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Evaluation of Color Stability and Remineralization Capacity of Dual Effect Desensitizing Agents on Bleached Enamel with Hydrogen Peroxide

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Research Article

History

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ABSTRACT

Objectives: The aim of the study was to evaluate the effects on enamel calcium and phosphorus content and color stability of double-acting desensitized varnishes applied to bovine tooth enamel bleached with 40% hydrogen peroxide.

Material and Methods: The coronal part of 10 newly extracted bovine teeth (approximately 10x12mm in size) was divided into 5 regions for mineral exchange measurements. To evaluate the color change, 50 newly extracted bovine teeth were divided into 5 groups. Group 1 unbleached (negative control group), Group 2 was bleached with 40%HP (positive control group), Groups 3, 4 and 5 were treated MI varnish, Clinpro White and Profluoroid varnish after bleaching, respectively. Then, all groups were exposed to the tea solution and enamel color measurements were made using the CIE Lab method with spectrophotometer. Mineral change measurements were determined by EDS and morphological changes were observed using SEM. One-way ANOVA, Tukey HSD tests and Tamhane's tests were used for statistical analysis.

Results: The content of calcium and phosphorus in enamel were like No bleaching>MI varnish>Clinpro White>Profluoroid>Bleaching. The most color stability after bleaching was determined in teeth which were applied MI varnish applied group ($p<0.05$). The color stability of Profluoroid and Clinpro White applied group were similar ($p>0.05$). There was a statistically significant difference between the groups in terms of $\triangle L$ and $\triangle b$ averages ($p<0.05$).

Conclusions: MI, Clinpro White and Profluoroid varnish used after bleaching were effective in preventing mineral loss from tooth enamel and reducing the susceptibility of tooth enamel to staining with tea.

Key words: Bleaching, Desensitizing Agents, Remineralization, SEM-EDS, Color Stability.

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Introduction

Today, individuals not only want to have healthy teeth, but also want to have a perfect smile.[1] White teeth are considered to be associated with both health and beauty and are preferred by patients. The demand for bleaching treatments is increasing with rising living standards and the increased aesthetic expectations.[2]

Dental bleaching is a safe, effective and minimally invasive treatment for tooth discoloration[3] and is a more conservative approach compared to porcelain crowns and composite laminate veneer restorations.[4] Dental bleaching can be performed by using home and in- office bleaching techniques. Home bleaching is usually applied to 10-22% carbamide peroxide or 4-10% hydrogen peroxide, while in-office bleaching is applied to 20-38% hydrogen peroxide or 35% carbamide peroxide.[5] In-office bleaching is preferred more in dentistry practice as it will provide faster whitening results with less application compared to home bleaching techniques.[6]

Although dental bleaching is a minimally invasive treatment, it causes some adverse effects on dental hard tissues. The most common adverse effect is tooth sensitivity.[7] The others are microscopic changes such as increased porosity and surface irregularities[8], an increase in surface roughness[9] and a decrease in hardness,[10] a decrease in mineral content of enamel.[11] Changes in the enamel surface after bleaching will create a porous structure and facilitate the precipitation of various color pigments in these pores. This will accelerate the discoloration process on the tooth surface exposed to bleaching. Therefore, this process can be prevented by removing the rough enamel surfaces.[12] For this purpose, using a dual-action product consisting of desensitizing and remineralizing agents during or after the bleaching treatment not only relieves tooth sensitivity but can also decrease the potential negative effects of peroxides on enamel structure.[13]

Fluoride varnish acts as a slow-release reservoir of fluoride, developed to keep fluoride on the tooth surface longer. Addition of calcium phosphate salts such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), tri-calcium phosphate (TCP) to increase the effect of the varnish can improve the remineralization of

enamel.[14,15] TCP is functionalized (fTCP) by ball milling beta-tricalcium phosphate with sodium lauryl sulfate. During the production process, a protective barrier is created that helps calcium coexist with fluoride ions around it but prevents unwanted reaction between them during storage. It releases calcium and fluoride when it comes into contact with saliva after application.[15] CPP-ACP buffers free calcium and phosphate ion activities, helping to maintain a stage of supersaturation relative to tooth enamel, which suppresses demineralization and strengthens remineralization.[16] One study reported that CPP-ACP application to the tooth surface exposed to 35% HP decreased the incidence and severity of tooth sensitivity.[17] Studies have reported that enamel surface hardness increases, and roughness decreases after CPP-ACP is applied to enamel whose roughness increases and hardness decreases because of bleaching.[13,18] For these reasons, the application of these agents after bleaching can reduce the absorption of pigments as well as promote remineralization and greater surface sealing, resulting in greater color stability.

