# **RESEARCH ARTICLE**

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# Evaluation of hydrogen peroxide permeability, color change, and physical-chemical properties on the in-office dental bleaching with different mixing tip

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#### Abstract

**Objective:** This study aims to assess hydrogen peroxide (HP) penetration into the pulp chamber, color change (CC), physical-chemical properties, and material wastage (MW) and material used (MU) in mixing tips when using in-office bleaching gels with two different mixing tips.

**Materials and Methods:** Forty teeth were divided into five groups (n = 8) based on the bleaching gels used (Pola Office +37.5% [PO+] and Whiteness HP Automixx Plus 35% [AM+]) and the mixing tip types (T-Mixer and Helical). A negative control group was treated with ultra-purified water. HP concentration was measured using UV-Vis, and CC was evaluated with a digital spectrophotometer. Initial concentration, pH, and viscosity were measured through Titration, a Digital pH meter, and Rheometer, respectively. MW and MU were measured using a precise analytical balance. Statistical analysis included two-way ANOVA, Tukey's, and Dunnett's test ( $\alpha = 0.05$ ). **Results:** A higher HP concentration was observed with PO+ with the Helical mixing tip in comparison with AM+ (p = 0.01). No significant differences in CC or MU were found for different mixing tips (p = 0.001). The T-mixer mixing tip resulted in significantly less MW (p < 0.00001) and improved mixture homogeneity and viscosity. **Conclusions:** Utilizing a T-mixer with self-mixing bleaching gels achieves comparable CC while reducing MW. Moreover, it decreases HP penetration when using PO+.

mixer mixing tip should be recommended, as it reduces the penetration of hydrogen peroxide into the pulp chamber when using PO+, while also minimizing gel wastage.

#### KEYWORDS

bleaching gels, dental enamel permeability, dentin permeability, hydrogen peroxide, tooth bleaching

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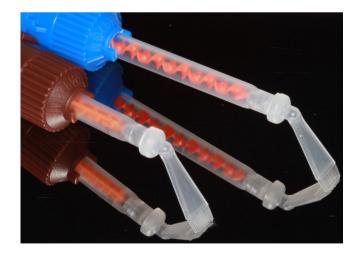
# 1 | INTRODUCTION

In-office dental bleaching has become increasingly popular for its ability to deliver impressive tooth whitening results in a single session,<sup>1</sup> significantly improving patients' quality of life.<sup>2</sup> This technique involves using high concentrations of hydrogen peroxide, which are supplied in two separate vials that need to be mixed just before application.<sup>3,4</sup>

One vial contains an acidic hydrogen peroxide solution, while the other contains alkaline thickeners,<sup>3</sup> such as carbomer, alkali swellable emulsion, modified sulfonic acid polymer, semisynthetic polysaccharide, and particulate colloids.<sup>3-5</sup> Once these components are mixed, the resulting gel is applied to the tooth surface. The manual manipulation of these materials depends on the dental clinician's expertise to ensure proper handling and achieve the desired viscosity.<sup>3,4,6,7,8</sup>

Recent advancements have introduced self-mixing syringes for inoffice bleaching gels, providing an innovative approach to mixing.<sup>9–16</sup> The commonly used Helical mixing tip (Figure 1) has its limitations due to its length and potential waste during manipulation. A novel internal design, the T-mixer mixing tip (Figure 1),<sup>17,18</sup> was recently developed and has already been employed in polyvinyl siloxane impression materials.<sup>19</sup> This design optimizes fluid element rearrangement within the mixer geometry,<sup>17,18</sup> resulting in a more efficient mixing process, despite having the same number of elements as the Helical mixing tip but with a considerably shorter length.<sup>19</sup>

The efficiency of the T-mixer mixing tip suggests a potential reduction in the gel's required amount. A recent study demonstrated that inoffice bleaching gels used with an applicator brush in combination with a Helical mixing tip reduce hydrogen peroxide reaching the pulp chamber.<sup>9</sup> Considering the potential impact of hydrogen peroxide on dental pulp, including changes in blood flow,<sup>20</sup> oxygen saturation,<sup>21</sup> and the initiation of inflammatory processes,<sup>22</sup> tooth sensitivity may occur as a



**FIGURE 1** Self-mixing tips employed in this study. The Helical mixing tip (blue, Sulzer) is a conventional tip commonly used in dentistry. On the other hand, the T-Mixer mixing tip (brown, Sulzer) features an innovative design that aims to break up and rearrange fluid elements within the mixer geometry. This design allows the T-mixer mixing tip to achieve a more efficient mixing process using the same number of elements but in a considerably shorter length.

common side effect.<sup>23</sup> Thus, a reduced gel quantity during therapy could potentially decrease hydrogen peroxide diffusion into the pulp chamber, leading to a reduction in postoperative tooth sensitivity<sup>24</sup>—an additional advantage of using the T-mixer mixing tip.

