

**Efficacy of Ozonized Sunflower Oil with Tea Tree Oil as Desensitizing Agents in Dental Bleaching: Randomized and Double-Blind Clinical Trial.**

**Abstract**

Dental bleaching is one of the most commonly prescribed procedures in aesthetic dentistry due to its effectiveness and minimally invasive nature. However, a recurrent and challenging side effect is dentin hypersensitivity after the procedure. This study aimed to evaluate whether the use of ozonized oil combined with tea tree oil is effective in controlling post-bleaching dentin sensitivity. A randomized, double-blind, split-mouth clinical trial was conducted. Participants were divided into two experimental groups (n=29) according to the desensitization protocol: NPFS (potassium nitrate and sodium fluoride) - control group; OM (ozonized sunflower oil combined with tea tree oil) - test group. Both groups were applied before dental bleaching with 35% hydrogen peroxide. The obtained data were subjected to statistical analysis using a non-parametric Friedman repeated measures ANOVA test ( $p < 0.05$ ), followed by the Durbin-Conover post hoc test ( $p < 0.05$ ). In the assessment of sensitivity level, there were no statistically significant differences between the OM and NPFS groups. A higher level of sensitivity was observed for the lower arch after one hour of bleaching agent removal in both groups, and the color change was statistically equal between the two groups. The study concluded that the OM paste was as effective in reducing dental sensitivity caused by dental bleaching as NPFS. Additionally, neither of the groups affected the bleaching capacity of hydrogen peroxide.

Keywords: Ozone; Dentin Sensitivity; Tooth Bleaching

**1 Introduction:**

Dental bleaching is an aesthetic dental procedure that reflects the contemporary culture of beauty appreciation. Its popularity stems from its ability to promote tooth whitening in a highly conservative manner [1].

Dental discoloration can be caused by a variety of factors, including intrinsic and extrinsic pigments. Intrinsic pigments are present within the tooth, resulting from natural aging or the use of certain antibiotics, such as tetracycline. On the other hand, extrinsic pigments result from dietary habits, such as frequent consumption of coffee, tea, dark sodas, and red wine, which can stain the tooth surface over time. Additionally, dental trauma and cavities can also contribute to dental discoloration [2,3].

In the dental bleaching process, hydrogen peroxide plays an important role. It acts through an oxidation-reduction reaction, breaking down the organic molecules responsible for dental pigmentation [3]. This reaction reduces the saturation of the teeth, resulting in a visible whitening effect [2]. Therefore, hydrogen peroxide is capable of combating both intrinsic and extrinsic pigments, contributing to effective teeth whitening. However, the possibility of dentin sensitivity is the main drawback of this technique [1]. The dentin sensitivity resulting from bleaching may indicate an inflammatory response in the dental pulp. Although dentin is not innervated, it has permeable dentinal tubules that house fluids. The hydrodynamic hypothesis suggests that under external stimuli, these fluids move within the tubules, activating pulp nociceptors and causing brief and acute pain [3]. For some authors, bleaching sensitivity results from the formation of oxygen bubbles in the dentinal tubules during the application of hydrogen peroxide. The release of this gas triggers the movement of dentinal fluid, activating the interdental nerves [4].

To minimize patient discomfort, systemic agents such as analgesics and anti-inflammatories, and/or topical desensitizing agents can be used. These topical agents can have a neural action, reducing the excitability of receptors, as is the case with potassium-based products. On the other hand, obliterating agents react with dentin components, precipitating crystals that block dentinal tubules [2]. The most commonly used agents in this category are based on sodium fluoride, calcium, GLUMA, and toothpaste, among others. However, none of these products are completely effective in controlling dentin sensitivity [5].

Currently, the use of ozone in the treatment of dentin sensitivity is being investigated. Ozone (O<sub>3</sub>) is a natural gas composed of three oxygen atoms and has been used in various pathological conditions due to its oxidation, circulatory stimulation, and analgesic properties, among others. In the dental field, ozone is indicated for the control

of dentin sensitivity and can be used in different forms, such as diluted in water, in the form of gas, or incorporated into oils [6].

