10.5937/bjdm2302073K

BALKAN JOURNAL OF DENTAL MEDICINE

ISSN 2335-0245

# In vitro Evaluation of the Effect of Ozonated Olive Oil Containing Dentifrice on Enamel Color Change and Microhardness

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SUMMARY

***Background/Aim:*** *Nowadays, people have an insatiable desire for white and beautiful teeth. The present in vitro study aimed to evaluate the effect of experimental toothpaste containing ozonated olive oil commercial whitening toothpaste and ozone-free olive oil toothpaste, on color change and enamel microhardness.* ***Material and Methods:*** *In this in vitro study, 48 bovine incisors were mounted in an acrylic resin. After the immersion of samples in tea solution, each sample was subjected to the Vickers microhardness test and colorimetry (T1). The samples were then randomly assigned to four groups (n=12) and brushed twice a day for two min with an electric toothbrush with toothpaste specific to each group; Group 1: commercial whitening toothpaste (positive control), Group 2: Experimental toothpaste containing ozone-free olive oil , Group 3: Experimental 10 µg/ml ozonated olive oil toothpaste, Group 4: Experimental toothpaste containing ozonated olive oil with an ozone concentration of 15 µg/ml. Surface microhardness measurements and color evaluation were repeated after two (T2) and four weeks (T3). Data were analyzed by, repeated measures analysis of variance, Friedman, ANOVA, and Kruskal-Wallis.* ***Results:*** *The highest mean of color change (ΔE) was related to toothpaste containing 15 µg/ml ozonated olive oil, and the lowest mean pertained to toothpaste containing ozone-free olive oil (P=0/019). In these four groups, the amount of microhardness increased after the end of the study (P=0.001); nonetheless, these changes were not significant.* ***Conclusions****: The addition of ozone to toothpaste in a specific concentration results in teeth whitening; however, it does not significantly affect enamel microhardness.*

**Keywords:** Color change, Dental enamel, Dentifrice, Enamel microhardness, Ozonated olive oil

**ORIGINAL PAPER (OP)**

**Balk J Dent Med, 2023;73-77**

## Introduction

Nowadays, people have an insatiable desire for white and beautiful teeth[1], therefore various chemical compounds have been tested to whiten teeth. The most effective one's release oxidizing agents, whether they are hydrogen peroxide, carbamide peroxide, sodium perborate, or other compounds[2]. Every day, various treatments are offered to improve the color of teeth; nonetheless, since patients show a great desire for over-the-counter medications, the use of whitening types of toothpaste is widespread and popular. Whitening toothpaste can cause serious complications, such as increased tooth sensitivity, gingival irritation, increased enamel surface roughness, demineralization, and discoloration of dental restorations[3].These dentifrices whiten teeth act with different physical and chemical mechanisms[4]. Toothpaste containing abrasives may contain mica, calcium carbonate, calcium pyrophosphate, dicalcium phosphate, sodium carbonate, hydrated silica, and perlite-alumina[5]. The mechanism of these materials is based on abrasiveness; that physically remove the external colors of the teeth. The regular use of these kinds of toothpaste (for example, twice a day) will make teeth

whiter after 2-4 weeks. Nevertheless, the abrasiveness of the toothpaste should be balanced to avoid excessive wear of the tooth surfaces[6]. A number of chemical components used in toothpaste formula include surfactants, calcium, enzymes, polymers, oxidizing agents (including carbamide peroxide 10% and hydrogen peroxide)[7], and blue covarine which help to remove colors or prevent the settling of colors with different mechanisms[8].

Ozone has been used in various fields of dentistry, including periodontology, endodontics, and maxillofacial surgery due to its strong antimicrobial properties without increasing drug resistance and facilitating wound healing in the oral cavity[9, 10-18]. Baysan *et. al* demonstrated the safe use of this compound in the oral environment[19]. The antimicrobial effects of ozone are focused on breaking the integrity of the cell membrane through the oxidation of phospholipids and lipoproteins, ozone penetration through the cell membrane, reaction with the cytoplasmic content, and the conversion of closed circular plasmid DNA to open circular DNA[20, 21].

Since ozone is in gas form, it must be used with a carrier in order to be used in the oral cavity. So far, various carriers have been used for ozone, with water and oil being more common than others[19,22.] When ozone dissolves in water, it quickly disintegrates and becomes unstable through a complex set of reactions; therefore, it cannot be stored, while the average lifespan of ozone dissolved in oil is so sufficient that it can be stored for up to 2 years[21]. Ozone chemically reacts with oil and converts the double bonds of carbons into single bonds and create long molecule and superoxide, which is stable, and its ozone is released over time in the vicinity of the tissue. Ozone oil is one of the relatively common methods used in ozone therapy[23].

