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ORIGINAL ARTICLE

Investigation of the bleaching efficiencies of different office type bleaching techniques and the changes caused on the enamel surface

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Abstract

The aim of this in vitro study is to evaluate the bleaching efficiency of 5 different office bleaching methods and the changes in enamel morphology after bleaching. In this study, 75 human molar teeth are used. The teeth are divided in half in the mesiodistal direction, and a total of 150 enamel samples are obtained. The obtained samples are split into 3 main groups to evaluate the bleaching efficiency, surface roughness, and enamel surface hardness, and each group is composed of 50 samples. Then, each main group was divided into 5 separate subgroups ($n=10$) containing 5 varied bleaching techniques. Before the bleaching, color measurement with a spectrophotometer, surface roughness with a profilometer, and microhardness measurement with Vickers test device are performed. After that, different bleaching procedures are applied to the 5 subgroups formed. As a result of the statistical evaluation, it is found that there is a significant level of bleaching in all groups ($p<0.05$). In the comparison between the groups, there is no remarkable divergence in terms of hardness and roughness levels ($p^{>}0.05$). In light of the findings obtained from our study, we suggest that ozone can be used as an alternative bleaching agent to hydrogen peroxide. In addition, it is discovered that the use of light activation is not necessary to increase bleaching effectiveness. Finally, we believe that enamel surface morphology may be affected after office bleaching methods; therefore, various precautions should be taken before and after bleaching.

Keywords Diode laser · Ozone · Bleaching efficiency · Microhardness · Surface roughness

Introduction

Esthetic dentistry's main goal is to give people an ideal smile. An ideal smile contributes to a better role in people's social lives and makes them psychologically more self-confident and happy. There are many components of an esthetic smile. The dimensions of the teeth to each other, the appearance of the lips, the relationship between the teeth and the gums, and the harmony of the teeth with the midline of the face can be listed. However, color changes that may occur in the teeth are another important factor affecting an esthetic smile. Many people have become increasingly interested in whiter teeth in recent years to achieve a healthier and younger appearance [1].

Tooth bleaching can be achieved through many methods and approaches. These include the use of different bleaching agents and the activation of bleaching agents at different concentrations and with different techniques [2].

In office bleaching applied in dental clinics under the supervision of a dentist, activation tools such as heat, light, and lasers are used to accelerate the chemical reaction to increase bleaching efficiency and shorten the procedure time [3]. Halogen polymerization lamps, plasma arc units, LEDs, and lasers are currently used as heat and light sources [4].

Although bleaching is a conservative and safe method, chemical agents can cause a decrease in hardness, roughness, and mineral change on the enamel surface during the oxidation and elimination of discolored tissues on the teeth [5]. These negative effects of bleaching agents on dental tissues are known to cause bacterial plaque to adhere to the tooth surface, resulting in increased caries and periodontal

Clinical significance Although ozone is an alternative to H_2O_2 in terms of whitening, it is similar in terms of the changes it creates on the enamel surface.

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diseases. Different bleaching methods have been developed in recent years to reduce these negative side effects. Ozone bleaching is one of these methods. Ozone, which is used in many fields in dentistry, can be used for bleaching due to its high oxidizing properties. Ozone is reported to be fast, effective, and harmless in tooth bleaching and not to cause negative changes on the enamel surface [6].

There are many options for assessing the efficiency of bleaching. The major ones are color scales, colorimeters, and computer-aided analysis methods. Color is perceived subjectively as color measurements are affected by environmental factors. Studies have shown that the three-dimensional color is more objective and gives more accurate results. For these reasons, studies mostly utilize three-dimensional digital applications [5].

The null hypothesis of this study was that bleaching procedures using different office bleaching techniques do not differ in their bleaching efficiency and in vitro changes on the enamel surface.

Materials and methods

Ethical committee approval was obtained from the non-interventional clinical research ethics committee before the study. The study protocol is designed as shown in Table 1.

Collection and preparation of samples

In our study, extracted 75 human molar teeth for surgical and periodontal reasons were used. Enamel surfaces were carefully examined for caries, fractures, cracks, and abrasions after the attachments on the teeth were removed with the help of a scaler. The obtained teeth were kept in + 4 degrees distilled water until the time of the study. The root parts of the teeth were removed with a diamond bur and aerator, and the crowns were divided in the mesiodistal direction with diamond separators under water cooling. In this way, we obtained 150 enamel samples from 75 teeth. The enamel samples obtained were embedded in cold acrylic in ready-made Teflon molds with the enamel surfaces exposed, and acrylic blocks were obtained. Smooth surfaces were obtained by sanding the samples in an acrylic block with an 800–1000-1200 grit sanding machine to standardize the enamel surfaces. To ensure standardization, the prepared samples were kept in coffee for 48 h.

Table 1 Sequencing of study steps

Study protocol	1. Collection and preparation of samples
	2. Formation of groups
	3. Surface roughness, color, and microhardness measurement of samples before whitening
	4. Application of whitening procedures
	5. Surface roughness, color, and microhardness measurement of samples after whitening
	6. Statistical analyses

Formation of groups

The design of our study was to evaluate the bleaching efficiency of 5 different bleaching protocols and the changes they produce on the enamel surface. To this end, 150 enamel samples were divided into 3 main groups bleaching efficiency, surface roughness, and enamel surface hardness, and each group consisted of 50 samples. Each main group was then divided into 5 separate subgroups ($n = 10$), including 5 different bleaching methods. Subgroups were formed as shown in Table 2.