Despite the popularity of in-office dental bleaching procedures, there is a current lack of literature investigating the potential effects of different mixing techniques on the diffusion of hydrogen peroxide molecules into the pulp chamber. To address this research gap, the present study was conducted to assess the concentration of hydrogen peroxide within the pulp chamber, resulting color change, various physical-chemical properties (initial concentration, pH, and gel viscosity), and material wastage in mixing tips, as well as the amount of bleaching gel used. The study evaluated two in-office bleaching gels mixed with distinct types of mixing tips: the T-Mixer mixing tip and the Helical mixing tip. The research hypothesis of this study was that high concentration hydrogen peroxide in-office bleaching gels applied with T-Mixer mixing tip compared Helical mixing tips, (1) reduce the concentration of hydrogen peroxide within the pulp chamber, (2) keep the resulting color change, (3) keep the initial concentration, pH, and viscosity, and (4) reduce the in-material waste into the mixing tip and the amount of bleaching gel used.

# 2 | MATERIALS AND METHODS

#### 2.1 | Ethics statement

The Ethics Committee of State University of Ponta Grossa, PR, Brazil approved this study under protocol number 4.647.755.

# 2.2 | Selection of teeth and inclusion and exclusion criteria

A total of 40 caries-free maxillary first premolars were obtained from the tooth bank of the State University of Ponta Grossa, PR, Brazil. They were cleaned, disinfected in 0.5% chloramine for 7 days, and stored in distilled water until use. The selection of teeth was carried out using a  $10 \times$  magnification microscope (Lambda LEB-3, ATTO Instruments). Teeth with fractures, enamel defects, and colors greater than >20 according to the Whiteness Index in Dentistry (WID)<sup>25</sup> (VITA Easyshade Advance 4.0, VITA Zahnfabrik, Germany) were excluded. Additionally, teeth with a buccal thickness less than 2.5 mm and greater than 3.5 mm, as determined through previously performed radiography (Timex 70C, Gnatus, Ribeirão Preto, SP, Brazil), as described in the specimen preparation section, were also excluded.<sup>26</sup>

Forty teeth were randomly distributed into five groups (n = 8) according to the study design, which aimed to evaluate two main factors: (1) Bleaching gels, subdivided into 2 levels (Pola Office + 37.5% [PO+, SDI, Bayswater, Australia] and Whiteness HP Automixx Plus 35% [AM+, FGM Dental Group, Joinville, SC, Brazil]); and (2) Different mixing tips, with 2 levels (T-Mixer mixing tip and Helical mixing tip [Sulzer, Haag, Switzerland, Figure 1]). A group treated with ultrapurified water served as the negative control.

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### 2.3 | Sample size calculation

The main focus of the present study was to measure the concentration of hydrogen peroxide within the pulp chamber. Consistent with previous research,<sup>9,11</sup> the average concentration of hydrogen peroxide quantified within the pulp chamber of teeth subjected to in-office bleaching with high-concentrated hydrogen peroxide, using a Helical mixing tip applied with an applicator brush (Sulzer), was found to be  $0.351 \pm 0.114 \mu$ g/mL. To detect a difference of  $0.175 \mu$ g/mL (50%) between the groups, a bilateral test with an alpha of 0.05 and a power of 80% required a sample size of seven teeth in each group. Additionally, one extra tooth was allocated to each group to account for potential sample losses during the experiment.

#### 2.4 | Specimen preparation

Prior to specimen preparation, X-ray radiographs were taken using the Timex 70C X-ray machine (Gnatus). For this purpose, the mesial face of the tooth was placed in contact with the X-ray film. Each radiograph was captured with an exposure time of 0.5 s and a 30-cm focus-object distance (70 kVp-7 mA). The central X-ray beam was focused at a 90° angle to the tooth's distal surface. After exposure, the images were digitally obtained, and the corresponding buccal tooth thickness was measured using New IDA software (Dabi Atlante, Ribeirão Preto).

Next, a low-speed diamond disk (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA) was utilized to remove approximately 3 mm apically to the cementum-enamel junction. Pulp tissue was carefully removed and flushed with deionized water.<sup>26</sup> A spherical bur (#1014, KG Sorensen, Serra, ES, Brazil) was then employed to expand access to the pulp chamber, allowing for the introduction of 25  $\mu$ L of the solution using a micropipette (LABMATE Soft, HTL Lab Solutions).

