Restor Dent Endod. 2023 Nov;48(4):e33
https://doi.org/10.5395/rde.2023.48.e33
eISSN 2234-7666

Restorative Dentistry & Endodontics RDE

Research Article

Check for updates

# Evaluation of at-home bleaching protocol with application on different surfaces: bleaching efficacy and hydrogen peroxide permeability

**Heloisa Forville**,[1] **Michael Willian Favoreto**,[1] **Michel Wendlinger**,[1] **Roberta Micheten Dias**,[1] **Christiane Philippini Ferreira Borges**,[2] **Alessandra Reis**,[1] **Alessandro D. Loguercio** [1*]

[1]Department of Restorative Dentistry, School of Dentistry, State University of Ponta Grossa, Ponta Grossa, PR, Brazil
[2]Department of Chemistry, State University of Ponta Grossa, Ponta Grossa, PR, Brazil

OPEN ACCESS

**Received:** Jul 2, 2023
**Revised:** Aug 4, 2023
**Accepted:** Aug 8, 2023
**Published online:** Oct 6, 2023

**Citation**
Forville H, Favoreto MW, Wendlinger M, Dias RM, Borges CPF, Reis A, *et al.* Evaluation of at-home bleaching protocol with application on different surfaces: bleaching efficacy and hydrogen peroxide permeability. Restor Dent Endod 2023;48(4):e33.

***Correspondence to**
**Alessandro D. Loguercio, DDS, MSc, PhD**
Department of Restorative Dentistry, School of Dentistry, State University of Ponta Grossa, Rua Carlos Cavalcanti, 4748 Bloco M, Sala 64-A, Uvaranas, Ponta Grossa, PR 84030-900, Brazil.
Email: aloguercio@hotmail.com



## ABSTRACT

**Objectives:** This study aimed to evaluate the bleaching efficacy and hydrogen peroxide permeability in the pulp chamber by the at-home bleaching gel in protocols applied on different dental surfaces.

**Materials and Methods:** Forty premolars were randomly into 4 groups: control group no bleaching, only application on the buccal surface (OB), only application on the lingual surface (OL) and application in buccal and lingual surfaces, simultaneously (BL). At-home bleaching gel (White Class 7.5%) was used for the procedure. The bleaching efficacy was evaluated with a digital spectrophotometer (color change in CIELAB [$\Delta E_{ab}$] and CIEDE 2000 [$\Delta E_{00}$] systems and Whitening Index for Dentistry [$\Delta WI_D$]). The hydrogen peroxide permeability in the pulp chamber (μg/mL) was assessed using UV-Vis spectrophotometry and data were analyzed for a 1-way analysis of variance and Tukey's test ($\alpha = 0.05$).

**Results:** All groups submitted to bleaching procedure showed bleaching efficacy when measured with $\Delta E_{ab}$ and $\Delta E_{00}$ ($p > 0.05$). Therefore, when analyzed by $\Delta WI_D$, a higher bleaching efficacy were observed for the application on the groups OB and BL ($p = 0.00003$). Similar hydrogen peroxide permeability was found in the pulp chambers of the teeth undergoing different protocols ($p > 0.05$).

**Conclusions:** The application of bleaching gel exclusively on the OB is sufficient to achieve bleaching efficacy, when compared to BL. Although the OL protocol demonstrated lower bleaching efficacy based on the $\Delta WI_D$ values, it may still be of interest and relevant in certain clinical scenarios based on individual needs, requiring clinical trials to better understand its specificities.

**Keywords:** Color shade; Dental enamel permeability; Hydrogen peroxide; Tooth bleaching; Tooth whitening

**Funding**

This study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grants 304817/2021-0 and 308286/2019-7 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

**Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

**Author Contributions**

Conceptualization: Forville H, Favoreto MW, Wendlinger M, Dias RM, Loguercio AD, Reis A; Data curation: Forville H, Favoreto MW, Wendlinger M; Formal analysis: Loguercio AD; Funding acquisition: Loguercio AD; Investigation: Forville H, Favoreto MW, Wendlinger M; Methodology: Forville H, Favoreto MW, Wendlinger M, Dias RM, Loguercio AD, Reis A, Borges CPF; Project administration: Loguercio AD; Resources: Loguercio AD; Software: Loguercio AD; Supervision: Loguercio AD; Visualization: Loguercio AD; Writing - original draft: Forville H, Favoreto MW, Wendlinger M; Writing - review & editing: Loguercio AD, Reis A.

**ORCID iDs**

Heloisa Forville
https://orcid.org/0000-0001-8327-2279
Michael Willian Favoreto
https://orcid.org/0000-0002-0800-7915
Michel Wendlinger
https://orcid.org/0000-0002-5282-500X
Roberta Micheten Dias
https://orcid.org/0009-0004-8174-6038
Christiane Philippini Ferreira Borges
https://orcid.org/0000-0002-7041-3084
Alessandra Reis
https://orcid.org/0000-0001-6798-2094
Alessandro D. Loguercio
https://orcid.org/0000-0001-9880-4856

# INTRODUCTION

In contemporary times, there has been a growing concern about aesthetic parameters, and one of the contributing factors is the appearance of the smile, with tooth color being a crucial aspect of overall satisfaction [1,2]. To enhance this parameter, dental bleaching is a technique that yields positive outcomes. Among the available methods, at-home bleaching is the preferred technique for patients seeking a minimally invasive treatment option, as it offers the convenience of application anywhere, provided it is done under proper supervision and recommendation by the dentist [3,4].

