The Influence of a Novel In-Office Tooth Whitening Procedure Using an Er,Cr:YSGG Laser on Enamel Surface Morphology

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Background and Objective: The purpose of this in vitro study was to evaluate the influence of a novel in-office tooth whitening procedure using Er,Cr:YSGG laser radiation on bovine enamel.

Study Design/Materials and Methods: Forty-eight enamel specimens were prepared from bovine canines and divided into four groups: Group 1 specimens (control) received no whitening treatment; Group 2 received whitening treatment with an at-home whitening agent (22% carbamide peroxide) for 7 days; Group 3 received whitening treatment with a novel in-office whitening agent (35% H2O2); Group 4 received the same in-office whitening therapy with Group 3 using Er,Cr:YSGG laser in order to accelerate the whitening procedure. The specimens were stored for 10 days after the whitening treatment in artificial saliva. Vickers hardness was determined using a microhardness tester and surface roughness was evaluated using a VSI microscope. Three specimens of each experimental group were examined under SEM and the mineral composition of the specimens was evaluated using EDS. Data were statistically analyzed using one-way ANOVA, Tukey’s post-hoc test, Wilcoxon signed rank and Kruskal-Wallis tests (α = 0.05).

Results: The surface microhardness of the enamel was reduced after the in-office whitening treatments (P < 0.05), but not influenced after the at-home whitening treatment (P > 0.05). Moreover, the surface roughness was not significantly changed after tooth whitening. EDS analysis did not show alterations in the enamel mineral composition, while SEM observations indicated changes in the surface morphology, especially after in-office tooth whitening (P < 0.05).


Key words: laser assisted whitening; mineral composition; surface microhardness; surface morphology; surface roughness; whitening agent

INTRODUCTION

In-office tooth whitening is a very popular method in dental esthetics, providing in many cases an appropriate alternative for at-home tooth whitening. It can be recommended for rapid therapy, especially in severe tooth discolorations, or as a boost therapy which might be followed by an at-home whitening technique [1]. In-office whitening procedures generally use highly concentrated whitening agents ranged from 25% to 40% hydrogen peroxide (HP) or from 35% to 38% carbamide peroxide (CP) for shorter application time compared to home-whitening procedures. On the other hand, home-whitening procedures typically contain lower levels of active agents (3–6% HP or 10–16% CP), which are applied to the enamel for a longer period [2].

It is important to mention that the knowledge regarding the tooth whitening mechanism is still limited. Possibly, when the molecules of H2O2 diffuse into enamel, dissociate and produce free radicals such as hydroxyl radicals (HO·), perhydroxyl radicals (HOO·), perhydroxyl anions (HOO·─), and superoxide anions (OO·─) which are highly unstable because they contain one or more unpaired electrons in their atomic orbital. To stabilize their molecular structure, they tend to get an electron from the adjacent compound acting as strong oxidative agents. Hence, they attack the double bonds of chromophore molecules within the tooth enamel resulting in smaller, less heavily pigmented constituents, and there will be a shift in the absorption spectrum of chromophore molecules leading to the reduction of tooth discoloration [3,4].

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In order to accelerate the speed of the clinical procedure and the comfort of the patient, the whitening agent can be additionally light-activated by various light sources such as plasma arc lamps, quartz-tungsten-halogen (QTH) lamps, light-emitting diodes (LED) and lasers. The chemical reaction of the decomposition of \( \text{H}_2\text{O}_2 \) is accelerated by a rise in temperature \( (\text{H}_2\text{O}_2 + 211 \text{kJ/mol} \rightarrow 2\text{H}_2\text{O}) \). This means that a temperature rise of 10°C leads to an increase in speed of decomposition of a factor of approximately 2.2 [5]. Thus, when the whitening agent takes up energy from the light-source a small fraction is absorbed and its energy is converted into heat, and as a result the breakdown of \( \text{H}_2\text{O}_2 \) molecules increases and the whitening effectiveness improves [6].

