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ORIGINAL ARTICLE

Analysis of enamel structure and mineral density after different bleaching protocols using micro-computed tomography

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ABSTRACT

Objective: This study aims to investigate the effect of three different bleaching applications on structural integrity, mineral volume (MV) and density of the enamel by using micro-computed tomography (Micro-CT) and evaluate the colour effectiveness using CIEDE2000.

Materials and methods: Twenty-four maxillary premolar teeth were divided into three groups ($n = 8$) (group 1: 40% HP gel with erbium, chromium: yttrium–scandium–gallium–garnet (Er,Cr:YSGG) laser activation; group 2: 40% HP gel with diode laser activation; group 3: 16% CP gel). Bleaching protocols were applied and colour alteration was obtained. Data were calculated with CIEDE2000 before and after bleaching. A Micro-CT was used to scan the specimens before and after the bleaching application. Statistical analysis was performed by ANOVA.

Results: No significant difference was observed between the groups for colour changes ($p > .05$). Micro-CT analysis showed significant differences in structural thickness, structural separation, mineral density and MV for the different ROIs before and after bleaching for all groups ($p < .05$).

Conclusions: All of the bleaching methods represented similar efficiency. However, bleaching with Er,Cr:YSGG laser was less harmful to enamel in comparison with other bleaching methods. The present results may be useful for establishing a numerical standard for the change in bleaching with laser systems in dental hard tissues.

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Introduction

Bleaching treatments, which have become very popular in recent years, are applied in two different ways including as at-home and in-office [1,2]. Office bleaching involves the application of gels with a concentration of 35–40% hydrogen peroxide (HP) to the surface of the tooth in a short period and can be accelerated with different light or heat sources [3]. On the other hand, the home bleaching process involves, direct contact of low concentration of HP or carbamide peroxidase (CP) agents to the enamel surface with the use of individualized trays for several hours per day within several weeks [4]. CP is an organic compound that decomposes to 30% HP and 70% urea HP, in turn, produces water, oxygen and free radicals, and these latter by-products cause the tooth whitening or bleaching effect [5,6]. Free radicals formed by the degradation of HP penetrate deep into enamel/dentine tissue to break down high molecular weight pigments [7]. However, bleaching mechanism is still unknown, and safety issues about bleaching treatments are controversial [1].

In recent years, lasers of various wavelengths such as CO_2, Er:YAG, erbium, chromium: yttrium–scandium–gallium–garnet (Er,Cr:YSGG) and diode have been used to accelerate the reaction [8–10]. 980 nm diode laser which absorbed in water is preferred more than other diode laser wavelengths for bleaching efficiency [11]. Although the use of a diode laser in the bleaching process increases the whitening efficiency, there are different statements regarding enamel surface changes caused by laser application [12]. Another laser used in bleaching treatment is Er,Cr:YSGG (2780 nm wavelength) which is effective in the treatment of dental hard tissues [10]. Dionysopoulos et al. [13] found that Er,Cr:YSGG laser-activated bleaching process did not significantly change the mineral content or surface morphology of enamel compared to the conventional chemical whitening procedure. However, its use for the bleaching process has not been adequately investigated.

Bleaching may cause alteration in tooth structure regardless of agents (HP or CP) and laser activation [14,15]. Morphological changes of teeth resulting from bleaching materials have been shown by several studies using a scanning electron microscope (SEM) and atomic force microscopy [16,17]. Also, mineral alterations have been shown on the enamel surfaces using energy dispersive spectroscopy, X-ray diffraction or infra-red spectroscopy [15,18]. Micro-computed tomography (Micro-CT) is a widely used visualizing technique for obtaining three-dimensional images of a sample,

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including its internal structure by X-ray attenuation. It has the advantage of being non-destructive, non-invasive [19]. Micro-CT permits to the corrected measuring of X-ray attenuation factor within objects liable for X-ray image contrast [20] and research of fields that are difficult to examine.