Although it is a simple technique, it requires the use of individually customized trays for 2 to 4 weeks, depending on the concentration of the bleaching gel [5,6]. However, extended treatment time is a drawback of the at-home bleaching procedure. In order to enhance the technique and promote greater bleaching efficacy and patient comfort, some studies have explored modifications, such as varying the concentration of the bleaching gel, different application times, and increasing the volume of bleaching gel in contact with the dental surface using reservoirs [7-12].

However, a very simple procedure, as applied the at-home bleaching on 1 more surface, as the lingual area, could improve the bleaching efficacy of at-home bleaching. Actually, Fick's Second Law has been used to better understand the mechanism of action of bleaching agents [13]. This theory states that the diffusion of molecules is directly proportional to the contact area within a structure [14]. Thus, when different surfaces of the tooth come into contact with the bleaching agent simultaneously, it is expected that hydrogen peroxide will diffuse into the enamel and dentin more easily. This could facilitate the acceleration of bleaching results, increasing the availability of free radicals for the bleaching reaction [14,15]. To the best of the authors' knowledge, no *in vitro* study has evaluated whether the association could enhance bleaching efficacy and to what extent this would impact the hydrogen peroxide permeability in the pulp chamber.

Therefore, this study aimed to evaluate the bleaching efficacy and penetration of hydrogen peroxide in the pulp chamber of human teeth subjected to at-home bleaching with a 7.5% hydrogen peroxide gel, employing protocols involving the application of bleaching gel on different tooth surfaces (buccal surface, lingual surface, and both buccal and lingual surfaces simultaneously). The null hypotheses tested were as follows: 1) there would be no difference in bleaching efficacy and 2) there would be no difference in hydrogen peroxide permeability within the pulp chamber when different tooth surfaces were bleached.

# MATERIALS AND METHODS

## Ethical approval protocol

This *in vitro* study was approved by the Research Ethics Committee of the State University of Ponta Grossa, PR, Brazil (protocol number 5.355.873).

## Selection of teeth and inclusion and exclusion criteria

Forty healthy premolars of similar size were utilized in this study, obtained from the Human Teeth Local Bank at the State University of Ponta Grossa, PR, Brazil (**Figure 1A**). To ensure standardization of the selected teeth, all of them were examined under a microscope at 10×

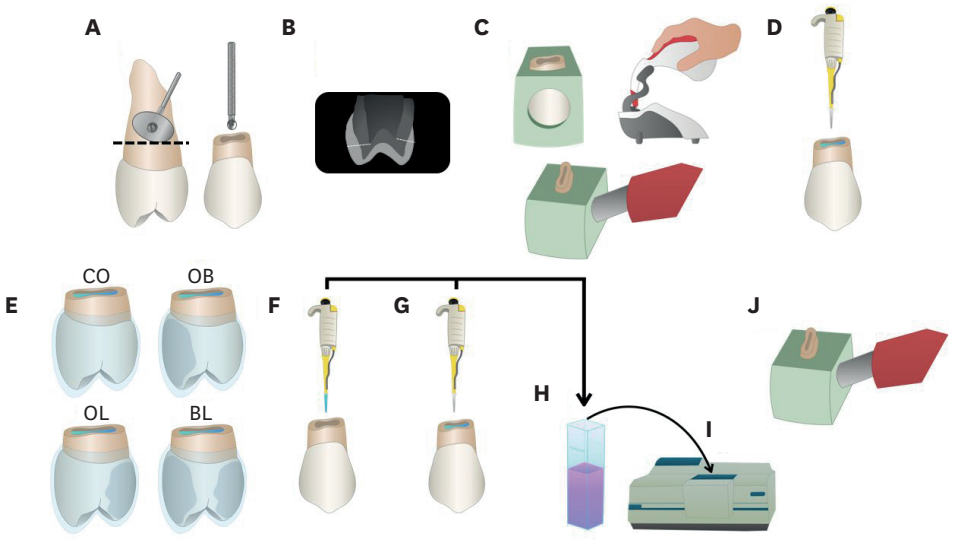


**Figure 1.** (A) In the forty premolars used, the roots were removed with a cut 3 mm from the cementoenamel junction and the access to the pulp chamber was slightly enlarged. (B) Measurement of enamel and dentin thickness on the buccal and lingual surfaces of the teeth using digital radiography. (C) Silicone guides were created for each specimen, and initial color measurements (pre-operative value) were taken using a digital spectrophotometer. (D) Acetate buffer solution was inserted into the pulp chamber of each specimen. (E) Acetate trays were made for all teeth and bleaching gel was applied according to each group (CO [no bleaching], OB, OL, and BL). (F, G) Acetate buffer solution was removed to the pulp chamber of each specimen. This process was repeated. (H) Leucocrystal violet solution, horseradish peroxidase enzyme, and ultrapurified water were added. (I) The final solution was read using UV–Vis spectrophotometer. (J)The same silicone guides were used, and final color measurements were taken using a digital spectrophotometer.CO, control group with no bleaching; OB, bleaching applied on the buccal surface only; OL, bleaching applied on the lingual surface only; BL, bleaching simultaneous applied on both buccal and lingual surfaces.

magnification (Lambda LEB-3, ATTO instruments, Hong Kong, China). Teeth exhibiting enamel cracks or morphological changes were excluded from the study. Additionally, teeth lighter than A2 were also excluded, as determined by digital spectrophotometry (Vita Zahnfabrik, Bad Säckingen, Germany). Teeth with a thickness of less than 2.5 mm or greater than 4.0 mm were excluded from the study [16-18].