The aim of this study is to evaluate and compare the effects on enamel Ca and P content and color stability of double-acting products applied to enamel bleached with 40% HP. The following null hypotheses were tested: 1) the bleaching treatment does not affect Ca and P content of the enamel; 2) dual-acting products applied after bleaching do not affect Ca and P content of the enamel; 3) dual-acting products applied after bleaching do not have a significant effect on reducing the susceptibility of tooth enamel staining with black tea.

Material and Methods

Specimen preparation

In this study, 50 freshly extracted bovine teeth without caries, cracks, or demineralized surfaces were used. Periodontal ligament and gingival tissues were cleaned using a scalpel. The teeth were stored in distilled water before use. The crown was removed from the root and buccal enamel specimens were obtained. 10 specimens (approximately 10x12mm in size) were divided into 5 parts (each part was used in a separate group n=10) to assess the effect of bleaching and postbleaching dual-acting desensitizing agents on enamel Ca and P concentration. 50 specimens were used to assess the effect of bleaching and postbleaching dual-acting desensitizing agents on color stability. The specimens were washed in an ultrasonic bath to eliminate the dirty of enamel surface and then were embedded in translucent acrylic resin.

Bleaching procedure and surface treatments

The specimens were divided into 5 groups (n=10).

Group A (negative control group): No bleaching procedure was performed.

Group B (positive control group): At first, in-office bleaching gel containing 40% HP (Opalesence Boost, Ultradent, South Jordan, Utah, USA) was applied twice, and each application time was 20 minutes.

Group C (MI Varnish): At first, in-office bleaching gel containing 40% HP (Opalesence Boost, Ultradent, South Jordan, Utah, USA) was applied twice, and each application time was 20 minutes. After the bleaching, MI (GC, Tokyo, Japan) varnish were applied to enamel blocks, dried with air water spray, and waited for 10 minutes.

Group D (Clinpro Varnish): At first, in-office bleaching gel containing 40% HP (Opalesence Boost, Ultradent, South Jordan, Utah, USA) was applied twice, and each application time was 20 minutes. After the bleaching, Clinpro White (3M ESPE, MN, USA) varnish was applied to enamel blocks, dried with air water spray, and waited for 10 minutes.

Group E (Profluoroid varnish): At first, in-office bleaching gel containing 40% HP (Opalesence Boost, Ultradent, South Jordan, Utah, USA) was applied twice, and each application time was 20 minutes. After the bleaching, Profluoroid (VOCO GmbH. Cuxhaven, Germany) varnish were applied to enamel blocks, dried with air water spray, and waited for 10 minutes.

Staining protocol

After bleaching protocols were completed, the specimens were immersed in black tea (Lipton Yellow Label, Unilever, Istanbul, Turkey) solution (prepared by soaking 1 bag of black tea in 200 ml of boiled water for 5 minutes) for 10 min per day for two weeks. The solution was prepared freshly at every day. After that washed and immersed in distilled water during all day. This cycle was repeated for 2 weeks.

Color measurement

Color measurements were performed using Vita Easyshade 5 spectrophotometer (Vita Zahnfabrik, Bad Sackingen, Germany) in accordance with CIELAB system. In this system, L* represents brightness (white-black range), a* represents red-green color range, and b* represents yellow-blue color range The spectrophotometer was calibrated in compliance with the manufacturer's instructions before each measurement. Removable plates were prepared on an acrylic block using an essix plate (1.0 mm Essix C; Dentsply, USA) on a vacuum press machine (Scheu Ministar, Germany) to standardize the color measurement site on the specimens. Then standard windows (R=7mm) were prepared on the plate corresponding to the areas to be measured on the specimens. The color parameters (L, a, and b values) of each sample were measured with a spectrophotometer from these opened windows. All color measurements for each specimen were made for each evaluation time point, at same room and standard light source by a single trained operator.

