v. 3, n. 42, 2023

International Journal of Health Science

EFFECT OF DIFFERENT WHITENING ORAL RINSES ON DENTAL ENAMEL DEMINERALIZED WITH A COLA BEVERAGE; IN VITRO

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Abstract: Objective: Determine the effect of three whitening mouthwashes on dental enamel demineralized with a cola beverage, evaluating surface roughness and color. **Materials and methods:** Ninety impacted third molars were sectioned mesiodistally to obtain 180 enamel surfaces. They were divided into 6 experimental groups: Scope-Crest® (SO), Luminous White Colgate® (LW), 3D-Whitening Oral-B® (3DW), Whitening-Extreme Listerine® (WE), Initial lesion group (IL), every group was submitted to an initial lesion (96h-pH 2.8), and a healthy enamel group (HE). All groups were put through a daily pH cycle, alternating between an immersion in a cola beverage for 1.5 hours at a pH of 2.8 and artificial saliva for 22.5 hours at a pH of 7.0, simulating oral conditions during 7, 14, 21, and 28 days. Roughness was measured using an optic profile-meter and color was evaluated with a Colormeter CIELAB®. Two-way ANOVA and Tukey (post-hoc) $p<0.05$ with Graphpad-8.0 were used for statistical analysis. **Results**: WE showed less roughness in mm (0.69), followed by LW (0.74), and 3DW (0.92), in color 3WE obtained the whitest color ΔE^* (10.83±0.1), followed by WE (11.9±1.1), then LW (12.2±1.5), and finally SO (38.3±3.1). **Conclusions**: 3WE had the most whitening effect, however, it also produced the most roughness due to demineralization of the enamel. In contrast LW obtained a less whitening effect but presented less roughness, that is to say, less affectation of the enamel.
Keywords: color, roughness, mouthwash, dental whitening, demineralization

INTRODUCTION

In today's day and age having whiter teeth is a priority for most patients. That is why many over the counter products have surfaced as an alternative to conventional bleaching done in the dental office. Products like toothpaste, gels, strips and oral rinses addition with whitening agents offer a simple, fast, and affordable way to achieve a whiter smile. However, there is not enough evidence that shows that these products work the same or better than the products used professionally in a clinical setting.

Tooth color is mainly determined by dentine and modified by the thickness and transparency of enamel. Pigments in a biofilm can also influence the overall color of teeth. These pigments can be classified as intrinsic or extrinsic stains depending on the source.

The process of whitening teeth consists in the application of a whitening agent over the dental enamel which can cause secondary effects such as hyper sensibility, gingivitis, throat irritation, gastric irritation, and changes in the surface of the enamel.

The use of mouthwash dates back to about 4000 years, apart from water they can contain, antimicrobials, salts and on occasion alcohol which can cause dehydration of the buccal mucosa, this can lead to a diminished flow of saliva and elevate the risk of developing caries.

Since then, other ingredients have been added to these mouthwashes, the most common, hydrogen peroxide which has shown has whitening properties, making it a very useful ingredient.44

A study concluded that a mouthwash containing hydrogen peroxide (Listerine whitening extreme) presented a whitening effect in comparison to those that did not have hydrogen peroxide in the formula.55

Other ingredients added are usually sodium fluoride, low concentrations of pyrophosphate, triphosphate, hexametaphosphate salts, and sodium bicarbonate. The purpose of these agents is to protect the tooth surface from staining and to remove stains already present. Polyphosphates are widely known to bind to proteins in dental biofilm, by doing so this chemical compound helps remove stains from teeth.41,20

Sodium hexametaphosphate is added to rinses to combat tartar and surface stains, which benefits prevention and elimination. One of the potential advantages of high molecular weight condensed phosphate analogues, such as sodium hexametaphosphate, is increased inhibitory activity to prevent crystallization or chromogenic staining.48

Sodium hexametaphosphate can alter the salivary biofilm on the tooth surface, creating a more hydrophilic tooth surface, this allows for greater desorption and diffusion of surface chromogen into saliva, thereby reducing overall extrinsic staining.49

Pyrophosphate ion is a salt with chelating activity, chelating agents are known to retard calculus formation and remove them once they have formed. In addition, chelating agents can, in principle, remove stains by attaching to surfaces of the teeth. 48

A cyclic pH model is a model that provides constant controlled changes, simulating the conditions present in the oral cavity, the purpose of the cyclical pH method is the reproduction of demineralization and remineralization conditions that can occur in the oral cavity.54

There is little evidence that these products and ingredients work, so it is important to determine the effect of these mouthwashes on surface enamel roughness and color with prolonged use, in an environment similar to the oral cavity.

