferentially absorbs light in the UV and near infrared portions of the electromagnetic spectrum. On the other hand, the combination between VR and CP resulted in color changes that were higher when compared to those promoted by CP alone (VR + CP > CP) in enamel stained with CF and NS. In a critical literature review, Joiner suggested that visible wavelengths are capable of increasing the internal temperature of bleaching gels and the number of molecular collisions [36]. As a consequence, this event could increase the chemical dissociation of HP and ROS generation. In this scenario, the addition of coloring in the gels could increase the efficacy of VR, as reported for photonic bleaching protocols with blue light [37]. However, future studies are necessary to determine the ideal coloration and necessary quantity to play a role in the efficacy of VR on bleaching with gels.

Other in vitro studies have demonstrated that VR was also capable of increasing the bleaching efficacy of commercially available gels with total peroxide concentrations lower than 35% (v/v) on teeth that were artificially stained with black tea [22,27]. Also, Geus et al., [38] demonstrated, using gas chromatography-mass spectrometry, that TS does not respond well to both prophylaxis and bleaching procedures, thereby corroborating the ΔE_{00} results. According to the authors, [38] TS contains numerous chemical components with different levels of susceptibility and bioaccumulation profiles. Despite these findings, color results after VR, CP and HP procedures indicated that the investigated bleaching procedures were able to significantly bleach specimens stained by TS, and displayed ΔWI_D values that were higher when compared to specimens stained by RW.

In general, the reported ΔL results showed that specimens stained by RW were more susceptible to an increase in the luminosity following bleaching protocols when compared to NS specimens, or those stained by CF or TS. On the other hand, Δb results indicated that specimens stained by RW displayed the lowest variations amongst all experimental groups tested, which could have impacted the ΔWI_D in these groups. These results are believed to precipitate from the intrinsic characteristics of RW (pH= 3.0), whose combination with high oxidative bleaching agents may increase the roughness and porosity levels of enamel [2]. Thus, the staining itself with RW could have adversely impacted the whiteness index changes conducted in the present study. However, it is important to highlight that ΔE_{00} in RW groups were similar or higher than CF and TS regardless of the bleaching protocol, which did not rule out the efficacy of the present protocols in this type of substrate.

When comparing the whiteness index changes after the investigated bleaching protocols, it becomes evident that specimens treated with VR/CP displayed ΔWI_D results that were comparable ($p > 0.05$) to those with HP or VR/HP, independently of the presence or type of staining. These findings are clinically meaningful because not only they suggest that VR + CP is as effective as HP protocols, but also because lower total peroxide concentrations are typically associated with low levels of dentin hypersensitivity and cytotoxicity [4]. The carbamide peroxide presents one-third of the total hydrogen peroxide found in the HP gels [4]. A recent study demonstrated that lower concentrations of peroxide (4· less) in the pulp were detected when the dental bleaching procedures were mediated by 37% CP and VR in comparison to 35% HP [22]. Since the VR/CP does not present the drawback of an extended protocol as for VR alone, a similar bleaching outcome to high-concentration HP, with reduced intrapulpal concentration of hydrogen peroxide by-products, could positively influence the tooth sensitivity [5]. Therefore, the photonic bleaching raises the possibility of an extremely effective esthetic outcome with reduced side effects by decreasing the concentration of the bleaching gel.

The secondary objective of the present study was to determine potential adverse effects of bleaching protocols with VR and peroxide-containing products on the mineral content (specifically Ca and P) of bovine enamel (NS, CF, TS, and RW). Previous reports have indicated that high-concentrated peroxides (either CP or HP) may decrease the enamel content of Ca and P [39-43], and that this condition may not be exacerbated by visible light radiation under the blue wavelength [43].