In the control of dentin sensitivity, a widely used approach is the dilution of ozone in vegetable oils, which provides greater safety in therapy. Furthermore, another promising alternative has been the combination of different oils for the treatment of various pathological conditions, as natural products have been a primary source of medicines throughout the centuries [7]. For example, tea tree oil, also known as melaleuca oil, demonstrates anti-inflammatory and antioxidant effects, making it relevant in this context. Therefore, the anti-inflammatory action of tea tree oil is being explored as a preventive measure to avoid pulpal inflammatory response during the whitening treatment. The water-soluble components present in tea tree oil have the ability to suppress the production of pro-inflammatory mediators, thus demonstrating its anti-inflammatory effect on dental structure. This property of tea tree oil may contribute to minimizing dentin sensitivity and promoting a more comfortable and safe whitening process [8].

Based on the scarcity of randomized clinical trials investigating the combined use of tea tree oil and ozonized sunflower oil in the management of post-whitening dental pain, the hypothesis of this study is that the combination of tea tree oil and ozonized sunflower oil will be as effective as the control group in reducing dentin sensitivity, a common adverse reaction to dental whitening treatment.

## **2 METHODS**

### ***2.1 Ethical Aspects and protocol registration***

The experimental design followed the statement CONSORT and was registered with the Brazilian Registry of Clinical Trials (RBR-88kny27). The study protocol was reviewed and accepted by the Local Ethics Committee on Investigations Involving Human Beings (64011722.0.0000.0107). All patients who met the selection criteria were informed about the objectives, procedures, risks, and benefits of the study and expressed their consent to participate by signing the Free and Informed Consent Form.

## ***2.2 Clinical study design, randomization, allocation, and Recruitment***

This was a randomized, prospective, double-blind, split-mouth study, in which the patient and evaluator were blinded to the distribution of the groups. This controlled clinical trial had an equal allocation rate to the groups. Simple randomization was performed using an open-access online system ([www.sealedenvelope.com](http://www.sealedenvelope.com)) by a third person not involved in the implementation and evaluation steps.

The distribution of the group to be assigned for the first time was recorded sequentially on numbered cards and placed in sealed envelopes. The information contained in the envelope determined the treatment to be assigned to the upper right arch, while the other arch received the alternative treatment. Once the participant was fit for the procedure and all evaluations were completed, the allocation assignment was revealed when opening the envelope immediately after implementation.

The recruitment of patients was carried out through disseminating research on the social network: Instagram. All participants were informed about the nature and objectives of the study. Before enrolling patients in the study, informed consent was obtained by asking the prospective patient to store and sign a form containing all information about the risks and benefits of treatment. The study was conducted from October 2022 to May 2023, at the Dentistry Clinics of the Local University.

This clinical trial evaluated the following variables: I- the intensity of sensitivity at different times in the same group; II- the intensity of sensitivity at different times in different groups; III- global sensitivity (GS) (sum of sensitivity throughout treatment, up to 48 hours); IV- worst pain (WP) and V- Bleaching effectiveness.

## ***2.3 Eligibility criteria***

Based on pre-established criteria, 40 volunteer patients were selected. general and oral health and aged between 18 and 50 years and had at least six caries-free upper anterior teeth, restorations, or endodontic treatment, with canine tone A2 or darker, according to the vita color scale (VITA ClassicalShade, VITA Zahnfabrik, BadSäckingen, Germany). Otherwise, participants with cognitive difficulties, that is,

patients who did not understand the correct way of filling out forms for the registration of dental sensitivity were not included in the study, just as patients with orthodontic appliances, dental prostheses, and severe internal tooth discoloration, such as tetracycline, fluorosis or pulped teeth stains were not included in the study. Pregnant and lactating women, patients with bruxism or any pathology that could cause tenderness, such as recession, dentin exposure, visible clefts in the teeth, and patients who use anti-inflammatory drugs or analgesics [9].

