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

ABSTRACT

Aims: to investigate whether the application of ozonized sunflower oil can producesuperior results compared to the conventional bleaching technique in relation to themicrohardnessofthe enamelsurface.
Studydesign:*invitro*study.
Place and Duration of Study: Department of Dentistry of the State University of WesternParaná-UNIOESTE betweenJanuary2023andDecember2023.
Methodology: 30 healthy bovine incisor crowns were stored in 0.01% thymol solution(4ºC/30 days). Blocks measuring 4x4x2.5mm were made. With the exceptionofthebuccal side, all sides were waterproofed and stored in artificial saliva and phosphatebuffer. The specimens were divided into 3 groups (n=10) - CT (control), NP + H_2O_2(desensitizing agent based on potassium nitrate and 35% hydrogen peroxide) and OL +H_2O_2 (ozonized sunflower oil and 35% hydrogen peroxide). The desensitizing agents wereapplied before the whitening gel. Color was recorded before and after the whiteningprocedure. Knoop surface microhardnesswas measured at 7, 14 and 21 days. The dataobtained was submitted to Shapiro Wilk statistical analysis, Friedman ANOVA ($p<0.05$),Durbin-Conover($p<0.05$) andKruskal-WallisANOVA ($p<0.05$).
Results: In the intra-group analysis, the groups tested showed a statistical difference inenamelsurfacehardness,exceptfortheOL+H_2O_2group,beforebleaching(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 nosignificant change in the period before and immediately after bleaching. In the analysis ofcolorsaturation,statisticalchangeswereobservedinthe bleachedgroups.
Conclusion: Ozonated sunflower oil did not influence the microhardness values of theenamelsurface,confirmingitssafetyasadesensitizingagentduringtreatment.

Keywords:whitening,desensitizing,microhardness,ozone.

1. INTRODUCTION

Chromogenicsubstancesareresponsiblefortoothdiscolorationor pigmentationoftheteethandarepresentaslargeorganiccompoundswithbonds orascompoundscontainingmetals.Hydrogenperoxideisacompound unstableanddecomposesinwaterandreactiveoxygenradicals,beinghighlysoluble andacidicwithapHthatdiffersaccordingtoconcentration.Thefreeradicalsreleased by hydrogenperoxidereactmoreeffectivelywithorganicchromogens throughanoxidizingprocessthatbreaksthestrongdoublebonds,destabilizingthem, and culminatinginachangeinthecolorofthetoothstructure[1,2,3].

Thehydrogenperoxidereactionproducesfreeradicals,predominantly oxygen,resultingintheoxidationoftheorganicandinorganiccomponentsoftheenamel. The exposure of tooth to bleaching agents mayleadtomorphologicalchanges,includingporosity, microcracks,and,inparticular,changesinthehardnessoftoothenamel.The bleachingagentswithacidicpHaffect hardness greatlywhencomparedto

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productswithaneutralorslightlyalkalinepH.Thus,themorphologicalchangesin the structureoftheenamelaredirectlyrelatedtothechemicalreactionandtheprocessof oxidation, considering theconcentrationandpHofthebleachingagentsusedintheprocess of toothwhitening[4,2,3].

Themostcommonlyuseddesensitizingagentsarefluoride-based,nitrate-based,and ni-trate-based potassiumandcalciumgluconate[5,6].Amongthevariousproductsstudied,ozone standsoutasanoxidizingagentcapableofeliminatingbacteria,fungi,viruses,andparasites [6].Italsohasimmunostimulant,analgesic,anddetoxifyingproperties, antimicrobial,biosynthetic,andbioenergetic.Therefore,ozonetherapy,whichuses oxygenandozoneadministeredviagasorinwateroroil-basedsolution,is recognizedasaneffectivebio-oxidativetherapyinthetreatmentoftoothsensitivity. Inaddition,itisanon-invasiveprocedurethatallowsthedepolarizationofthefibers nevus,reducingsensitivitythroughneuralaction[5,6].Thus,this study aimedto tocheckwhethertheuseofozonizedsunfloweroilinfluencessurfacehardnessvalues oftheenamelcomparedtothe conventionalwhiteningtechnique.