## Sample size calculation

The sample size calculation was conducted based on the primary outcome of this study, which aimed to evaluate the bleaching efficacy of teeth subjected to at-home bleaching with a 7.5% hydrogen peroxide gel, utilizing different surface protocols (4 groups) for gel application. In a pilot study (data not shown), 10 teeth underwent at-home bleaching with a 7.5% hydrogen peroxide gel using a conventional protocol (application on the buccal surface only), resulting in an $\Delta E_{ab}$ value of 7.7. This value ($\Delta E_{ab}$ = 7.7 ± 2.0) was used as the reference for the control group. To consider a procedure effective in terms of bleaching efficacy, the changes must reach acceptable limits. According to Paravina *et al.* [19], the acceptable value for color change ($\Delta E_{ab}$) is 2.7. Therefore, an $\Delta E_{ab}$ equal to or higher than 10.4 was expected in the experimental group.

Using an online calculator (sealedenvelope.com) for continuous outcomes and determining sample size for superiority, it was determined that 9 samples per group were necessary to achieve an 80% probability of detecting a significant increase of $\Delta E_{ab}$ by 2.7 among the groups, with a significance level of 5%. However, to account for potential sample loss, an additional 10% more teeth were included. Therefore, the final sample size was 10 teeth for each group.


https://doi.org/10.5395/rde.2023.48.e33

### Specimen preparation

To introduce a 25 µl solution using a micropipette (LABMATE Soft, HTL Lab Solutions, Warsaw, Poland), the tooth roots were removed approximately 3 millimeters from the cemento-enamel junction using a low-speed diamond disc (**Figure 1A**; Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA). Additionally, the pulp tissue was extracted through an apical access, and the cavity was rinsed with deionized water. Subsequently, access to the pulp chamber was slightly enlarged with the assistance of a spherical drill nº 1014 (**Figure 1A**; KG Sorensen, Cotia, SP, Brazil), while ensuring no contact was made with the inner buccal and lingual regions of the cavity, in order to maintain the previously measured enamel and dentin structure.

The thickness of the teeth was assessed via radiography (Timex 70C, Gnatus, Ribeirão Preto, SP, Brazil), using an exposure time of 0.5 seconds and a 30-cm focus-object distance (70 kVp-7 mA) (**Figure 1B**). The central X-ray beam was positioned at a 90° angle to the tooth's lateral surface. Following exposure, digital images were obtained, and the corresponding buccal and lingual tooth thicknesses (from the external point of enamel to the innermost point of dentin, corresponding to the pulp horn) were measured using New IDA software (Dabi Atlante, Ribeirão Preto, SP, Brazil).

### Initial color evaluation

The color change was assessed using a digitally calibrated spectrophotometer (VITA Easyshade Advance 4.0, VITA Zahnfabrik) at baseline (pre-operative value). To ensure consistent measurement, guides were created using dense condensation silicone (Coltoflax and Cub Kit Profile, Vigodent, Rio de Janeiro, RJ, Brazil) based on the spectrophotometer's tip diameter (6 mm × 6 mm) (**Figure 1C**). Measurements were taken on the middle one-third of the buccal surface of the specimens as it is considered the most suitable area for evaluating bleaching effects due to the diffusion process involved in bleaching (**Figure 1C**).

The spectrophotometer measurements were used to record the color parameters (L*, a*, and b*). The L* value indicates the luminosity, with 0 representing black and 100 representing white. The a* value represents the position on the green-red axis, with negative values indicating green and positive values indicating red. The b* value represents the position on the blue-yellow axis, with negative values indicating blue and positive values indicating yellow [20].

### Analytical curve

The products utilized in this study were used without prior purification, and deionized water was employed to prepare the solutions. To establish the analytical curve pattern, a 5,000 µg/mL stock solution was utilized. This stock solution was prepared from a concentrated solution (50% hydrogen peroxide, Pharma Efficacy, Ponta Grossa, PR, Brazil) and subsequently diluted in an acetate buffer solution (pH = 4). The diluted solution was titrated with a potential permanganate solution to determine the precise concentration, ensuring analytical grade accuracy. Dilutions ranging from 0.000 µg/mL to 0.403 µg/mL were prepared using the initial concentration to generate the analytical curve. The known concentration of hydrogen peroxide was determined using a Cay UV-Vis 100 spectrophotometer (Varian, Palo Alto, CA, USA). This methodology successfully established a standard reference line that could be applied to the results of the study samples ($R = 0.998$; data not shown).

## Experimental groups and treatment protocols

Before the bleaching procedure, a 25 μL aliquot of acetate buffer solution (pH = 4) was inserted into the pulp chamber of each tooth to absorb any hydrogen peroxide that might have entered during the bleaching procedures (**Figure 1D**). A single experienced and calibrated operator conducted all treatment protocols in this study. Custom trays were created to ensure consistent application of the bleaching gel and maintain a uniform film thickness. The forty selected specimens were randomly assigned to treatment groups ($n$ = 10). The control group did not undergo any bleaching procedure (**Figure 1E**). In the 3 experimental groups, a 7.5% hydrogen peroxide bleaching gel (White Class, FGM Dental Group, Joinville, SC, Brazil) was applied according to specific protocols: application on the buccal surface only (OB, **Figure 1E**), application on the lingual surface only (OL, **Figure 1E**), and simultaneous application on both buccal and lingual surfaces (BL, **Figure 1E**). Further details about the bleaching gel, including concentration, composition, and batch number, can be found in **Table 1**. Prior to the bleaching procedure, the initial concentrations of the bleaching gel were verified and compared to the manufacturer's information by titrating the gel with a standardized potassium permanganate solution [18,21,22].