#### 2.5 | Initial color change

To standardize the position of the spectrophotometer, individual impressions were taken using a dense silicone paste (Coltoflax and Perfil Cub Kit, Vigodent, Rio de Janeiro, RJ, Brazil) through a 6-mm diameter window located at the tip of the spectrophotometer. This process was carried out with the aid of a metal device placed in the middle third of the buccal surface for each specimen.<sup>27</sup>

Before the treatments, the initial color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) were measured using a digital spectrophotometer (VITA Easyshade Advance 4.0, VITA Zahnfabrik, Germany). The  $L^*$  value represents lightness, with values ranging from 0 for black to 100 for white. The  $a^*$  value represents the color along the red-green axis, and the  $b^*$  value represents the color along the yellow-blue axis.

### 2.6 | Obtaining the study calibration curve

The analytical products used in this study were not pre-purified, and all solutions were prepared with deionized water. Initially, a typical reference line was plotted using a 5.000 µg/mL stock solution prepared from a concentrated solution (35% hydrogen peroxide, Pharmacy Eficácia, Ponta Grossa, PR, Brazil). Subsequently, this solution was diluted in an acetate buffer solution (pH 4) and calibrated using conventional methods. To determine the analytical grade and actual concentration of the solution, a potassium permanganate solution was used for titration.<sup>26</sup>

Based on the verified initial concentration, serial volumetric dilutions ranging from 0.000 to 0.464 µg/mL were performed to construct the calibration curve. Known hydrogen peroxide concentrations were added to glass tubes and placed in a Cary 100 UV–Vis spectrophotometer (Varian, Palo Alto, CA, USA). This procedure resulted in a standard reference line used for extrapolating the results of the study samples (R = 0.996, data not reported).<sup>26</sup>

# 2.7 | Treatment protocols and hydrogen peroxide within the pulp chamber

For all groups, the specimens were vertically fixed to a wax plate with the occlusal surface facing the plate. Prior to the application of the bleaching agent, the vestibular surface of each specimen was isolated by applying a light-cured resin barrier, enclosing an area of 6 mm  $\times$  6 mm (Topdam, FGM Dental Products). To retain any hydrogen peroxide that entered the pulp chamber during the bleaching procedures, a 25-µL aliquot of acetate buffer (pH = 4) was inserted into the pulp chamber of each tooth.

A single, calibrated, and experienced operator was responsible for applying the materials. After mixing, the bleaching agent was applied to the vestibular enamel area according to the different experimental groups. Both bleaching gels were applied until the vestibular area of the teeth to be bleached was completely covered. After 50 min in each session, the bleaching gel was removed with gauze and thoroughly rinsed with deionized water (Table 1). The negative control group was kept isolated from contact with bleaching agents.

Following that, the acetate buffer solution inside the pulp chamber of each tooth was removed and transferred to a glass tube using a mechanical micropipette. This process involved rinsing the pulp chamber of each tooth four times with 25  $\mu$ L of acetate buffer and transferring the solution to the same glass tube. Subsequently, 2.725  $\mu$ L of distilled water was added to the glass tube, along with 100  $\mu$ L of 0.5 mg/mL Leucocrystal Violet (Sigma Chemical Co, St. Louis, MO, USA) and 50  $\mu$ L of 1 mg/mL horseradish peroxidase enzyme (Peroxidase Type VIA, Sigma Chemical Co.). This procedure was repeated separately for each specimen.

The resulting solution exhibited a violet color with a maximum absorbance peak at 590 nm, which was measured using a Cary 100 UV–Vis spectrophotometer (Varian). The absorbance recorded was that of the highest absorption peak resulting from the reaction between hydrogen peroxide and Leucocrystal Violet (Crystal Violet – 590 nm). According to Beer's Law, absorbance directly corresponds to concentration. Consequently, the concentration of hydrogen peroxide ( $\mu$ g/mL) was determined by comparing it with the previously obtained calibration curve.<sup>26</sup>

TABLE 1 Commercial bleaching gel used in the study (manufacturer, composition, and application method).

