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# LASER-INDUCED BREAKDOWN SPECTROSCOPY (LIBS) USED TO ANALYZE THE EVOLUTION OF MINERAL CONTENT OF ENAMEL AFTER WHITENING AND ITS RECOVERY WITH A DESENSITIZING GEL

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## ABSTRACT

**Objective:** To analyze the mineral composition of enamel after a whitening treatment using hydrogen peroxide at 35% (HP) and a subsequent recovery treatment with desensitizing gel containing potassium nitrate at 3% and sodium fluoride at 0.25%, using the LIBS technique.

**Study design:** Thirteen healthy premolars were exposed to in vitro tooth whitening using HP at 35% (WhiteGold, Dentsply, USA) and subsequently treated with a desensitizing gel containing potassium nitrate at 3% and sodium fluoride at 0.25% (UltraEZ, Ultradent, South Jordan, UT, USA) to assess the recovery of mineral content lost because of the treatment with HP. The content of Ca, Mg, Na, C and CaF was repeatedly evaluated using laser-induced breakdown spectroscopy of the control enamel (M1), enamel exposed to HP (M2), enamel treated with potassium nitrate and sodium fluoride at 24 hours (M3), 7 days (M4) and 14 days (M5) after whitening.

**Results:** The presence of elements like Ca, Mg, Na, C and the compound CaF, was detected, proving there is no mineral recovery after applying potassium nitrate at 3% and sodium fluoride at 0.25%.

**Conclusions:** The use of a desensitizing gel containing potassium nitrate at 3% and sodium fluoride at 0.25% does not recover the mineral content of the enamel treated with HP at 35%.

  

The LIBS technique allowed to verify the presence of elements such as Ca, Mg, Na, C and the compound CaF, as well as the temporal modifications of their proportions in enamel.

**Keywords**: enamel mineral content, dental bleaching, laser induced breakdown spectroscopy, desensitizing agents.

## INTRODUCTION

Tooth bleaching is widely used to heighten the aesthetic aspect of vital teeth. One of the most used methods is the treatment with Hydrogen Peroxide (HP) (1, 2), and a concentration of 35% is the most widely used (2-4). However, HP may alter some features of enamel such as hardness and surface appearance (5), besides, its efficacy depends on the exposure time (3, 6). It has been reported that HP at 35%, combined with light irradiation coming from an halogen, LED or laser lamp, reduces the mineral content, primarily of Ca, of human enamel (7). Also, it has been reported possible loss of other elements such as K, P, Na, Mg, and F; when enamel is exposed to home bleaching Ca and K decrease while Na, F, and oxygen levels increase (8). On the other hand, it has also been documented an increase of dental sensitivity as a direct consequence of the HP bleaching process (9).

Measurements of enamel's mineral content have been acquired using computerized microtomography (10), atomic absorption spectrophotometry (FT-Raman spectroscope) (7, 11) and energy dispersive spectrometry using an Scanning Electron Microscope (SEM) (8). This latter method limits the study of the mineral content to a single assessment, while the computerized microtomography is an expensive method. Therefore, the search for precise and fast techniques to analyze the composition of teeth continues to be a necessity. It would be ideal to have a technique able to generate immediate information, in order to make accurate decisions during tooth whitening treatments.

An analytical technique, which has had an increasing development during past years, is the laser-induced breakdown spectroscopy (LIBS). This technique has several advantages, for example, it does not require previous preparation of the sample, it uses a small quantity of material, can be used to determine the composition of a broad range of elements and it gives the results *in situ* and on real time. LIBS has had a limited application in odontology, being limited to discrimination of dental caries, metallic traces, elements present in the enamel of primary teeth and to detection of toxic elements present in smoker's teeth (12-16), yet, there are no reports in which this technique is used to identify chemical elements present in enamel treated with HP. This method is non-expensive, easy to learn and to handle, it has had multiple uses among other areas (17, 18) allowing repeated measurements of the chemical elements present in the same sample and, therefore, allowing to observe changes in the mineral content during different periods since enamel exposed to HP presents changes in its hardness and surface appearance as days go by (19).

Due to negative consequences of the whitening process, there are post-whitening processes used to palliate them. Particularly, in previous studies, it has been reported that the use of potassium nitrate and fluoride significantly decrease dental sensitivity (20). Besides, it is known that the use of gel to decrease this sensitivity does not alter the whitening's efficacy (21).