Experimental measurements before bleaching

Color measurement

A spectrophotometer (Lovibond RT400, UK) was used for color measurement. After placing the samples on a natural gray background, color measurements were taken from the middle third of the teeth under daylight with the end surface of the device positioned parallel to the ground. One measurement was taken for each sample, and the L^*, a^*, and b^* values were recorded for comparison with the values obtained after bleaching.

Surface roughness measurement

For surface roughness, the prepared samples were subjected to a surface roughness test with a profilometer (Surftest SJ-301, Mitutoyo, Kawasaki, Japan) before bleaching.

Table 2 Subgroups formed in the study

Group I	Whitening is implemented only with 40% hydrogen peroxide
Group II	The activation process is implemented with 40% hydrogen peroxide plus an LED light source
Group III	The activation process is implemented with 40% hydrogen peroxide plus a diode laser source
Group IV	Whitening is implemented with ozone gas
Group V	40% hydrogen peroxide and ozone gas are implemented in combination

The profilometer device was standardized with a cut-off of 0.25 mm, a reading length of 1.25 mm, and a speed of 0.05 mm/s. Surface roughness was measured with a needle-scanning tip, and for each sample, measurements were recorded at three separate points, and the average surface roughness (Ra) was determined.

Microhardness measurement

Before the bleaching procedure, the samples prepared for microhardness measurement were measured with a Vickers microhardness tester (EMCO TEST, Durasan, Germany). The enamel surface was exposed to 200 g of force for 10 s, and a pyramid-shaped scar was formed on the tooth surface. The two parallel lines on the microscope in the device were adjusted to be tangent to the corners of the formed scar, and the microhardness value was calculated. It was measured inversely proportional to the size of the indentation formed on the tooth surface. From 3 different points, measurements were taken, and Vickers microhardness value (VHN) was calculated for each sample by averaging the values obtained.

Application of bleaching procedures

All of the bleaching procedures were performed at the Department of Restorative Dental Treatment, Faculty of Dentistry, Adıyaman University. Following bleaching, we grouped all samples and kept them in distilled water until the time of measurement. The bleaching procedures applied to each group are as follows.

Group I (40% hydrogen peroxide)

A 40% hydrogen peroxide containing opalescence boost (Ultradent, South Jordan, UT, USA) bleaching agent was applied according to the manufacturer's instructions. Hydrogen peroxide and an activator in two adjacent syringes were mixed just before the procedure to activate the bleaching agent. An opalescence boost was applied 1–2 mm thick on the enamel surface after activation. The procedure was repeated 2 times by renewing the gel every 10 min. The H_2O_2 gel was in contact with the teeth for a total of 20 min.

Group II (40% hydrogen peroxide plus activation with LED light source)

With a thickness of 1–2 mm, an opalescence boost (Ultradent, South Jordan, UT, USA) containing 40% hydrogen peroxide was applied to cover the entire surface of the samples. They were then activated for 10 min with a 200–300 mW/cm^2 intensity LED (cold light bleaching, Guangzhou, China) bleaching light source with a wavelength of 460–490 nm. The procedure was repeated 2 times, each time replacing the gel with reduced activity. The bleaching process took 20 min in total.

Group III (40% hydrogen peroxide plus activation with diode laser source)

With 1–2 mm thickness, a freshly prepared opalescence boost gel (Ultradent, South Jordan, UT, USA) containing 40% hydrogen peroxide was applied evenly to the enamel surfaces with an applicator. The bleaching gel was then activated for 30 s with a diode laser set to bleaching mode (7 W–940 nm). After waiting for 5 min, laser activation was performed again for 30 s. Laser activation was performed for 2 min for a total of 20 min by repeating the procedure 2 times.

Group IV (ozone bleaching)

Ozone gas was applied to the surface of the prepared samples for 20 min at the sixth power level with the flat probe tip number 3 of the Ozone DTA generator (Ozone DTA, Apoza, New Taipei, Taiwan) providing a gas concentration of 235 ppm (Fig. 1).

Group V (bleaching with the combined use of ozone and hydrogen peroxide)

The sample surface was first treated with 40% hydrogen peroxide, as described in group 1. Ozone gas (Ozone DTA, Apoza, New Taipei, Taiwan) was then applied with flat probe tip number 3, providing a gas concentration of 235 ppm to the tooth surface for 1 min. Peroxide on the tooth surface, whose activity decreases after 10 min, was removed, and the procedure was repeated in the same way with the new gel. In total, the bleaching procedure was completed by applying H_2O_2 for 20 min and ozone gas for 2 min.

The samples after bleaching were measured for color, roughness, and hardness after bleaching, and the data obtained were recorded.