The purpose of this work is to determine the effect of different mouthwashes addition with sodium fluoride, hydrogen peroxide and other ingredients that help prevent stains on dental enamel. Using a cola beverage as a demineralizing solution in a cyclic pH cycle to determine change in color and roughness, during and after treatment. The null hypothesis of these research is that the use of whitening mouthwashes will not modify roughness or color of dental enamel demineralized with cola beverage in cyclic pH conditions.

MATERIALS AND METHODS

SAMPLE PREPARATION

The samples were obtained by maxillofacial surgeons extracted either for preventive or orthodontic purposes. 90 retained inferior third molars without caries or structural defects were selected and stored in deionized water in an incubator at 36°C until ready to use.

Once the samples were obtained, they were sectioned in a mesiodistal direction with a fine diamond disc (Germany Brasseler Diamond 910 <20,000 rpm, California, U.S.™) under constant irrigation, obtaining a total of 180 working surfaces. The work surfaces were then conditioned, removing soft tissue with Gracey curettes (7/8,11/12,13/14). The pulp space was blocked out with wax in order to obtain a flat working surface, acid resistant varnish (Revlon™) was applied to the lingual and buccal working surfaces and covered from the cervical line to the apical third of different colors to distinguish each group.

Prophylaxis was carried out on all enamel surfaces with a non-fluoridated prophylactic toothpaste.

INITIAL LESION

An initial lesion was made on 90 work surfaces by immersing the samples in a demineralizing solution (Coca-Cola®) with a pH of 2.8, corroborated by a pH-meter (Science Med SM-25CW Meter) for 96 hours. They were stored in an incubator at a controlled temperature. After 96 hours the samples were removed from the incubator, with a noticeable change in color in all of them.

ARTIFICIAL SALIVA

Artificial saliva was made every 4 days, using one liter of deionized water in a beaker while stirring with a magnetic

stirrer (SCILOGEX®, MS-H280-Pro). Vijaya Lakshmi´s composition for artificial saliva was followed, until a pH of 6.9 was obtained and then stored in an amber glass bottle at room temperature.29

PH CYCLE

A pH cycle consists in immersing the samples in a sequence of remineralizing and demineralizing solutions:

- For 1 hour and 30 minutes: The samples were immersed in a demineralizing solution (Coca-Cola®), at a pH of 2.8 at a temperature of 36°C.
- For 60 seconds: The samples were immersed in mouthwash, Luminous White (Colgate®), Whitening extreme (Listerine®), 3DWhite (Oral B®), and Scope (Crest®).
- For 22 hours and 30 minutes: They were stored in artificial saliva (remineralizing solution) with pH 6.75 at a temperature of 36°C.

After each change of solution, the samples were rinsed with deionized water.

IMMERSION IN MOUTHWASH

20 ml of each mouthwash was added into 40 ml beakers, then submerged twice a day with intervals of 6 hours, simulating the time of each meal, and rotating movements were performed in a clockwise direction simulating mouth movements when using a mouthwash. The treatment was applied following this cycle every 24 hours for 7,14,21 and 28 days.

As a demineralizing solution, 40 ml of cola soft drink (Coca-Cola®) was used, submerging the samples for 1 hour and 30 minutes. The soft drink was emptied into each group, taking special care that the samples did not turn over, and that they all received the demineralizing solution equally.

At the end of the Coca-Cola® application, the samples were rinsed with deionized water to continue with the second immersion of mouthwashes.

At the beginning and end of the experiment of 7, 14, 21 and 28 days, 16 LW surfaces, 16 3DW surfaces, 16 WE surfaces, 16 SO surfaces, 16 LI surfaces and 16 ES surfaces, were stored in deionized water in the incubator (Memmert™) at 36°C, the samples were placed and fixed individually in acrylic squares to avoid movement. Subsequently, the work surfaces corresponding to 7, 14, 21 and 28 days of treatment were randomly selected to obtain the roughness of the dental enamel of each of the experimental groups.

DATA ANALYSIS

The Shapiro-Wilk test was performed to analyze the distribution of the data of the roughness and color variables.

Levenes test was performed for the homogeneity of our data. ($p<0.05$)

Two-way ANOVA was performed with treatment and time as factors (levels).

All statistical analyzes and graphs were performed with the GraphPad Software Prism 8.0® program for information processing with a value of $p\leq0.05$.