The measurements were performed after baseline (T_0), bleaching (T_1), and staining protocol (T_2). Baseline is defined as the time before bleaching protocols applied. The color differences between T_1-T_0, T_2-T_0 were

represented by ΔE_{ab}*(T1), ΔE_{ab}* (T2) respectively and calculated as follows:

ΔE_{ab}*(T1)=[(ΔL1-L0*)^2+(Δa1-a0*)^2+(Δb1-b0*)^2]$^{1/2}$

ΔE_{ab}*(T2)=[(ΔL2-L0*)^2+(Δa2-a0*)^2+(Δb2-b0*)^2]$^{1/2}$

ΔE_{ab} =ΔE_{ab} *(T2)- ΔE_{ab} *(T1)

Scanning Electron Microscopy (SEM) Energy Dispersive Spectrometry (EDS)

After the sample was air-dried, it was fixed on aluminum plates and gold plated. Then, surface topography images were taken with a Scanning Electron Microscope (FE-SEM, Hitachi SU5000, Hi-Tech., Ltd.Japan) at 5000X magnifications with an acceleration voltage of 10 kV. Calcium and phosphorus content of enamel surfaces in each group were determined by EDS.

Statistical Analysis

In this study, IBM SPSS Statistics 22 program was used for statistical analysis. Kolmogorov-Smirnov and Shapiro-Wilks tests were used to evaluate the suitability of the parameters to the normal distribution. It was determined that the parameters in this study were suitable for the normal distribution. While evaluating the datas, to compare between groups the Oneway Anova test was used, and the Tukey HDS test was used if the variances of the groups were homogeneous, and Tamhane's T2 test was used if they were not homogeneous. Significance was evaluated at the p<0.05 level.

Results

Color Stability Measurement

There was a statistically significant difference between the groups in terms of △E averages (p:0.001; p<0.05). As a result of Tamhane's T2 test performed to determine which groups the significance originates from; although there was no significant difference between the △L mean of the non-bleaching group and the MI varnish group (p>0.05), it was significantly higher than the bleaching group (p:0.012; p:0.007; p<0.05). There was no statistically significant difference between Clinpro White and Proflouoroid groups (p>0.05).

There was a statistically significant difference between the groups in terms of △L averages (p:0.012; p<0.05). As a result of the Tukey HSD test performed to determine which groups the significance originated from; although there was no significant difference between the △L mean of the unbleached group and the MI varnish group (p>0.05), it was significantly higher than the bleaching group (p:0.006; p:0.000; p<0.05). There was no statistically significant difference between Clinpro White and Profluoroid groups (p>0.05). The procedures used in this study did not effect △a values. There was no statistically significant difference between the groups in terms of mean △a (p:0.349; p>0.05).

There was a statistically significant difference between the groups in terms of △b averages (p:0.001; p<0.05). As a result of Tamhane's T2 test performed to determine which groups the significance originates from; although there was no significant difference between the △b mean of the non-bleaching group and the MI varnish group (p>0.05), it was significantly higher than the bleaching group (p:0.001; p:0.002; p<0.05). There was no statistically significant difference between Clinpro White and Profluoroid groups (p>0.05).

SEM EDS Analysis

Evaluation of Ca and P values of the groups are shown in Table 2. The graphical comparison is also shown in Figure 1. There was a statistically significant difference in the groups of calcium averages (p:0.001; p<0.05). As a result of Tamhane's T2 test performed to determine which groups the significance originates from; the amount of calcium and phosphorus in MI varnish group were significantly higher than the Bleaching, Clinpro White and Profluoroid (p:0.001; 0.001; 0.001; respectively p<0.05). The amount of calcium and phosphorus in the unbleached group were significantly higher than the Bleaching, Clinpro White and Profluoroid (p:0.001; 0.001; 0.001; respectively p<0.05). The amount calcium and phosphorus of the Clinpro White group were significantly higher than the Bleaching and Profluoroid groups (p:0.001; 0.001; respectively p<0.05). The amount of calcium and phosphorus in Profluoroid group were significantly higher than the Bleaching group (p:0.001; p<0.05). There was no statistically significant difference between the non-bleaching and MI varnish groups (p>0.05).