Shapiro–Wilk and Kolmogorov–Smirnov methods were used to test whether the data were normally distributed. While the paired sample *t*-test was used to compare the

Fig. 1 Ozone device used in the study

Table 3 Mean L^*, a^*, b^*, and ΔE values of the groups before and after whitening

Bleaching groups	L^*		a^*		b^*		ΔE
	Before whitening	After whitening	Before whitening	After whitening	Before whitening	After whitening	
H2O2	64.3 ± 2.87	70.97 ± 2.3	2.63 ± 1.28	1.81 ± 1.24	16 ± 1.85	13.11 ± 2.82	7.61 ± 2.87
H2O2 + LED	68.62 ± 4.8	77.18 ± 4.72	2.01 ± 1.29	2.57 ± 1.72	14 ± 1.95	9.08 ± 2.91	6.84 ± 2.92
H2O2 + DİYOT	68.11 ± 2.62	73.12 ± 2.62	2.12 ± 1.63	2.16 ± 1.86	15.85 ± 1.6	9.6 ± 5.21	6.64 ± 2.04
OZON	67.72 ± 3.78	72.27 ± 3.42	2.16 ± 1.15	1.17 ± 1.33	16 ± 2.2	9.6 ± 5.21	7.14 ± 2.55
OZON + H2O2	67.72 ± 3.78	72.27 ± 3.42	2.5 ± 1.6	1.84 ± 1.77	14.43 ± 3.04	10.72 ± 2.26	6.82 ± 1.76

Table 4 Comparison of ΔL^* measurements before and after whitening within groups

ΔL^* (L_A-L_B)	Groups	Mean	SD	SE	t	p
	H2O2	6.58300	2.49008	0.78743	8.360	0.000
	H2O2 + LED	8.55700	5.80782	1.83659	4.659	0.001
	H2O2 + DİYOT	5.00600	2.04708	0.64734	7.733	0.000
	OZON	5.32250	2.80260	0.88626	6.006	0.000
	Ozon + H2O2	4.54600	2.24983	0.71146	6.390	0.000

means of two dependent variables with normal distribution, the Wilcoxon test was used to compare the means of variables with normal distribution. For comparisons of more than two independent groups that conform to the normal distribution, one-way ANOVA was used, and for comparisons of more than two independent groups that differ from the normal distribution, the Kruskal–Wallis H test was used. Post hoc Tukey and H tests were preferred for multiple comparisons between groups. Analyses were performed using SPSS 25.0 package program. $p < 0.05$ was accepted as statistically significant.

Results

In our study, 150 enamel samples were subjected to 5 different vital bleaching treatments. The bleaching efficiency, surface roughness, and microhardness values of the samples before and after the bleaching process were measured, and the data obtained were statistically analyzed.

Evaluation of L*, a*, b*, and ΔE values of spectrophotometric data

After bleaching with different bleaching methods, the color analysis of the samples was performed before and 24 h after the bleaching process using the CIE L^*, a^*, and b^* formulation using a Lovibond RT400, UK spectrophotometer. The average L^*, a^*, b^*, and ΔE values of the groups are shown in Table 3.

When L^* values before and after bleaching were compared, a statistically significant increase was observed in L^* values after bleaching in all groups ($p < 0.05$). When ΔL^* values were compared between groups, no statistically significant difference was found between bleaching methods ($p > 0.05$). Descriptive statistics of L^* values are shown in Tables 4 and 5. Statistical data of a^* values of the groups are shown in Tables 6, 7, and 8.

Table 5 Comparison of ΔL^* values between groups

	Sum squares	SD	Mean squares	F	p
Between-group	104.393	4	26.098	2.288	0.075
Within-group	513.343	45	11.408		
Total	617.735	49			

Comparison of Δa^* values between the groups statistically revealed a significant difference between the groups ($p < 0.05$). Post hoc Tukey test was performed to find out between which groups there was a significant difference. By multiple comparisons, Δa^* values in group 4 were found to be statistically significantly higher than group 2. Comparing the * values before and after intra-group bleaching, a statistically significant difference was found only in group 1 and group 4. Group 1 and group 4 showed a significant decrease in a^* values after bleaching, while no significant change was observed in the other groups.

The findings of b^* values obtained from the study groups are shown in Tables 9 and 10. When the b^* values obtained as a result of the experimental measurements before and after bleaching were compared, it was observed that there was a statistically significant decrease in b^* values after bleaching in all groups ($p < 0.05$). Comparison of Δb^* values between the groups showed that there was no statistically significant difference between the bleaching methods ($p > 0.05$).

Table 6 Multiple comparisons of Δ*a** values between groups

(I) Groups	(J) Groups	Average difference (I–J)	SH	*p*
	H2O2 + LED	1.38200	0.49080	0.053
	H2O2 + DİYOT	0.85400	0.49080	0.421
H2O2	OZON	− 0.18000	0.49080	0.996
	Ozon + H2O2	0.15500	0.49080	0.998
	H2O2	− 1.38200	0.49080	0.053
	H2O2 + DİYOT	− 0.52800	0.49080	0.818
H2O2 + LED	OZON	− 1.56200*	0.49080	**0.021***
	Ozon + H2O2	− 1.22700	0.49080	0.109
	H2O2	− 0.85400	0.49080	0.421
H2O2 + DİYOT	H2O2 + LED	0.52800	0.49080	0.818
	OZON	− 1.03400	0.49080	0.235
	Ozon + H2O2	− 0.69900	0.49080	0.616
	H2O2	0.18000	0.49080	0.996
OZON	H2O2 + LED	1.56200*	0.49080	**0.021***
	H2O2 + DİYOT	1.03400	0.49080	0.235
	Ozon + H2O2	0.33500	0.49080	0.959
	H2O2	− 0.15500	0.49080	0.998
	H2O2 + LED	1.22700	0.49080	0.109
Ozon + H2O2	H2O2 + DİYOT	0.69900	0.49080	0.616
	OZON	− 0.33500	0.49080	0.959