RESULTS

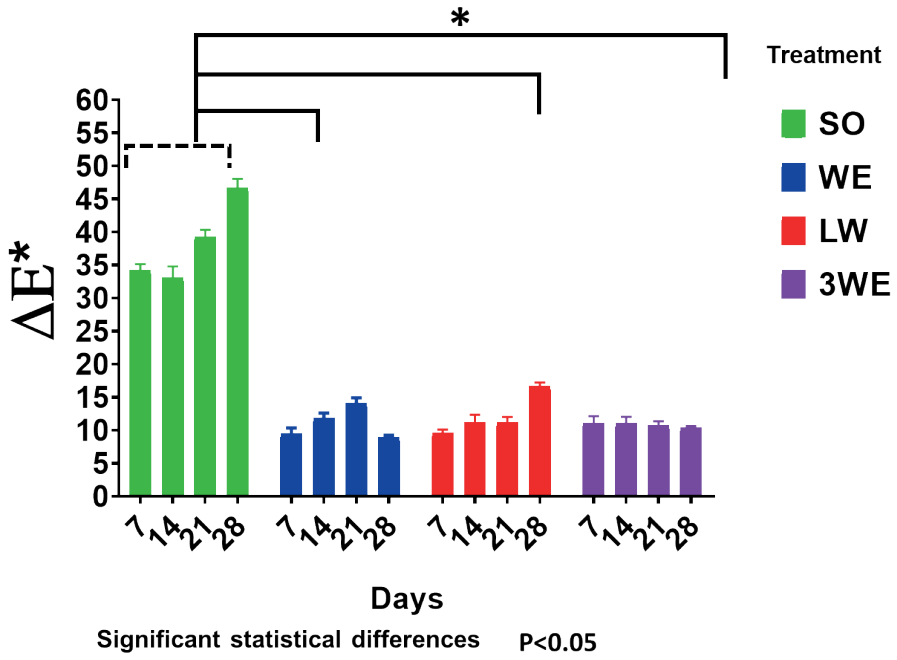
COLOR

The color results were obtained at 7, 14, 21 and 28 days of treatment, taking the color of the samples by placing the colorimeter on the sample, each group was always compared to the healthy enamel group (ES). The coordinates were recorded using the colorimeter software, which represents the Cielab® color system. Both standard coordinates of the healthy enamel group and of the tested samples were observed.

To obtain the color difference, the numerical comparison of a sample with the standard coordinates must be made, indicating the differences in absolute color coordinates,

Luminous White COLGATE®	Sodium fluoride 0.05%, water. Glycerin, Propylene Glycol, Sorbitol, Tetrapotassium Pyrophosphate, Polyphosphate 20, Tetrasodium Pyrophosphate, Zinc Citrate, Copolymer, Benzyl Alcohol, Sodium Saccharin, Acid Blue, Dipentene.
3DWhite Oral B®	Water, glycerin, sodium hexametaphosphate, poloxamer 407, sodium bezolate, sodium lauryl sulfate, phosphoric acid, sucralose, saccharin, cinnamon, eugenol.
Whitening Extreme Listerine®	Water, 2.5% hydrogen peroxide, aroma, poloxamer 407, sodium saccharin, menthol, phosphoric acid, 100ppm sodium fluoride, sucralose
Scope Crest®	Active ingredient: 0.05 cetylpyridine hydrochloride, water, alcohol, glycerin, aroma, polysorbate 80, sodium saccharin, sodium benzoate, benzoic acid.

Table 1: Mouthwashes and Ingredients



Graph 1 shows the results of ΔL, Δa, Δb and ΔE of the changes presented by the samples during the cyclical pH for 7,14,21 and 28 days.

known as Delta (Δ). Deltas by L* (ΔL*), a* (Δa*) and b* (Δb*) which can be positive (+) or negative (-). The final result that indicates the total color difference is Delta E (ΔE*), the result of which will always be positive.

The mouthwash used without a whitening agent (SO), obtained values of more than 35, having statistically significant differences with the rinses that do have an added whitening ingredient. The WE mouthwash produced a lower color change when performing the cyclical pH during the 28 days, being positive from the first 7 days of the cyclical pH, followed by the 3DW mouthwash, which remained stable in its ΔE values. Group LW had a whitening effect for the first 14 days, but did not on days 21 and 28, on day 28 beginning to decrease its effectiveness.

In graph 2 the behavior of all experimental groups and control groups, that is, the Healthy Enamel group and the group with Initial Lesion can be observed. The color changes substantially due to the immersion in cola and it can be seen that the whitening mouthwashes have the effect of reducing extrinsic pigmentation compared to the initial lesion, but even so they do not resemble healthy enamel.

ROUGHNESS

Roughness was measured using an optical profilometer, the enamel surfaces of all experimental groups were randomly tested, and 6 observations were obtained from each of the experimental groups; as well as the mean roughness (Ra) and its standard deviation.

All experimental groups presented a loss of superficial enamel, and all differed from each other. Group SO did not have statistically significant differences with the WE group, in contrast it did have them with groups LW and 3DW, followed by group WE, which did not have statistically significant differences with group 3DW. Groups LW and 3DW did present statistically significant differences.

