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Effect of Desensitizing Agent on Enamel Microhardness: *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author BFD did took part in the laboratory phase and wrote the Manuscript. Authors VC and JKU conceived the study and carried out the statistical analysis. Author ALZ did took part in the laboratory phase. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Investigate possible changes in the microhardness of tooth enamel associated with the use of ozonized sunflower oil during the whitening procedure.
Study Design: *In vitro* study.
Place and Duration of Study: Department of Dentistry of the State University of Western Paraná - UNIOESTE between January 2023 and December 2023.

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Methodology: 30 healthy bovine incisor crowns were stored in 0.01% thymol solution (4ºC/30 days). Blocks measuring 4x4x2.5mm were made. With the exception of the buccal side, all sides were waterproofed and stored in artificial saliva and phosphate buffer. The specimens were divided into 3 groups (n=10) - CT (control), NP + H2O2 (desensitizing agent based on potassium nitrate and 35% hydrogen peroxide) and OL + H2O2 (ozonized sunflower oil and 35% hydrogen peroxide). The desensitizing agents were applied before the whitening gel. Color was recorded before and after the whitening procedure. Knoop surface microhardness was measured at 7, 14 and 21 days. The data obtained was submitted to Shapiro Wilk statistical analysis, Friedman ANOVA ($p<0.05$), Durbin-Conover ($p<0.05$) and Kruskal-Wallis ANOVA ($p<0.05$).
Results: In the intra-group analysis, the groups tested showed a statistical difference in enamel surface hardness, except for the OL + H2O2 group, before bleaching (234 + 95) and after bleaching (200 + 99). In the inter-group analysis, there was a significant statistical difference between the groups in the periods of 14 and 21 days after bleaching and no significant change in the period before and immediately after bleaching. In the analysis of color saturation, statistical changes were observed in the bleached groups.
Conclusion: Ozonated sunflower oil did not influence the microhardness values of the enamel surface, confirming its safety as a desensitizing agent during treatment.

Keywords: Whitening; desensitizing; microhardness; ozone.

1. INTRODUCTION

Chromogenic substances are responsible for tooth discoloration or pigmentation of the teeth and are present as large organic compounds with bonds or as compounds containing metals. Hydrogen peroxide is a compound unstable and decomposes in water and reactive oxygen radicals, being highly soluble and acidic with a pH that differs according to concentration. The free radicals released by hydrogen peroxide react more effectively with organic chromogens through an oxidizing process that breaks the strong double bonds, destabilizing them, and culminating in a change in the color of the tooth structure [1-3].

The hydrogen peroxide reaction produces free radicals, predominantly oxygen, resulting in the oxidation of the organic and inorganic components of the enamel. The exposure of tooth to bleaching agents may lead to morphological changes, including porosity, microcracks, and, in particular, changes in the hardness of tooth enamel. The bleaching agents with acidic pH affect hardness greatly when compared to products with a neutral or slightly alkaline pH. Thus, the morphological changes in the structure of the enamel are directly related to the chemical reaction and the process of oxidation, considering the concentration and pH of the bleaching agents used in the process of tooth whitening [4, 2, 3].
The most commonly used desensitizing agents are fluoride-based, nitrate-based, and ni-trate-based potassium and calcium gluconate [5, 6]. Among the various products studied, ozone stands out as an oxidizing agent capable of eliminating bacteria, fungi, viruses, and parasites [6]. It also has immunostimulant, analgesic, and detoxifying properties, antimicrobial, biosynthetic, and bioenergetic. Therefore, ozone therapy, which uses oxygen and ozone administered via gas or in water or oil-based solution, is recognized as an effective bio-oxidative therapy in the treatment of tooth sensitivity. In addition, it is a non-invasive procedure that allows the depolarization of the fibers nevus, reducing sensitivity through neural action [5, 6]. Thus, this study aimed to to check whether the use of ozonized sunflower oil influences surface hardness values of the enamel compared to the conventional whitening technique.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Thirty healthy, clean bovine incisors without periodontal tissues were collected and adhered. Subsequently, blocks measuring 4x4x2.5mm were made and the crowns were separated from the roots by sectioning with a double-sided diamond blade (KG Sorensen, Cotia, São Paulo - Brazil) 2mm below the joint and stored in a 0.01% thymol solution at 4ºC for 30 days.