The bleaching agents were applied, at room temperature (21ºC), according to the manufacturer's instructions using custom trays specific to each experimental group. The application period for all bleaching gels was 60 minutes, according to the manufacturer. Subsequently, the gel was carefully removed using gauze, and the surfaces were thoroughly rinsed with deionized water. During the intervals between the applications of the bleaching gel, the specimens were immersed in artificial saliva at a controlled temperature of 37°C.

## Hydrogen peroxide permeability evaluation

After the time of the first bleaching session, the bleaching gel was removed using cotton and tweezes, and the acetate buffer solution within the pulp chamber of each sample was promptly removed using a mechanical micropipette and transferred to a glass tube (**Figure 1F**). To ensure the complete elimination of hydrogen peroxide, this process was repeated 4 times using 25 μL of acetate buffer, and the collected solutions were combined in the same glass tube (**Figure 1G**). Next, 100 μL of 0.5 mg/mL Leucocrystal Violet (Sigma Chemical Co., St. Louis, MO, USA), 50 μL of 1 mg/mL horseradish peroxidase enzyme (Peroxidase Type VI-A, Sigma Chemical Co.), and 2.725 μL of deionized water were added to the glass tube (**Figure 1H**). This sequence was individually repeated for all samples. The resulting solution was analyzed using a Cary 100 UV-Vis spectrophotometer (Varian) (**Figure 1I**). According to Beer's Law, there is

**Table 1.** Characteristics of the bleaching gel used: concentration, components, and batch number

| Bleaching gel | The initial concentration of HP* | Components | Batch number | Mode of application |
|---|---|---|---|---|
| White Class 7.5% (FGM Dental Group, Joinville, SC, Brazil) | 7.8 ± 0.2* (% HP) | Hydrogen peroxide gel, neutralized carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water, and surfactant | 071220 (2002-04-06) | 1 hour a day (OB) |
| White Class 7.5% (FGM) | 7.8 ± 0.2* (% HP) | Hydrogen peroxide gel, neutralized carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water, and surfactant | 071220 (2002-04-06) | 1 hour a day (OL) |
| White Class 7.5% (FGM) | 7.8 ± 0.2* (% HP) | Hydrogen peroxide gel, neutralized carbopol, potassium nitrate, sodium fluoride, calcium gluconate, stabilizer, deionized water, and surfactant | 071220 (2002-04-06) | 1 hour a day (BL) |

HP, hydrogen peroxide; OB, bleaching applied on the buccal surface only; OL, bleaching applied on the lingual surface only; BL, bleaching simultaneous applied on both buccal and lingual surfaces.
*Measured hydrogen peroxide amount assessed in triplicate ($n$ = 3).


https://doi.org/10.5395/rde.2023.48.e33

a direct proportional relationship between concentration and absorbance. Therefore, the hydrogen peroxide permeability in concentration (μg/mL) was determined by comparing it with the previously established calibration curve. Upon quantifying the hydrogen peroxide permeability, the 14-day bleaching protocol was concluded.

### Bleaching efficacy evaluation

After that, the color alteration was measured 1 week after bleaching treatments, using a digital spectrophotometer (VITA Easyshade Advance 4.0, VITA Zahnfabrik) (**Figure 1J**).

The final color parameters (L*, a*, and b*) were measured after treatments, using a digital spectrophotometer (VITA Easyshade Advance 4.0, VITA Zahnfabrik). The bleaching efficacy was provided by the difference between the colors evaluated before and after the treatment with the spectrophotometer, through the $CIEL_{AB}$ formula $\Delta E_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ [23]. Also, we calculated the bleaching efficacy using the $CIEDE_{00}$ formula $\Delta E_{00} = [(\Delta L/kLSL)^2 + (\Delta C/kCSC)^2 + (\Delta H/kHSH)^2 + RT\ (\Delta C^*\Delta H/SC^*SH)]^{1/2}$ and Whiteness Index for Dentistry ($WI_D$) $WI_D = 0.551xL - 2.324 \times a - 1.1 \times b$, which is more recently reported in bleaching studies [24-32]. Furthermore, the changes in $WI_D$ resulting from each step were determined by subtracting the observed values at each assessment time from those calculated in the previous step ($\Delta WI_D$). For a procedure to be considered efficacy in terms of color change, the changes must reach acceptable limits of $\Delta E_{ab} > 2.7$; $\Delta E_{00} > 1.2$; and $\Delta WI_D > 2.6$ [19,33].

### Statistical analysis

Data presented normality according to the Kolmogorov-Smirnov test. Statistical analysis for all variables was performed using 1-way analysis of variance. Tukey's test was applied for a paired analysis ($\alpha = 0.05$).

## RESULTS

The mean thickness of the teeth employed in this study was 3.3 ± 0.4 mm on the buccal surface and 3.1 ± 0.3 mm on the lingual surface, with no difference between groups ($p > 0.49$). The baseline values of coordinates L* ($p = 0.78$), a* ($p = 0.91$), and b* ($p = 0.92$) were similar with no significant differences among groups. Therefore, the initial color of the specimens among different groups was similar (**Table 2**).