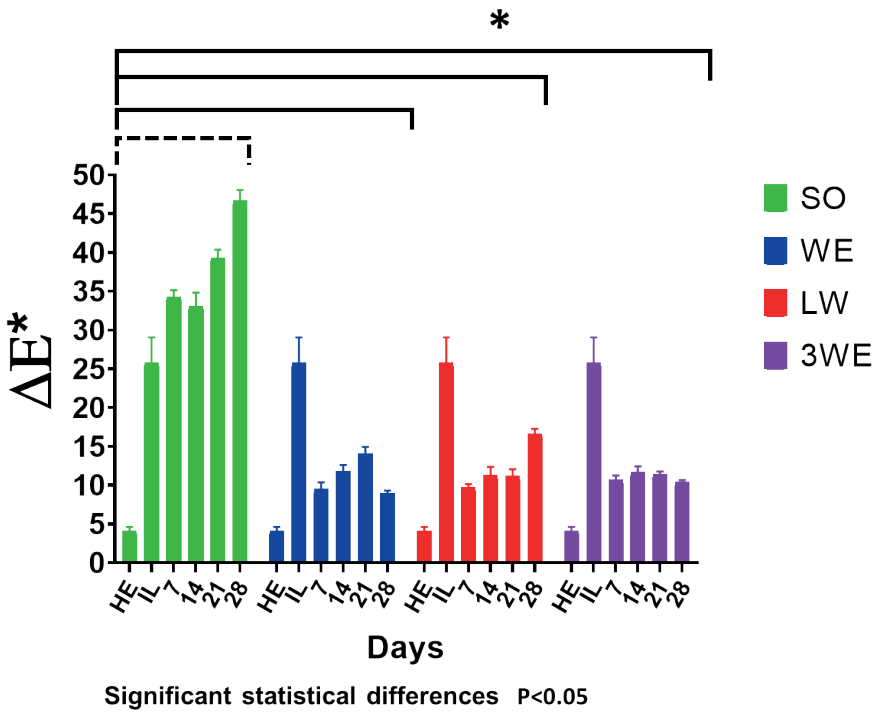
DISCUSSION

The results of this study supported the hypothesis that the use of whitening mouthwashes will modify surface roughness and color of demineralized dental enamel.

In previous studies, such as the one carried out by Muhammet Karadas (71), he used 80 bovine teeth for his study, determining that they had a larger work area; however, in our study, retained mandibular third molars were used, as we intended to obtain the greatest similarity to the oral cavity.

Many substances have been used to produce stains in vitro, reviewing the literature Jaime et al (47) used an Orange II dye (methyl orange) to stain teeth, and thus determine the efficacy of hydrogen peroxide. Roncal Espinoza (74), in his study, applied black tea for three days to homogenize the color of the samples, and to evaluate different whitening rinses. Muhammet Karadas (75) immersed his samples in coffee for 24 hours, to analyze the effect of whitening mouthwashes and toothpastes, in this research, Coca-Cola soft drink was used, as it is the most consumed cola beverage in the world.

In some articles, different methods have been used to simulate the demineralization of dental enamel under cyclic pH conditions. In the study by Cavali (74), the cyclical pH consisted in immersing the specimens for 16 hours in a remineralizing solution with a pH of 7.0 and 8 hours in a demineralizing solution adjusting the pH to 4.5, for 8 days. In this study, wanting to have the greatest similarity to the oral cavity, the cyclical pH consisted of immersing the samples in remineralizing solution for 22.5 hours at a pH of 7 with artificial saliva, since in the oral cavity saliva acts as a remineralizing agent, and in demineralizing solution for 1 hour 30 minutes at a pH of 2.8. Due to the lower pH



Graph 2 shows the multiple comparisons with 2-way ANOVA and the Tukey post-hoc test by groups, the SO rinse did have statistically significant differences with the rest of the rinses, 3DW remained stable from day 7 to day 28, followed by WE and LW.