#### ***2.4 Sample size calculation***

The sample calculation was performed based on probability distributions of the t-test family (Wilcoxon and Mann-Whitney tests for comparison of two groups). The effect size used of 0.50, error type 1( $\alpha$ ) of 0.05, and power of analysis (error  $\beta$ ) of 0.90 resulted in a total of 26 volunteers per group. For convenience, due to the possible dropout of patients, 30 patients were selected per group. The sample calculation was performed in the GPower program, version 3.1.9.2 - University of Düsseldorf.

#### ***2.5 Study intervention***

After the insertion of a lip reformer (Arcflex, FGM Dental Products, Joinville, Brazil), a light-curing gingival barrier (Top Dam Angelus Dam, FGM Dental Products, Joinville, Brazil), was placed in the gingival tissue of the teeth to be bleached (from the second left premolar to the second right premolar of the upper arch). The gingival barrier was light curing using a light curing machine with a power of 1250 mW/cm<sup>2</sup> (Emitter NOW, Schuster Dental Equipment, Santa Maria, Brazil) according to the manufacturer's recommendations. After that, the right and left sides of the dental arch were separated with a Mylar matrix (Superdent, United States). In hemiarch, the process of the test group (GT) was performed. It involved the application of a paste that combined 5ml of ozonized sunflower oil (Philozon, Balneário Camburiú, Santa Catarina, Brazil), 5 drops of tea tree oil (Biovitarré, São José do Rio Preto – SP, Brazil), and 5mL of ozonized toothpaste (Philozon, Balneário Camburiú, Santa Catarina, Brazil). The paste was applied for 2 minutes using a low-speed rubber cup, followed by an additional 5 minutes of application solely on the dental surface. In the other hemiarch, the control group (CG), a desensitizing gel based on potassium nitrate (Desensibilize KF, FGM Dental Products, Joinville, Brazil) was applied for 10 minutes,

and subsequent removal with water for one minute. Then, both arches were bleached with 35% hydrogen peroxide gel manipulated with carbopol (Chamomilla Pharmacy, Cascavel, Paraná, Brazil), for 45 minutes and removed with a saliva ejector, gauze, and rinse with water for 1 minute. After seven days, all participating patients were reassessed. The composition of the materials used in the study is described in Table 1.

Table 1 - Composition of the materials used

Material	Manufacturer	Composition
Hydrogen Peroxide	Chamomilla Pharmacy	Hydrogen Peroxide 35% with Carbopol
Top Dam	FGM/ Dental Products, Joinville, Brazil	HEMA, Urethane Dimethacrylate Monomer, Inert fillers, Pigments, and Photoinitiators
Desensitize KF	Joinville, Brazil	5% Potassium Nitrate with 2% Sodium Fluoride
Ozonized Sunflower Oil	Philozon// Balneário Camburiu, Santa Catarina, Brazil	Ozonized Sunflower Seed Oil
Tea Tree Oil	Biovitarte Compounding Pharmacy, São José do Rio Preto - SP, Brazil.	A-pinene (2.5%), B-pinene (1%), myrcene (1%), A-terpinene (8%), G-terpinene (18%), p-cymene (3.9%), limonene (2%), terpinolene (3%), p-phellandrene, sabinene hydrates (trans and cis), and trans-piperitol
Ozonized sunflower oil	Philozon// Balneário Camburiu, Santa Catarina, Brazil	Ozonized Olive Oil; Tea Tree Oil; Calendula Extract; Grapefruit; Tahiti Lemon; Xylitol

## 2.6 Tooth sensitivity evaluation

Each patient received a form to evaluate the sensitivity experienced by them. This data collection instrument form for dental sensitivity registration every 5 minutes during the action of the bleaching gel, after 1 hour, 24 hours, and 48 hours after bleaching treatment. Patients were instructed in detail on how to record their most intense pain experience each day, in each hemiarch (right and left), based on the visual analogue scale (VAS) [10].

In addition, messages were sent daily to all research participants via WhatsApp Messenger, version 17.2.443 (WhatsApp Messenger, Social Networks. Facebook Inc.,

Menlo Park, CA, USA), informing them about completing the form, to ensure that the pain level was assessed correctly each day. All were instructed not to use any analgesic medication; if they did, they should notify the person responsible for the treatment. At the end of the treatment, the form was delivered by the patient to the researcher in charge.