In the past, many dental laser systems with various applications in dentistry have been used for tooth whitening treatments [5, 7, 8]. The effectiveness of laser systems indicated for whitening procedures depends on the wavelength and the power. Wavelengths with a high absorption coefficient in water and in hydroxyapatite are mainly absorbed at the tooth surface, where heat conversion occurs. In particular, wavelengths around 3,000 nm hardly penetrate deeper into tooth tissues and as a consequence, there is limited threat for the pulp of the tooth [5, 9]. The wavelength of \( \text{Er, Cr:YSGG} \) (yttrium-scandium-gallium-garnet) laser is 2780 nm and has a high affinity for tooth hydroxyapatite and the nearly highest absorption of water in any dental laser wavelength [10]. Although \( \text{Er, Cr:YSGG} \) is one of the lasers of choice for hard dental tissue treatment, but its use for tooth whitening is not sufficiently investigated.

Therefore, the purpose of this study was to evaluate in vitro the effect of a novel in-office laser-assisted whitening procedure using \( \text{Er, Cr:YSGG} \) laser on bovine enamel surface microhardness, roughness, morphology and mineral composition and to compare it with conventional in-office and at-home whitening procedures. The first null hypothesis (Ho1) of the study was that there are no significant differences in enamel surface microhardness among the experimental groups; the second null hypothesis (Ho2) was that there is no effect of the tested whitening procedures on surface roughness of the exposed enamel; the third null hypothesis (Ho3) was that there are no alterations in mineral composition of the enamel, while the forth null hypothesis (Ho4) was that the surface morphology of the enamel is not influenced after whitening treatments.

**MATERIALS AND METHODS**

**Preparation of the Enamel Specimens**

Twenty-four sound bovine incisors were stored in a 0.5% chloramines T solution at 6°C for up to 3 months. The crowns were separated from the roots, and each crown was sectioned into two halves (each specimen measuring approximately 4 mm long, 4 mm wide and 1.5 mm height) using a water-cooled diamond disc (Isomet, Buehler, Lake Bluff, IL). The 48 tooth fragments were not allowed to be dehydrated and examined by means of optical microscope under \( \times 10 \) magnification for any surface structural damage or deflection. Subsequently, the enamel specimens were randomly distributed into 4 groups \((n = 12)\) and were embedded in epoxy resin (Epoxy resin, Struers Tech A/S, Denmark) with the facial or lingual surface facing up. The enamel surfaces were ground and polished on a polishing machine (Jean Wirtz TG 250, Dusseldorf, Germany) using up to 1200 grit silicon carbide abrasive papers and a 0.4 μm alumina polishing suspension, in order to form parallel planar surfaces. After polishing, the specimens were checked for the absence of dentin areas on the polishing surfaces, immersed in an ultrasonic bath to remove any impurities from the past and stored in artificial saliva for 24 hours at 37°C before whitening treatment. The composition of artificial saliva was: 0.103 g/l of \( \text{CaCl}_2 \), 0.019 g/l \( \text{MgCl}_2 \cdot 6\text{H}_2\text{O} \), 0.544 g/l \( \text{KH}_2\text{PO}_4 \), 2.24 g/l KCl and buffer (TCP–KOH) was added to adjust the pH to 7 [11].

**The Tooth Whitening Techniques**

The four experimental groups submitted to one of the following treatments: Group 1 specimens (control) received no whitening treatment. Specimens of Group 2 received a 22% CP at-home whitening agent (Polanight, SDI Limited, Victoria, Australia), which was applied to a layer of 0.5–1 mm in thickness for 45 min each day, totally for 7 days, according to manufacturer’s instructions. Before the application of the whitening agent, the specimens were rinsed with distilled water and air-dried thoroughly. After the end of the procedure, the whitening agent was removed gently using a paper towel and the specimens were thoroughly rinsed with distilled water, air dried and stored in artificial saliva, which renewed daily for 10 days, at 37°C.

Specimens of Group 3 received a whitening treatment with a recently introduced whitening agent (Laser-White20, BIOLASE) for in-office tooth whitening with a concentration of 35% HP. The mixing conditions of the gel complied with the manufacturer’s instructions. The whitening agent was applied on the enamel surface in a layer of approximately 1 mm thickness, for 15 min and then removed from the teeth with high-power dry suction. This procedure was repeated twice and after the last application the specimens rinsed with distilled water.