However, the results of this investigation demonstrated that neither bleaching nor staining protocols combined or not with the VR light alter the atomic percentage weight of Ca, P, and the Ca/P ratio. These findings agree with more recent reports that evaluated bleaching application protocols similar to those of this study. A randomized clinical trial conducted enamel microbiopsies and demonstrated that VR did not adversely impact the Ca and P concentrations or the molecular ratios between the elements [1]. Also, a □Raman evaluation has shown that bleaching protocols with VR do not affect the phosphate and carbonate concentrations of enamel selected after surface microhardness evaluation and 37% CP and 35% HP bleaching [28]. Therefore, the results of this and previous studies strongly suggest that VR does not impose potential side effects on enamel surface, as can be typically observed during bleaching techniques by high-concentrated CP or HP [39,44,45].

One limitation of this study was that the energy-dispersive X-ray spectroscopy (EDS), a semi-quantitative analysis to detect Ca, P, and Ca/P ratio, was performed only after bleaching because of the need for carbon-coating to obtain the images. Therefore, the specimens were not selected based on the initial chemical composition. Although this constitutes a limitation, the primary goal of this research was to evaluate the color change of stained teeth after bleaching protocols combined or not with VR. Besides, the EDS results confirm the findings of recent in vitro study (28) and a randomized clinical trial (1), in which the bleaching protocols tested did not change enamel Ca/P ratio. Although this may indicate a positive bleaching outcome, we should bear in mind that it refers to the bleaching protocol tested in these studies and should be carefully extrapolated to other bleaching products or protocols.

Finally, this study indicates that VR combined or not with peroxide-containing gels (either CP or HP) was more efficient to bleach specimens that were artificially stained as compared to non-stained specimens. The combination of VR and CP was as effective as bleaching protocols mediated by HP alone. The results allowed us to infer that VR may be combined with gels containing lower total concentration of hydrogen peroxide, thereby maintaining excellent colorimetric outcomes and, possibly, attenuating the adverse effects of dental bleaching procedures caused by high-concentrated HP.

5. Conclusions:

Based on the conditions and limitations of the present study, all bleaching protocols investigated achieved clinically perceivable color and whiteness changes, regardless of the utilization of peroxide-containing gels (either carbamide or hydrogen peroxide) or the presence and type of pigments. The reported findings indicated that organic pigments (coffee, tobacco smoke and red wine) displayed distinct susceptibility levels to bleaching procedures, and coffee was the most susceptible pigment. Violet radiation increased the bleaching efficacy of carbamide peroxide (37%) in specimens stained with coffee or in non-stained ones. Bleaching protocols using violet radiation did not adversely impact the mineral content of enamel and peroxide-containing gels.

REGULATORY STATEMENT

This research was conducted respecting the rules of the local Ethics Committee on the Use of Animals and the National Council for Animal Experimentation.

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3 CONCLUSÃO

De acordo com os resultados obtidos no presente estudo, foi possível concluir que:

- Foram observadas alterações de cor clinicamente perceptíveis em todos os protocolos de clareamento, independentemente da utilização dos géis de peróxido e da presença e do tipo de pigmentação extrínseca.
- Pode-se considerar que a ação do LED violeta sem os agentes químicos é influenciada pela presença e pelo tipo de pigmentação extrínseca do esmalte, e foi mais evidenciada em café e fumaça de tabaco.
- A eficácia do PC 37% foi aumentada com a associação do LED violeta (LED/CP) para os grupos pigmentados com café ou com a ausência do pigmento.
- O protocolo LED/CP apresentou eficácia clareadora similar a PH sem ativação luminosa, independentemente do tipo de pigmentação extrínseca.
- Não foram encontradas diferenças significativas no conteúdo mineral do esmalte, tanto para cálcio, quanto para fósforo, em todos os protocolos clareadores.

