

EFFECT OF DESENSITIZING AGENT ON SURFACE MICROHARDNESS: AN IN VITRO STUDY

ABSTRACT

Aims: to investigate whether the application of ozonized sunflower oil can produce superior results compared to the conventional bleaching technique in relation to the microhardness of the enamel surface.

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.

Methodology: 30 healthy bovine incisor crowns were stored in 0.01% thymol solution (4°C/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 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 represented 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 [1,2,3].

The hydrogen peroxide reaction produces free radicals, predominantly oxygen, resulting in the oxidation of the organic and inorganic components of the enamel. This exposure to the bleaching agent can lead to morphological changes, including porosity,

microcracks, and, in particular, changes in the hardness of tooth enamel. The bleaching agents with acidic pH cause greater reductions in hardness 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, i.e. 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 nitrate-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 check whether the use of ozonized sunflower oil influences surface hardness values of the enamel compared to the conventional whitening technique.

2. MATERIAL AND METHODS

2.1 Sample preparation

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

2.2 Making the specimens

Blocks measuring 4x4x2.5 mm were made with 1 mm of enamel and 1.5 mm of dentin, obtained from the middle third of the buccal surface of each tooth. All the faces of the sample, except the vestibular surface, were water proofed and then the specimens were stored in artificial saliva, a solution of base of 1.5 mM Ca, 0.9 mM MP, 150 mM KCl, 0.05 µg F/mL, and phosphate-buffered saline (PBS) 0.01 M, pH 7.2. Table 01 shows the composition of the products that were used in the study, according to the manufacturer.

Table 01 – Composition of the products used in the study.

Produtos	Fabricantes	Composição
Gel Clareador Whiteness HP 35%	FGM, Joinville, Santa Catarina, Brasil	Peróxido de hidrogênio 35%, espessante, corante vermelho, glicol e água
Dessensibilize	FGM, Joinville, Santa Catarina, Brasil	Nitrato de potássio 5%, fluoreto de sódio 2%, água deionizada, glicerina, agentes neutralizantes e espessante
Óleo de girassol ozonizado	Philozon, Camboriú, Santa Catarina, Brasil	Contém compostos oxigenados, na forma de ozonídeos e peróxidos, adquiridos durante o processo de ozonização

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. Table 02 shows information on the study design.

Table 02 – Division of experimental groups.

Grupos	Protocolos de Uso
Controle (CT)	Dentes hígidos imersos em saliva artificial
Nitrato de potássio + Peróxido de hidrogênio 35% (NP + H ₂ O ₂)	Aplicação do dessensibilize por 10 minutos, lavar, secar e aplicar o peróxido de hidrogênio 35% em três vezes de 15 minutos
Óleo de girassol ozonizado + Peróxido de hidrogênio 35% (OL + H ₂ O ₂)	Aplicação do óleo de girassol ozonizado por 10 minutos com auxílio de taça de borracha e baixa rotação, lavar, secar e aplicar o peróxido de hidrogênio 35% em três vezes

2.4 Analysis of the degree of whitening

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 03.

Table 03 – 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

2.5 Surface microhardness analysis

Five readings were taken on each specimen, with a distance of 100 μm between them, 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.

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 + H₂O₂ and OL + H₂O₂ for the 14-day period and between the CT group and OL + H₂O₂ for the 21-day period. The data is shown in Table 04.

Table 04 - 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.

Grupos	Antes	Após	7 Dias	14 Dias	21 Dias
CT	185 ± 81 Aa	178 ± 45 Aab	192 ± 65 Aab	177 ± 43 ABab	164 ± 42 Ab
NP + H ₂ O ₂	202 ± 78 Aac	179 ± 38 Ab	217 ± 67 Aa	128 ± A65 c	211 ± 68 ABab
OL + H ₂ O ₂	234 ± 95 Aa	200 ± 99 Aa	191 ± 174 Aa	214 ± 35 Ba	227 ± 68 Ba

*Different lowercase letters on the line represents 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 the Kruskal-Wallis ANOVA test ($p < 0.05$).

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 05). 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 06).

Table 05 - Median values and interquartile deviation of the initial and final color score for the CT, NP + H₂O₂ and OL + H₂O₂ groups.

Cor	CT	NP + H ₂ O ₂	OL + H ₂ O ₂
Inicial	7.0 ± 1.75 Aa	8.0 ± 1.75 Aa	5.0 ± 3.0 Ba
Final	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 using Friedman's repeated measures ANOVA test followed by the Durbin-Conover post-test ($p < 0.05$).
 **Different capital letters in the line shows 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 06 - Median values and interquartile deviation of the difference between initial and final color for the CT, NP + H₂O₂ and OL + H₂O₂ groups.

CT	NP + H ₂ O ₂	OL + H ₂ O ₂
0.0 ± 0.0 a	4.5 ± 2.75 b	1.0 ± 0.0 c

*Different lowercase letters in the line shows 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 ($p < 0.05$).

Whitening gels 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]. This difference in the change in enamel microhardness can be seen in Table 04 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].