If the participant scored 0 (without sensitivity) in all time evaluations of both bleaching sessions, this participant was considered insensitive to the whitening protocol. In all other circumstances, participants were considered to have whitening-induced dental sensitivity.

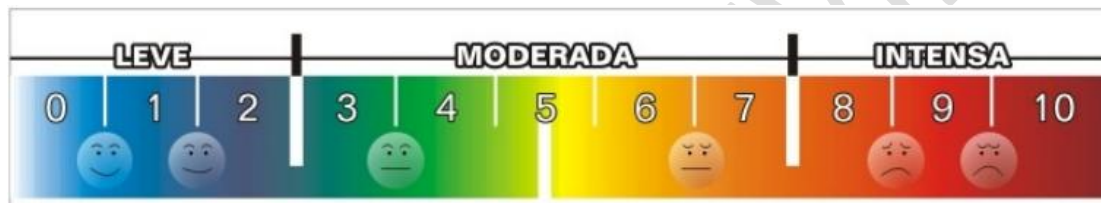


Figure 1 – Visual analog scale (VAS).

### **2.7 Bleaching effectiveness**

The color assessments were performed during the bleaching treatment, using the upper central incisors as a reference. An operator, previously calibrated, conducted these using the visual comparison method with the aid of the Vitapan Classical color scale (Vita, Bad Säckingen, Germany). Before the initial application of the bleaching gel and seven days after treatment completion, color assessments were performed. The shade guide was mounted in a sequence of increasing luminosity, from the most luminous shade (B1) to the least luminous (C4). Each shade received a score in this sequence: B1, score 1; A1, score 2; and so on, with A3 being a score of 9. The scores are shown in Table 2.

**Table 2** - Scores for color assessment

B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3,5	B4	C3	A4	C4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

The bleaching effectiveness ( $\Delta B$ ) was calculated by the difference between the color assessment initial ( $\Delta I$ ) and the color assessment final ( $\Delta F$ ), in each patient, according to the following formula:

$$\Delta B = (\Delta I) - (\Delta F)$$

### **2.8 Statistical analysis**

The results were tabulated and subjected to statistical analysis using JAMOVI software, version 1.2.24. For the analysis of data related to the degree of sensitivity of patients in the intragroup evaluation, the Friedman repeated measures ANOVA test was performed, followed by the Durbin-Conover post hoc test ( $p < 0.05$ ). On the other hand, for the intergroup analysis, comparing the same time interval, the Wilcoxon test was performed ( $p < 0.05$ ).

## **3. RESULTS**

During the period between October 2022 and May 2023, 40 patients were examined. After applying the eligibility and exclusion criteria, 30 patients were included as per the CONSORT (Consolidated Standards of Reporting Trials) flowchart, as depicted in Figure 2.