### Bleaching efficacy evaluation

The values of bleaching efficacy measurements are shown in **Table 3**. For all color change measurements, a significant difference was observed among groups ($p = 0.00001$ for $\Delta E_{ab}$, $p = 0.005$ for $\Delta E_{00}$ and $p = 0.00003$ for $\Delta WI_D$). All groups submitted to bleaching showed significant bleaching efficacy when measured with $\Delta E_{ab}$ and $\Delta E_{00}$ when compared to the

**Table 2.** Means and standard deviations in the baseline values of L*, a*, and b* from different groups, as well as statistical analysis

| Experimental groups | Baseline values | | |
|---|---|---|---|
| | L* | a* | b* |
| Control | 82.7 ± 2.7A | −1.1 ± 1.2a | 27.1 ± 5.6A |
| Buccal only | 83.8 ± 4.2A | −2.0 ± 1.3a | 25.5 ± 4.8A |
| Lingual only | 87.2 ± 3.6A | −3.0 ± 0.8a | 25.3 ± 4.3A |
| Buccal + lingual | 86.7 ± 3.6A | −2.3 ± 0.9a | 26.7 ± 1.5A |

Different capital and lower-case letter, superscript or not in each column indicate statistically similar means (1-way analysis of variance and Tukey's test for each column, $\alpha = 0.05$).


https://doi.org/10.5395/rde.2023.48.e33

**Table 3.** Means and standard deviations of the color change $\Delta E_{ab}$, $\Delta E_{00}$, and $\Delta WI_D$, from different groups, as well as statistical analysis

| Experimental groups | Color measurements | | |
|---|---|---|---|
| | $\Delta E_{ab}$ | $\Delta E_{00}$ | $\Delta WI_D$ |
| Control | 1.1 ± 0.9B | 0.7 ± 0.6b | 0.7 ± 1.0$^{C}$ |
| Buccal only | 6.3 ± 4.3A | 3.9 ± 2.8a | 6.0 ± 4.7$^{A}$ |
| Lingual only | 5.7 ± 2.9A | 3.6 ± 2.0a | 3.5 ± 1.7$^{B}$ |
| Buccal + lingual | 7.2 ± 3.2A | 4.0 ± 1.7a | 8.4 ± 6.4$^{A}$ |

Different capital and lower-case letter, superscript or not, in each column indicate statistically similar means (1-way analysis of variance and Tukey's test for each column, α = 0.05).

**Table 4.** Means and standard deviations of the HP concentration (μg/mL) detected inside the pulp chamber from different groups, as well as statistical analysis

| Experimental groups | HP concentration (μg/mL) |
|---|---|
| Control | 0.004 ± 0.006$^{B}$ |
| Buccal only | 0.027 ± 0.015$^{A}$ |
| Lingual only | 0.027 ± 0.010$^{A}$ |
| Buccal + lingual | 0.029 ± 0.014$^{A}$ |

HP, hydrogen peroxide.
Different superscript letters in indicate statistically similar means (1-way analysis of variance and Tukey's test, α = 0.05).

control group (**Table 3**; $p < 0.005$). Regarding the color measurement in $\Delta WI_D$, the group applying only on the buccal surface showed a similar bleaching efficacy in comparison with the group applying on the buccal and lingual simultaneously. Both groups showed a significant and higher bleaching efficacy when compared to the group applying only to the lingual surface (**Table 3**; $p = 0.00003$).

### Hydrogen peroxide permeability evaluation

The values of the hydrogen peroxide permeability inside the pulp chamber are shown in **Table 4**. A significant difference was observed among groups ($p = 0.0007$). All groups submitted to bleaching showed significant amounts of hydrogen peroxide permeability inside the pulp chamber when compared to the control group (**Table 4**; $p = 0.0007$). However, no significant difference in the amounts of hydrogen peroxide permeability was evaluated in the pulp chamber of the different bleaching protocols (**Table 4**).

## DISCUSSION

All experimental groups exhibited varying degrees of bleaching efficacy compared to the control group. According to Paravina *et al.* [19] and Pérez *et al.* [33] values exceeding the 50:50 acceptability threshold indicate a noticeable difference that is generally acceptable to most individuals. As seen in **Table 2**, the values of color change for the different color parameters are superior to the 50:50 acceptability threshold ($\Delta E_{ab} = 2.7$, $\Delta E_{00} = 1.2$, and $\Delta WI_D = 2.6$).

No significant difference was observed between the experimental groups when evaluating bleaching efficacy based on $\Delta E_{ab}$ and $\Delta E_{00}$. However, a significant difference was observed when assessing $\Delta WI_D$. The OL group exhibited lower $\Delta WI_D$ values compared to the OB and BL groups. This difference could be attributed to variations in the assessment systems used. The $CIEL_{ab}$ formula has been improved with the introduction of the $CIEDE_{00}$ formula, which better aligns with the human visual perception of color changes by considering adjustments in hue, chroma, and lightness parameters [19]. Although $CIEDE_{00}$ is a more advanced formula recommended by the CIE, that should be considered for use in clinical instrumental

analysis, most studies evaluating tooth whitening still use the $CIEL_{ab}$ system formula, which is why we present both data [8,11,32].

While $\Delta E_{ab}$ and $\Delta E_{00}$ are capable of measuring overall color change, they do not provide information regarding the direction of the change, specifically whether the values are shifting towards whiter or darker shades. Conversely, $\Delta WI_D$ indicates the extent of whitening towards either the lighter or darker end of the spectrum [25]. This indicates that the results obtained from $\Delta WI_D$ should be considered as the most crucial formula for evaluating the whitening effect. Consequently, this leads the authors to partially reject the first null hypothesis.