2. MATERIAL AND METHODS

2.1 Sample preparation

Thirtyhealthy,cleanbovineincisorswithoutperiodontaltissueswerecollected and adhered.Subsequently, blocks measuring 4x4x2.5mm were made and thecrownswereseparatedfromtherootsbysectioningwith a double-sideddiamondblade(KGSorensen,Cotia,SãoPaulo-Brazil)2mmbelowthejoint andstoredina0.01%thymolsolutionat4ºCfor30days.

2.2 Making the specimens

Blocksmeasuring4x4x2.5mmweremadewith1mmofenamel and1.5mmofdentin,obtainedfromthemiddlethirdofthebuccalsurfaceofeachtooth. Alltheaspectsofthesample,exceptthevestibularsurface,werewaterproofed andthenthespecimenswerestoredinartificialsaliva,asolutionof baseof1.5mMCa,0.9mMP,150mMKCl,0.05µgF/mL,andphosphate-bufferedsaline(PBS) 0.01M,pH7.2.Table01showsthecompositionoftheproductsthatwere usedinthestudy,accordingtothemanufacturer.

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

Products	Manufacturers	Composition
HP Whiteness Whitening Gel 35%	FGM, Joinville, Santa Catarina, Brasil	35% hydrogen peroxide, thickener, red dye, glycol and deionized water
Dessensibilize	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 ozonization process

2.3 Division of experimental groups

The30specimenswereallocatedinto3experimentalgroupsaccordingtothe desensitizationprotocol, theapplicationtechniqueofthewhiteninggelandagent. Thedesensitizingagentwasappliedfollowingthemanufacturer'sinstructions. As shown in table (2).

Table 02 – 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 minutos
Ozonated Sunflower Oil + Hydrogen Peroxide 35% (OL + H_2O_2)	Application of ozonated sunflower oil for 10 minutes with the aid of a rubber bowl and low rotation, wash, dry and apply 35% hydrogen peroxide in three times of 15

2.4 Analysis of the color change

Thecolorwasrecordedbeforeandimmediatelyafterthebleachingtreatmentbycompariso nwiththeVitaClassicalcolorscale(Vita,BadSäckingen,Germany). Thecolorscalewasassembledinascendingorderintermsofluminosity,hue,andcolor. brightest-B1-toleastbright-C4[3].Inthissequence,eachhuereceivesa score:B1thescore1;A1thescore2,andsoon,whichmakesthehueA3. ThescoresareshowninTable03.

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 μmin between in the central region of the block using a Knoop-type penetrator (HMV-2, Shimadzu,Tokyo,Japan)withastaticloadof25gramsfor15secondstocalculatetheinitialmicrohardness.Aftertheendofthebleachingtreatments,thespecimensweresubmittedtoanewfinalsurfacemicrohardnessreading,followingthesameprotocols.Theevaluationwasrepeated7,14,and21days aftertreatment.

2.6 Statistical analysis

Theresultsweretabulatedandsubjectedtostatisticalanalysisusing JAMOVIsoftware,version 1.2.24.

Initially, the data was assessed for the requirement of normal distribution using theShapiro-Wilk test, with a negative result. After analyzing this pre-requisite, statistical testswere 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-Conoverfollow-uptest($p<0.05$)andfortheinter-groupanalysistheKruskal-WallisANOVAtest($p<0.05$).

To analyze the data related to the degree of whitening, the test performed wasFriedman'srepeatedmeasuresANOVA,followedbytheDurbin-Conoverfollow-uptest($p<0.05$).

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 theKruskal-WallisANOVA test($p<0.05$)for inter-group comparisons.