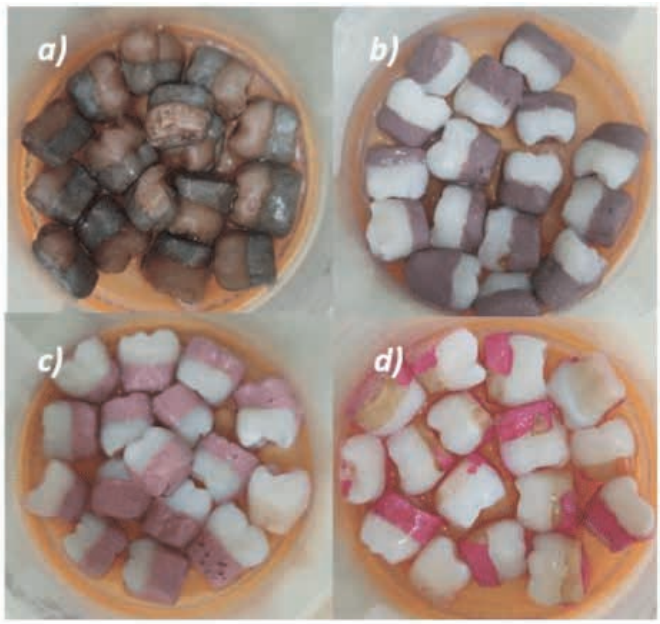


Fig. 1 Photograph of the four groups of oral rinses after treatment A) group Scope Crest (SO), B) group Colgate® Luminous White (LW), C) group Oral B® 3DWhite (3DW), and D) group Listerine® Whitening Extreme(WE).

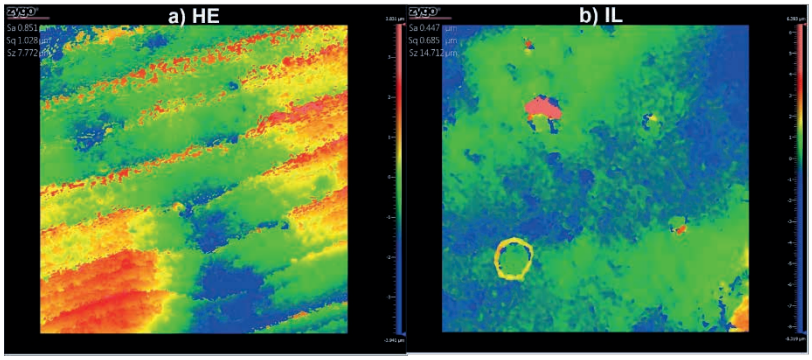


Fig. 2 Roughness image a) healthy enamel (HE), b) initial lesion (IL) or demineralized enamel with cola drink. Direct source.

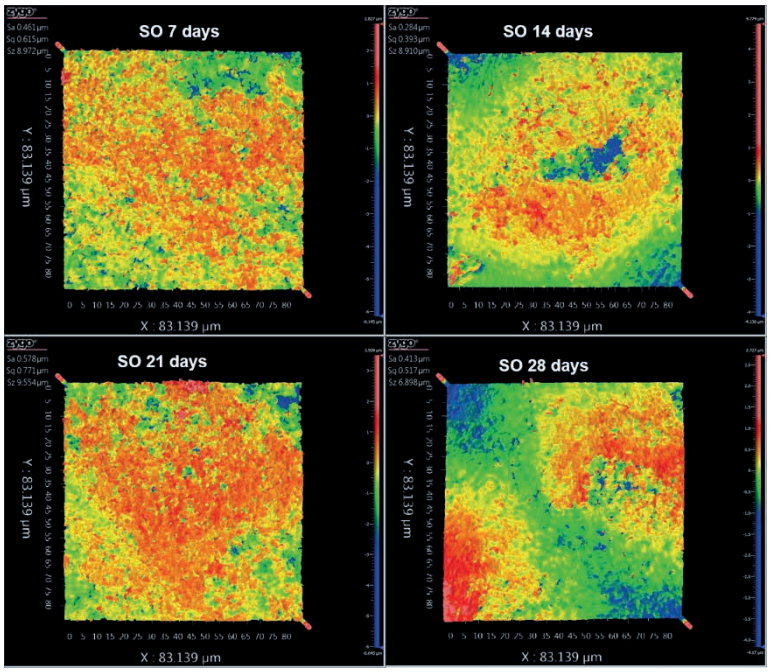


Fig.3 Surface roughness of dental enamel treated with Scope Crest® mouthwash 7, 14, 21 and 28 days. direct source