This can likely be explained by the fact that the bleaching gel was applied exclusively to the lingual surface, which has a smaller surface area compared to the buccal surface, limiting its interaction with the bleaching agents [14,15]. In addition, although the same thickness between enamel and dentin was measured in the present study, there is usually a higher amount of dentin on the lingual surface compared to the buccal surface [14]. The less whitening that occurred when the bleaching agent was applied to the lingual surface could be related to the distance that hydrogen peroxide needs to travel to reach the target areas on the buccal surface, being this distance longer when compared to the direct application of bleaching gel on the buccal surface [13,14]. However, it is important to note that the color evaluation was conducted on the buccal surface for all groups, even though the bleaching procedure was performed exclusively on the lingual surface in 1 group. Actually, due to the measurement of color on the lingual surface group being performed on the opposite surface region (buccal), it is not possible to guarantee that bleaching did not occur on the lingual surface. It can only be affirmed that when using bleaching solely on the lingual surface, a lesser bleaching effect was observed on the buccal surface compared to using bleaching directly on the buccal surface. Although this should be considered a limitation of the present study, measuring the color change on the lingual surface is clinically irrelevant since only the buccal area is visualized for the patients during the bleaching treatment, and this was the most important factor guiding the decision to measure the bleaching effect solely on the buccal surface for all groups.

When the bleaching gel was applied to the groups OB or BL, significant $\Delta WI_D$ values were observed compared to when the gel was applied to the OL group. This suggests that a higher degree of whitening occurred when the bleaching gel was applied to the buccal surface. Based on the aforementioned characteristics, it appears that the buccal surface plays a more significant role in the bleaching procedure, while the association with the lingual surface seems less relevant. Therefore, achieving the desired whitening effect primarily relies on the application of the bleaching gel to the buccal surface.

As the hydrogen peroxide whitens the teeth, its low molecular weight allows it to penetrate the dental pulp chamber, causing pulp reactions [34]. The results of the present study confirm the ability of hydrogen peroxide to penetrate the enamel and dentin structure and to reach the pulp chamber immediately after a home bleaching session when compared to the control group, as previously observed in other studies [17,18,35]. The average amount of hydrogen peroxide measured inside the pulp chamber after a 7.5% hydrogen peroxide session was similar to that observed previously [21]. However, despite different techniques being tested, no significant difference was observed in terms of hydrogen peroxide permeability in the groups. This leads the authors to accept the second null hypothesis.

The fact that the same thickness of enamel and dentin was measured in the buccal and lingual surfaces helps to understand why the same amount of hydrogen peroxide was measured inside the pulp chamber when the bleaching gel was applied in the OB or OL groups. It is expected that, when applied to the BL protocol, due to the higher amount of gel available, higher amounts of hydrogen peroxide would reach the pulp chamber. However, the present study showed that the amount of hydrogen peroxide inside the pulp chamber was not proportional to the extent of the tooth surfaces involved in the application of the gel. Applying bleaching gel to the BL protocol achieved similar values of hydrogen peroxide permeability inside the pulp chamber when compared to groups for which the bleaching gel was applied to one surface. These results agree with findings from a previous laboratory study where the interaction of peroxide with the pulp was similar when more bleaching gel was applied through reapplication compared to a single application [36]. In addition, this was confirmed clinically, where clinical studies found no higher tooth sensitivity after using greater amounts of bleaching gel [12,37].

Although the present study did not demonstrate the same level of bleaching efficacy when the OL protocol was employed, it is important to note that this technique may still be feasible in certain cases where veneers are utilized [13]. However, it is likely that a longer bleaching time will be required to achieve an equivalent whitening effect when the bleaching gel is applied solely to the lingual surface as compared to the buccal surface [8]. Further clinical studies are needed to substantiate this hypothesis.

Although the results are similar between the OB and BL groups, most studies have applied bleaching gel only on the buccal surface and found positive results in bleaching efficacy [8,38,39]. This indicates that the utilization of the alternative protocol (applying to both buccal and lingual surfaces) can lead to increased material costs without yielding superior outcomes, making it an unfavorable alternative to traditional bleaching treatment. If it is taking into consideration that, despite the use of premolars for bleaching evaluation has been considered a feasible and reproducible method, their results should be taken into account carefully, because no direct correlation could be done with clinical findings [17,18, 21,35,40]. Therefore, it is very important that clinical trials should be carried out to validate these findings under real-life conditions.

Lastly, it is important to acknowledge the limitations of this study. Simulating pulp fluid pressure and accounting for the actual conditions and temperature within the oral cavity could potentially influence the concentration of hydrogen peroxide permeability and its impact on bleaching efficacy. Additionally, the use of a single concentration and manufacturer might have implications, and further investigations exploring different concentrations and manufacturers are warranted.

## CONCLUSIONS

At-home bleaching with 7.5% hydrogen peroxide on buccal and lingual surfaces simultaneously does not provide any additional benefits to the technique compared to the buccal application alone, as it results in the same bleaching efficacy. Although the lingual protocol demonstrated lower bleaching efficacy based on the whitening index values, it may still be of interest and relevant in certain clinical scenarios based on individual needs, requiring clinical trials to better understand its specificities. All protocols showed the same

hydrogen peroxide permeability concentration within the pulp chamber, regardless of the protocol used.

## ACKNOWLEDGEMENTS

The authors would like to thank FGM Dental Products for the donation of the bleaching gel used in this investigation. They would also like to thank the enriching partnership with the physical-chemical characterization laboratory of the Chemistry Department of the State University of Ponta Grossa. The authors would like to thank Bleaching&Bond group (Brazil).