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APÊNDICE 1 - METODOLOGIA ILUSTRADA

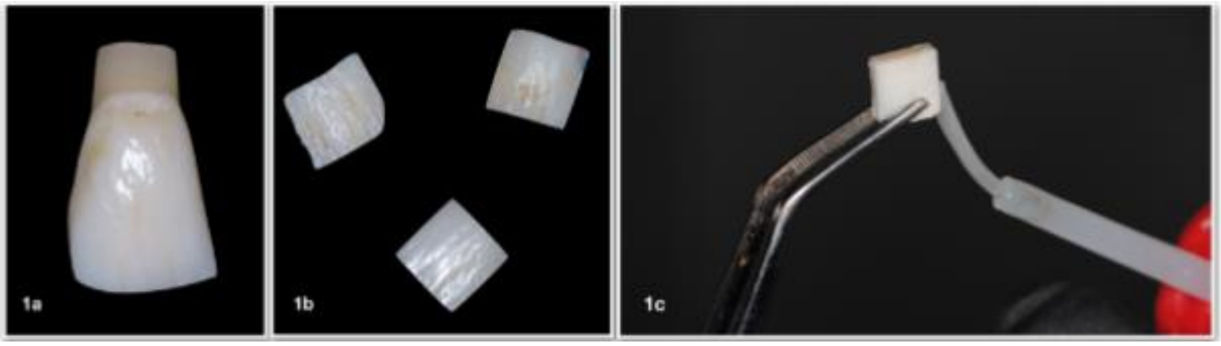

Figura 1 – a: Dentes bovinos obtidos com as raízes seccionadas; b: Blocos obtidos (6mm x 6mm x 3mm). Fig 1c: aplicação de esmalte incolor na área correspondente a dentina

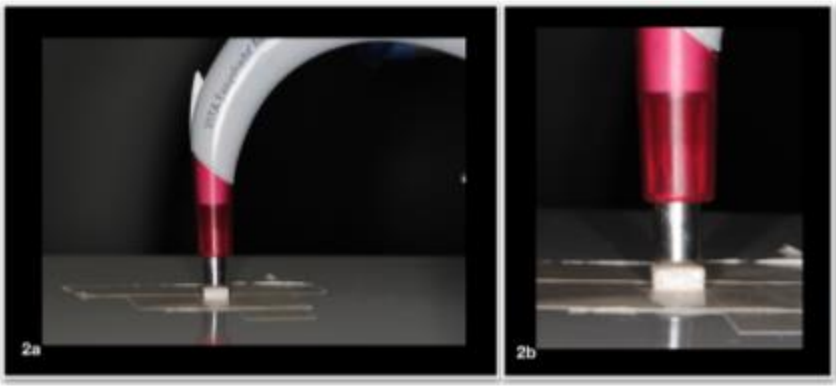

Figura 2 – a: Medição da cor dos espécimes; b: Detalhe da ponta do aparelho perpendicular ao bloco

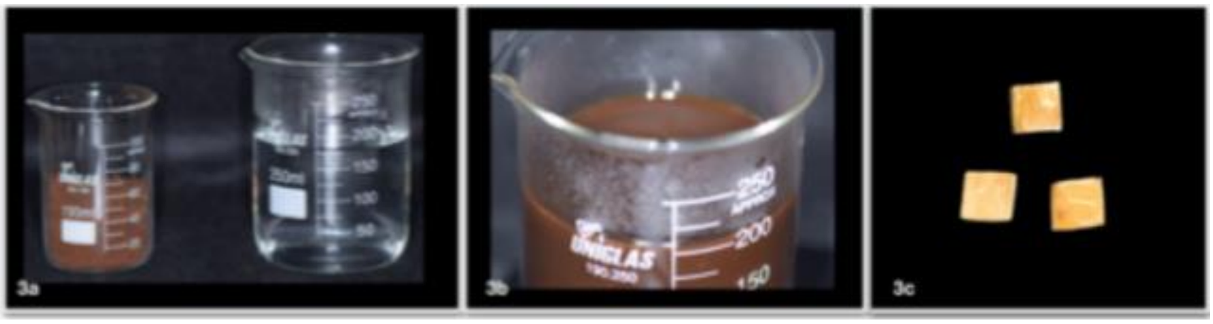

Figura 3 – a: 16 gramas de cáfe solúvel pesado em balança de precisão; b: Solução de café preparada com 200 ml de água destilada; c: Espécimes após ciclo de pigmentação com café