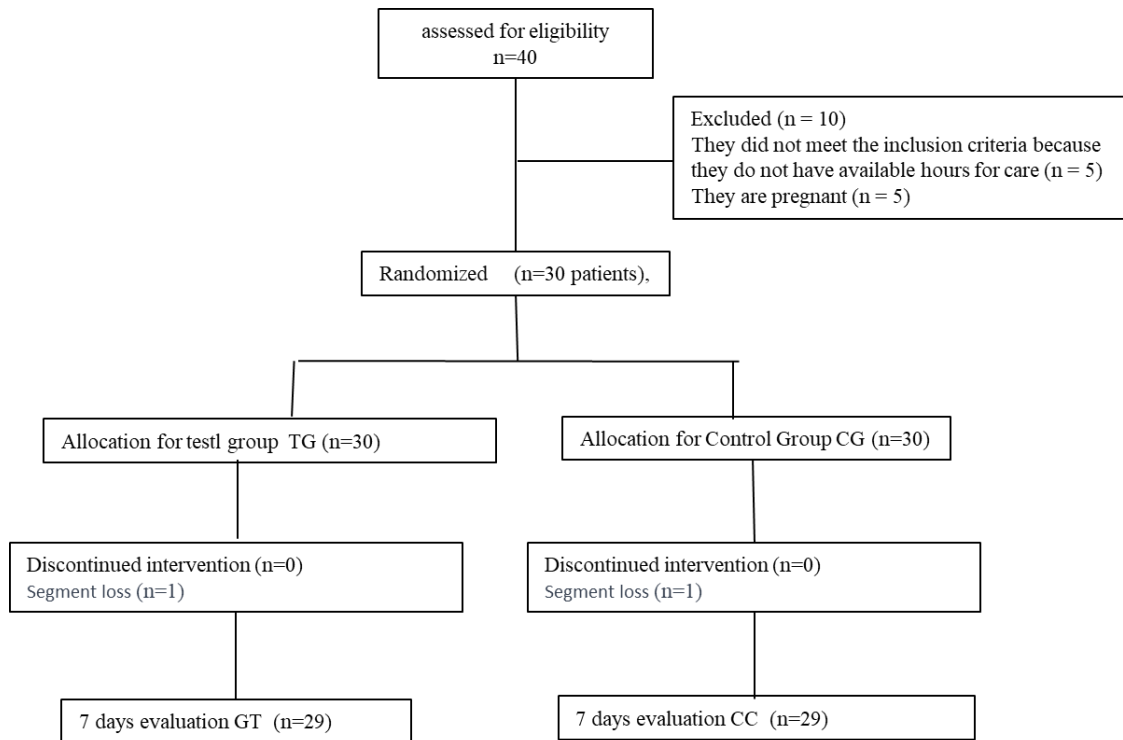


Figure 2 - Distribution and dynamics of the groups.

The statistical analysis was conducted by a blinded researcher who did not know which treatment protocol had been applied to each experimental group. The obtained results were subjected to statistical analysis using the non-parametric Friedman repeated measures ANOVA test ( $p < 0.05$ ), followed by the Durbin-Conover post hoc test ( $p < 0.05$ ).

It can be observed that the use of OM did not result in statistically significant differences when compared to the NPFS desensitizer. The assessment of sensitivity level for the upper quadrant revealed no statistically significant difference among the evaluated time points in the intragroup analysis, except for a statistically significant difference at the 1 hour. As for the intergroup analysis, there was no statistically significant difference among the groups at all reassessment time points. The data are presented in Table 3.

Table 3 - Degree of sensitivity during the execution of the whitening protocol using desensitizing agents in Group T and Group C at different evaluation times for the upper arch

Group	Reassessment period											
	5min	10min	15min	20min	25min	30min	35min	40min	45min	1hr	24hr	48hr
<b>Test</b>	0.213	0.282	0.355	0.379	0.517	0.4068	0.4655	0.486	0.4689	0.879	0.0689	0 ± 0
	793 ±	759 ±	172 ±	3 ±	241 ±	97 ±	17 ±	207 ±	66 ±	31 ±	66 ±	<b>Ca</b>
	0.489	0.589	0.624	0.607	1.030	0.8811	0.9251	0.984	0.9798	1.057	0.2205	
	697	23AB	835	324	627	24 Da	55 Da	786	34 Da	753	57 Ba	
	<b>ABCa</b>	<b>Da</b>	<b>Da</b>	<b>Da</b>	<b>Da</b>			<b>Da</b>		<b>Ea</b>		
<b>Control</b>	0.344	0.362	0.362	0.534	0.5 ±	0.4034	0.4551	0.544	0.5793	1.048	0.2310	0.051
	828 ±	069 ±	069 ±	483 ±	0.731	48 ±	72 ±	828 ±	1 ±	276 ±	34 ±	724 ±
	0.695	0.479	0.625	0.812	925	0.6726	0.6977	0.808	0.8694	1.269	0.5958	0.204
	694	891	308	085	<b>ABCa</b>	72	44	255	74 CDa	032	81 EGa	626
	<b>AEFa</b>	<b>ABEa</b>	<b>ABEa</b>	<b>ABCa</b>		<b>ABCEa</b>	<b>ABCEa</b>	<b>BCa</b>		<b>Da</b>		<b>FGa</b>

\*Different uppercase letters in the row indicate significant differences with  $p < 0.05$  in the intragroup analysis using Friedman's repeated measures ANOVA test. Different lowercase letters in the column indicate significant differences with  $p < 0.05$  in the intergroup analysis using the Wilcoxon test.