In order to evaluate changes, using LIBS technique, in the elemental composition of the teeth during the whitening process, in the present work we studied changes in the composition of C, Na, Mg, C and CaF present in dental enamel treated with HP at 35% and, subsequently, with potassium nitrate at 3% and sodium fluoride at 0.25% which are used to recover elements lost as consequence of the whitening process.

## MATERIALS AND METHODS

This study was performed using a sample of 13 premolars extracted non-traumatically for orthodontics reasons from healthy patients with ages ranging from 18 to 25. Patients had had no enamel alterations at least 1 month prior to the extraction. The teeth were cleaned removing the remaining tissue and disinfected using NaOCL at 5.25% for 15 minutes. Then, they were affixed in an acrylic resin (NicTone, MDC, Guadalajara, Jal.) contained in metallic cylinders of 1.5 cm diameter x 1 cm height. The position of the teeth was secured on acrylic bases using a dental surveyor to align the flattest zones of the vestibular enamel, which was oriented facing the top. The teeth were affixed at the same height ensuring the enamel's surface was positioned at 1.3 cm height regarding the superior plane of the acrylic base.

## EXPERIMENTAL SET-UP

Figure 1 shows a diagram of the experimental set-up. As an excitation source we use a Nd:YAG laser, equipped with a Cr:YAG passive Q-Switch.

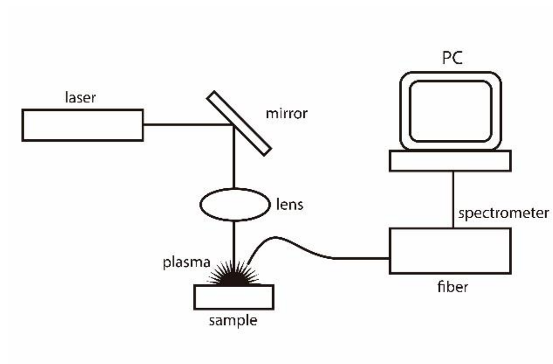


**Figure 1: Schematic diagram of the experimental system for LIBS measurements.**

The laser emits shots at a wavelength of 1064 nm laser shots, each one of up to 4 pulse trains with a minimum energy of 80 mJ. Laser beam is focused on the sample through a lens with a focal distance of 10 cm. The temporal structure of the laser shot

  

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was monitored using a RIGOL DS-1102E digital oscilloscope. Upon impinging on the sample's surface, the laser causes the formation of plasma which emission is collected and introduced into an optical fiber with a 200 µm diameter. The fiber conveys the radiation to a spectrometer supplied by OceanOptics, HR2000 model, within a detection range of 200 – 1100 nm, spectral resolution of 1.5 nm and integration time of 3.8 ms. Integration time was controlled by the software given by the supplier, while the delay between the pulse and the reading is controlled by a delay generator; this delay was of 1 µs for every experiment. For the computerized detection and capture of the spectra, we used the Spectra Suite Software. Identification of spectral lines was performed using the database published by the National Institute of Standards and Technology (NIST) as a reference. We carried out 5 repeated measures on human enamel in the same experimental conditions: the basal measurement (relative intensity) of elements content in the control enamel (M1), the measurement taken after the enamel was exposed to HP at 35% (WhiteGold, Dentsply, USA) for 35 minutes according to the manufacturer specifications (M2), the measurements taken after 24 hours (M3), 7 days (M4) and 14 days (M5) of a treatment with desensitizing gel (potassium nitrate at 3% and sodium fluoride at 0.25%) on enamel previously exposed to HP at 35%. Once the whitening was done, the teeth were stored in an incubator (Shellab Mod. 1500E) at 37ºC inside plastic containers filled with artificial saliva (Biotene, Glaxo SmithKline). Application of desensitizing gel (UltraEZ, Ultradent, South Jordan, UT, USA) was carried out immediately after the mineral content of the bleached enamel was evaluated using LIBS (M2). Desensitizing gel containing potassium nitrate at 3% and sodium fluoride at 0.25%, was applied daily on the enamel for 1 hour according to the manufacturer indications; this was made daily for 14 days to perform evaluations M3, M4 and M5.

## STATISTIC ANALYSIS

Data were analyzed obtaining descriptive for each element at the different moments of the study and were shown in graphs using the visual representation of the identified spectra in all evaluations. Likewise, the assumptions of normality and homogeneity of variances were checked through the Kolmorogov-Smirnov and the Levene tests, fulfilling the parametric assumptions, was chosen a Multivariate Anova followed by multiple post-hoc comparisons. All statistical tests have been handled at an alpha value of .05 in the IBM SPSS STATICS 23 statistical package.