Product (manufacturer)	Composition	Application method
Pola Office +37.5% (SDI) (6.5) <sup>a</sup>	37.5% Hydrogen peroxide (final concentration of Hydrogen Peroxide 28.0% <sup>b</sup> ) and potassium nitrate	<ol> <li>With the self-mixing pointer with applicator brush (T-Mixer mixing tip or Helical mixing tip) duly coupled to the double-body syringe that contains the bleaching gel.</li> <li>Press the piston until the phases (peroxide and thickener) are slowly mixed.</li> <li>A small quantity of gel was dispensed into a container prior to applying the product to the tooth surface, to assure that the applied product is properly homogenized.</li> <li>The bleaching gel was applied until it completely covered the area of the teeth that were bleached, with a gel layer as thin as possible, using the self-mixing without an applicator brush or with an applicator brush, according to the experimental group.</li> <li>After application, the gel was left in contact with the tooth for 50 min.</li> <li>In the end, the gel was removed with a suction cannula and washed with deionized water only on the vestibular surface</li> </ol>
Whiteness HP AutoMixx Plus 35% (FGM Dental Product) (7.7) <sup>a</sup>	35% Hydrogen peroxide (final concentration of Hydrogen Peroxide 32.7% <sup>b</sup> ) and digluconate calcium	<ol> <li>With the self-mixing pointer with applicator brush (T-Mixer mixing tip or Helical mixing tip) duly coupled to the double-body syringe that contains the bleaching gel.</li> <li>Press the piston until the phases (peroxide and thickener) are slowly mixed.</li> <li>A small quantity of gel was dispensed into a container prior to applying the product to the tooth surface, to assure that the applied product is properly homogenized.</li> <li>The bleaching gel was applied until it completely covered the area of the teeth that were bleached, with a gel layer as thin as possible, using the self-mixing without an applicator brush or with an applicator brush, according to the experimental group.</li> <li>After application, the gel was left in contact with the tooth for 50 min.</li> <li>In the end, the gel was removed with a suction cannula and washed with deionized water only on the vestibular surface</li> </ol>

<sup>a</sup>Measured pH assessed in triplicate (n = 3).

<sup>b</sup>Measured hydrogen peroxide amount assessed in triplicate (n = 3).

# 2.8 | Final color change evaluation

Afterward, the final color change was measured 1 week after the bleaching treatments, using a digital spectrophotometer (VITA Easy-shade Advance 4.0, VITA Zahnfabrik), as previously described. Throughout this period, the specimens were immersed in artificial saliva, with daily changes of artificial water maintained at a controlled temperature of 37°C.

The color change before (baseline) and after 1 week of bleaching was determined by calculating the difference between the measurements with the spectrophotometer. This calculation was performed using the CIELab formula ( $\Delta E_{ab}$ ),<sup>28</sup> CIEDE 2000 formula ( $\Delta E_{00}$ ),<sup>29</sup> and Whiteness Index for Dentistry (WI<sub>D</sub>).<sup>25</sup> Furthermore, changes in WI<sub>D</sub> caused by each step were calculated by subtracting the values observed at each assessment time from those calculated in the previous step ( $\Delta WI_D$ ).<sup>25</sup> Perceptual changes were considered significant when the differences in the initial and post-bleaching colors presented  $\Delta E_{ab} > 2.7$  and  $\Delta E_{00} > 1.8^{30}$  and  $\Delta WI_D > 2.9.^{31}$ 

### 2.9 | Initial concentrations of bleaching agents

The bleaching gels used in the study were titrated with a standardized potassium permanganate solution before the bleaching procedure, following the procedure described in the literature.<sup>27,32</sup> This titration was performed to determine the initial concentrations within the bleaching gel and compare them with the information provided by the manufacturer. The analyses were performed in triplicate to ensure accuracy and

consistency. Acceptable limits of variation of up to -30% and +10% of the original concentration stated by the manufacturer were considered.<sup>33</sup>

#### 2.10 | pH measurements of bleaching agents

The pH of each bleaching agent was measured using a pH meter (Extech pH 100, Extech Instruments, Nashua, NH, USA) placed directly in contact with the bleaching gel on a tooth.<sup>9,12,27</sup> Measurements were taken at various time points, starting immediately after application and then at 10-min intervals. The analyses were performed in triplicate at each time point to ensure accuracy and reliability.

### 2.11 | Viscosity of the bleaching gel

The viscosity of the bleaching gels was measured based on their shear rate using a controlled-strain rheometer (DHR-2, TA Industries, New Castle, DE, USA) with a 2° geometry in a conical plate 40 mm in diameter. The rheometer was equipped with a Peltier accessory integrated with a heating/cooling system, ensuring consistent sample temperature at 37°C, which is equivalent to mouth temperature. We conducted all tests at this constant temperature, following the manufacturer's recommended application time, both immediately after mixing and after 1 week. To assess the thixotropic behavior of the gels, we subjected them to a permanent flow for 50 min at a constant shear rate of 5 s<sup>-1</sup>. The analyses were performed in triplicate to ensure accuracy and consistency of the results.