Evaluation △L, △a, △b and △E of the groups are shown in Table 3. The graphical comparison is also shown in Figure 2. The amount of phosphorus in Clinpro White group was significantly higher than Bleaching and Profluoroid groups (p:0.001; 0.010; respectively p<0.05). Phosphorus mean of the Profluoroid group was significantly higher than that of the Bleaching group (p:0.001; p<0.05). There was no statistically significant difference between the non-bleaching and MI varnish groups (p>0.05).

SEM images were taken at 5000X magnifications to compare differences in the surface topography of groups (Figure 3). SEM image of unbleached enamel (control) (A) with smooth surface morphology and a small incidence of porosities, bleached enamel (B) with serious surface changes such as complete removal of the aprismatic layer and increased depth of enamel irregularities, enamel treated with MI varnish after bleaching (C) with the formation of a superficial mineral layer and organized nanocluster, enamel treated with Clinpro White varnish after bleaching (D) with the presence of a superficial mineral layer, but with the presence of numerous and various sizes of pores in some areas, and enamel treated with Profluoroid varnish after bleaching with the presence of a superficial mineral layer, but with the prism centers not closing completely in some regions.

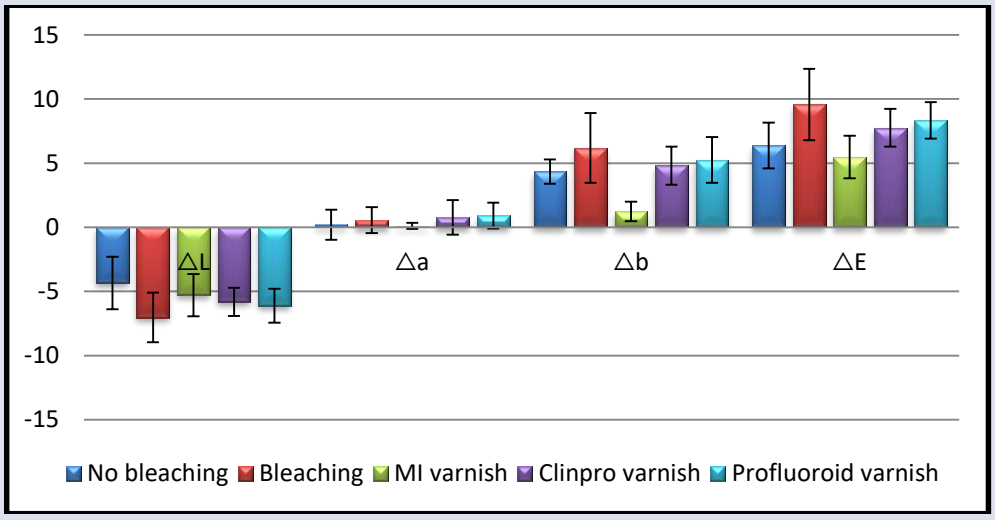

Figure 1. Evaluation of the groups in terms of △L, △a, △b and △E.