In the literature, there are quite limited bleaching application studies that analysed the mineral volume (MV), density and enamel structure difference with micro CT [21–23]. However, to the best of our knowledge, there is no publishing study about the effect of bleaching with laser activation on mineral and structural alteration of the tooth using micro-CT.

This study aimed to investigate the effects of different bleaching methods on enamel structure, mineral density and MV using micro-CT, and evaluate colour changes by using CIEDE2000 formula. The null hypotheses were as follows:

i. Laser-activated bleaching did not alter on enamel structure, mineral density or MV.
ii. Laser-activated bleaching did not enhance bleaching efficiency.

Materials and methods

This *in vitro* study was approved by the Ethics Committee of the Gaziantep University (decision no.: 2018/108).

Preparation of samples and bleaching procedure

Twenty-four freshly extracted maxillary human premolar teeth, which were extracted for periodontal reasons that do not include caries, restoration, previous endodontic treatment, cracks and hypoplastic lesions were taken for this study. The samples were stored in a saturated thymol solution until the bleaching application. The teeth were randomly divided into three different groups ($n = 8$). Three different bleaching protocols were used as follows:

Group 1 (bleaching with Er,Cr:YSGG laser): First, teeth were embedded in a silicone mould. Then, the 40% HP gel (Opalescence Boost, Ultradent, South Jordan, UT) was prepared by mixing the content of two different syringes the manufacturer's instructions. The gel was applied uniformly over the buccal surface of the teeth at a thickness of 2 mm. Er,Cr:YSGG laser (Waterlase MD Turbo, Biolase, Irvine, CA) was used for activation with the special handpiece (Z-type glass tip, MZ8) at a distance of 2.5 cm to the enamel surfaces. The selected laser settings were an average power of 1.25 W, a pulse repetition rate of 10 Hz, and a pulse duration of 700 μs while water and air were off (energy density = 21.6 J/cm^2, power density = 250 W/cm^2). Irradiation was performed two times of 10 seconds per tooth. The total bleaching procedure lasted for 20 minutes.

Group 2 (bleaching with diode laser): All steps for group 2 were the same for group 1 with one difference that activated laser tip. Bleaching gel was kept on the teeth for 10 minutes while the 980 nm diode laser (Gigaa Dental Laser Cheese, Wuhan, China) activation was performed for each quadrant by using a special bleaching handpiece (5.85 cm^2 spot size) at an output power of 7 W for 12 s from 5 mm distant to surface. Laser irradiation cycle was repeated times per bleaching sessions for each quadrant (energy density = 14.3 J/cm^2, power density = 1.19 W/cm^2). The total bleaching procedure lasted for 15 × 2 minutes.

Group 3 (home bleaching): Silicone material was shaped as a dental arch and teeth were embedded in this arch according to 1 cm above the enamel–cement connection. Alginate impressions of the arch were obtained to prepare the stone model. Then, arch-tray was prepared by using soft vinyl material at a vacuum-forming machine (Vacuum Forming Machine, Keystone İndustries, Myerstown, PA). Bleaching procedures were performed at 37 °C for 14 days. Teeth were exposed to a 16% CP (Opalescence, Ultradent, South Jordan, UT) agent for six hours daily.

Table 1. Parameters of laser application.

Laser system	Diode	Er,Cr:YSGG
Wavelength (nm)	980	2780
Probe	Bleaching handpiece	Z-type glass tip, MZ8
Tip area	5.85 cm^2 (spot size)	5×10^{-3} cm^2 (spot size)
Irradiation time (s)	12	10
Output power (W)	7	1.25
Energy density (J/cm^2)	14.3	21.6
Frequency of treatment	2 times	2 times

After the whitening treatment performed, the samples were kept in artificial saliva containing 2190 mg sodium bicarbonate, 1270 mg potassium phosphate, 441 mg magnesium chloride, 820 mg potassium chloride, 4.5 mg sodium fluoride, 100 mg nipazole, 10 mg nipagim, 24 mg sorbitol, 8 mg carboxymethylcellulose, 1000 mL distilled water (pH 7.0). This composition was prepared at Gaziantep University, Faculty of Biochemical.