The asterisk emphasizes that there is a statistically significant difference between the two compared groups ($p < 0.05$)

Values in bold indicate a statistically significant difference ($p < 0.05$)

Table 7 Statistical comparison of Δ*a** values between groups

	Sum squares	SD	Mean squares	*F*	*p*
Between-group	17.180	4	4.295	3.566	0.013
Within-group	54.199	45	1.204		
Total	71.379	49			

The *L**, *a**, and *b** values of the samples measured at the beginning and after the bleaching process were fitted to the formula $\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$. The Δ*E* value indicates the total color change; a larger value means a larger color change.

There was no statistically significant difference between the groups in terms of color change (Δ*E*) after bleaching ($p > 0.05$). Δ*E* values obtained from the study groups are shown in Table 11. Figure 2 shows Δ*E* values between groups.

Table 8 Comparison of *a** values before and after whitening within groups

	H2O2	0.81900	0.96285	0.30448	2.690	0.025*
Δ*a** (*a*S-*a*Ö)	H2O2 + LED	− 0.56300	1.33515	0.42221	− 1.333	0.215
	H2O2 + DİYOT	− 0.03500	1.13727	0.35964	− 0.097	0.925
	OZON	0.99900	0.88756	0.28067	3.559	0.006*
	Ozon + H2O2	0.66400	1.10964	0.35090	1.892	0.091

*There is a statistically significant difference ($p < 0.05$)

Table 9 Statistical comparison of Δ*b** between groups

	Sum squares	SD	Mean squares	*F*	*p*
Between-group	23.822	4	5.956	0.880	0.483
Within-group	304.528	45	6.767		
Total	328.350	49			

Surface roughness results

A statistical evaluation of the roughness values obtained before and after bleaching showed a significant increase in roughness values after treatment in all groups ($p < 0.05$). However, there was no significant difference between the groups when the difference between the roughness values before and after bleaching was compared ($p > 0.05$). Considering the average values, it was determined that the highest roughness increase was in group 1.

Surface roughness values and statistical data measured by profilometer before and after bleaching are shown in Tables 12, 13, and 14. Figure 3 shows ΔRa values of the groups.

Microhardness results

Tables 15, 16, and 17 show the mean and statistical data of the Vickers microhardness (VSD) values of the groups whose microhardness was measured before and after bleaching. Analysis of the difference between the hardness values obtained before and after bleaching showed no significant difference between the bleaching methods in terms of microhardness change ($p > 0.05$). In the intra-group comparison, a significant decrease in microhardness values was found in all groups after bleaching treatments ($p < 0.05$). Considering the mean VSD values, the highest decrease was in group 5, and the lowest decrease was in group 4. Figure 4 shows ΔVSD values before and after the bleaching procedures.

Discussion

In the last century, there has been a growing demand for esthetics. Teeth are also part of facial esthetics. On the other hand, white teeth and a pleasant smile come to mind when it comes to dental esthetics. Bleaching agents are available in various concentrations and methods to meet the increasing demand for bleaching. In our study, the

Table 10 Comparison of b^* values before and after whitening within groups

Paired sample test									
Differences									
Groups		Average	SS	SH	95% GA		*t*	SD	*p*
					ALT	ÜST			
H2O2	bSM_Ö-b_SM_S	2.89200	3.07265	0.97166	0.69396	5.09004	2.976	9	0.016
H2O2 + LED	bSM_Ö-b_SM_S	4.91400	3.25297	1.02868	2.58697	7.24103	4.777	9	0.001
H2O2 + DİYOT	bSM_Ö-b_SM_S	4.07300	1.77163	0.56024	2.80565	5.34035	7.270	9	0.000
OZON	bSM_Ö-b_SM_S	4.47900	1.49101	0.47150	3.41240	5.54560	9.500	9	0.000
Ozon + H2O2	bSM_Ö-b_SM_S	3.70800	2.90717	0.91933	1.62834	5.78766	4.033	9	0.003

Table 11 Statistical comparison of color change between groups

	Sum squares	SD	Mean squares	*F*	*p*
Between-group	5.761	4	1.440	0.235	0.917
Within-group	275.943	45	6.132		
Total	281.704	49			

bleaching efficiency and changes in the enamel surface caused by bleaching procedures using different office bleaching techniques were investigated in vitro.