For the lower quadrant, a similarity was observed between the initial evaluation periods and the 24 and 48-hour reevaluations. However, the highest sensitivity was observed at the 1-hour time point, both in the test and control groups. Regarding intergroup analysis, there were no statistically significant differences between the groups at all reevaluation times. The data are presented in Table 4.

Table 4 - Degree of sensitivity during the execution of the bleaching protocol using desensitizers from the test group and control group at different evaluation times for the lower arch

Group	Reassessment period											
	5min	10min	15min	20min	25min	30min	35min	40min	45min	1hr	24hr	48hr
<b>Test</b>	0.189	0.293	0.3551	0.5862	0.824	0.758	0.913	0.9206	1.051	1.6827	0.317	0.044
	655 ±	103 ±	72 ±	07 ±	138 ±	621 ±	793 ±	9 ±	724 ±	59 ±	241 ±	828 ±
	0.541	0.700	0.7302	0.9984	1.132	1.184	1.210	1.1681	1.256	1.0039	0.538	0.191
	39	985	57	72	839	88	585	42 EFa	02	45 Ga	562	956
	<b>ACa</b>	<b>ABC</b>	<b>ABCa</b>	<b>BCDa</b>	<b>DEa</b>	<b>DFa</b>	<b>EFa</b>		<b>Fa</b>		<b>Ca</b>	<b>Aa</b>
<b>Control</b>	0.224	0.396	0.3793	0.5862	0.706	0.713	0.851	1.0758	1.086	1.9655	0.386	0.044
	138 ±	552 ±	1 ±	07 ±	897 ±	793 ±	724 ±	62 ±	207 ±	17 ±	207 ±	828 ±
	0.576	0.724	0.7518	0.9264	1.056	1.030	1.066	1.1797	1.268	1.2169	0.712	0.191
	105	314	45	85	589	161	577	87 Fa	217	27 Ga	503	956
	<b>AHa</b>	<b>ABH</b>	<b>ACHa</b>	<b>BCDa</b>	<b>DEa</b>	<b>Ea</b>	<b>Ea</b>		<b>Ea</b>		<b>Aa</b>	<b>Ha</b>

\*Different uppercase letters in the row indicate significant differences with  $p < 0.05$  in the intragroup analysis using the Friedman repeated measures ANOVA test. Different lowercase letters in the column indicate significant differences with  $p < 0.05$  in the intergroup analysis using the Wilcoxon test.

For the evaluation of whitening degree in the intragroup analysis, both tested groups showed statistically significant differences between the initial and final color, indicating a reduction in color saturation for both groups (Table 5). However, when comparing the difference between the initial and final color achieved after the whitening procedure, there was no statistically significant difference between the groups (Table 6).

Table 5 - Mean values and standard deviation of color score for the control and test groups

	Control (NPFS)	Test (OM)
Initial	8.76 ± 2.49 a	8.76 ± 2.49 a
Final	3.14 ± 1.51 b	3.14 ± 1.51 b

\*In the column, different lowercase letters indicate statistically significant differences by the Wilcoxon test ( $p < 0.05$ ).

Table 6 - Mean values and standard deviation of the difference between initial and final color for groups X and Y

Control	Test
5.62 ± 1.76 a	5.62 ± 1.76 a

\*In the row, different lowercase letters indicate statistically significant differences according to the Mann-Whitney test ( $p < 0.05$ ).

### Discussion:

The hypothesis that the combination of ozonized tea tree and sunflower oils would be as effective as the control group in reducing dentin sensitivity was confirmed in the present study.