# 2.12 | Evaluation of the waste of material in into mixing tip and amount of bleaching gel used

To measure the amount of material wasted into mixing tips, the T-Mixer and Helical mixing tips were weighed using a precision analytical digital scale (AUX220, Shimadzu, Tokyo, Japan) before and immediately after the application of the bleaching agents. The disparity between the weights before and after mixing was considered as the amount of waste material in the mixing tip.

In order to quantify the volume of bleaching gel utilized with the T-Mixer and Helical mixing tips for both bleaching gels, some specimen (tooth) was individually weighed using a precise analytical digital balance (AUX220, Shimadzu) before and directly after the administration of the bleaching agents, following the methodology described in previous literature.<sup>9,11,19</sup> The variation in weight observed between the measurements taken before and immediately after the application of the bleaching agents was deemed indicative of the amount of bleaching gel used with the respective mixing tips. Both analyses were conducted in triplicate to ensure accuracy and consistency of the results.

# 2.13 | Statistical analysis

The data underwent statistical analysis, which included the Kolmogorov–Smirnov test to assess normality and the Barlett test for equality of variances to examine the assumption of equal variances (unreported data). As the data exhibited a normal distribution, a two-way ANOVA (bleaching gels vs. mixing tips) was performed to analyze the concentration of hydrogen peroxide within the pulp chamber ( $\mu$ g/mL), the color change in different parameters ( $\Delta E_{ab}$ ,  $\Delta E_{00}$ , and  $\Delta$ WI<sub>D</sub>), the amount of material wasting (g) and the amount of material used (g) using both in-office bleaching gels. Subsequently, Tukey's post-hoc test was employed to compare different bleaching techniques.

Furthermore, a one-way ANOVA was conducted, followed by Dunnett's post-hoc test to compare the values obtained from different bleaching techniques with those of the control group for the concentration of hydrogen peroxide within the pulp chamber ( $\mu$ g/mL) and the color change ( $\Delta E_{ab}$ ,  $\Delta E_{00}$ , and  $\Delta$ Wl<sub>D</sub>). The initial concentrations and pH data were evaluated by *t*-test for independent samples ( $\alpha = 0.05$ ). The results of viscosity were only subjected to descriptive analysis, without undergoing further statistical testing.

# 3 | RESULTS

# 3.1 | Hydrogen peroxide concentration within the pulp chamber

The specimens in all groups exhibited standardized thicknesses ranging from 3.1 to 3.4 mm (p = 0.07). Table 2 presents the average values of hydrogen peroxide concentration within the pulp chamber. All in-office bleaching materials showed a significant and higher amount of hydrogen peroxide within the pulp chamber compared to the control (Table 1; p < 0.0000001; Dunnett's post hoc test).

Regarding the two-way ANOVA, a significant difference was observed in the interaction between factors (p = 0.01). When both inoffice gels were mixed with the T-mixer mixing tip, a similar amount of hydrogen peroxide was observed within the pulp chamber. However, the use of the Helical mixing tip resulted in an increased amount of hydrogen peroxide within the pulp chamber for both in-office gels, with significant differences detected only when PO+ was used (p = 0.01).

### 3.2 | Color change

Table 3 presents the baseline  $WI_D$  color and color change measured by  $\Delta E_{ab}$ ,  $\Delta E_{00}$ , and  $\Delta WI_D$ . Regarding the  $WI_D$  baseline color evaluation, no significant difference among experimental groups was observed (p = 0.61).

All in-office bleaching materials showed a significant and higher color change compared to the control (Table 2; p < 0.0001 for all parameters; Dunnett's post-hoc test). However, some differences were observed when different color parameters were considered. When the color change was evaluated by  $\Delta E_{ab}$  and  $\Delta E_{00}$ , a significant difference was only observed for the factor bleaching gel, with AM+ showing higher color change compared to PO+ (p < 0.015). On the

Experimental groups	HP concentration (µg/mL)	Amount of bleaching gel wasted (g)	Amount of bleaching gel used (g)
Control	0.002 ± 0.003 ≠	-	-
PO+			
T-Mixer	0.128 ± 0.048 B	0.241 ± 0.012 A	0.045 ± 0.04 A
Helical	0.193 ± 0.057 A	0.305 ± 0.016 B	0.048 ± 0.03 A
AM+			
T-Mixer	0.107 ± 0.021 B	0.257 ± 0.031 A	0.041 ± 0.04 A
Helical	0.136 ± 0.027 B	0.312 ± 0.022 B	0.049 ± 0.03 A

**TABLE 2** Means ( $\pm$  standard deviations) of the hydrogen peroxide (HP) concentration ( $\mu$ g/mL) detected within the pulp chamber, of the amount of gel wasted for bleaching (g) and of the amount of gel used for bleaching (g) in different experimental groups (\*).