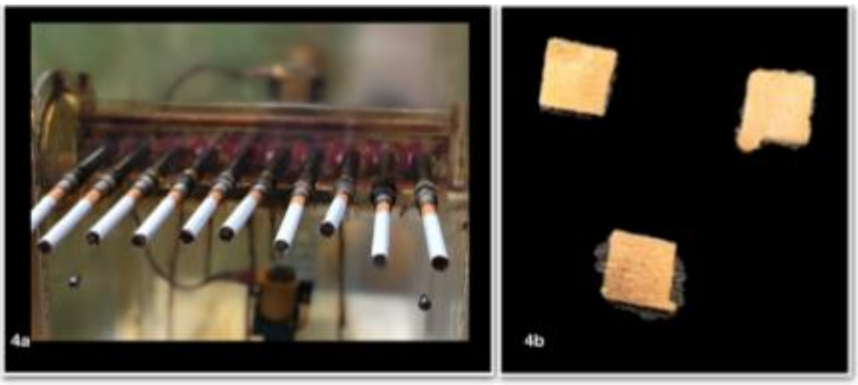

Figura 4 - a: Máquina de inalação de cigarro; b: Espécimes após término da pigmentação com fumaça de cigarro

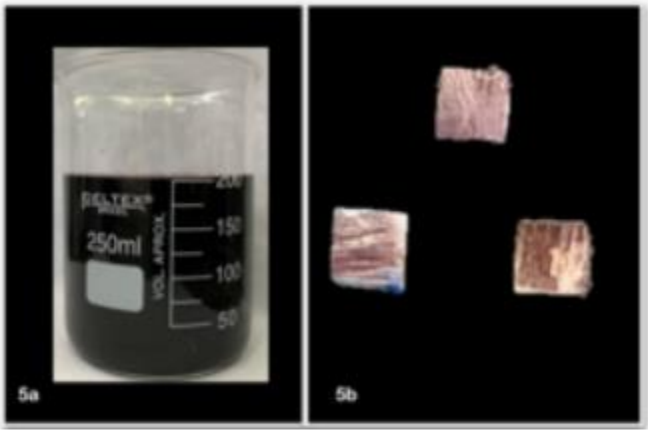

Figura 5 - a: 200ml de vinho tinto; b: Espécimes após término da pigmentação com vinho tinto

Figura 6 - Esquema representativo dos géis clareadores e fonte de luz utilizados nos protocolos adotados por este estudo

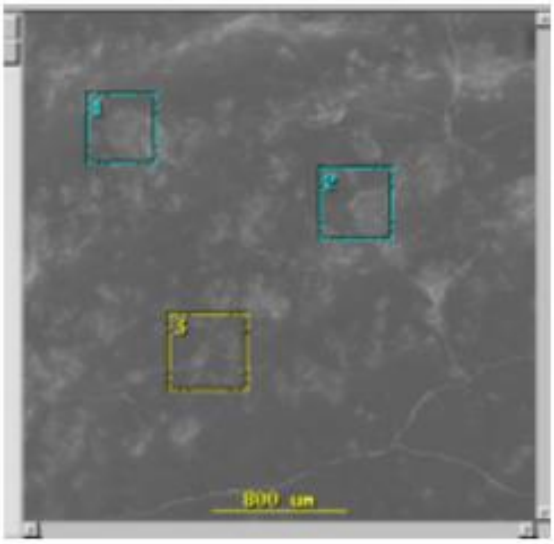

Figura 7 - Imagem representativa da coleta de dados do conteúdo de Ca e P em áreas pré-determinadas da superfície de esmalte coberto por carbono