Specimens of Group 4 received the same whitening procedure utilizing a \( \text{Er, Cr:YSGG} \) laser to accelerate the breakdown of HP molecules. The \( \text{Er, Cr:YSGG} \) laser (2780 nm, Waterlase MD Turbo, BIOLASE) has a Z-type glass tip (MZ8) with a 800 μm diameter and 6 mm length, used with the gold handpiece of the laser system. The laser parameters that were used for this case were an average output power of 1.25 W, pulse duration of 700 μs (S-mode) and a pulse repetition rate of 10 Hz. The laser parameters are adjusted for the whitening treatments so that the laser fluence of every laser pulse is below 0.5 J/cm² which is significantly below the ablation threshold of dental tissues. Since the ablation threshold for enamel of \( \text{Er, Cr:YSGG} \) is
in the range of 10−14 J/cm² [12] there is no risk of accidentally damaging the hard dental tissue. The activation of the whitening agent made for two intervals of 15 second for each specimen, keeping the handpiece of the laser device at a distance of 2.5 cm from the surface of the specimens with the use of a custom made spacer and positioned perpendicularly to the enamel surfaces. The selected distance was based on trigonometry calculations and a known total angle of divergence (α total = 12°) of the fiber tip to reduce the fluence mentioned above.

Surface Microhardness Evaluation

Surface microhardness of the specimens was assessed 10 days after the whitening treatment. Vickers hardness at a load of 200 g, with an indentation time of 10 second (Vickers pyramid: diamond right pyramid with a square base and an angle of α = 136° between the opposite faces at the vertex), was determined using a microhardness tester (HMV-2000, Shimadzu, Tokyo, Japan). Four indentations were made for each specimen (≥100 μm from each other), and were independently averaged and reported in Vickers Hardness Number (VHN).

Surface Roughness Evaluation

Measurements were done using a Vertical Scanning Interference (VSI) microscope (Bruker, ContourGT, Berlin, Germany). VSI is based on light interferometry and operates as a non-contact optical profilier in vertical scanning mode to produce 3D topography maps of the sample surface. For each specimen, three images were obtained in the four quadrants of the enamel surface. The software Vision64™ was used to acquire the data and compute the mean surface roughness (Ra, expressed in μm) on each image. For each specimen, the values were averaged on the 12 images and the mean value was obtained.

Energy Dispersive X-Ray Spectroscopy Analysis and SEM Observations

Three specimens for each experimental group were mounted on aluminum stubs, sputter coated with carbon to a thickness of approximately 200 Å in a vacuum evaporator (at low vacuum) and examined under Scanning Electron Microscope (JEOL Ltd, JSM-840, Tokyo, Japan) at 19 KV. Photomicrographs were performed using different magnifications up to 2000x in the surface area of the enamel specimens in order to detect any alterations in surface morphology. The mineral composition of the enamel specimens was evaluated using Energy Dispersive X-ray Spectroscopy (EDS). EDS is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum [13]. In the present investigation the elemental analysis was focused on the levels of calcium (Ca) and phosphorus (P), which are the main elements of the inorganic part of the enamel and form the molecules of hydroxyapatite.

Statistical Analysis

The enamel surface microhardness and surface roughness data were statistically analyzed using one-way ANOVA test. The Tukey’s post-hoc test was used at a 5% level of significance. The Kolmogorov-Smirnov test was applied to verify if the data were normally distributed. Moreover, the mineral composition of the enamel were analyzed using Wilcoxon signed rank and Kruskal–Wallis tests and the level of significance was preset at α = 0.05.

RESULTS

Surface Microhardness

The means and standard deviations of the surface microhardness (VHN) of enamel, recorded from the four experimental groups, are shown in Table 1. The Vickers hardness values ranged from 279.28 ± 24.30 VHN (control) to 250.00 ± 12.54 VHN (in-office and laser). The highest surface hardness exhibited the specimens of control group which was statistically significantly higher than that of the specimens which were received in-office whitening procedures (P < 0.05). However, control group specimens did not exhibit significant difference in surface microhardness compared to Group 2 specimens, which were received at-home whitening treatment (P = 0.16). Furthermore, there was no significant difference in surface hardness values between the two in-office bleached experimental groups (P = 0.64). The reduction of surface microhardness after whitening procedures was 3.88% for Group 2, 9.31% for Group 3 and 10.48% for Group 4.