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 by the use of light sources to accelerate the chemical reaction, as observed by [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, it inhibits the formation of hydroxyapatite due to its high calcium-binding capacity, resulting in a decrease in enamel microhardness, as described by [2]. The results of this study revealed no significant difference between the NP + H₂O₂ and OL + H₂O₂ 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, stimulated by contact with 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 there is a decrease in enamel surface microhardness. This change is directly related to the action of hydrogen peroxide, as corroborated by [8,9,10]. 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 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 is in agreement with the results found by [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 in order 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 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 can be 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 a desensitizing protocol, when it comes to changes in the microhardness of the surface of the tooth enamel.

REFERENCES

1. Eimar H, Siciliano R, Abdallah MN, Nader SA, Amin WM, Martinez PP, Celemin A, Cerruti M, Tamimi F. Hydrogen peroxide whitens teeth by oxidizing the organic structure. *Journal of Dentistry*. 2012;40(2):e25-e33.
2. Alkahtani R, Stone S, German M, Waterhouse P. A review on dental whitening. *Journal of Dentistry*. 2020;100(1):103423.
3. Godoy CE, Consani S, Guimarães AT, Laurindo BM, Mendonça MJ, Camilotti V. Effect of two desensitizing agents applied previous to in-office bleaching on the degree of whitening and dentin sensitivity: A randomized, controlled, double-blind clinical trial. *American Journal of Dentistry*. 2021;34(2):70-74.
4. Pasquali EL, Bertazzo CA, Anziliero L. Study of the effects of dental bleaching on the enamel: a review about the evidences for the clinical indication. *Revista Perspectiva*. 2014;38(141):99-108.
5. Al-Omiri MK, Al Nazeh AA, Kielbassa AM, Lynch E. Randomized controlled clinical trial on bleaching sensitivity and whitening efficacy of hydrogen peroxide versus combinations of hydrogen peroxide and ozone. *Scientific reports*. 2018;8(1):2407.
6. Veenah R, Afigith Mathew C, Daniel RA, Shubha P, Sreeparvathy R, Pradhan N. An in vitro analysis of the effect of adjunctive use of ozonated oil with a desensitizing agent on dentinal tubule occlusion. *Journal of oral biology and craniofacial research*. 2020;10(4):727-732.
7. Carvalho RR, Carlos NR, de Campos FUF, Turssi CP, Júnior WFV, do Amaral FLB, Basting RT. Ozone gas therapy for tooth bleaching preserves enamel microhardness, roughness and surface micromorphology. *Acta Odontologica Latinoamericana*. 2023;36(1):15-23.
8. Gomes MN, Francci C, Medeiros IS, De Godoy Fores Salgado NR, Rieh IH, Marasca JM, Muench A. Effect of light irradiation on tooth whitening: enamel microhardness and color change. *Jornal Esthet Restor Dent*. 2009;21(6):387-394.
9. Araújo F de O, Baratieri LN, Araújo E. In situ study of in-office bleaching procedures using light sources on human enamel microhardness. *Oper Dent*. 2010;35(2):139-146.
10. Lia Mondelli RF, Garrido Gabriel TR, Piola Rizzante FA, Magalhães AC, Soares Bombonatti JF, Ishikiriama SK. Do different bleaching protocols affect the enamel microhardness? *Eur Journal Dent*. 2015;9(1):25-30.

11. ItoY, MomoiY. Bleaching using 30% hydrogen and sodium hydrogen carbonate. *Dent Mater Journal*. 2011;30(2):193-198.
12. BorgesAB, ZanattaRF, BarrosAC, SilvaLC, PucciCR, TorresCR. Effect of hydrogen peroxide concentration on enamel color and microhardness. *Oper Dent*. 2015;40(1):96-101.
13. RoqueJVO, VanolliRS, CamposFUF, CologneseMRV, Silva MP, UedaJK, MendonçaMJ, CamilottiV. Comparison of desensitizing agents for tooth bleaching: evaluating the influence of ozonated sunflower oil and potassium nitrate/sodium fluoride on sensitivity and shade change. *Journal of Advances in Medicine and Medical Research*. 2023;35(17):19-31.
14. WiggersIF, MendonçaMJ, VanolliRS, CamposF, UedaJK, CologneseMRV, CamilottiV. Ozonated oil as desensitizing protocol in external dental bleaching: a clinical, randomized, and double-blind study. *Journal of Advances in Medicine and Medical Research*. 2023;35(15):10-20.
15. HolmesJ. Clinical reversal of root caries using ozone, double-blind, randomized, controlled 18-month trial. *Gerodontology*. 2003;20(2):106-114.
16. TiwariS, AvinashA, KatitarS, Aarthi IyerA, JainS. Dental applications of ozone therapy: a review of literature. In *Saudi Journal for Dental Research*. 2017;8(1):105-111.
17. ReddyS, AssociateA, ReddyN, Dinapadu S, ReddyM, PasariS. Role of ozone therapy in minimal intervention dentistry and endodontics - a review. *Journal of international oral health*. 2013;5(3):102-108.