2.2 Making the Specimens

Blocks measuring 4x4x2.5mm were made with 1mm of enamel and 1.5mm of dentin, obtained

from the middle third of the buccal surface of each tooth. All the aspects of the sample, except the vestibular surface, were waterproofed and then the specimens were stored in artificial saliva, a solution of base of 1.5 mM Ca, 0.9 mM P, 150 mM KCl, 0.05 µg F/mL, and phosphate-buffered saline (PBS) 0.01 M, pH 7.2. Table 1 shows the composition of the products that were used in the study, according to the manufacturer.

2.3 Division of Experimental Groups

The 30 specimens were allocated into 3 experimental groups according to the desensitization protocol, the application technique of the whitening gel and agent. The desensitizing agent was applied following the manufacturer's instructions. As shown in Table 2.

2.4 Analysis of the Color Change

The color was recorded before and immediately after the bleaching treatment by comparison with the Vita Classical color scale (Vita, Bad Säckingen, Germany). The color scale was assembled in ascending order in terms of luminosity, hue, and color. brightest - B1 - to least bright - C4 [3]. In this sequence, each hue receives a score: B1 the score 1; A1 the score 2, and so on, which makes the hue A3. The scores are shown in Table 3.

2.5 Surface Microhardness Analysis

Five readings were taken on each specimen, with a distance of 100 µm in between in the central region of the block using a Knoop-type penetrator (HMV-2, Shimadzu, Tokyo, Japan) with a static load of 25 grams for 15 seconds to calculate the initial microhardness. After the end of the bleaching treatments, the specimens were submitted to a new final surface microhardness reading, following the same protocols. The evaluation was repeated 7, 14, and 21 days after treatment.

2.6 Statistical Analysis

The results were tabulated and subjected to statistical analysis using JAMOVI software, version 1.2.24.

Table 1. Composition of the products used in the study

Products	Manufactures	Composition
HP Maxx Whiteness Whitening Gel 35%	FGM, Joinville, Santa Catarina, Brasil	35% hydrogen peroxide, thickener, red dye, glycol and deionized water
Desensibilize KF 2%	FGM, Joinville, Santa Catarina, Brasil	Potassium nitrate 5% sodium fluoride 2%, deionized water, glycerin, neutralizing and thickening agents
Ozonated sunflower oil	Philozon, Camboriú, Santa Catarina, Brasil	Contains oxygenated compounds, in the form of ozonides and peroxides, acquired during the ozonation process

Table 2. Division of experimental groups

Groups	Usage Protocols
Control (CT)	Stood teeth immersed in artificial saliva
Potassium Nitrate + Hydrogen Peroxide 35% (NP + H_2O_2)	Application of desensitize for 10 minutes, wash, dry and apply 35% hydrogen peroxide in three times of 15 minutes
Ozonated Sunflower Oil + Hydrogen Peroxide 35% (OL + H_2O_2)	Application of ozonated sunflower oil for 10 minutes with the aid of rubber bowl and low rotation, wash, dry and apply 35% hydrogen peroxide in three times of 15

Table 3. Color evaluation scores

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

Initially, the data was assessed for the requirement of normal distribution using the Shapiro-Wilk test, with a negative result. After analyzing this pre-requisite, statistical tests were carried out to assess the existence of statistically significant differences in the intra-group analysis using the Friedman ANOVA test ($p<0.05$), followed by the Durbin-Conover follow-up test ($p<0.05$) and for the inter-group analysis the Kruskal-Wallis ANOVA test($p<0.05$).