All information of the laser system included is summarized in Table 1.

Colour change calculation

The colour of the specimens was measured using a spectrophotometer (VITA Easyshade Advance, Zahnfabrik H. Rauter, Bad Säckingen, Germany). The spectrophotometer was calibrated with a standard tile supplied by the manufacture manufacturer's instructions. The colour measurements were performed before the application (baseline) and after the application, respectively. Total colour changes were calculated using CIEDE2000 formula (International Commission on Illumination 2000 colour-difference formula) as follows [24]:

$$\Delta E_{00} = [(\Delta L/K_L S_L)^2 + (\Delta C/K_C S_C)^2 + (\Delta H/K_H S_H)^2 + R_T(\Delta C/K_C S_C)(\Delta H/K_H S_H)]^{1/2}$$

In the formula, ΔL, ΔC and ΔH represent the differences in lightness, chroma and hue, respectively. The rotation function R_T captures the interaction between the chroma and hue differences in the blue region. The weighting functions S_L, S_C and S_H allow the adjustment of the total colour difference for the changes in the colour difference pairs on the L*, a* and b* axes. The parametric factors K_L, K_C and K_H are correction terms used for the experimental conditions. For this study, the parametric factors of the CIEDE2000 colour-difference formula were set to 1 and the discolouration was evaluated based on perceptibility and clinical acceptability thresholds [25].

Micro-CT evaluation

A high-resolution, desktop micro-CT system (Bruker Skyscan 1275, Kontich, Belgium) was used to scan the specimens. The scanning conditions were: 100 kVp, 100 mA beam current, 0.5 mm Al/Cu filter, 9.1 µm pixel size and rotation at 0.5 steps. To minimize ring artefacts, the air calibration of the detector was carried out before each scanning. Each sample was rotated 360° within an integration time of 5 min. The mean time of scanning was around 2 h. Other settings included beam-hardening correction, as described, and input of optimal contrast limits according to the manufacturer's instructions, based on prior scanning and reconstruction of the teeth. Each tooth scanned two times before and after the application of bleaching procedures to ensure the standardization of the study with the same scanning parameters.

Micro-CT image analysis

The NRecon software (ver. 1.6.10.4, SkyScan, Kontich, Belgium) and CtAn (ver. 1.17.7.2, SkyScan) were used for the visualization and quantitative measurements of the samples, which used the modified algorithm described by Feldkamp et al. [26] to obtain axially, two-dimensional (2D), 1000 × 1000 pixel images. For the reconstruction parameters, ring artefact correction and smoothing were fixed at zero and the beam artefact correction was set at 40%. Contrast limits were applied following SkyScan's instructions. By using the NRecon software (Skyscan, Kontich, Belgium), the images obtained by the scanner were reconstructed to show 2D slices of the roots. In total, 1560 cross-sectional images were reconstructed from the whole volume. Moreover, the CTAn (Skyscan, Kontich, Belgium) software was used for the 3D volumetric visualization, analysis and volume of the root canal measurement. The reconstructed images were also further processed in Skyscan CTVox (ver. 3.3.0, SkyScan, Kontich, Belgium) for visualization (Skyscan, Kontich, Belgium). All reconstructions were made with a 21.3-inch flat-panel colour-active matrix TFT medical display (NEC MultiSync MD215MG, Munich, Germany) with a resolution of 2048–2560 at 75 Hz and 0.17-mm dot pitch operated at 11.9 bits.

The Skyscan CT-analyzer software (CTAn) provides for an integrated calibration of datasets into these two density scales, Hounsfield unit (HU) and Mineral density (MD). For this purpose, two different mineral concentration conical MD phantoms (rods) of 0.25 and 0.75 gHAp cm^3 were used for appropriate calibration phantom scans and measurements. Thus, to aid calibrations, the samples placed and scanned with MD phantom rods placed into an identical tube for the calibrating scan which allowed the density value to be independently calibrated for each scan. After scanning, the greyscale values were converted to mineral density values with a linear calibration curve based on the greyscale values obtained from the two different mineral concentration conical phantoms of 0.25 and 0.75 gHAp cm^3. All the scan parameters, such as pixel size, rotation step, frame averaging, voltage, filter, etc. were identical for all scans.