Today, ozone compounds are widely used in dentistry. Therefore, in light of the aforementioned issues, the present study aimed to evaluate the effect of ozonated olive oil containing dentifrice on enamel color change and microhardness in vitro.

## Material and Methods

This in vitro study was conducted on freshly extracted, undamaged, and intact 48 bovine incisors which were assigned to four groups (n= 12). The selected teeth, evaluated under a stereomicroscope (Dino lite Pro; Anmo Electronics; Taiwan) in terms of cracks, enamel defects, and caries. They were stored in 0.1% thymol solution until required.

Firstly, the crown of each tooth was separated from the root by a trimmer, and each specimen was mounted in self-curing acrylic resin (Acropars, Marlic Co., Tehran, Iran) in such a way that the surface of the tooth was parallel to the horizon. Sheets of sandpaper (100 to 600 grit) were used along with water flow to create a smoother and more polished enamel surface.

The mounted samples were placed in tea bag solution (Golestan, Iran) for two weeks to cause color change; subsequently, the samples were numbered, and each sample was tested with the Vickers microhardness test. The color evaluation was performed by calorimetry (T1). In order to increase accuracy, the microhardness test was repeated three times for each sample, and their average was recorded; moreover, color evaluation was recorded with a, b, and L variables.

After the initial recording of surface microhardness (H1) and initial color (E1), each group was brushed by Oral-B professional care 3000 electric toothbrush with a pea size amount of special toothpaste for each group twice a day (morning and evening, about 8 hours apart) for two min each time. This toothbrush rotates at a speed of 8800 revolutions per min with a vibration speed of 40000. It has a timer, and every 30 sec., it will announce with a pulse to remind the time to change the brushing point.

The toothpaste used in each group was as follows:

Group 1: Aquafresh intense clean whitening commercial toothpaste (positive control)

Group 2: Experimental toothpaste containing ozone-free olive oil (negative control)

Group 3: Experimental toothpaste containing ozonated olive oil with an ozone concentration of 10 µg/ml

Group 4: Experimental toothpaste containing ozonated olive oil with an ozone concentration of 15 µg/ml

Experimental toothpaste was manufactured by Rajabi Pharmaceutical Company with registration number 48430. Before and after brushing, the teeth were washed with water and kept in artificial saliva with neutral pH in the intervals between brushing. Surface microhardness measurement and color evaluation were repeated after two weeks (H2 – E2) and four weeks (H3- E3). The overall color change (ΔE) was calculated using the formula $\Delta E=\sqrt{(a2 - a1)2 + (b2 - b1)2 + (L2 - L1)2}$ for each tooth.

### *Statistical Analyses*

Shapiro-Wilk tests, analysis of variance with repeated measures, Friedman, one-factor analysis of variance, and Kruskal-Wallis were used in data analysis. ($\alpha$= 0.05)

## Results

In this in vitro study, 48 dental samples were assigned to four groups of 12 in terms of tooth color components L, a, and b, and overall color changes. The changes in each variable were compared over time and also at any time between the four groups. We defined the

calculated ΔE as follows: $\Delta E_{1-2}$: delta of color changes between the first and second stages, $\Delta E_{1-3}$: delta of color changes between the first and third stages, and $\Delta E_{2-3}$: delta of color changes between the second and third stage. The normality of data distribution was checked by the Shapiro-Wilk test.

A) Comparison of L, a, b

The data of L, a and b component were presented in table 1. Although, in groups 1, 3 and 4 the mean L significantly increased from E1 to E3 (P<0.001), in the toothpaste containing ozone-free olive oil, the mean L was not significantly increased from E1 to E3 (P= 0.097) (Table1).

In groups 1,3 and 4, the mean E1, E2 and E3 consistently decreased, and the mean "a" had significant changes among the three time periods (P<0.001). In the toothpaste containing ozone-free olive oil, the mean "a" decreased from E1 to E2 but increased from E2 to E3; moreover, the mean had significant changes among the three time periods (P<0.001) (Table 1).

In group1, 2 and 3 the mean of b was reduced from E1 to E3; moreover, the mean of b had significant changes among the three time periods (P<0.001 for each). In group 4, the mean of b decreased from E1 to E2 and then increased from E2 to E3; moreover, the mean of b had significant changes among the three time periods (P<0.001) (Table1).