Home bleaching has some advantages, such as the use of low concentrations of bleaching gels and cost-effectiveness, but it also has some disadvantages, such as long application time, soft tissue irritation, the taste of the gel, and the use of trays may not be tolerated by some patients [7]. In the bleaching method applied by the physician in the clinic, although the use of highly concentrated costly gels seems to be a disadvantage, advantages such as protection of the surrounding and soft tissues, obtaining effective results in a very short time and high patient satisfaction and motivation, cause office bleaching to be preferred more frequently

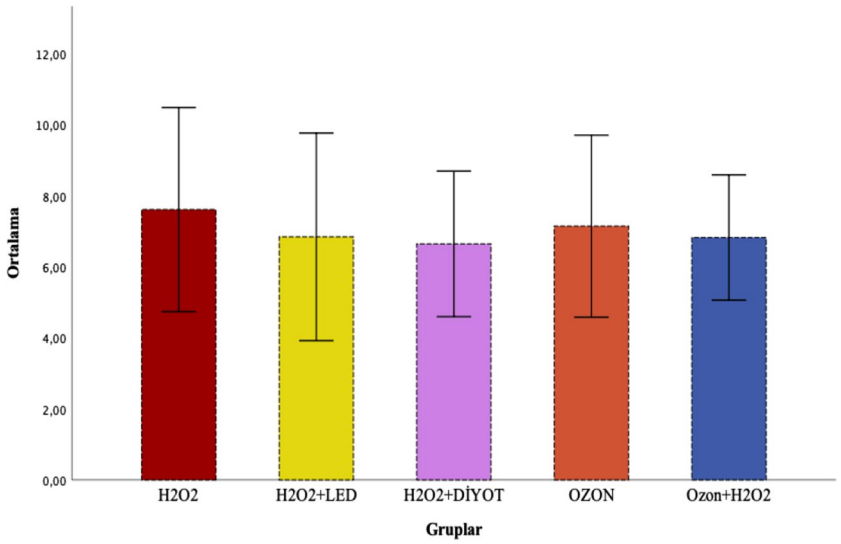

Fig. 2 Graphical representation of ΔE values between groups

Table 12 Average surface roughness values before and after whitening

Groups	Ra values before whitening			Ra values after whitening		
	Mean	Min	Max	Mean	Min	Max
Group 1 ($n=10$)	0.65 ± 0.2	0.41	1.08	0.79 ± 0.25	0.5	1.14
Group 2 ($n=10$)	0.71 ± 0.18	0.48	0.94	0.77 ± 0.21	0.52	1.05
Group 3 ($n=10$)	0.53 ± 0.12	0.38	0.74	0.60 ± 0.11	0.42	0.74
Group 4 ($n=10$)	0.64 ± 0.22	0.42	1.2	0.74 ± 0.3	0.43	1.54
Group 5 ($n=10$)	0.67 ± 0.23	0.46	1.14	0.73 ± 0.22	0.49	1.15

Table 13 Comparison of roughness values before and after in-group whitening

	Groups	AVG	SS	SH	*t*	*p*
ΔRa (RaA-RaB)	H2O2	0.1420	0.10623	0.03359	4.227	0.002*
	H2O2 + LED	0.06400	0.05602	0.01771	3.613	0.006*
	H2O2 + DİYOT	0.07400	0.06769	0.02141	3.457	0.007*
	OZON	0.06500	0.04790	0.01515	4.291	0.002*
	Ozon + H2O2	0.06500	0.04790	0.01515	4.291	0.002*

*There is a statistically significant difference ($p < 0.05$)

Table 14 Statistical comparison of ΔRa values between groups

	Sum squares	SD	Mean square	*F*	*p*
Between-group	0.045	4	0.011	1.511	0.215
Within-group	0.336	45	0.007		
Total	0.381	49			

by patients [7]. We, therefore, preferred the office bleaching method in our study.

Throughout the visible spectrum, spectrophotometers measure the amount of light energy reflected from an object at 1–25 nm intervals and can convert the measured spectral reflectance into color coordinates (CIEXYZ, CIELAB, or CIELCH) and various tooth color values [8]. Measurements

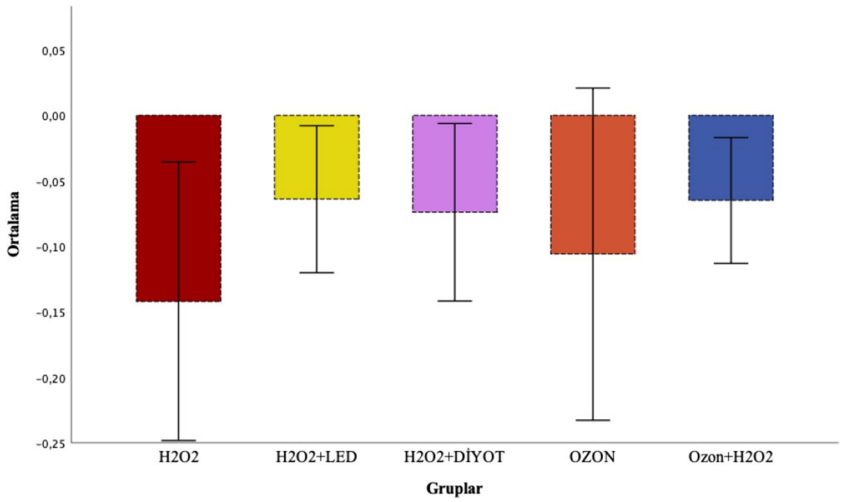

Fig. 3 Graphical representation of ΔRa values of the groups

Table 15 Average microhardness values before and after whitening

Groups	Before whitening			After whitening		
	Mean	Min	Max	Mean	Min	Max
Group 1 ($n = 10$)	410.5 ± 27.41	366	468	353.2 ± 39.5	299	412
Group 2 ($n = 10$)	420.4 ± 49.14	337	484	381.2 ± 62.51	258	463
Group 3 ($n = 10$)	442.9 ± 19.08	417	482	379.6 ± 40.31	329	455
Group 4 ($n = 10$)	388.5 ± 30.97	349	448	350.8 ± 13.78	324	372
Group 5 ($n = 10$)	440.3 ± 27.58	391	475	371.4 ± 18.73	339	404