In general, in the intra-group analysis for the control group, there was a statisticallysignificant difference only between the pre-bleaching period and the period of 21 days afterbleaching. For the NP + H2O2 group, there was a statistically significant difference for allperiods, except between the periods (before bleaching and 7 days after), (before bleachingand21days after),(immediatelyafterbleachingand21days after)and(7days afterbleaching and 21 days after). In the intra-group analysis for the OL + H2O2group, there wasnostatisticallysignificantdifference.

Forinter-groupanalysis,therewasastatisticallysignificantdifferencebetweenNP + $H_2O_2$and OL + $H_2O_2$for the 14-day period and between the CT group and OL + $H_2O_2$for the21-dayperiod. The dataisshown in Table04.

Table 04 - Median values and interquartile deviation of tooth structure microhardness before,immediately after,7 days,14 days,and 21days afterbleachingfor theCT, NP+ H_2O_2,andOL + H_2O_2 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
$OL + H_2O_2$	234 ± 95 Aa	200 ± 99 Aa	191 ± 174 Aa	214 ± 35 Ba	227 ± 68 Ba

*Differentlowercaselettersonthelinerepresentsignificantdifferenceswithp<0.05intheintra-groupanalysisusingFriedman'srepeatedmeasures ANOVAtestfollowedbythe Durbin-Conoverpost-test(p<0.05).
**Different capital letters in the column show significant differences with p<0.05 in the inter-group analysis using theKruskal-WallisANOVA test (p<0.05).

When evaluating the degree of whitening in the intra-group assessment, except forthe control group, there was a statistically significant difference between the initial and finalcolor, 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 colorbetween the groups, there was a statistically significant difference between the OL + H2O2group and the other groups (Table 05). In turn, when evaluating the difference between theinitial and final color in the inter-group analysis, there was a statistically significant differencebetweenallthe groups(Table06).

Table 05 - 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	$NP + H_2O_2$	$OL + H_2O_2$
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'srepeatedmeasuresANOVA testfollowedbytheDurbin-Conoverpost-test(p<0,.05).
**Differentcapitallettersintheline-showsignificantdifferenceswithp<0.05intheinter-groupanalysisusingFriedman'srepeatedmeasuresANOVAtest followedbytheDurbin-Conoverpost-test(p<0.05).

Table 06 - Medianvaluesandinterquartiledeviationofthedifferencebetweeninitialandfinalcolorfor the CT,NP +$H_2O_2$and OL +H_2O_2 groups.

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

*Differentlowercaselettersintheline-showsignificantdifferenceswithp<0.05intheinter-groupanalysisusingFriedman'srepeatedmeasuresANOVA testfollowedbytheDurbin-Conoverpost-test(p<0.05).

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 moresusceptible to deformation and fracture, since the process of oxidation of the organic andinorganic components of enamel occurs, culminating in changes in tissue morphology [4, 2].As shown in table (4) in thecomparisonbeforeandaftertoothwhitening,butitispossibletoseethatintheparametersof07,14, and21daysafterwhiteningthereisanincreaseinthevalues. Hardnessofthesurface,sincethesaliva,whenincontactwiththestructure canremineralizeteeth[4,2].

Themechanicalmodificationofenamelafterbleachingisinfluencedbythereaction chemicalandoxidationprocess andisdirectlyrelatedto the concentrationand pHofthebleachingagentsused.Thisphenomenonisnotaffectedusinglightsourcestoacceleratet h echemicalreaction,asobservedby[4,2].Carbopolis oftenusedasathickeningagentandischaracterizedasapolymer acidthatintensifiestheprocessofdemineralizationofthe enamelsurface.Inaddition, inhibitstheformationofhydroxyapatiteduetoitshighcalcium-bindingcapacity, resultinginadecreaseinenamelmicrohardness,asdescribedby[2]. Theresultsof thisstudyrevealedno significantdifferencebetweenthe NP + H_2O_2 and OL+ H_2O_2groupsat7,14,and21daysaftertoothwhitening.Thesevariationsintheperiods mentionedareassociatedwiththeremineralizationpotentialofsalivaandtheresponseofthe dentaltissuetocalciumandphosphateions.Theremineralizationoftoothstructure,induced by exposure tosalivarichincalciumandphosphateions,facilitatestherestorationof the integrity ofthetissuebyclosingtheintercrystallinespaces,resultingfromtheformationof newcrystalsortheprecipitationofsalivarycomponents[4,2,7].