To distinguish enamel from other structures, a suitable threshold is required. Therefore, global thresholding was applied using CTAn software, all procedure was semi-automatic using the same software. For analysing dentinal mineral density in 3D volumes, the original greyscale images were processed with a Gaussian low-pass filter for noise reduction and an automatic segmentation threshold was used. A thresholding (binarization) process was used, which entails processing the range of grey levels to obtain an imposed image of black/white pixels only. Then, separately for each slice, a region of interest was chosen to contain a single object entirely to analyse the mineral density (Figure 1).

After reconstruction, three regions of interest (ROI) were drawn to include the entire specimen within the sample using CTAn software, in which all specifications of the programme were used to analyse the mineral density. Three ROIs were from the outer enamel surface extending to the app. 150 µm in-depth (ROI-1 = 50.1 µm thickness; ROI-2 = 100 µm thickness and ROI-3 = 150 µm thickness). All ROIs were enamel regions, and changes in the structure could occur after bleaching using the aforementioned techniques (Figure 2).

Calculations of mineral loss difference (ΔZ; gHAp cm^3) of each specimen were made by calculating the MD values from the entire demineralized area as a mean of loss of surface and inner part of the demineralized area. The mineral loss was defined after subtracting the demineralized MD values from baseline phantom rod MD values to correct alignment inaccuracies from the specimen.

Moreover, structural parameters were also calculated by CTan 2D/3D (Bruker micro-CT, Kontich, Belgium) in 3D, based on model volume. CT parameters were the following: structure thickness (plate model) – ST (µm) means of structure thickness; structure separation (plate model) – SS (µm) mean distances of structure separation; fragmentation index – FI determines the higher or lower connectivity of the structure calculated for each ROI. Lower fragmentation index (FI) indicates a better-connected structure while higher indicates a more disconnected structure. The FI is calculated in 3D, by comparing the volume and surface of binary solid before and after image dilation following the method purposed by Hahn et al. [27] and Gomes et al. [28].

Statistical analysis

The analysis was performed using SPSS software for Windows version 22.0 (IBM, Armonk, NY). Before statistical analysis, the normality of the data was analysed with the Shapiro–Wilk test. One-way ANOVA with a post hoc LSD test

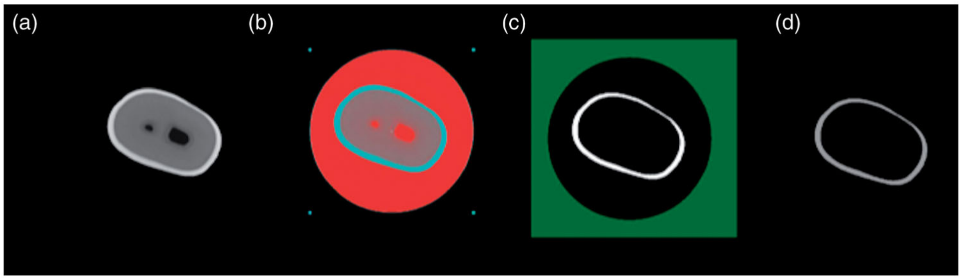

Figure 1. Micro CT images showing (a) axial image of tooth, (b) ROI selection on images, (c) thresholding and (d) binarization of the image by the grey-level histogram.

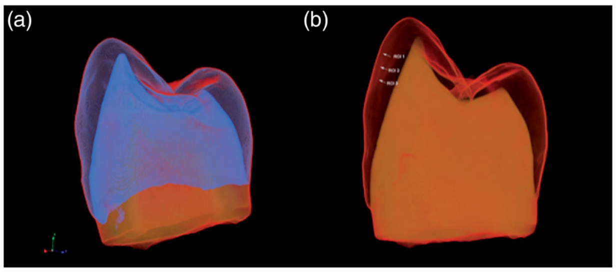

Figure 2. (a) 3D representation of enamel and dentine prior to bleaching and (b) 3D representation of Micro-CT image shows ROIS after bleaching.