Table 16 Comparison of hardness values before and after whitening within groups

Variable	Groups	Mean	SD	SE	*t*	*p*
ΔVSD (VSD_B-VSD_A)	H_2O_2	57.30000	28.33157	8.95923	6.396	0.000*
	H_2O_2 + LED	39.20000	35.68940	11.28598	3.473	0.007*
	H_2O_2 + DİYOT	63.30000	36.86627	11.65814	5.430	0.000*
	Ozone	37.70000	34.80437	11.00611	3.425	0.008*
	Ozone + H_2O_2	68.90000	28.03946	8.86685	7.771	0.000*

Asterisk (*) symbol indicates a statistically significant difference ($p < 0.05$)

Table 17 Statistical comparison of ΔVSD values between groups

	Sum squares	SD	Mean squares	*F*	*p*
Between-group	8015.280	4	2003.820	1.844	0.137
Within-group	48,897.800	45	1086.618		
Total	56,913.080	49			

by spectrophotometer have also been reported to be easy, reproducible, and consistent [9]. In our study, a Lovibond RT Series (The Tintometer® Group, Lovibond House, UK) spectrophotometer with digital color measurement and visible light and CIELAB measurement value were used to measure bleaching efficiency.

The ΔE value is often used to indicate perceptible tooth color change. The perceptibility threshold $\Delta E^* = 1$ is used by more than half of the studies, and a third of the studies refer to $\Delta E^* = 3.7$ as the threshold at which 50% of observers accept the color difference [10]. The threshold value for ΔE^* was accepted as 3.7 in this study. According to the CIE $L^*a^*b^*$ system, the ΔE^* value of all groups in our study was greater than 3.7 and bleaching occurred in all groups, and no statistically significant difference was detected between the groups in terms of color.

Since the introduction of in-office bleaching treatments using highly concentrated gels, various heat and light sources have been used to increase the speed and effectiveness of the bleaching gel. The activation of hydrogen peroxide increases, and the formation of free radicals increases when the light at the appropriate wavelength is sent to the photoinitiators in the bleaching gel. Besides, the heat increase resulting from activation accelerates the reaction and shortens the bleaching time [11, 12].

Historically, quartz tungsten halogen lamps, plasma arc lamps, LEDs, and different types of laser light sources with various wavelengths have been used to activate the bleaching agent used in office bleaching [13]. Used for different purposes in dentistry, these light sources have been given special settings for bleaching over time. Recently, due to some advantages, LED and diode lasers are frequently preferred in bleaching treatments [14].

In the literature reviews, light activation does not seem to have a consensus on the effect of light activation on bleaching efficiency. Some researchers have shown in their studies that light activation increases the bleaching efficiency [15–17], while others have shown that light activation does not affect the results of office bleaching [18–20].

In an in vitro study comparing the bleaching efficacy of 35% hydrogen peroxide activated with different light sources such as LED, diode laser, and halogen by Dominguez et al., light activation has been reported to increase the bleaching efficiency compared to the control group [15]. In another bleaching study in which a halogen light lamp and different types of lasers were used to activate hydrogen peroxide, it was reported that the use of light sources increased the bleaching effect of hydrogen peroxide and reduced the processing time [16]. Similarly, in an in vitro study by Torres et al., it was reported that activation of hydrogen peroxide with different light sources increased the bleaching efficiency, especially in the LED and diode laser group [17].

Similar to our study, Shahabi et al. reported that activation of 40% hydrogen peroxide with LED and diode laser did not have a significant effect on color change [20].

Papathanasiou et al. reported that when they compared the groups in which they activated 35% HP gel with halogen light and the groups in which they applied 35% HP alone in terms of bleaching efficiency, they obtained bleaching in both groups, but there was no difference between the groups in terms of efficiency, and the use of halogen light source would not benefit the physician or the patient [19]. In line with these studies, we concluded that light activation did not change the bleaching efficiency. Our opinion is that the

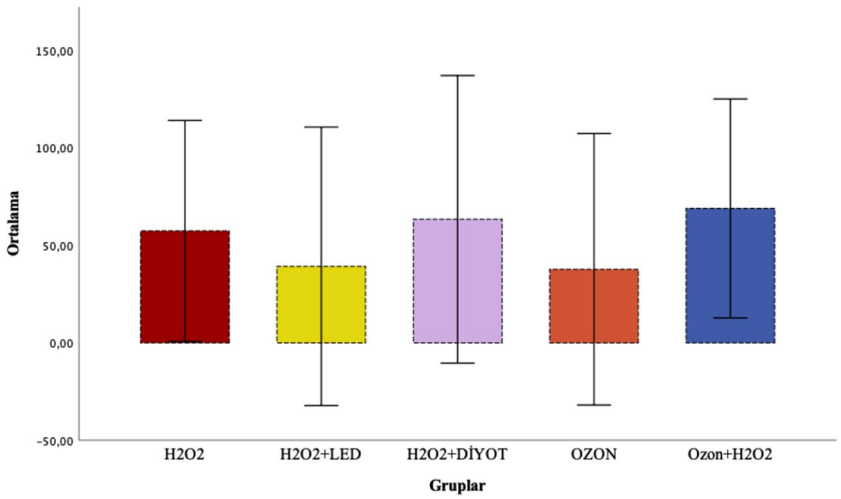

Fig. 4 Graphical display of ΔVSD values before and after bleaching

conflicting results on the bleaching efficacy of light activation applications are due to the different properties of the agents and devices used and the differences in the application methods.