To analyze the data related to the degree of whitening, the test performed was Friedman's repeated measures ANOVA, followed by the Durbin-Conover follow-up test($p<0.05$).

3. RESULTS AND DISCUSSION

The results were statistically analyzed using the Friedman ANOVA test ($p<0.05$), followed by the Durbin-Conover follow-up test ($p<0.05$) for intra-group comparisons and the Kruskal-Wallis ANOVA test ($p<0.05$) for inter-group comparisons.

In general, in the intra-group analysis for the control group, there was a statistically significant difference only between the pre-bleaching period and the period of 21 days after bleaching. For the NP + H2O2 group, there was a statistically significant difference for all periods, except between the periods (before bleaching and 7 days after), (before bleaching and 21 days after), (immediately after bleaching and 21 days after) and (7 days after bleaching and 21 days after). In the intra-group analysis for the OL + H2O2 group, there was no statistically significant difference.

For inter-group analysis, there was a statistically significant difference between NP + H_2O_2 and OL + H_2O_2 for the 14-day period and between the CT group and OL + H_2O_2 for the 21-day period. The data is shown in Table 4.

When evaluating the degree of whitening in the intra-group assessment, except for the control group, there was a statistically significant difference between the initial and final color, i.e. there was a reduction in the degree of color saturation in the groups. For the inter-group analysis evaluating the same moment, when comparing the initial and final color between the groups, there was a statistically significant difference between the OL + H2O2 group and the other groups (Table 5). In turn, when evaluating the difference between the initial and final color in the inter-group analysis, there was a statistically significant difference between all the groups (Table 6).

All bleaching gels affect hardness of tooth enamel, leaving enamel deformed and more susceptible to fracture cause changes in the hardness of tooth enamel, making it more susceptible to deformation and fracture, since the process of oxidation of the organic and inorganic components of enamel occurs, culminating in changes in tissue morphology [4, 2]. As shown in Table (4) in the comparison before and after tooth whitening, but it is possible to see that in the parameters of 07, 14, and 21 days after whitening there is an increase in the values. Hardness of the surface, since the saliva, when in contact with the structure can remineralize teeth [4, 2].

Table 4. Median values and interquartile deviation of tooth structure microhardness before, immediately after, 7 days, 14 days, and 21 days after bleaching for the CT, NP + H2O2, and OL + H2O2 groups

Groups	Before	After	7 Days	14 Days	21 Days
CT	185 ± 81 Aa	178 ± 45 Aab	192 ± 65 Aab	177 ± 43 ABab	164 ± 42 Ab
NP + H_2O_2	202 ± 78 Aac	179 ± 38 Ab	217 ± 67 Aa	128 ± A65 c	211 ± 68 ABab
OIL + H_2O_2	234 ± 95 Aa	200 ± 99 Aa	191 ± 174 Aa	214 ± 35 Ba	227 ± 68 Ba

**Different lowercase letters on the line represent significant differences with p<0.05 in the intra-group analysis using Friedman's repeated measures ANOVA test followed by the Durbin-Conover post-test (p<0.05)*

***Different capital letters in the column show significant differences with p<0.05 in the inter-group analysis using theKruskal-Wallis ANOVA test (p<0.05)*

Table 5. Median values and interquartile deviation of the initial and final color score for theCT, NP + H_2O_2 and OL + H_2O_2 groups

Color	CT	NP + H_2O_2	OIL + H_2O_2
Initial	7.0 ± 1.75 Aa	8.0 ± 1.75 Aa	5.0 ± 3.0 Ba
End	7.0 ± 1.75 Aa	3.5 ± 1.5 Ab	1.5 ± 0.5 Bb

**Different lowercase letters in the column show significant differences with p<0.05 in the intra-group analysis usingFriedman's repeated measures ANOVA test followed by the Durbin-Conover post-test (p<0,.05); **Different capital letters in the line - show significant differences with p<0.05 in the inter-group analysis using Friedman's repeated measures ANOVA test followed by the Durbin-Conover post-test (p<0.05)*