Table 2. Means and standard deviations of the ΔE_{00} for different groups.

	Group 1	Group 2	Group 3
ΔE_{00} (mean ± SD)	5.72 ± 1.26^a	5.32 ± 3.09^a	4.08 ± 1.20^a

Different letters within lines indicate statistically significant differences.

was used to compare differences in more than two independent groups. A paired *t*-test was used for the difference between pairs of measurements. The statistical significance level was set to $\alpha = 0.05$.

Results

All the tested bleaching systems resulted in statistically significant colour change compared to baseline colours ($p<.05$). Colour changes (ΔE^*±SD) values for each group are shown in Table 2. Comparison between intergroup, before and after the colour change was similar ($p>.05$).

Micro-CT analysis showed that statistically significant differences were found in the structural thickness, structural separation, mineral density and MV of enamel for the different ROIs before and after bleaching for all groups ($p<.05$).

After bleaching applications for intra groups:

- *Structure thickness (ST):* In ROI-1, there were significant differences except for Er,Cr:YSGG group ($p<.05$). For ROI-2 and ROI-3, there were significant differences for all groups ($p<.05$). and
- *Structure separation (SS) and mineral density, MV (g/cm^3):* All of the ROI values, there were significant differences for all groups ($p<.05$).
- *Fragmentation index:* In ROI-1 and ROI-2, there were no significant differences for all groups ($p>.05$), but in ROI-3, significant differences were detected in group 1 ($p<.05$).

For intra groups, structural parameters are represented in Table 3.

After bleaching applications for inter groups:

- *Structure thickness:* When the groups were compared with each other immediately after bleaching, there were significant differences in the structural thickness of enamel between Er,Cr:YSGG and other groups for the ROI-1 and ROI-3 ($p<.05$), but no significant difference was found between diode and home group ($p>.05$). In ROI-2, there was no significant difference for all groups ($p>.05$).
- *Structure separation:* Significant differences were detected among groups for the structural separation of enamel in ROI-1 ($p<.05$). For ROI-2 and ROI-3, a significant difference was determined between the home group and other groups ($p<.05$).
- *Fragmentation index:* There were no significant discrepancies among the groups for the FI in the ROI-1 and ROI-2 ($p>.05$), but a significant difference was found between the home group and other groups in the ROI-3 ($p<.05$).
- *Mineral density:* Significant differences were determined for the mineral density of enamel between the groups in ROI-1 ($p<.05$), but no significant discrepancy was found between the groups in ROI-2 ($p>.05$). For ROI-3 values, there was a significant difference among the diode group with other groups ($p<.05$).
- *Mineral volume (g/cm^3):* There were no significant differences among the groups for the MV of the enamel in the ROI-2 and ROI-3 ($p>.05$), but the significant discrepancy was found between the home group with other groups in the ROI-1 ($p>.05$).

For inter groups, structural parameters are represented in Table 4.

Discussion

The first null hypothesis was rejected because the laser-activated bleaching techniques caused partial alterations in enamel structure, mineral density and MV. However, the

Table 3. Descriptive analysis of structural parameters before and after different bleaching applications.