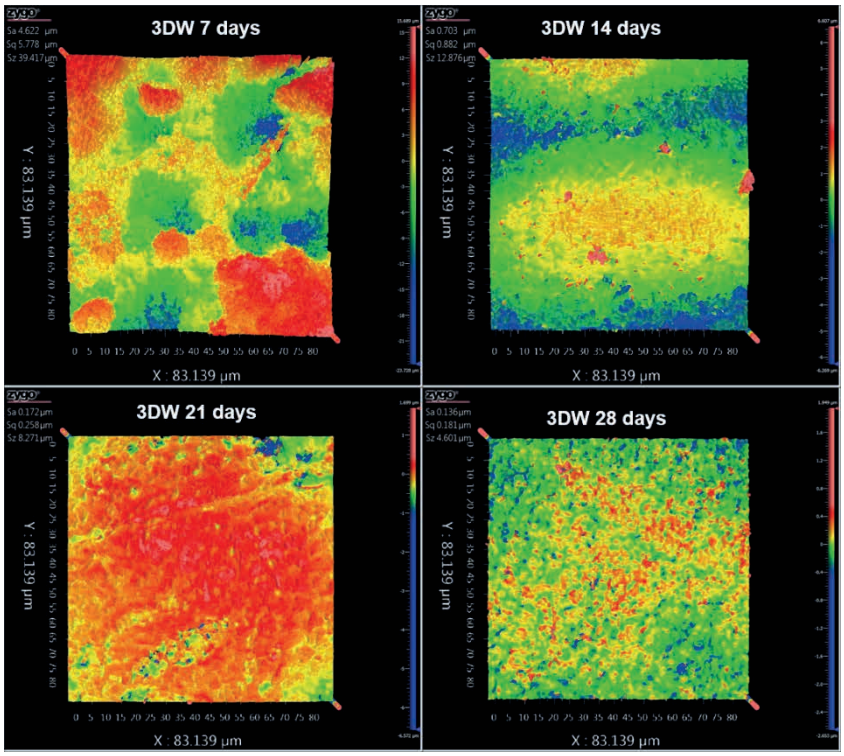


Fig 3 Roughness of dental enamel treated with 3DWhite Oral B® mouthwash 7, 14, 21 and 28 days of treatment. Direct source.

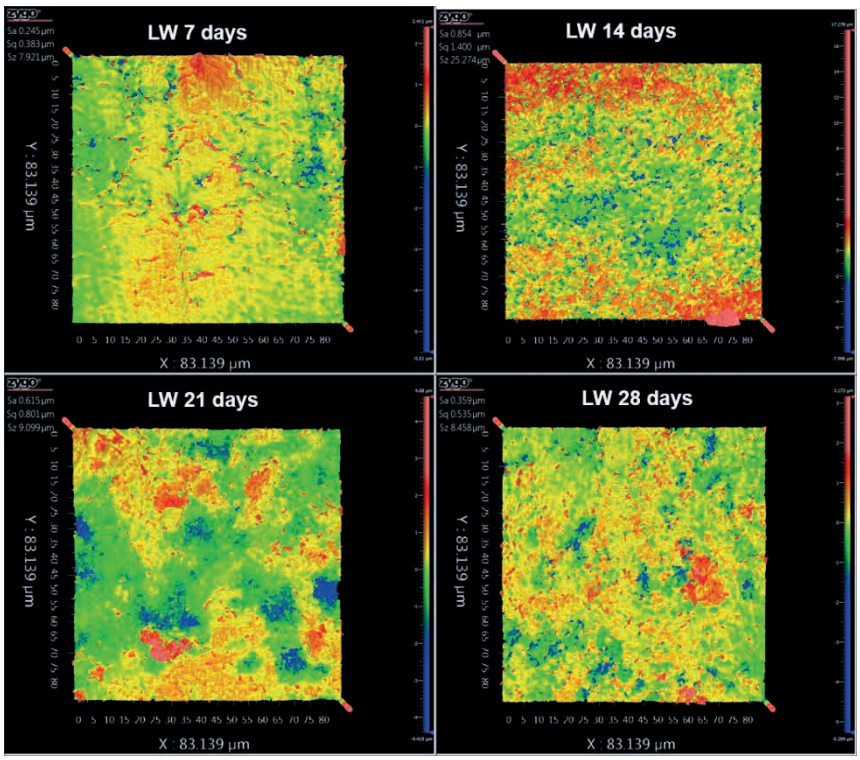


Fig. 5 Roughness of samples treated with Luminous White Colgate® mouthwash 7,14, 21 and 28 days of treatment. direct source.