## RESULTS

Descriptive of the relative intensity of the studied elements shown in Table 1. The Figure 2 shows a typical LIBS spectrum captured from an enamel sample before and after the whitening process. We identified the presence of Ca, C, Mg, Na and CaF. The characteristic peaks belong mostly to neutral species, although we identify a peak of CaII ionized at 392.78 nm. Figure 2 shows a spectrum captures immediately after the whitening process was concluded.

Figure 3 show the relative intensities of Ca, Na, Mg, C and CaF. We identified a significant reduction of the elements of study regarding the relative intensity of the enamel's original condition. ($p < 0.001$).

**Table 1: Relative intensity of elements contained in enamel treated with HP at 35% and a desentizing agent at different moments.**

| Calcium (392.78nm) | Mean | SD | 95% IC Mean lower | 95% IC Mean upper | Median |
|---|---|---|---|---|---|
| Before whitening | 56092.49 | 10774.92 | 49581.27 | 62603.71 | 64756.25 |
| Immediately after whitening | 30234.06 | 11829.92 | 23085.31 | 37382.81 | 28832.07 |
| 24 hours after whitening | 30257.98 | 11126.70 | 23534.18 | 36981.77 | 32970.80 |
| 7 days after whitening | 29721.33 | 5073.21 | 26655.62 | 32787.04 | 30289.82 |
| 14 days after whitening | 23906.57 | 9913.97 | 17915.62 | 29897.52 | 22153.50 |
| Sodium (589.05nm) | | | | | |
| Before whitening | 17806.18 | 9648.43 | 11975.69 | 23636.67 | 20844.40 |
| Immediately after whitening | 2294.72 | 3639.19 | 95.57 | 4493.86 | 356.76 |
| 24 hours after whitening | 1323.33 | 1676.62 | 310.14 | 2336.50 | 509.27 |
| 7 days after whitening | 2812.12 | 2899.42 | 1060.01 | 4564.22 | 1295.09 |
| 14 days after whitening | 1393.40 | 3029.89 | -437.54 | 3224.35 | 131.69 |
| Magnesium (518.39nm) | | | | | |
| Before whitening | 8762.35 | 9170.88 | 3220.44 | 14304.26 | 7237.70 |
| Immediately after whitening | 68.11 | 209.30 | -58.37 | 194.59 | 4.27 |
| 24 hours after whitening | 76.11 | 101.92 | 14.52 | 137.70 | 35.90 |
| 7 days after whitening | 192.13 | 510.87 | -116.58 | 500.86 | 40.81 |
| 14 days after whitening | 128.39 | 402.31 | -114.72 | 371.50 | 20.85 |
| Carbon (373.42nm) | | | | | |
| Before whitening | 28915.15 | 21162.27 | 16126.92 | 41703.39 | 20509.99 |
| Immediately after whitening | 5355.97 | 3398.89 | 3302.04 | 7409.90 | 4082.05 |
| 24 hours after whitening | 4875.76 | 4100.39 | 2397.92 | 7353.61 | 3884.03 |
| 7 days after whitening | 6562.90 | 2552.07 | 5020.70 | 8105.10 | 6158.71 |
| 14 days after whitening | 3236.11 | 3727.45 | 983.63 | 5488.58 | 1345.73 |
| CaF (605.24nm) | | | | | |
| Before whitening | 24579.29 | 10734.19 | 18092.68 | 31065.89 | 28718.14 |
| Immediately after whitening | 1650.87 | 3639.91 | -548.70 | 3850.45 | 300.21 |
| 24 hours after whitening | 778.25 | 1089.74 | 119.73 | 1436.78 | 198.38 |
| 7 days after whitening | 3396.11 | 4483.52 | 686.75 | 6105.47 | 1051.08 |
| 14 days after whitening | 1785.20 | 4083.69 | -682.55 | 4252.95 | 99.74 |

  