*Table 1. Mean and standard deviation of L, a, b component in each group according to different study times*

| Toothpaste | Time | L component | | A component | | B component | |
|---|---|---|---|---|---|---|---|
| | | Mean | Sd | Mean | Sd | Mean | Sd |
| Aquafresh | E1 | 55.89 | 2.26 | 3.46 | 1.46 | 15.62 | 4.39 |
| | E2 | 64.36 | 4.83 | 0.43 | 1.82 | 13.73 | 4.26 |
| | E3 | 62.51 | 5.48 | 0.13 | 1.53 | 10.70 | 2.64 |
| Ozonated10 micrograms per milliliter | E1 | 56.17 | 2.05 | 3.17 | 1.59 | 14.13 | 3.54 |
| | E2 | 64.29 | 3.76 | -0.02 | 1.63 | 13.56 | 3.37 |
| | E3 | 65.20 | 3.11 | -0.017 | 1.41 | 10.56 | 2.34 |
| Ozonated 5 micrograms per milliliter | E1 | 55.45 | 2.05 | 3.77 | 1.54 | 15.40 | 3.76 |
| | E1 | 61.53 | 2.39 | 0.22 | 0.88 | 10.21 | 1.28 |
| | E3 | 64.62 | 1.81 | -0.12 | 0.77 | 10.28 | 1.46 |
| Contains olive oil without ozone | E1 | 55.71 | 2.64 | 3.55 | 1.54 | 14.62 | 3.64 |
| | E2 | 554.99 | 5.40 | 0.64 | 1.42 | 10.40 | 2.13 |
| | E3 | 57.47 | 5.14 | 0.70 | 1.32 | 9.68 | 1.79 |

*Table 2. Comparison of color change at different times among different types of toothpaste*

| Variable | Toothpaste | n | Mean | Standard deviation | Result of ANOVA test |
|---|---|---|---|---|---|
| $\Delta E_{12}$ | Aquafresh | 12 | 9.45 | 4.46 | F=1.04 P=0.385 |
| | Ozonated 10 µg/ml | 12 | 9.20 | 3.97 | |
| | Ozonated 15 µg/ml | 12 | 9.31 | 2.27 | |
| | Contains olive oil without ozone | 12 | 7.32 | 2.40 | |
| $\Delta E_{13}$ | Aquafresh | 12 | 10.18 | 2.78 | F=3.68 P= 0.019 |
| | Ozonated 10 µg/ml | 12 | 10.72 | 2.96 | |
| | Ozonated 15 µg/ml | 12 | 11.50 | 2.34 | |
| | Contains olive oil without ozone | 12 | 8.15 | 2.18 | |
| $\Delta E_{23}$ | Aquafresh | 12 | 3.83 | 1.84 | F=2.57 P=0.066 |
| | Ozonated 10 µg/ml | 12 | 3.61 | 1.46 | |
| | Ozonated 15 µg/ml | 12 | 3.16 | 1.58 | |
| | Contains olive oil without ozone | 12 | 2.23 | 1.18 | |

### *Comparison of color changes (ΔE)*

The mean of color changes in $\Delta E_{1-2}$, as well as $\Delta E_{2-3}$, were not significantly different. In a two-by-two comparison of the types of toothpaste, it was found that the mean of $\Delta E_{1-3}$ in the toothpaste containing 15 µg/ml ozone was significantly higher than that in the toothpaste containing olive oil without ozone (P= 0.019). Moreover, no significant difference was observed between other types of toothpaste (P= 0.385 and P= 0.066, respectively). The mean color changes in $\Delta E_{1-3}$ were significantly different among the different types of toothpaste (P= 0.019) (Table 2).

### *C) Comparison of microhardness*

In the Aquafresh toothpaste group (group 1) and in 10 µg/ml and 15 µg/ml ozonated types of toothpaste, (group 3, 4) the mean of microhardness increased from H1 to H3; moreover, in each group, the mean of microhardness displayed significant changes among the three periods (P< 0.001 for each, Table 3).

*Table 3. Mean and SD of the micro hardness component in each study group according to different study times*

| Toothpaste | Time | n | Mean | Standard deviation |
|---|---|---|---|---|
| Aquafresh | Before experiment | 12 | 243.83 | 25.53 |
| | Two weeks later | 12 | 295.50 | 19.25 |
| | Four weeks later | 12 | 310.79 | 33.50 |
| Ozonated10 µg/ml | Before experiment | 12 | 224.85 | 35.73 |
| | Two weeks later | 12 | 298.00 | 20.90 |
| | Four weeks later | 12 | 281.13 | 14.68 |
| Ozonated15 µg/ml | Before experiment | 12 | 262.53 | 39.13 |
| | Two weeks later | 12 | 306.88 | 25.28 |
| | Four weeks later | 12 | 287.60 | 29.28 |
| Contains olive oil without ozone | Before experiment | 12 | 226.42 | 44.13 |
| | Two weeks later | 12 | 309.99 | 16.65 |
| | Four weeks later | 12 | 283.12 | 35.06 |

## Discussion

As evidenced by the results of the present study, the overall color changes (ΔE) increased in all groups. The highest mean of color change pertained to toothpaste containing ozonated olive oil with a concentration of 15 µg/ml, while the lowest mean of color change was related to ozone-free olive oil toothpaste. In this study, the amount of microhardness increased after the end of the experiment in all groups; nonetheless, these changes were not significant. As a result, in general, these types of toothpaste did not have a substantial effect on the enamel microhardness.