Ozone, which is used as a non-invasive method in many areas of dentistry due to its high oxidation power, circulatory stimulation, analgesic, and anti-inflammatory properties, can be used as a fast-acting and harmless agent in tooth bleaching thanks to its high oxidizing capacity [21].

It is also stated that the bleaching efficiency of ozone will depend on the concentration of ozone, application time, concentration of the gel used, and gas flow rate. However, there is no consensus in the literature on the most appropriate implementation protocol [22]. An Ozone DTA generator (Ozone DTA, Apoza, New Taipei, Taiwan) was used to produce ozone gas in our study. With flat probe tip number 3, which provides a gas concentration of 235 ppm, the bleaching process was performed for 20 min at the sixth power level. Similar to the study of Al-Omiri et al. [23], in the group bleaching with the combined use of hydrogen peroxide and ozone, ozone gas was applied for 60 s after every 10 min of hydrogen peroxide application and the procedure was repeated twice for a total of 20 min of HP and 2 min of ozone application.

Grundlingh et al. reported that ozone could whiten teeth as effectively as 45% carbamide peroxide [24]. In a study by Zanjani et al. evaluating the bleaching effect of 35% hydrogen peroxide, ozone gas, and the combined use of both agents, it was reported that a 4-min ozone-bleaching application showed significantly lower efficacy compared to other groups. It has also been reported that hydrogen peroxide and hydrogen peroxide + ozone groups have similar bleaching efficiency [25]. Similarly, in an in vitro study by Tavares et al. comparing different in-office bleaching procedures, it was reported that the combined use of hydrogen peroxide and ozone was significantly superior to the use of ozone alone [26].

In in vitro and in vivo studies examining the effect of combined bleaching application, including 38% hydrogen peroxide and 60 s ozone application with hydrogen peroxide on discoloration, it was reported that hydrogen peroxide + ozone treatment provided more effective bleaching than hydrogen peroxide treatment alone [27, 28]. The data obtained in our study are partially consistent with these studies. In our study, the bleaching efficiency of 5 different office bleaching methods was examined, and adequate bleaching occurred in all groups. We also found that different light activation did not affect the bleaching efficiency, and the combination of ozone and hydrogen peroxide showed similar results with the other groups. In contrast to other studies, we found that the 20-min application of ozone in our study showed similar results with other groups. We believe that the difference here may be due to the duration of the ozone application. On the other hand, Al-Omiri et al. indicated that numerous factors, such as the ozone device employed, application time, and different study techniques, may have contributed to the disparate outcomes seen in the scientific literature [23].

In the literature, studies examining the effects of peroxide-based materials on the enamel surface have reported that the bleaching agent causes changes in the inorganic and organic composition of the enamel depending on the application method, concentration, and duration. These structural changes result in a decrease in enamel hardness and an increase in surface roughness [29]. Nevertheless, there are different results in the studies evaluating surface roughness and hardness after bleaching. Some researchers have stated that the bleaching process affects the surface roughness and hardness [30, 31], while others have stated that the surface roughness and microhardness are not affected [32, 33].

Microhardness and surface roughness studies require the samples to have smooth and polished surfaces to make accurate measurements. For this reason, it has been recommended in many studies to sand the surfaces to 150 nm before treatment to ensure the reliability of the measured data [34]. In our study, the samples were sanded to obtain smooth surfaces before the measurements.

It has been stated that the solutions in which the samples are stored in microhardness and roughness studies may cause chemical changes on the mineralized tissue surface, and this may affect the measurement reliability [35]. In studies where samples were kept in saliva, the increase in surface roughness and decrease in enamel surface hardness after bleaching were reversed by the passage of calcium phosphate between the saliva and the samples [36]. In studies where the samples were kept in distilled water, no ion transfer between the medium and the samples was observed, and therefore, more reliable results were obtained. Studies have shown that there is no ion migration in samples placed in distilled water [37].

Mondelli et al. examined the enamel surface of the teeth after bleaching bovine teeth with 35% HP-hybrid light, 35% HP-halogen light, and 16% KP. According to the results of their study, it was reported that there was no significant change in the enamel roughness value [38]. Suresh et al. evaluated the surface roughness by SEM after bleaching with different light sources (35% hydrogen peroxide, 37.5% HP + LED light activation, and 45% HP + diode laser). In light of the data they obtained, the highest roughness value was reported in the group without any light activation, and the lowest roughness values were reported in the group activated with LED [39].