	ST	*p* Value	SS	*p* Value	FI	*p* Value	MD	*p* Value	MV	*p* Value
ROI-1										
Er,Cr:YSGG group	29.81 ± 0.48^{b}	.325	59.37 ± 0.52^{b}	.005*	0.063 ± 0.01^{b}	.187	2.83 ± 0.02^{b}	.010*	2.80 ± 0.35^{b}	.009*
	29.55 ± 0.48^{a}		59.25 ± 0.67^{a}		0.057 ± 0.02^{a}		2.61 ± 0.04^{a}		2.49 ± 0.35^{a}	
Diode group	29.53 ± 0.12^{b}	.008*	59.49 ± 0.13^{a}	.005*	0.060 ± 0.01^{b}	.731	2.82 ± 0.01^{b}	.007*	2.78 ± 0.34^{a}	.007*
	28.63 ± 0.12^{a}		58.60 ± 0.13^{a}		0.058 ± 0.02^{a}		2.65 ± 0.01^{a}		2.59 ± 0.34^{a}	
Home group	29.63 ± 0.37^{b}	.010*	59.20 ± 0.47^{b}	.005*	0.056 ± 0.01^{b}	.183	2.81 ± 0.01^{b}	.011*	3.03 ± 0.23^{b}	.023*
	28.86 ± 0.37^{a}		58.07 ± 0.22^{a}		0.049 ± 0.01^{a}		2.74 ± 0.00^{a}		2.97 ± 0.23^{a}	
ROI-2										
Er,Cr:YSGG group	60.95 ± 0.76^{b}	.010*	95.32 ± 0.58^{b}	.011*	0.025 ± 0.00^{b}	.157	2.96 ± 0.15^{b}	.010*	2.90 ± 0.25^{b}	.013*
	60.04 ± 0.76^{a}		92.70 ± 0.19^{a}		0.020 ± 0.00^{a}		2.80 ± 0.15^{a}		2.76 ± 0.35^{a}	
Diode group	59.97 ± 0.12^{b}	.009*	95.56 ± 0.12^{b}	.009*	0.020 ± 0.00^{b}	.157	2.98 ± 0.27^{b}	.026*	2.77 ± 019^{b}	.013*
	58.82 ± 0.12^{a}		92.60 ± 0.12^{a}		0.017 ± 0.00^{a}		2.71 ± 0.18^{a}		2.64 ± 0.06^{a}	
Home group	63.65 ± 0.09^{b}	.007*	95.72 ± 0.35^{b}	.008*	0.022 ± 0.00^{b}	.183	2.96 ± 0.07^{b}	.011*	2.92 ± 0.14^{b}	.014*
	62.43 ± 0.09^{a}		91.74 ± 0.34^{a}		0.018 ± 0.00^{a}		2.81 ± 0.09^{a}		2.74 ± 0.16^{a}	
ROI-3										
Er,Cr:YSGG group	81.70 ± 0.43^{b}	.010*	102.84 ± 0.07^{b}	.011*	0.025 ± 0.01^{b}	.705	2.91 ± 0.13^{b}	.010*	2.93 ± 0.05^{b}	.010*
	80.77 ± 0.43^{a}		99.94 ± 0.17^{a}		0.021 ± 0.01^{a}		2.12 ± 0.10^{a}		2.31 ± 0.05^{a}	
Diode group	81.61 ± 0.62^{b}	.011*	102.84 ± 0.17^{b}	.010*	0.019 ± 0.00^{b}	.014*	2.86 ± 0.01^{b}	.010*	2.85 ± 0.00^{b}	.007*
	79.75 ± 0.40^{a}		99.93 ± 0.11^{a}		0.011 ± 0.00^{a}		2.01 ± 0.00^{a}		2.29 ± 0.20^{a}	
Home group	81.69 ± 0.37^{b}	.010*	102.90 ± 0.13^{b}	.005*	0.015 ± 0.00^{b}	.046*	2.91 ± 0.08^{b}	.009*	2.88 ± 0.04^{b}	.009*
	79.77 ± 0.37^{a}		98.97 ± 0.13^{a}		0.010 ± 0.00^{a}		2.07 ± 0.08^{a}		2.25 ± 0.04^{a}	

ST: structural thickness; SS: structural separation; FI: fragmentation index; MD: mineral density; MV: mineral volume; group 1: Er,Cr:YSGG group; group 2: diode group; group 3: home group.
Superscript b, before; superscript a, after.
*$p<.05$.

Table 4. *p* Values of structural parameters among after different bleaching applications for different ROIs.