ANEXOS

Anexo 1 – Verificação de originalidade e prevenção de plágio

TCC Roberta Kobayashi

RELATÓRIO DE ORIGINALIDADE

8%	5%	7%	4%
ÍNDICE DE SEMELHANÇA	FONTES DA INTERNET	PUBLICAÇÕES	DOCUMENTOS DOS ALUNOS

FONTES PRIMÁRIAS

1. R. Banu Ermis, Esra Uzer CELIK, Gul YILDIZ, Basak YAZKAN. "Effect of tooth discoloration severity on the efficacy and color stability of two different trayless at-home bleaching systems", Journal of Dental Research, Dental Clinics, Dental Prospects, 2018 — 1%
   Publicação

2. Jéssica D. Theobaldo, Waldemir F. Vieira-Junior, Anderson Catelan, Maria do Carmo A. Mainardi et al. "Effect of Heavy Metals Contamination from Cigarette Smoke on Sound and Caries-Like Enamel", Microscopy and Microanalysis, 2018 — <1%
   Publicação

3. Leticia Cunha Amaral Gonzaga de Almeida, Diana Gabriela Soares, Fernanda Almeida Azevedo, Marjorie de Oliveira Gallinari et al. "At-Home Bleaching: Color Alteration, Hydrogen Peroxide Diffusion and Cytotoxicity", Brazilian Dental Journal, 2015 — <1%
   Publicação

Anexo 2 – Iniciação Científica

pibic 2020 pibiti

Programa Institucional de Bolsas de Iniciação Científica e Tecnológica

Relatório Final

Eficácia do clareamento com diferentes protocolos clareadores do Led violeta no esmalte escurecido com fumaça de cigarro, café e vinho tinto.

Versão enviada em 11/08/2019 23:05:23

ver relatório (../arquivos/rel_final/AlunoCod_19677_1-RelFinal_2018.pdf)

— **Parecer do orientador emitido em 13/08/2019 07:53:58**

Desempenho do aluno no projeto: A aluna Roberta Kobayashi surpreendeu durante todo o período de IC. Embora possua perfil discreto, esteve presente em todas as etapas do projeto, e desempenhou grande parte das etapas da pesquisa, em finais de semana, feriados e férias. Desta forma, por se tratar de um projeto extenso (com 200 corpos de prova analisados e diferentes fases de repetição de análises) foi capaz de cumprir com suas obrigações. Ainda, nos surpreendeu a postura da aluna na apresentação do trabalho em congresso e sua preocupação em realizar um trabalho bem feito. Estou muito satisfeita com o desempenho da aluna e com a responsabilidade que conduziu essa pesquisa.

Desempenho acadêmico do aluno: Acompanhei a aluna em clínica integrada e o mesmo comportamento responsável foi demonstrado em clínica. Foi uma das primeiras alunas a finalizar as metas de produção e as executou com qualidade e comprometimento. Sem dúvida, uma aluna com excelente perfil de pesquisa.

— **Parecer do Assessor dado em 27/09/2019 10:59:22**

A aluna desenvolveu o projeto de pesquisa de acordo com o cronograma proposto inicialmente. Teve uma melhora no rendimento acadêmico ao longo da execução da iniciação científica. A professora orientadora se mostrou satisfeita com o desempenho da aluna e com os resultados obtidos. De acordo com o exposto, o parecer e favorável a aprovação do relatório final.

● Aprovado

Anexo 4 – Comprovante de submissão do Artigo

Photodiagnosis and Photodynamic Therapy

Effects of dental bleaching protocols with violet radiation on the color and chemical composition of stained bovine enamel

--Manuscript Draft--

Manuscript Number:	PDPDT-D-20-00504R1
Article Type:	Research Paper
Keywords:	Keywords: Violet radiation, dental bleaching, hydrogen peroxide, carbamide peroxide, minimally invasive, ultra-conservative
Corresponding Author:	Vanessa Cavalli University of Campinas, Piracicaba Dental School Piracicaba, BRAZIL
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