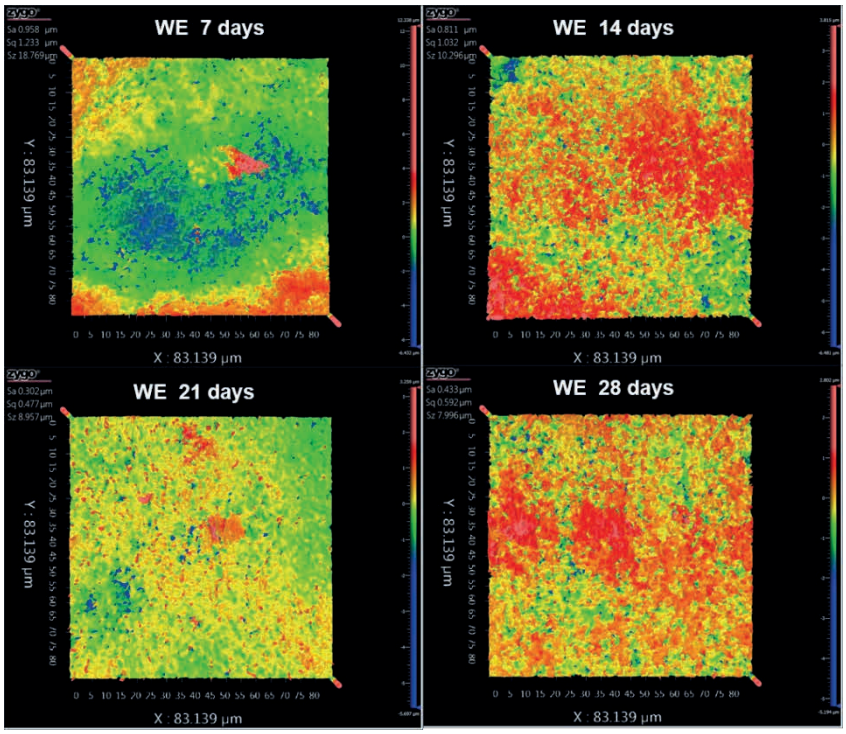
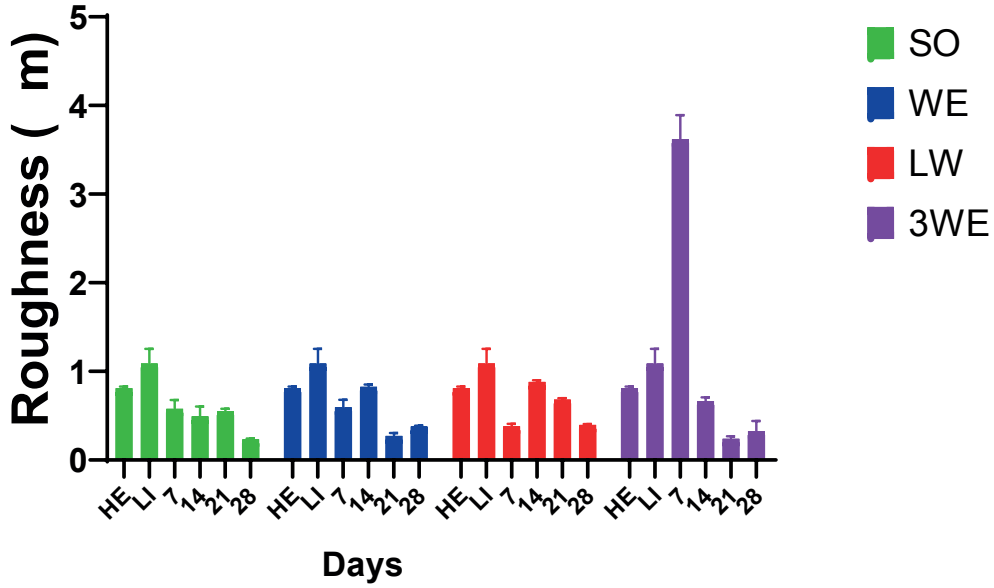
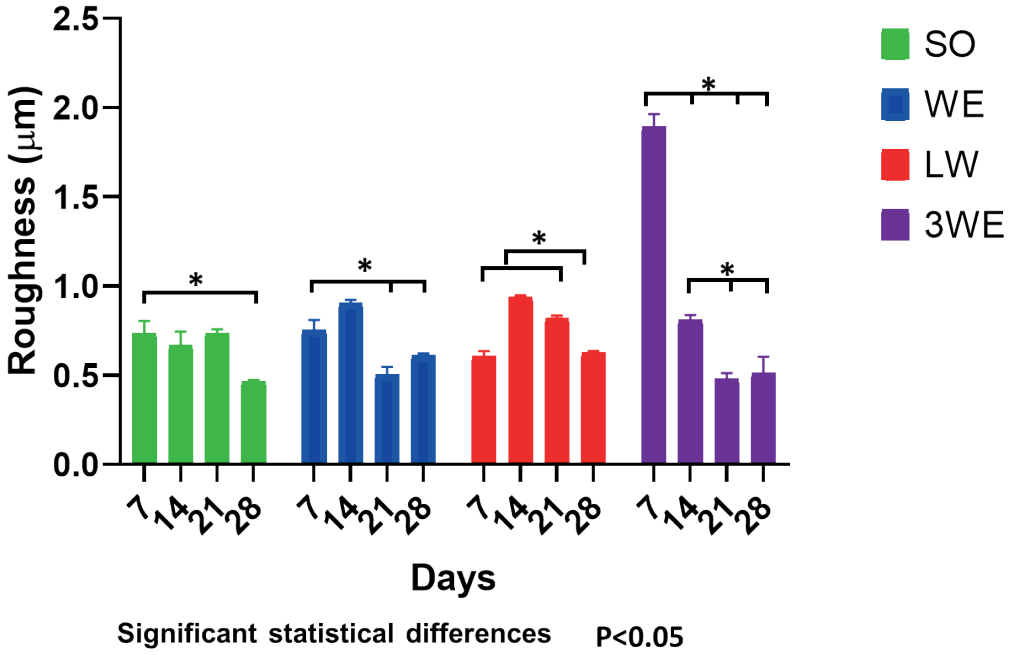


Fig 6. Roughness of samples treated with Whitening Extreme Listerine® mouthwash 7, 14, 21 and 28 days. Direct source.