## REFERENCES

1. Bernardon JK, Ferrari P, Baratieri LN, Rauber GB. Comparison of treatment time versus patient satisfaction in at-home and in-office tooth bleaching therapy. J Prosthet Dent 2015;114:826-830.
PUBMED | CROSSREF
2. Mailart MC, Sakassegawa PA, Santos KC, Torres CRG, Palo RM, Borges AB. One-year follow-up comparing at-home bleaching systems outcomes and the impact on patient's satisfaction: randomized clinical trial. J Esthet Restor Dent 2021;33:1175-1185.
PUBMED | CROSSREF
3. Joiner A. The bleaching of teeth: a review of the literature. J Dent 2006;34:412-419.
PUBMED | CROSSREF
4. Haywood VB. Nightguard vital bleaching: current concepts and research. J Am Dent Assoc 1997;128 Supplement:19S-25S.
PUBMED | CROSSREF
5. Matis BA. Tray whitening: what the evidence shows. Compend Contin Educ Dent 2003;24:354-362.
PUBMED
6. Luque-Martinez I, Reis A, Schroeder M, Muñoz MA, Loguercio AD, Masterson D, *et al.* Comparison of efficacy of tray-delivered carbamide and hydrogen peroxide for at-home bleaching: a systematic review and meta-analysis. Clin Oral Investig 2016;20:1419-1433.
PUBMED | CROSSREF
7. Meireles SS, Heckmann SS, Leida FL, dos Santos IS, Della Bona A, Demarco FF. Efficacy and safety of 10% and 16% carbamide peroxide tooth-whitening gels: a randomized clinical trial. Oper Dent 2008;33:606-612.
PUBMED | CROSSREF
8. Chemin K, Rezende M, Loguercio AD, Reis A, Kossatz S. Effectiveness of and dental sensitivity to at-home bleaching with 4% and 10% hydrogen peroxide: a randomized, triple-blind clinical trial. Oper Dent 2018;43:232-240.
PUBMED | CROSSREF
9. Matis BA, Yousef M, Cochran MA, Eckert GJ. Degradation of bleaching gels *in vivo* as a function of tray design and carbamide peroxide concentration. Oper Dent 2002;27:12-18.
PUBMED
10. López Darriba I, Novoa L, de la Peña VA. Efficacy of different protocols for at-home bleaching: a randomized clinical trial. Am J Dent 2017;30:329-334.
PUBMED
11. Martini EC, Favoreto MW, Coppla FM, Loguercio AD, Reis A. Evaluation of reservoirs in bleaching trays for at-home bleaching: a split-mouth single-blind randomized controlled equivalence trial. J Appl Oral Sci 2020;28:e20200332.
PUBMED | CROSSREF
12. Cordeiro D, Toda C, Hanan S, Arnhold LP, Reis A, Loguercio AD, *et al.* Clinical evaluation of different delivery methods of at-home bleaching gels composed of 10% hydrogen peroxide. Oper Dent 2019;44:13-23.
PUBMED | CROSSREF
13. Silva LM, da Costa Lacerda ÍA, Dos Santos DB, Herkrath FJ, da Silva KL, Loguercio AD, *et al.* Is the at-home bleaching treatment applied only on the lingual surface as effective as that on the buccal surface? A randomized clinical trial. Clin Oral Investig 2022;26:1551-1560.
PUBMED | CROSSREF

14. Fick AV. On liquid diffusion. Lond Edinb Dublin Philos Mag J Sci 1855;10:30-39.
CROSSREF

15. Kwon SR, Li Y, Oyoyo U, Aprecio RM. Dynamic model of hydrogen peroxide diffusion kinetics into the pulp cavity. J Contemp Dent Pract 2012;13:440-445.
PUBMED | CROSSREF

16. Fugaro JO, Nordahl I, Fugaro OJ, Matis BA, Mjör IA. Pulp reaction to vital bleaching. Oper Dent 2004;29:363-368.
PUBMED

17. Bernardi LG, Favoreto MW, de Souza Carneiro T, Borges CPF, Pulido C, Loguercio AD. Effects of microabrasion association to at-home bleaching on hydrogen peroxide penetration and color change. J Esthet Restor Dent 2022;34:335-341.
PUBMED | CROSSREF

18. Favoreto MW, Madureira MP, Hass V, Maran BM, Parreiras SO, Borges CP, *et al.* A novel carbamide peroxide polymeric nanoparticle bleaching gel: Color change and hydrogen peroxide penetration inside the pulp cavity. J Esthet Restor Dent 2021;33:277-283.
PUBMED | CROSSREF

19. Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, *et al.* Color difference thresholds in dentistry. J Esthet Restor Dent 2015;27 Supplement 1:S1-S9.
PUBMED | CROSSREF

20. Robertson A, Lozano R, Alman D, Orchard S, Keitch J, Connely R, *et al.* CIE recommendations on uniform color spaces, color-difference equations, and metric color terms. Color Res Appl 1977;2:5-6.
CROSSREF

21. Parreiras SO, Favoreto MW, Cruz GP, Gomes A, Borges CPF, Loguercio AD, *et al.* Initial and pulp chamber concentration of hydrogen peroxide using different bleaching products. Braz Dent Sci 2020;23:1-8.
CROSSREF