Table 6. Median values and interquartile deviation of the difference between initial and final color for the CT, NP + H_2O_2 and OL + H_2O_2 groups

CT	NP + H_2O_2	OIL + H_2O_2
0.0 ± 0.0 a	4.5 ± 2.75 b	1.0 ± 0.0 c

**Different lowercase letters in the line - show significant differences with p<0.05 in the inter-group analysis using Friedman's repeated measures ANOVA test followed by the Durbin-Conover post-test (p<0.05)*

The mechanical modification of enamel after bleaching is influenced by the reaction chemical and oxidation process and is directly related to the concentration and pH of the bleaching agents used. This phenomenon is not affected using light sources to accelerate the chemical reaction, as observed by Pasquali et al [4, 2]. Carbopol is often used as a thickening agent and is characterized as a polymer acid that intensifies the process of demineralization of the enamel surface. In addition, inhibits the formation of hydroxyapatite due to its high calcium-binding capacity, resulting in a decrease in enamel microhardness, as described by Alkahtani et al [2]. The results of this study revealed no significant difference between the NP + H_2O_2 and OL + H_2O_2 groups at 7, 14, and 21 days after tooth whitening. These variations in the periods mentioned are associated with the remineralization potential of saliva and the response of the dental tissue to calcium and phosphate ions. The remineralization of tooth structure, induced by exposure to saliva rich in calcium and phosphate ions, facilitates the restoration of the integrity of the tissue by closing the intercrystalline spaces, resulting from the formation of new crystals or the precipitation of salivary components [4, 2, 7].

Regardless of the type of desensitizing agent used, it is observed that decrease in enamel surface microhardness. This change is directly related to the action of hydrogen peroxide, as corroborated by Gomes et al [8-10]. On the other hand On the other hand, the authors [11, 12] did not identify significant changes in the microhardness of the enamel. However, this discrepancy can be attributed to differences in methods used, exposure time, composition, pH, and concentration of the bleaching agent, as well as as well as variations in the treatment evaluation intervals, which differ from those of the methodology adopted in this chapter.

About the degree of color saturation, none of the agent desensitizers had an impact on the activity of hydrogen peroxide. This finding agrees with the results found by Borges et al [13, 14]. These researchers, in a clinical study, observed that the use of ozonized sunflower oil did not affect the degree of tooth whitening. Evidence from studies investigating the efficacy of ozone in dental materials supports its use before tooth whitening to preserve the structure of the enamel [15]. No changes were observed in the physical properties of enamel, including Knoop surface microhardness and angle of contact, when ozone was applied before the bleaching procedure. In addition, the ozone gas has been shown to have a powerful bactericidal effect on microorganisms present in the dentinal tubules, which may contribute to the preservation of the dentin structure enamel and the clinical success of whitening [16].

Ozone therapy associated with in-office tooth whitening does not induce changes in enamel microhardness and surface micromorphology, demonstrating the safety of ozonized sunflower oil about the surface properties of the enamel, with no statistically significant changes before and after the teeth whitening [7]. As for the mechanism of action, [17] elucidated that ozone, by coming into contact with dentin, widens the diameter of the dentinal tubules and, by precipitating calcium and fluoride ions, reduces sensitivity by blocking the outflow of fluids. through the dentinal tubules, without presenting adverse effects on the dental enamel that could compromise its hardness. However, it is important to note that this study was conducted *"in vitro"*, which limits its direct applicability to real clinical situations. Controlled clinical studies are necessary to validate these findings and confirm their relevance in dental practice.

4. CONCLUSION

It was concluded that ozonized sunflower oil does not influence the values of microhardness of the enamel surface, when compared to potassium nitrate, during in-office tooth whitening, as well as being a safe product for use as desensitizing protocol, when it comes to changes in the microhardness of the surface of the tooth enamel.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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