In a bleaching study using 40% HP (Opalescence Xtra Boost) and diode laser + heydent gel, Anaraki et al. evaluated the enamel surface roughness. As a result of the study, it was reported that both in-office bleaching systems significantly increased enamel micro-roughness [40]. In another

study using bovine teeth, three different light sources (Er-YAG laser, diode laser, LED) were used to activate 35% hydrogen peroxide gel. Using enamel surface roughness after bleaching, the researchers found a significant increase in roughness in all groups [20]. In our study, in accordance with these studies, it was found that a significant degree of roughness occurred on the enamel surfaces in the groups in which 40% HP and 40% HP were whitened with LED and diode laser activation, but this degree of roughness did not differ between the groups.

According to the researchers, these different results on enamel surface roughness are due to differences in the content, concentration, application times, storage conditions, pH, and application methods of the agent used [41].

In many studies conducted, high concentrations of hydrogen peroxide used for bleaching purposes have been reported to cause adverse effects on enamel tissue, such as roughness, increased demineralization, and decreased mechanical properties [42, 43]. To mitigate these negative effects, low concentrations of peroxide, high Ph content, and the use of remineralizing agents such as calcium and fluoride are recommended by researchers. Some researchers in recent years, however, have reported that ozone can be used as an alternative bleaching agent to hydrogen peroxide due to its oxidizing properties [22, 23]. A review of the literature reveals that there are few studies examining the effect of ozone bleaching on enamel surface roughness.

In the study by Elsayad, it was reported that 1 min of ozone exposure does not cause changes on the tooth surface, but a 2-min exposure causes significant changes in enamel surface morphology in terms of roughness [44]. Wagner and Błaszczyk reported that the use of ozone caused an increase in roughness on the enamel surface of deciduous teeth [45]. In another study evaluating the bleaching efficacy of ozone and the physicochemical changes on the teeth, it was reported that the combined use of 35% HP, ozone, and ozone and HP showed similar properties in terms of surface roughness [26]. When the roughness values before and after bleaching were compared in our study, the surface roughness showed a statistically significant increase in all groups. Although there was no significant difference between the groups in terms of roughness, surface roughness increased more in the ozone-bleaching group. Compared to other studies, we associate this increase with the application of ozone for a longer period in our study.

In a study designed to evaluate the effects of 35% H_2O_2 bleaching gel and its activation with an LED light source on enamel hardness, Parreiras et al. reported no significant change in enamel hardness in both groups [46]. It was reported by Vanderstricht et al. that peroxide activation with KTP laser, diode laser, and LED lamp caused similar microhardness changes in enamel [47].

Araújo et al. evaluated the microhardness change after 35% HP application in combination with LED, halogen lamps, and argon laser and in conclusion that HP application led to a decrease in microhardness after 14 days, while light activation did not affect this value [48]. Berger et al. used 35% hydrogen peroxide, halogen light, and an LED/diode laser and showed a significant decrease in surface microhardness values regardless of the type of bleaching light used [49].

In light of these contradictory results in the literature, there was a significant decrease in the microhardness values measured before and after the procedure in all groups in our study. However, no difference was observed between the groups in terms of hardness change.

As in roughness tests, differences in the pH, concentration, different components, and application times of the agent used to play a role in the contradictory results were found in microhardness studies after bleaching. Besides, sample storage conditions (artificial saliva, distilled water, de-ionized water, etc.) may also affect the results obtained. For example, it is known that the occurrence of demineralization and remineralization events in teeth stored in artificial saliva may change the results to be obtained [12].

In our study, ozone gas and hydrogen peroxide combined with ozone gas showed a decrease in the microhardness value similar to the other groups. Tahmassebi et al. reported that there was no significant difference in the surface hardness value in the ozone-treated group in the microhardness experiment [50]. We associate the difference in the results from our study with the different ozone generators used and the different duration.

Duggal et al. examined the effect of ozone on the inhibition of mineral loss from enamel and dentin using a microhardness test. They concluded that ozone had no significant effect on the inhibition of dental hard tissue demineralization [51]. Santana et al. showed that ozone significantly reduced the Knoop hardness values of enamel in an in vitro study evaluating the effects of ozone on bleaching efficacy and enamel microhardness (52). In our study, a statistically significant decrease in the Vickers microhardness values of the enamel was observed after ozone bleaching, which is consistent with this study.

In the literature, there is no study examining the microhardness values after bleaching with the combined use of ozone and hydrogen peroxide. However, our data showed that the use of ozone in combination with hydrogen peroxide reduced the microhardness, and this reduction was similar to other office bleaching methods in our study.

Conclusion

In line with these results, all methods showed similar bleaching efficacy, while the LED light source and the laser did not have a positive effect on bleaching efficacy and enamel

surface changes. On the other hand, although ozone may be an alternative bleaching agent to hydrogen peroxide, we believe that more controlled in vitro and in vivo studies are needed to support this conclusion.

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Investigation: Rahime Zeynep Erdem.
Formal analysis: Ömer Çellik.
Writing—original draft preparation: Rahime Zeynep Erdem.
Writing—review and editing: Ömer Çellik, Rahime Zeynep Erdem.
Resources: Ömer Çellik, Rahime Zeynep Erdem.
Supervision: Rahime Zeynep Erdem.

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Data availability Not applicable.

Declarations

Ethics approval All procedures performed in studies involving humans were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This research was approved by the Clinical Research Ethics Committee.

Competing interests The authors declare no competing interests.

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