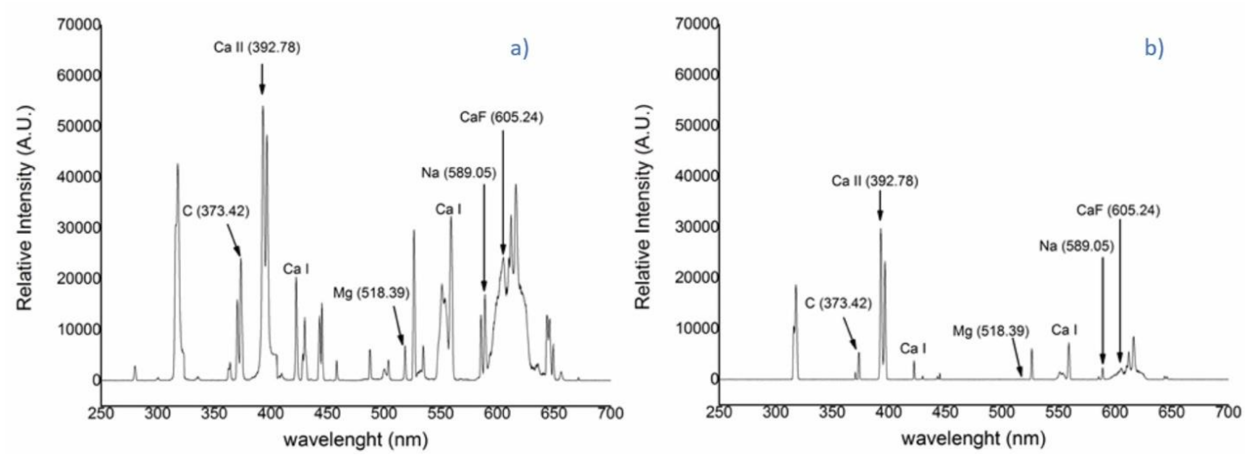


**Figure 2: Spectra from dental enamel, obtained with LIBS before (a) and after (b) whitening using hydrogen peroxide at 35%.**