	ROI-1 ST	ROI-1 SS	ROI1 FI	ROI-1 MD	ROI-1 MV	ROI-2 ST	ROI-2 SS	ROI-2 FI	ROI-2 MD	ROI-2 MV	ROI-3 ST	ROI-3 SS	ROI-3 FI	ROI-3 MD	ROI-3 MV
Group 1-2	0.000*	0.006*	0.904	0.000*	0.531	0.666	0.422	0.374	0.229	0.291	0.000*	0.822	0.002*	0.012*	0.246
Group 1-3	0.001*	0.000*	0.285	0.000*	0.006*	0.400	0.000*	0.665	0.078	0.856	0.000*	0.000*	0.001*	0.274	0.985
Group 2-3	0.206	0.017*	0.236	0.000*	0.026*	0.209	0.000*	0.665	0.060	0.379	0.913	0.000*	0.696	0.023*	0.968

ST: structural thickness; SS: structural separation; FI: fragmentation index; MD: mineral density; MV: mineral volume; group 1: Er,Cr:YSGG group; group 2: diode group; group 3: home group.
*$p<.05$.

second null hypothesis was accepted because the laser-activated bleaching methods did not enhance bleaching efficiency compared to the home bleaching group (without laser).

In-office bleaching offers more effective and faster treatments, which can be performed with different types of light sources and various laser devices with different wavelengths, such as Er:YAG, Er,Cr:YSGG, and diode laser [8–10]. The advantage of laser activation for tooth whitening treatments is related to transformation of laser energy into heat. Laser irradiation causes the release of hydroxyl radicals and increases the penetration depth of the whitening agent [29]. Thus, the duration of application decreases while the treatment effectiveness increases [30].

Besides, it has been reported that negative effects can be prevented by laser-activated bleaching [29]. However, some studies are reported that laser irradiation causes morphological and chemical changes in dental hard tissues. These modifications can differ, based on the type of laser and the dental tissue [31].

In the literature, it is controversial regarding the effect of bleaching products and methods on dental hard tissues. Some studies showed no effects on the mechanical properties of dental hard tissues after tooth bleaching [32,33], while in other studies about different concentrations of CP and HP have shown that bleaching treatment disrupts the surface integrity of enamel [15], increases the roughness [34], changes the inorganic composition [35], reduces microhardness and mineral content [36,37] and causes morphological changes [38]. These changes occurring after bleaching applications vary depending on the concentration of the agent used, [39], pH [40], contents [41], the application time of the agent [42] and method [30,39]. In our study, we compared the current laser bleaching techniques using the HP agent and home bleaching technique using CP to found the most compatible bleaching technique.

Erosive enamel surface analysis methods can be listed as: measuring SEM, energy-dispersive X-ray spectroscopy (EDX), elemental analysis of solid samples, iodine permeability test, Raman spectrophotometer, profilometer, surface microhardness, chemical analysis, microradiography, Micro-CT and atomic force microscope [43]. The latest use of micro-CT for measuring mineral concentration has a sensitivity of better than 1% and a resolution between 5 and 30 μm [20]. The most important advantage of the newly developed three-dimensional imaging system, Micro-CT; is measuring the mineral changes in the demineralization and remineralization process without damaging the material. Also, it is possible to obtain three-dimensional images repeatedly and allowing volumetric calculations [44]. Tanaka et al. [45] used micro-CT to examine the change and mineralization processes occurring in enamel after bleaching with HP application and stated that this device can display the mineral density without causing any damage to the samples. There are limited studies on the effect of laser bleaching applications especially Er,Cr:YSGG laser [13,46], also no data are available with an evaluation of Micro-CT.

CIELab and CIEDE2000 have been developed to identify the colour differences of objects [47]. In the CIEDE2000 system, unlike CIELab formula, it is better correlated with visual perception than the CIELab the parameters [48]. Due to these positive properties, the CIEDE2000 formula is recommended in dental *in vitro* research as well as *in vivo* dental colour measurement. Nevertheless, there are a few studies on colour changes after dental bleaching using this CIEDE2000 formula [49]. The detectability threshold value was considered to be $\Delta E_{00}>1.8$ for CIEDE2000 [24]. In the present study, we recorded $\Delta E_{00}>1.8$ after whitening all treatment. Data obtained with CIED2000 are difficult to compare with other studies as its use is not as prevalent as CIELAB.