Note: Symbol " $\neq$ " means that the control group was significantly different when compared to all experimental groups (Dunnet's test; p < 0.05). (\*) Identical letters, in each column, indicate statistically similar means (Tukey's test; p > 0.05).

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other hand, no significant changes in color were observed when different mixing tips were evaluated (p = 0.001). However, when  $\Delta WI_D$  values were considered, no significant difference was observed, regardless of the bleaching gel or mixing tips (p = 0.46).

# 3.3 | Initial concentrations of hydrogen peroxide in the bleaching agents

The initial concentration of hydrogen peroxide in PO+ was 28.0  $\pm$  3.4, instead 37.5% as described by the manufacturer, while in AM+ it was 32.7  $\pm$  2.3, instead 35% as described by the manufacturer, with a significant difference between them (*p* < 0.0001). The concentration of the former was below that indicated by the manufacturer, while the concentration of the latter was closer to the manufacturer's indication.

### 3.4 | pH measurements of bleaching agents

After mixing, PO+ had an initial pH of 6.5, which remained slightly acidic and stable, reaching a pH of 6.7 at the end of the application time. On the other hand, for AM+, an initial pH of 7.5 was measured

after mixing. This pH remained neutral and stable until reaching a pH of 7.4 at the end of 50 min of application.

# 3.5 | Viscosity of the bleaching gel

Figure 2 shows the thixotropies of the bleaching gels. For PO+, a greater fluctuation of viscosity was observed when using the Helical mixing tip. Clinically, this was evident by the heterogeneity and flow-ability of the mixture obtained for PO+. However, when using the T-mixer mixing tip, there was a significant improvement in the homogeneity and viscosity of the mixture, as evidenced by the reduced fluctuation observed in Figure 2. In comparing the two mixing tips, AM+ exhibited a slight decrease in thixotropy when the T-mixer mixing tip was utilized (Figure 2).

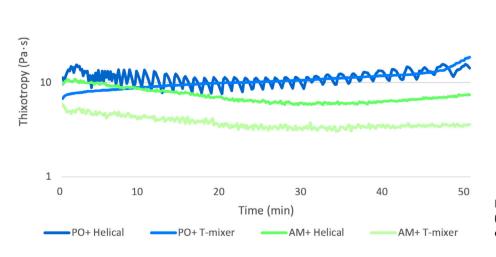
# 3.6 | Evaluation of the waste of material in into mixing tip and the amount of material used

Table 2 presents the amount of material wasted and the amount of material used. Regarding the two-way ANOVA, a significant

Experimental groups	WI <sub>D</sub> baseline (*)	$\Delta E_{ab}$	$\Delta E_{00}$	$\Delta WI_D$
Control	$18.4 \pm 4.6 =$	2.3 ± 1.0 ≠	1.5 ± 0.7 ≠	0.1 ± 2.3 ≠
PO+				
T-Mixer	18.0 ± 2.4 A	5.9 ± 1.9 a	$3.3 \pm 1.0^{\text{A}}$	7.4 ± 3.5 <sup>a</sup>
Helical	18.5 ± 4.8 A	5.8 ± 2.3 a	3.3 ± 1.3 <sup>A</sup>	8.7 ± 4.5 <sup>a</sup>
AM+				
T-Mixer	18.6 ± 4.4 A	8.4 ± 1.5 b	$5.0 \pm 0.9$ <sup>B</sup>	8.7 ± 4.4 <sup>a</sup>
Helical	17.6 ± 4.6 A	7.1 ± 3.4 b	4.0 ± 1.9 <sup>B</sup>	7.2 ± 5.5 <sup>a</sup>

**TABLE 3** Means (± standard deviations) of the baseline color (WI<sub>D</sub> baseline), as well as a color change in different objective assessments ( $\Delta E_{ab}$ ,  $\Delta E_{00}$ , and  $\Delta WI_D$ ) in different experimental groups (\*).

*Note*: Symbol " $\neq$ " means that control group was significantly different when compared to all experimental groups and symbol "=" means that control group was not significantly different when compared to all experimental groups (Dunnett's test; p < 0.05). (\*) Identical capital and lowercase letters, superscript or not, in each column, indicate statistically similar means (Tukey's test; p > 0.05).