Surface Roughness

The mean values and standard deviations of surface roughness of the enamel specimens expressed in Ra (μm) are due in large part to the fundamental principle that each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum [13]. In the present investigation the elemental analysis was focused on the levels of calcium (Ca) and phosphorus (P), which are the main elements of the inorganic part of the enamel and form the molecules of hydroxyapatite.

TABLE 1. Means and Standard Deviations of Surface Microhardness (VHN) and the % Reduction of Microhardness after Bleaching Procedures for Each Experimental Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Surface microhardness (VHN)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No bleaching treatment</td>
<td>279.28 ± 24.30a</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Bleaching with 22% CP</td>
<td>268.43 ± 15.66a</td>
<td>3.88%</td>
</tr>
<tr>
<td>3</td>
<td>Bleaching with 35% HP</td>
<td>253.26 ± 13.29b</td>
<td>9.31%</td>
</tr>
<tr>
<td>4</td>
<td>Bleaching with 35% HP + laser</td>
<td>250.00 ± 12.54b</td>
<td>10.48%</td>
</tr>
</tbody>
</table>

Values followed by different lowercase superscripts are statistically significantly different (P < 0.05).
values are presented in Table 2. Representative topographic surface maps and surface analysis of the enamel for each group are shown in Figure 1 (a–d) at a magnification of 20x. The mean Ra values ranged from 0.230 ± 0.017 (group 2) to 0.190 ± 0.022 (group 3). There were no statistically significant differences in Ra values among the experimental groups (P > 0.05). The increase in Ra values after at-home whitening procedure was negligible for Group 2 (6.96%), while for in-office whitening groups a slight reduction (11.21% for Group 3 and 3.27% for Group 4) was observed.

### Energy Dispersive X-Ray Spectroscopy and SEM Analysis
The mineral composition of the enamel for each experimental group is exhibited in Table 3. Additionally, the EDS spectrum of each element of the enamel structure appears below the SEM images in Figure 2 (a–d). EDS analysis revealed a slight reduction in the Ca levels (%wt) following whitening procedures but this reduction was not significant (P > 0.05). Moreover, the two in-office whitening treatments did not cause changes in the Ca composition of the enamel when compared with each other (P = 0.82). Regarding the phosphorus (P) levels of the enamel no significant changes were detected after whitening treatments (P > 0.05). The changes in wt % of the other elements (K, Na, Mg, and Cl) of the enamel did not have any clinical significance because of their very low levels in enamel composition. Concerning the fluoride content there is a significant increase of F but in the field of tooth whitening the higher fluoride content can be neglected.

Surface morphology changes in enamel surface after the whitening procedures are displayed in Figure 2 (a–d). When compared with the control group (Fig. 2a), which exhibited smooth and uniform surface without any morphological characteristics, the bleached groups showed apparent alterations in the enamel surface morphology. In particular, varying degrees of surface changes in terms of porosities, depressions, and superficial irregularities were observed for the bleached groups as shown in Figure 2(b–d). More specifically, these surface characteristics appeared in greater extent after in-office whitening treatment in comparison with those appeared after at-home whitening treatment. Nevertheless, between the in-office bleached groups the surface alterations were similar.

### DISCUSSION
The safety of the enamel structure when high-concentrated whitening agents are applied is of great importance. Although in the past many researchers investigated the effect of in-office tooth whitening on dental tissues, there is no consensus regarding the safety of this technique. It has been reported a negative effect on the integrity of organic enamel structures, such as proteins and collagen [14] and reduction in the mineral content [15], which may lead to changes in surface microhardness, surface roughness, surface morphology and mineral composition [16–18]. In contrast, there are many studies which did not confirm these observations [19–21].

The results obtained from this study demand rejection of the Ho1 which states that there are no significant differences in enamel surface microhardness among the experimental groups. The results of the present study agree with previous investigations which reported reduction in enamel surface microhardness after in-office tooth whitening [16,22,23]. Nevertheless, other studies demonstrated no changes in surface microhardness of the enamel [21,24]. On the other hand, the home-whitening procedure did not change the enamel surface microhardness significantly. Although this finding is supported by previous studies [21,25], some investigators reported significant reduction in surface microhardness after home-whitening procedures [26]. The outcomes of the present study agree with Lewinstein et al. [27] who found that an in-office whitening treatment decreased the enamel surface microhardness significantly more than an at-home whitening treatment.