In agreement with the results of the studies by Al-Omiri *et al*.[7,24], in the present research, the mean of L in all types of toothpaste generally increased; therefore, the color of the teeth has become lighter, except for the toothpaste containing ozone-free olive oil that does not significantly change L. This can be ascribed to the presence of whitening compounds in ozone and Aquafresh types of toothpaste and their durability on the tooth surface not only after brushing and mechanical wear but even after a month.

The values of Aquafresh and ozone types of toothpaste with a concentration of 10 µg/ml and 15 µg/ml in each step were reduced compared to the previous step. This finding is in line with those reported by Al-Omiri *et al*.[25] and inconsistent with another study conducted by these researchers in the same year in which a factor did not change in the ozonated group[26].

In accordance with the studies by Al-Omiri *et al.*[7,26,27], the b factor in all types of toothpaste decreased from each stage to the next, except for the toothpaste containing olive oil. Ozone with a concentration of 15 µg/ml increased slightly from the second to the third stage. However, in general, component b decreased during the study; so, the color of the teeth became brighter.

The comparison of ΔE at the beginning and end of the study pointed to a significant difference between the group of toothpaste containing 15 µg/ml ozonated olive oil toothpaste and ozone-free olive oil. This difference is the result of the sum of the differences immediately after bleaching and the changes made after a time. The difference in ΔE between these two groups was about 3.5 units, which is clear and obvious[28]. Although there was no statistically significant difference among the group of 15 µg/ml ozonated olive oil toothpaste, the group of 10µg/ml ozonated olive oil toothpaste, and Aquafresh, the overall color change in the group of 15 µg/ml ozonated olive oil toothpaste was more than one unit of the Aquafresh group. This finding is contrary to that reported by Mousavi *et al*., who indicated that toothpaste containing ozonated olive oil and Aquafresh had similar whitening effects[2]. This discrepancy in results can be attributed to differences in the concentration of ozone in the types of toothpaste assessed in these two studies.

In this study, microhardness increased in all steps only in Aquafresh toothpaste, and in other toothpastes, an increase in microhardness was seen only from the first to the second steps, and this factor decreased from the second to the third steps, but still the average was higher than at the beginning of the study.

In spite of the fact that in some past studies, most of the samples were kept in normal saline and water in the follow-ups, in this study, the samples were kept in artificial saliva solution with neutral pH, which caused different results in the amount of changes in the microhardness component. This has made the study conditions more similar to the oral environment and clinical conditions. So, although it may reduce the effect of toothpaste, it is more valuable from a clinical point of view.

The deposition of fat particles was observed in types of toothpaste containing olive oil due to their greasy properties, exerting an effect on the color of the teeth. The results would have been more accurate if we had selected larger sample size. It would be better to design a device as a holder for the toothbrush so that the force applied by the toothbrush is the same on the surface of all samples.

## Conclusions

The whitening role of ozone in the composition of toothpaste made with the formulated concentration was demonstrated in this study, and it was found that ozone, like other tooth whitening agents, is able to be effective in removing pigments with the help of its oxidizing agent. The whitening role of the toothpaste containing ozonated olive oil cannot be attributed only to its surface wear. In the ozone-free toothpaste, the application method was utterly similar to the toothpaste containing ozonated olive oil; nonetheless, the whitening degree and its effects were different. The addition of ozone to toothpaste at doses of 10 and 15 µg/ml did not significantly affect enamel microhardness based on this research.

**Acknowledgments.** This study was derived from a student thesis and supported financially by the vice chancellor for research of the Mashhad University

of Medical Sciences (grant number: 971766). It also was approved by the ethics committee of the Mashhad University of Medical Sciences, Mashhad, Iran.

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**Received on March 10, 2023.**
**Revised on Jun 11, 2023.**
**Accepted on Jun 20, 2023.**

**Conflict of Interests:** Nothing to declare.
**Financial Disclosure Statement:** Nothing to declare.
**Human Rights Statement:** All the procedures on humans were conducted in accordance with the Helsinki Declaration of 1975, as revised 2000. Consent was obtained from the patient/s and approved for the current study by national ethical committee. This research was presented on 4/16/2018 in the Ethics Committee of the Faculty of Dentistry, Mashhad University of Medical Sciences, with the code of ethics committee (IR.MUMS.DENTISTRY.REC.1398.035).
**Animal Rights Statement:** None required.

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