**FIGURE 2** Average viscosity (Thixotropy in Pa·s) according to different experimental groups. difference was observed only for the factor mixing tips when the amount of material wasted was evaluated (p < 0.00001). The T-mixer mixing tip showed a lower amount of material wasted compared to the Helical mixing tip (p < 0.00001), regardless of the in-office bleaching gel used. On the other hand, when the quantity of material utilized was assessed, there was no notable difference observed in the interaction between the factors or for each main factor (p > 0.54). A comparable amount of material was used irrespective of the mixing tip or bleaching gel.

# 4 | DISCUSSION

Conventional spatula-mixing (or hand-mixing) of in-office bleaching gels has been commonly used, but it often leads to problems like contamination, voids, and altered physical properties. To address these issues and simplify the mixing process, auto-mix in-office bleaching gels with mixing tips have been introduced to the market.<sup>9-16</sup> The Helical mixing tip has been widely used for these self-mixing products, but recently, the T-mixer mixing tips have gained popularity, especially in impression materials.<sup>19</sup> This study is the first to compare the T-mixer mixing tip with bleaching materials.

In this study, the results showed that the T-mixer mixing tip provided a lower concentration of hydrogen peroxide within the pulp chamber compared to the conventional Helical mixing tip when using PO+. This finding led the authors to partially accept the first hypothesis. While both mixing tips are considered statical, the Helical mixing tip is typically helically shaped and has spaces between the elements along the mixing tip, allowing for the division and merging of the composition multiple times as it passes the mixer. On the other hand, the T-mixer mixing tip, although shorter in length (Figure 1), allows for a permanent compressing and elongating of the fluid elements due to the shear stresses caused by radial velocity gradients. Additionally, the flow in the T-mixer is divided into halves between two alternating elements.<sup>17-19</sup>

Taking these differences into account, it appears that the Helical mixing tip produces a bleaching gel with higher oscillations of viscosity, resulting in a bleaching gel with heterogeneous behavior. This increased fluidity of the PO+ gel during application negatively impacts the higher amount of hydrogen peroxide that enters the pulp chamber, as observed in the present study.

On the other hand, the T-mixer tip improves the phase mixing of PO+, producing a more homogeneous and viscous gel, as seen in Figure 2. Moreover, it leads to an improvement in the final viscosity of the PO+ gel, which consequently reduces the amount of peroxide in the pulp compared to the Helical mixing tip, partially reject the third hypothesis. The viscosity of gels holds significant importance in clinical practice as it guarantees that the gel maintains direct contact with the tooth surface,<sup>8</sup> thereby preventing undesirable side effects by preventing runoff into the oral cavity. Nevertheless, contingent upon the type and quantity of thickener employed, the viscosity of gels can potentially impact the penetration of hydrogen peroxide into the pulp chamber. This is due to the fact that the gel's viscosity plays a

substantial role not only in the liberation of hydrogen peroxide that permeates hard tissues but also in the degradation of radicals.<sup>8</sup>

Before entering the tooth, the peroxide molecules within the applied bleaching gel layer on the enamel must find their way out of the intricate three-dimensional structure formed by the thickener.<sup>3,4,7</sup> Consequently, in gels with higher viscosity, hydrogen peroxide molecules necessitate more time for their release. Previous studies have also reported that gels with higher viscosity tend to have greater penetration, while gels with medium and low viscosity show lower penetration, which supports this idea.<sup>4,7</sup> Additionally, the increased fluidity of the PO+ bleaching gel, when mixed with a Helical mixing tip, affects the manufacturer's recommended application time, which specifies the need to renew the application of PO+ bleaching gel every 8 minutes.

However, the same effect was not observed when AM+ gel was mixed with both mixing tips in terms of hydrogen peroxide penetration within the pulp chamber. This can be explained by the fact that the final mixture obtained by AM+ bleaching gel showed similar viscosity and thixotropic capacity, regardless of the mixing tip used (Figure 2). Additionally, a homogeneous mixture was clinically observed when AM+ was mixed with both mixing tips.

Another hypothesis to account for these results is that, since the quantity of hydrogen peroxide within the pulp chamber remains contingent upon the utilized material if a larger volume of material is applied using one of the mixing tips, a reduced amount of hydrogen peroxide within the pulp chamber would be anticipated, as previously observed.<sup>9,11</sup> Nonetheless, upon closer examination of the outcomes, it was evident that no significant difference emerged when comparing the two mixing tips. This observation can be elucidated by the fact that both the Helical and T-mixer tips employ a brush-like applicator (as depicted in Figure 1), ensuring the application of a thinner and uniform layer onto the buccal surface of each tooth.