Regarding the use of Er,Cr:YSGG laser, no significant differences in surface microhardness compared with the conventional in-office whitening treatment were detected. This is in agreement with previous reports [4,24]. The decrease of the surface microhardness of the enamel of in-office bleached groups may be attributed to the oxidative abilities of the whitening agents. Jiang et al. [18] who investigate the effect of 30% H₂O₂ on enamel surface using Raman scattering and laser-induced fluorescence found that the mineral and the organic matter of human tooth enamel might be greatly affected by H₂O₂, which was also supported by the results of microhardness measurements.

On the basis of the results reported in the current study, the Ho2, stating that there is no effect of the tested whitening procedures on surface roughness of the exposed
Fig. 1. (A) Vertical scanning interference microscopy of the enamel surface of the control Group 1 (magnification 20x). Each image corresponds to a $0.634 \times 0.475$ mm surface area. The colored scale bar (ranging from 0.178 to 0.251 $\mu$m) represents the surface irregularities depth. (B) Vertical scanning interference microscopy of the enamel surface of the Group 2 (magnification 20x). Each image corresponds to a $0.634 \times 0.475$ mm surface area. The colored scale bar (ranging from 0.195 to 0.253 $\mu$m) represents the surface irregularities depth after whitening. (C) Vertical scanning interference microscopy of the enamel surface of the Group 3 (magnification 20x). Each image corresponds to a $0.634 \times 0.475$ mm surface area. The colored scale bar (ranging from 0.173 to 0.238 $\mu$m) represents the surface irregularities depth after whitening. (D) Vertical scanning interference microscopy of the enamel surface of the Group 4 (magnification 20x). Each image corresponds to a $0.634 \times 0.475$ mm surface area. The colored scale bar (ranging from 0.160 to 0.215 $\mu$m) represents the surface irregularities depth after whitening.
enamel, can be accepted. The results of this study agree with those of previous investigations, which did not find alterations in surface roughness induced by in-office [21,28] or at-home [29,30] whitening agents. However, other investigations revealed increase [16,31] or decrease [32] in enamel surface roughness after tooth whitening due to the oxidative and erosive actions of the whitening agents. Nevertheless, Sulaiman et al. [19] who focused on the safety concerns with whitening procedures by investigating the effects of a high concentration H₂O₂ in-surgery whitening product on enamel, concluded that studies which reported adverse effects on enamel reflect not the whitening agent itself but the pH of the formulation used.

In the present investigation the use of Er,Cr:YSGG laser did not change the surface roughness of the enamel. It is interesting to mention that in a recent study [33] which investigated the effects of a conventional versus a laser-assisted whitening treatment on enamel surface roughness, a less surface roughness increase was observed after laser-assisted whitening.

This in vitro study supports the third null hypothesis that there are no alterations in mineral composition of the enamel after the whitening treatments. This coincides with the results of previous studies which investigate the effect of in-office [34] and at-home [20] whitening procedures on enamel composition. Notwithstanding there are many studies which support our results, some other investigations do not confirm these observations [26,35,36]. Severcan et al. [36] revealed that in-office tooth whitening causes deleterious alterations in the composition and structure of the enamel that significantly affects its crystallinity and mineralization. Therefore, at-home tooth whitening seems to be much safer than in-office in terms of molecular variations. Moreover, Xu et al. [37] reported that whitening agents with lower pH values resulted in more significant erosion of enamel. According to this, different factors (i.e., pH, concentration, exposure time and whitening procedures as at-home or in-office) seem to be responsible for erosion processes.

The results of EDS analysis provide positive evidence to support the use of Er,Cr:YSGG for in-office tooth whitening. Parreira et al. [23] demonstrated that light activation during in-office whitening does not produce significant changes in the enamel composition compared to a non-light-activated technique. In contrast, Berger et al. [15] concluded that in-office whitening agents can alter the mineralization level of the dental enamel surface and sub-surface regardless of what type of whitening light is used. It is interesting to mention that Son et al. [38] found that in-office tooth whitening with H₂O₂ and diode laser activation improves not only the whitening effect but also protects the change of enamel structure compared to the treatment with only gel.