To increase the interaction of visible light and bleaching gel, which can stimulate the maximum absorption of light and dyes or pigments included in the product, the gels are manufactured with colours that can convert it to heat [50]. Colouring agents with one or more wavelength selective adsorbent feature acts as a filter or reflects other wavelengths. This process occurs for the pigment or colouring agent, which is in a position that is diametrically opposite of the complementary colour and can be regarded as the chromatic circle. This agent must have a specific colour, known as a complementary colour [51]. Gurgan et al. showed that Opalescence Boost including a carotene pigment with diode laser irradiation provides higher whitening efficiency in a shorter time [29]. However, no studies with Er,Cr:YSGG activated bleaching using an Opalescence Boost gel exist. The bleaching efficiency of the Opalescence Boost gel was found to be the same with both laser activations.

In the present study, ST did not change in Er,Cr:YSGG group, while the values were significantly decreased in-home and diode groups for ROI-1. In terms of ST, there was a significant decrease in all groups while the least decrease was in Er,Cr:YSGG for ROI-3. For SS, a significant decrease was detected in all ROIs after bleaching. The highest decrease was in the diode group, the lowest was in the Er,Cr:YSGG group in almost all ROIs. Also, in ROI-3, Er,Cr:YSGG and diode groups represent the same level of decrease, while the most decrease was in the home group. For the FI, the ROI-3 values decreased significantly in diode and home groups. In ROI-2, Er,Cr:YSGG group showed significantly more change than other groups, and the diode group showed significantly less change than other groups. Lower FI demonstrates a better-connected structure while higher demonstrates a more disconnected structure.

Within all these results, the group that caused the least change in superficial enamel in all structural change indexes was Er,Cr:YSGG group. Er,Cr:YSGG laser represents affinity to hydroxyapatite and water more than other laser systems [10]. This feature provides safety on bleaching because laser energy leads to the heating of bleaching agent which contains a high percentage of water. In this way, it is not transmitted to deeper layers of tooth structures [43]. The diode laser, which has a 980 nm wavelength, is absorbed by water more than other wavelengths [11]. However, because the diode laser is barely absorbed by hydroxyapatite, leading to minimal transmission and scattering of the laser's light, the anticipation of laser-induced enamel surface changes seems unreasonable [52]. Thus, it can be stated that preferring Er,Cr:YSGG seems safer than diode for the activation of bleaching agents.

Based on the results of previous studies [40,53], higher concentrations of HP provide easy enamel demineralization compared to lower concentrations. Efeoglu et al. [21] concluded that the enamel was significantly demineralized and the highest reduction in mineral content was in the outer layer of the enamel surface after bleaching with 35% CP. Also, Li et al. [54] found that the demineralization effect of 38% HP bleaching treatment led to a decrease in the concentration of the mineral content in the outer layer. Our results are following those previous ones reporting that any kind of bleaching application results in demineralization. For mineral density, there were different levels of reduction in all groups, while for MV, Er,Cr:YSGG showed significantly less change than other groups for ROI-3. On the other hand, all groups represented a similar level of reduction in ROI-1 and ROI-2 for mineral density. In ROI-3, the highest decrease was in the diode group and it is different from the other groups. For MV, all ROIs values showed a significant decrease in all groups. The highest decrease was in the home group in ROI-2 and ROI-3, and the lowest decrease was in the diode group in ROI-3. Thus, it can be stated that Er,Cr:YSGG laser-activated office bleaching seems to have a slight advantage in terms of demineralization.

Within the limitations of the present study, different bleaching applications using 40% HP caused a decrease in structural thickness, structural separation, FI, mineral density and MV in enamel. Histomorphometric parameters in a three-dimensional analysis, such as structural thickness, structural separation, fragmentation, and mineral density and volume can be useful for future mineralizing and remineralizing studies.

Conclusions

All the tested tooth bleaching methods presented similar effect on colour change. Within the limitation of this study, Er,Cr:YSGG laser was less harmful to enamel compared to other bleaching treatments.

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