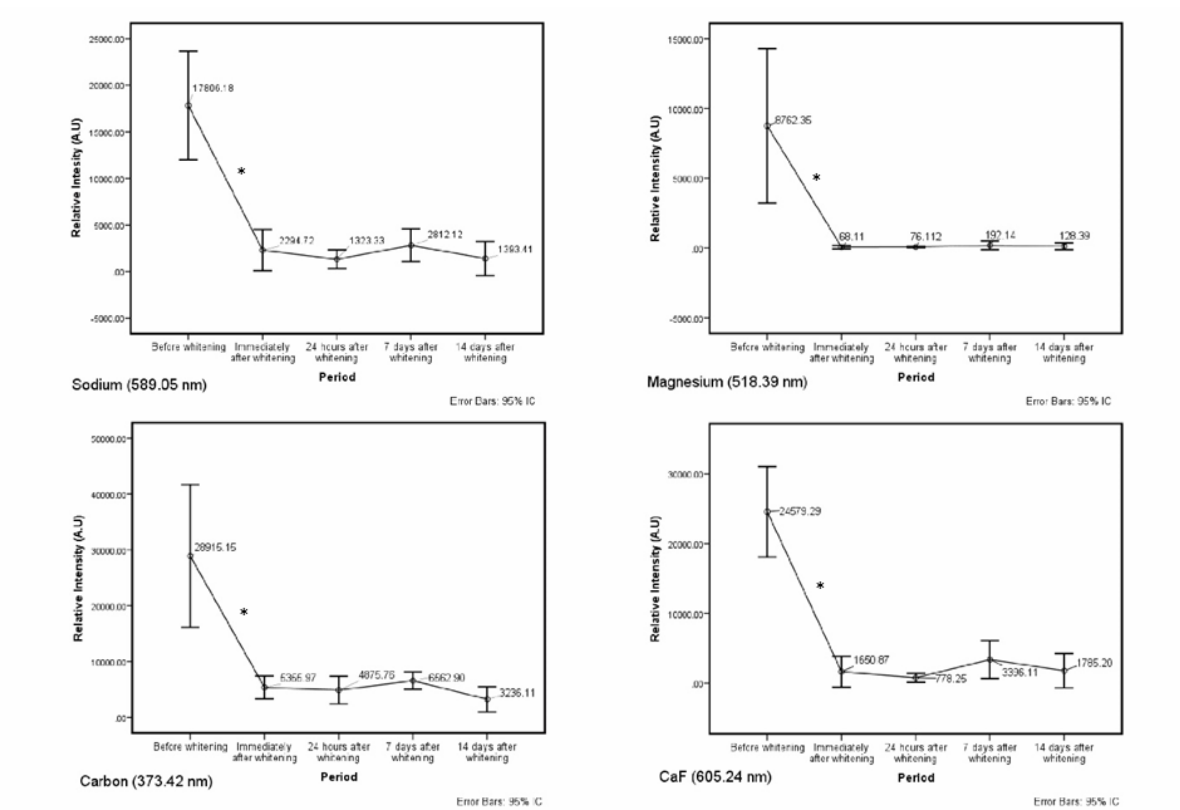


**Figure 3: Confidence intervals of 95% of the relative intensity of Na, Mg, C and CaF exposed to HP at 35% and to a desensitizing containing potassium nitrate at 3% and sodium fluoride at 0.25%. * Statistically significant difference.**

  

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## DISCUSSION

Nowadays, tooth whitening is a widely requested procedure asked by patients who want a more aesthetic smile (22, 23). HP is the most used agent to perform such procedure due to its easy penetration into the enamel; this agent breaks down water and oxygen and releases perhydroxyl radicals leading to oxidation, which destroys chromophore radicals altering enamel's pigments and leading to a whitening effect (6, 24). However, this reaction may modify the enamel's structure; have noted that HP concentrations above 30% minimally reduce superficial microhardness, which is related to the loss or gain of mineral content (25). On the other hand, it is important to stress that low pH hydrogen peroxide leads to important topographic changes on the enamel. The product used in our study had a neutral pH; therefore, we consider this aspect may not have a relevant role in enamel alterations.

Our study shows that exposure of enamel to HP at 35% results on reduced presence of elements like Ca, Mg, Na, C and CaF. So, we consider that the ability of HP to penetrate into the enamel modifies the mineral content and this could be a contributing factor to the appearance of sensitivity after tooth whitening. However, our results differ from those reported by Park *et al.* (26) and Goo *et al.* (27) who didn't find significant changes in the mineral content of enamel exposed to HP at 35% and enamel exposed to carbamide peroxide at 10%; this discrepancy may be due to the fact that they used bovine teeth and that we used different whitening agents. Besides, in those studies they used a FT-Raman spectrophotometer, this biomedical analytical method emits Raman signals that have information related to the composition and structure of materials at a molecular level (28) while, in our study, we used the LIBS method; this method has an excitation source that uses a Nd:YAG laser, the laser causes the formation of a plasma which is collected and introduced into an optical fiber, this fiber conveys the radiation to a spectrometer obtaining information about the elements found. An advantage of this method is that the laser eliminates the fluorescence created due to the electronic absorption of the excitation radiation and it has proven to be an efficient method to perform a qualitative evaluation of the elemental composition.

A limitation of our study was that the enamel places from which the spectra were obtained were very close, yet not the same ones, so this fact could represent variations of the studied minerals content.

In this study, we used desensitizing agents such as potassium nitrate at 3% and sodium fluoride at 0.25%, applied immediately after the exposure to HP at 35%; our results show these desensitizing agents do not recover mineral content of the enamel at any of the evaluated moments (24 hours, 7 days or 14 days), this interpretation is based on the relative intensities observed in the enamel's

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elements, which is very similar to those observed in enamel treated with HP at 35% and there is no recovery of the relative intensity of the lines corresponding to Ca I, Ca II, Mg, Na, C and CaF as time goes by. Saliva plays an important role in the evaluation of the mineral content of the obtained measurements, since it is capable of promoting enamel remineralization (29). In this study, except for when the measurements were taken, the sample remained inside an incubator at 37°C in containers filled with artificial saliva in order to recreate conditions of temperature, moisture and saliva presented in the oral cavity. Artificial saliva has inorganic components of calcium and phosphate able to form a protective saliva barrier (30); this factor, along with the desensitizing agent, seemingly had no important role regarding the recovery of mineral content of enamel treated with HP at 35%.

## CONCLUSION

We demonstrate that using LIBS technique is possible to study the temporal evolution of the enamel's composition once it has gone through a whitening process. We confirmed that decrease of mineral content is detectable immediately after the HP treatment with no subsequent modifications, at least after 14 days, verified by experimental measures.

The LIBS technique allowed verifying the presence of elements such as Ca, Mg, Na, C and the compound CaF, as well as the temporal modifications of their proportions in enamel.

Under this study's conditions, we can conclude that the use of potassium nitrate at 3% and sodium fluoride at 0.25% doesn't cause the recovery of mineral content of enamel treated with HP at 35%.

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