SEM observations of the enamel surface indicated that whitening procedures may change the surface morphology of the specimens. Consequently, Ho4 of the study which states that the surface morphology of the enamel is not influenced after whitening treatments is rejected. The changes in surface morphology, in terms of porosities, depressions, and superficial irregularities were depended on the concentration of the tested whitening agents. This evidence concerning alterations in enamel surface morphology after in-office [16,24] or at-home [39] tooth whitening has been found before. Severcan et al. [36] claimed that after whitening, demineralization, decrease in protein and polysaccharide concentrations, mineral-to-protein ratio, and the strength of hydrogen bonds around NH groups, as well as a change in protein secondary structure may occur leading to changes in surface morphology of the enamel. However, other researchers reported that in-office [19,28] or at-home [40] whitening procedures do not influence surface morphology of the enamel.

The SEM observations confirms the safety of the use of Er,Cr:YSGG laser for in-office tooth whitening because no differences in enamel surface morphology were detected in comparison with the conventional whitening treatment. This is in agreement with previous reports which found that the laser-assisted tooth whitening does not produce significant changes in the enamel compared to a non-light-activated treatment [15,23].

This inconsistency of the results of the studies which investigated the influence of the whitening procedure on enamel surface morphology might be due to differences in study design, such as the composition and concentration of elements.

### Table 3. Means and Standard Deviations of %wt for Each Element of the Enamel Surface of the Experimental Groups

<table>
<thead>
<tr>
<th>Elements</th>
<th>No bleeding</th>
<th>Bleaching 22%CP</th>
<th>Bleaching 35%HP</th>
<th>Bleaching 35% HP + laser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>39.40 ± 3.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.21 ± 3.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.27 ± 3.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.21 ± 3.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>15.51 ± 2.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.74 ± 2.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.40 ± 2.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.82 ± 2.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>K</td>
<td>0.09 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na</td>
<td>0.37 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.77 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mg</td>
<td>0.13 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cl</td>
<td>0.96 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.91 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.83 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>0.72 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.91 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.14 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>O</td>
<td>39.18 ± 2.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.54 ± 2.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.24 ± 2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.61 ± 2.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in rows followed by different lowercase superscripts are statistically significant different (P<0.05).
Fig. 2. (A) Representative SEM image of the enamel surface of the control Group 1 (magnification 2000x). The black bar stands for 20 μm. The surface is smooth and uniform without any morphological characteristics. Also, the EDS spectrum of each element of the enamel structure appears below the image. (B) Representative SEM image of an enamel surface of the Group 2 (magnification 2000x). The black bar stands for 20 μm. Slight surface porosities, depressions and irregularities could be observed after at-home whitening treatment. The EDS spectrum of each element of the enamel structure appears below the image. (C) Representative SEM image of the enamel surface of the Group 3 (magnification 2000x). The black bar stands for 20 μm. Surface porosities, depressions and irregularities could be revealed in greater extent after in-office whitening treatment in comparison with those revealed in Group 2. The EDS spectrum of each element of the enamel structure appears below the image. (D) Representative SEM image of the enamel surface of the Group 4 (magnification 2000x). The black bar stands for 20 μm. Surface porosities, depressions and irregularities could be observed after in-office whitening treatment using Er,Cr:YSGG laser similar to those of Group 3. The EDS spectrum of each element of the enamel structure appears below the image.
the tested whitening agents, the selected whitening technique, the enamel substrate, the duration of storage of the specimens, the storage solution, the selected test for the measurements, the different types of laser devices with different technical characteristics and settings and the conditions of the experiment (in vitro, in vivo or in situ). In the present study, the changes in enamel surface after the whitening procedures are superficial and refer to a microscopic level. As a matter of fact, these changes possibly are reversible with the presence of human saliva and may not have a clinical significance [26].

CONCLUSIONS
Within the limitation of this in vitro study, it can be concluded that:

1. The safety of the use of Er,Cr:YSGG laser in order to accelerate the whitening procedure on enamel surface is confirmed.
2. The surface microhardness of the enamel is reduced after whitening procedures and in greater extent after in-office tooth whitening.
3. The surface roughness of the enamel is not influenced after whitening procedures, regardless the selected whitening technique.
4. There are no alterations in the mineral composition of the enamel after whitening procedures.
5. The surface morphology of the enamel changes according to the applied whitening treatment.

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