The main objective of this study was to evaluate dental sensitivity during and after an in-office tooth whitening procedure using 35% hydrogen peroxide as the bleaching agent. In the control group, a 5% potassium nitrate with 2% sodium fluoride desensitizing gel (NPFS) was used. This gel is the most commonly used control in the literature but still presents contradictory results. In a study conducted by Rezende et al. [11], evaluating the effectiveness of 10% sodium fluoride as a desensitizing agent, no significant reduction in post-bleaching dentin sensitivity was observed. However, in a previous study by Tay et al. [12], using 5% potassium nitrate and 2% sodium fluoride, a positive effect on reducing dentin sensitivity was found. These findings are in line with a recent systematic review conducted by Krishnakumar et al. [4], which compared

different desensitizing agents and concluded that NPFS was the most effective agent in controlling sensitivity immediately after the procedure and in the first 24 hours.

In this research, no significant differences were found between the control group and the test group. This highlights the ability of the association of oils (OM) to reduce dental sensitivity after bleaching, as the test group showed similar results to NPFS, which is the most commonly used in the literature [2]. Ozonized oil was selected due to its advantage of allowing prolonged contact of ozone with the dental surface, as its half-life is significantly longer [13]. In a randomized clinical trial, Wiggers et al. [14] found a significant reduction in post-bleaching sensitivity using ozonized sunflower oil associated with glutaraldehyde. Supporting the efficacy of this oil, Veena H. R. et al. [13] reported that it can contribute to the opening of dentinal tubules, providing an anti-inflammatory action in dental pulp [15]. Initially, ozone causes a reduction in cell viability as transient oxidative stress, but later it increases the potential for a cellular response, stimulating ATP production and reducing the inflammatory process [13]. This finding is corroborated by the study of Holanda A.J.S. in 2020, which, in their literature review, mentions the anti-inflammatory and analgesic properties of local ozone application, countering the hypothesis that hydrogen peroxide generates a reversible inflammatory process in dental pulp. Additionally, ozone has analgesic properties that can prevent the activation of nerve branches present in dentin [9]. However, more studies are needed to confirm its effectiveness in the dental pulp and clarify its mechanism of action. Considering the anti-inflammatory, healing, analgesic, and antimicrobial properties of ozonized sunflower oil [16,17], its wide application in dentistry becomes evident. This characteristic provides a biological, practical, and economical advantage, as it allows obtaining a product with a broader spectrum of action compared to NPFS.

The objective of this study was to evaluate the association of ozonized sunflower oil with tea tree oil. Tea tree oil is known for its antimicrobial action, but there is also evidence of its anti-inflammatory activity, as its components can inhibit the production of inflammatory mediators [18]. In the context of this study, the focus was on the anti-inflammatory action of tea tree oil in relation to the possible inflammatory reaction in dental pulp caused by the bleaching procedure, and the results indicated a positive effect. Supporting this finding, the research by Stea S. et al [19] addressed terpinene-4-

ol, the main component of tea tree oil, which demonstrated the suppression of inflammatory mediators' production and reduction of inflammation. However, there is a scarcity of studies investigating the use of tea tree oil as a desensitizing agent in dental bleaching, and more research is needed to understand its efficacy and mechanism of action. Additionally, further studies are required to determine the capacity of each oil (ozonized sunflower oil vs. tea tree oil) as a desensitizing agent.

The bleaching capability of 35% hydrogen peroxide was evident, as all patients showed a reduction in color saturation. Furthermore, none of the protocols used negatively influenced the initial color stability after the 7-day evaluation period. In this regard, both the NPFS and OM groups achieved lighter shades, and there were no significant differences between them. However, to gain a more comprehensive and thorough understanding, it is highly recommended to conduct additional studies that track color stability over longer periods. This would allow for a more precise evaluation of the durability of the obtained results and provide valuable insights into the longevity of dental bleaching.

## **Conclusion**

Despite the limitations of this study, it can be concluded that the combination of ozonized sunflower oil with tea tree oil has a positive effect as a desensitizing agent in dental bleaching. Furthermore, it was observed that the bleaching gel made with 35% hydrogen peroxide is effective for tooth whitening, and it is important to note that none of the desensitizing agents interfered with the color change during dental bleaching.

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