Graph 3 shows the behavior of the experimental groups with the control groups, Healthy Enamel and Initial Lesion, and the results of the whitening mouthwashes.



Graph 4 shows the results only of the experimental groups and their significant differences between them. The distribution of the results of the roughness of the different experimental groups, where there were significant differences in the 3DW group at 7 days, and WE at 28 days are observed.

the samples were exposed for shorter times, this cyclical pH was carried out for 7,14,21 and 28 days.

Torres A. (74) mentioned that dental enamel samples in a demineralizing solution such as lactic acid at pH 3 artificially formed the incipient lesion of caries or clinically known as white spot in a period of 3 hours; while Chicaiza and Navarrete, (43), reported that their enamel samples in demineralizing solution of lactic acid at pH 2 artificially formed the white stain in a period of time of 50 min; However, when examining the dental enamel, they observed a total crumbling of the surface.

In the article by Santos (44), he mentions that all enamel samples used in the experimental study were demineralized with acid hydroxyethylcellulose, following the cyclical pH model. In this work, cola was used as it is the most consumed soft drink in the world. In addition, the whitening mouthwashes most purchased in shopping centers by consumers who, in addition to having the habit of ingesting carbonated beverages, also smoke.

Yamamoto (27) in his in vitro experimental work compared three types of drinks Coca Cola®, Coca Cola Cero® and Gatorade® on the surface of dental enamel, concluding that Coca-Cola® showed greater erosive effects and the greater the number of cycles exposed to Coca-Cola®, the greater the influence on the dental erosive process. In a study carried out by Van Eygen et al (30) they determined that even for a brief time the consumption of soft drinks can affect the structure of tooth enamel. In this experiment, Coca-Cola was used as a demineralizing solution due to its pH of 2.8, causing staining of the dental enamel and affecting the Scope mouthwash group the most, which did not contain any bleaching agent.

Favaro (36) in his study evaluated the color, roughness and microhardness of the enamel, where he used various mouthwashes including LW and WE and determined that all groups had a loss of superficial enamel. In his study he used distilled water to store samples, because if artificial saliva was used, a protective salivary film could form, which could influence the roughness values. In this study, artificial saliva was used because the objective was to reproduce oral conditions, in a pH cycle.

In her roughness results, Favaro (36) mentions that the greatest loss of superficial enamel was caused by the WE, followed by the LW. In contrast, in these results, the greatest loss of superficial enamel was caused by the 3DW mouthwash followed by of the WE and LW.

It would be decisive to continue with the investigation on some properties such as the hardness of the enamel treated with whitening rinses, since, although the color improves, that is, it eliminates the extrinsic pigmentation of the enamel, the change in the roughness of the surface is an indicator of erosion, consequence of the use of whitening mouthwashes.

CONCLUSIONS

The use/application of the three mouth rinses with whitening ingredients such as hydrogen peroxide, sodium pyrophosphate and sodium hexametaphosphate do eliminate extrinsic pigmentations, that is, they whiten demineralized dental enamel with cola soda.

The mouthwash with the greatest whitening effect was Listerine® Whitening Extreme (LW) which contains hydrogen peroxide at 2.5%, followed by 3DWhitening oral B® (3DW) with sodium hexametaphosphate, which is used for dental whitening, preventing extrinsic stains on the enamel, which maintained its efficacy from day 7 to day 28.

The mouthwash with sodium pyrophosphate Luminous White Colgate®

(LW) is effective in dental whitening only the for the first 14 days, losing effectiveness on day 28.

The use of whitening mouthwashes does modify the surface roughness of the dental enamel. The 3DW mouthwash obtained a constant whitening effect from day 7 to day 28 of treatment, however, it was the roughest due to the mineral loss of the enamel.

The mouthwash WE obtained a similar whitening effect to 3DW, but it obtained less roughness, followed by the LW mouthwash, which presented a minor whitening effect and less roughness, that is, less affectation on the enamel.

DISCLOSURE STATEMENTS

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

ACKNOWLEDGEMENTS

This work was supported by the DGAPA-PAPIIT IA-200421 and IA-202423 project.

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