22. Marson FC, Gonçalves RS, Silva CO, Cintra LT, Pascotto RC, Santos PH, *et al.* Penetration of hydrogen peroxide and degradation rate of different bleaching products. Oper Dent 2015;40:72-79.
PUBMED | CROSSREF

23. Gómez-Polo C, Portillo Muñoz M, Lorenzo Luengo MC, Vicente P, Galindo P, Martín Casado AM. Comparison of the CIELab and CIEDE2000 color difference formulas. J Prosthet Dent 2016;115:65-70.
PUBMED | CROSSREF

24. Luo MR, Cui G, Rigg B. The development of the CIE 2000 colour-difference formula: CIEDE2000†. Color Res Appl 2001;26:340-350.
CROSSREF

25. Pérez MM, Ghinea R, Rivas MJ, Yebra A, Ionescu AM, Paravina RD, *et al.* Development of a customized whiteness index for dentistry based on CIELAB color space. Dent Mater 2016;32:461-467.
PUBMED | CROSSREF

26. Esteves L, Fagundes TC, Dos Santos PH, da Silva L, de Alcântara S, Cintra L, *et al.* Does the bleaching gel application site interfere with the whitening result? A randomized clinical trial. Oper Dent 2022;47:20-30.
PUBMED | CROSSREF

27. de Melo Oliveira I, Santana TR, Correia ACC, Fontes LS, Griza S, Faria-E-Silva AL. Color heterogeneity and individual color changes in dentin and enamel bleached in the presence of a metallic orthodontic bracket. J Esthet Restor Dent 2021;33:262-268.
PUBMED | CROSSREF

28. Schwarzbold CG, Cuevas-Suárez CE, Pacheco RR, Ribeiro JS, Carreño NLV, Lund RG, *et al. In vitro* efficacy of commercial and experimental proteolytic enzyme-based whitening dentifrices on enamel whitening and superficial roughness. J Esthet Restor Dent 2021;33:849-855.
PUBMED | CROSSREF

29. Favoreto MW, Vochikovski L, Terra RMO, Campos VS, Santos ME, Meireles SS, *et al.* Topical application of Otosporin® before in-office bleaching: a split mouth, triple-blind, multicenter randomized clinical trial. Clin Oral Investig 2022;26:2555-2564.
PUBMED | CROSSREF

30. Lilaj B, Dauti R, Agis H, Schmid-Schwap M, Franz A, Kanz F, *et al.* Comparison of bleaching products with up to 6% and with more than 6% hydrogen peroxide: whitening efficacy using BI and WI **D** and side effects - an *in vitro* study. Front Physiol 2019;10:919.
PUBMED | CROSSREF

31. Cavalli V, Silva BGD, Berger SB, Marson FC, Tabchoury CPM, Giannini M. Decomposition rate, pH, and enamel color alteration of at-home and in-office bleaching agents. Braz Dent J 2019;30:385-396.
PUBMED | CROSSREF

32. Carneiro TS, Favoreto MW, Bernardi LG, Sutil E, Wendlinger M, Centenaro GG, *et al.* Gingival irritation in patients submitted to at-home bleaching with different cutouts of the bleaching tray: a randomized, single-blind clinical trial. Clin Oral Investig 2022;26:4381-4390.
PUBMED | CROSSREF

33. Pérez MM, Herrera LJ, Carrillo F, Pecho OE, Dudea D, Gasparik C, *et al.* Whiteness difference thresholds in dentistry. Dent Mater 2019;35:292-297.
PUBMED | CROSSREF

34. Gökay O, Yilmaz F, Akin S, Tunçbìlek M, Ertan R. Penetration of the pulp chamber by bleaching agents in teeth restored with various restorative materials. J Endod 2000;26:92-94.
PUBMED | CROSSREF

35. Mena-Serrano AP, Parreiras SO, do Nascimento EM, Borges CP, Berger SB, Loguercio AD, *et al.* Effects of the concentration and composition of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber. Oper Dent 2015;40:E76-E82.
PUBMED | CROSSREF

36. Soares DG, Basso FG, Pontes EC, Garcia LF, Hebling J, de Souza Costa CA. Effective tooth-bleaching protocols capable of reducing H(2)O(2) diffusion through enamel and dentine. J Dent 2014;42:351-358.
PUBMED | CROSSREF

37. Esteves LMB, Dos Santos PH, Fagundes TC, de Oliveira Gallinari M, de Mello Antonaccio GB, Cintra LTÂ, *et al.* Effect of bleaching gel volume on color change and postoperative sensitivity: a randomized clinical study. Clin Oral Investig 2022;26:2527-2536.
PUBMED | CROSSREF

38. Donassollo SH, Donassollo TA, Coser S, Wilde S, Uehara JLS, Chisini LA, *et al.* Triple-blinded randomized clinical trial comparing efficacy and tooth sensitivity of in-office and at-home bleaching techniques. J Appl Oral Sci 2021;29:e20200794.
PUBMED | CROSSREF

39. Terra R, da Silva KL, Vochikovski L, Sutil E, Rezende M, Loguercio AD, *et al.* Effect of daily usage time of 4% hydrogen peroxide on the efficacy and bleaching-induced tooth sensitivity: a single-blind randomized clinical trial. Oper Dent 2021;46:395-405.
PUBMED | CROSSREF

40. da Silva KL, Favoreto MW, Centenaro GG, Bernardi LG, Borges CPF, Reis A, *et al.* Can all highly concentrated in-office bleaching gels be used as a single-application? Clin Oral Investig 2023;27:3663-3671.
PUBMED | CROSSREF