Although there was no statistically significant distinction in the amount of bleaching gel utilized with respect to the mixing tip employed, a smaller volume of gel was wasted for both bleaching gels when combined with the T-mixer as opposed to the Helical mixing tip. This observation lends support to the fourth hypothesis. Even though both mixing tips have the same number of internal mix elements, the T-mixer mixing tip is significantly shorter than the Helical mixing tip (Figure 1). As a result, each application with the T-mixer can lead to a reduction of around 20%, which could ultimately increase profitability for clinicians using both in-office gels with the T-mixer mixing tip. This finding aligns with a previous study that evaluated waste material from polyvinyl siloxane impression materials. In that study, when the T-Mixer mixing tip was used, the amount of waste material was, on average, 27% lower than when the conventional Helical mixing tip was used.<sup>19</sup>

Regarding the color change, no significant differences were observed when comparing both mixing tips. This observation can be attributed to the fact that the entire bleaching procedure was conducted by one calibrated and experienced operator, ensuring that the same amount of gel was used for both mixing tips during the process, thereby explaining the similar color change achieved regardless of the mixing tip used.

Interestingly, a better color change was achieved with AM+ compared to PO+, leading to a accept of the second hypothesis. It is noteworthy that the initial concentration of hydrogen peroxide was measured, revealing that PO+ contained around 28% hydrogen peroxide, instead 37.5% as described by the manufacturer, while AM+contained approximately 33%, while in AM+ it was 32.7 ± 2.3, instead 35% as described by the manufacturer. These values are in accordance with a previous study.<sup>9</sup> This lower concentration of hydrogen peroxide in PO+ directly contributes to the reduced color change compared to AM+. Although PO+ (HP37.5%) deviated by 25% from the labeled concentration, it still falls within the acceptable limits defined by ISO 28399:2021,<sup>33</sup> which allows for an acceptable variation of up to -30% and +10% of the original concentration stated by the manufacturer. Despite this level of regulation, the lack of standardization or significant differences in concentration may still impact the whitening results, whether tested in vitro or clinically.

However, the mean color change of both in-office bleaching evaluated for  $\Delta E_{ab}$  and  $\Delta E_{00}$  fell within the range of perceptibility and acceptability thresholds ( $\Delta E_{ab}$  2.7 and  $\Delta E_{00}$  1.8),<sup>30</sup> regardless of the type of mixing tip used. This finding is in agreement with previous studies, indicating that although some significant differences were observed between the groups, the research findings cannot be fully interpreted in terms of real-life relevance without considering perceptibility and acceptability tolerances.<sup>30</sup> Therefore, based on these thresholds, it can be concluded that clinically significant differences in the whitening effect are not expected.

On the other hand, when considering  $\Delta WI_D$ , a statistically similar color change was observed, regardless of the initial concentration of hydrogen peroxide and the type of mixing tip used. This indicates that the bleaching effect is not influenced by the concentration of hydrogen peroxide available in the bleaching gel or the type of mixing tip used. Furthermore, the color change of both in-office bleaching gels evaluated was above the acceptability threshold limit for  $\Delta WI_D > 2.9$ ,<sup>31</sup> regardless of the type of tip used.

In fact, the Whiteness Index for Dentistry is the most recent and appropriate method for objectively assessing the degree of dental bleaching. This new formula offers a lower probability of error by utilizing the CIELab color space.<sup>25</sup> The WI<sub>D</sub> has demonstrated a stronger correlation with visual perception compared to other indices of whiteness or yellowness tested under laboratory and clinical conditions.<sup>25</sup>

To the best of our knowledge, this study is the first to evaluate the effects of the amount of hydrogen peroxide in the pulp chamber, the bleaching effect, and the waste resulting from different manipulations of a whitening gel. Further, in vivo studies are necessary to assess the influence of different mixing techniques on bleaching efficacy and tooth sensitivity.

### 5 | CONCLUSIONS

Based on the results of this in vitro study, it is possible to conclude that utilizing a T-mixer mixing tip for the application of self-mixing bleaching gels appears to offer several advantages: (1) using PO+ gel,

reduces the amount of hydrogen peroxide within the pulp chamber, (2) achieving the same level of color change, (3) using PO+ gel, improves the mixing of phases, and (4) reduce the amount of gel wasted, despite the similar amount of bleaching gel was used.

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#### CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any financial interest in the companies whose materials are included in this article.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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