Regardlessofthetypeofdesensitizingagentused,itisobservedthat decreaseinenamelsurfacemicrohardness.Thischangeisdirectly relatedtotheactionofhydrogenperoxide,ascorroboratedby[8,9,10].Ontheotherhand On the other hand, the authors [11, 12] did not identify significant changes in the microhardnessofthe enamel.However,thisdiscrepancycanbeattributedtodifferencesinmethods used,exposuretime,composition,pH,andconcentrationofthebleachingagent,aswellas aswellasvariationsinthetreatmentevaluationintervals,whichdifferfromthoseofthe methodologyadoptedinthischapter.

Aboutthedegreeofcolorsaturation,noneoftheagent desensitizershadanimpactontheactivityofhydrogenperoxide.Thisfinding agreeswiththeresultsfoundby[13,14].Theseresearchers,in aclinicalstudy,observedthattheuseofozonizedsunfloweroildidnotaffectthedegreeof toothwhitening.Evidencefromstudiesinvestigatingtheefficacyof ozoneindentalmaterialssupportsitsusebeforetoothwhiteningtopreservethestructureoftheenam el[15].Nochangeswereobservedinthe physicalpropertiesofenamel,includingKnoopsurfacemicrohardnessandangleof contact,whenozonewasappliedbeforethebleachingprocedure.Inaddition,the ozonegashasbeenshowntohaveapowerfulbactericidaleffectonmicroorganisms presentinthedentinaltubules,whichmaycontributetothepreservationofthedentinstructure enamelandtheclinicalsuccessofwhitening[16].

Ozonetherapyassociatedwithin-officetoothwhiteningdoesnotinduce changesinenamelmicrohardnessandsurfacemicromorphology,demonstratingthe safetyofozonizedsunfloweroilaboutthesurfacepropertiesofthe enamel,withnostatisticallysignificantchangesbeforeandafterthe teethwhitening[7].Asforthemechanismofaction,[17]elucidatedthatozone,by comingintocontactwithdentin,widensthediameterofthedentinaltubules and,by precipitatingcalciumandfluorideions,reducessensitivitybyblockingtheoutflowoffluids. throughthedentinaltubules,withoutpresentingadverseeffectsonthedentalenamelthat couldcompromiseitshardness.However,itisimportanttonotethatthisstudywas conducted*"invitro"*,whichlimitsitsdirectapplicabilitytorealclinicalsituations.Controlledclinicalstu diesarenecessarytovalidatethesefindingsand confirmtheirrelevanceindental practice.

4. CONCLUSION

It was concludedthatozonizedsunfloweroildoesnotinfluencethevaluesofmicrohardness of the enamel surface, when compared to potassium nitrate, during in-officetoothwhitening,aswellasbeingasafeproductforuseas desensitizingprotocol,whenitcomestochangesinthemicrohardnessofthesurfaceofthetoothenamel.

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COMPETING INTERESTS

The authorshavedeclaredthatnocompetinginterestsexist.

AUTHORS' CONTRIBUTIONS

AuthorBrunoFongaroDalboscotookpartinthelaboratoryphaseandwrotethe manuscript.AuthorsVeridianaCamilottiandJúlioKatuhideUedaconceivedthestudyand carriedoutthestatisticalanalysis.AuthorAdilvoLuizZanchetatookpartinthelaboratoryphase. Alltheauthorshavereadandapprovedthefinalmanuscript.

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