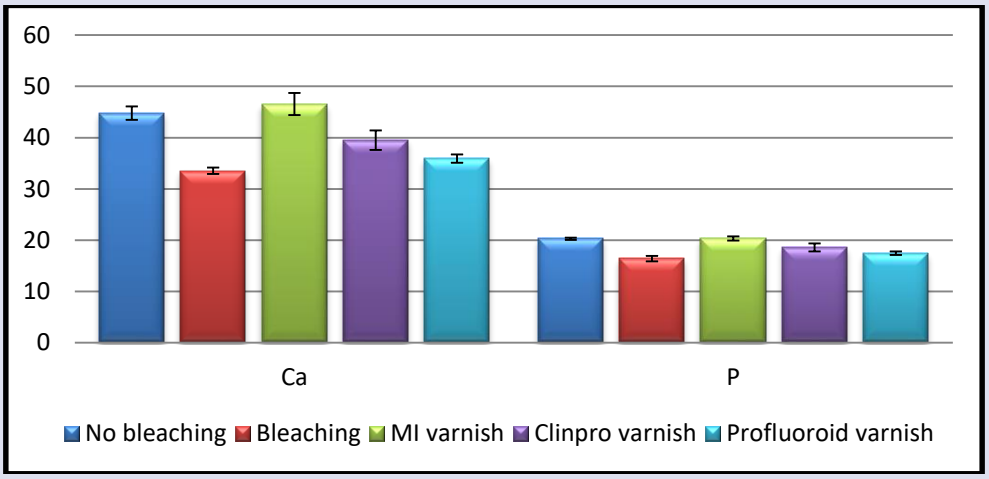

Figure 2. Evaluation of groups in terms of calcium (C) and phosphorus (P).

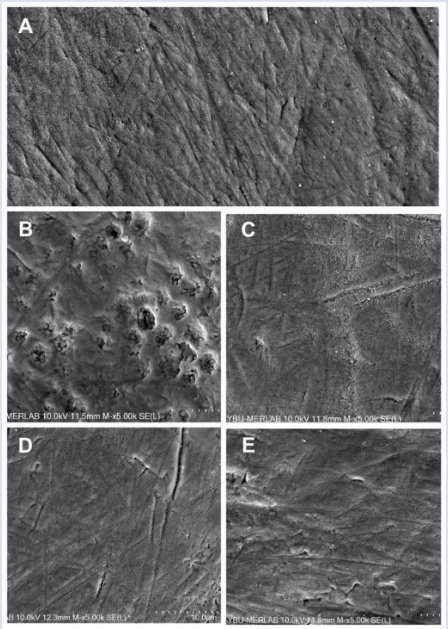

Figure 3. Representative scanning electron microphotographs of enamel surfaces(5000X) (A) Unbleached enamel, (B) Bleached enamel, (C) MI varnish after bleaching, (D)ClinPro varnish after bleaching, (E) Profluoroid varnish after bleaching

Discussion

Bleaching agents lighten the discolored tooth by the decomposition of peroxides into free radicals. These radicals convert big pigmented molecules of enamel tissue into less pigmented smaller molecules through reduction and oxidation reactions.[19] Although studies on the effect of bleaching agents on dental tissue are conflicting,[20, 21] it is widely agreed that peroxides can alter the mineral content of teeth.[22] Lee *et al.* reported a significant decrease in the Ca/P ratio of bovine enamel bleached with 30% HP. In another study, the effects of 10% CP on mineral change and surface properties of enamel were investigated, and it was reported that as a result of bleaching, the Ca/P ratio and the Ca, P concentrations of the enamel decreased, and local changes resembling the initial tooth decay occur in the tooth enamel.[11] In this study, Ca and P concentrations of enamel bleached with 40% HP were statistically significantly lower compared to the control group consistent with the results of previous studies. Therefore, the first hypothesis of the study was rejected.

Morphological defects and change in mineral content caused by bleaching can be treated by the application of remineralizing agents. Considering that the incidence of tooth sensitivity after bleaching is very high,[7] dual-acting products containing both desensitizing and remineralizing agents can be used after bleaching. In this study, Profluoroid, Clinpro White and MI varnish were used from these products. The lack of using saliva as a remineralizing agent after in-office bleaching may be the limitation of this study. Because saliva plays an important role in protecting enamel from mineral loss and allows enamel remineralization.[23, 24] Vargas *et al.*[25] report that saliva acts as an alkalizing agent and helps reverse the effects of bleaching. However, in this study, the specimens were stored in distilled water instead of artificial saliva during the study period to analyze the effects of both the bleaching agent and the double-acting products used after bleaching on the chemical composition and surface morphology of the enamel.

Cochrane *et al.*[26] reported that MI varnish released higher levels of fluoride and calcium ions compared to other varnishes containing Ca and F, and thus had a better remineralization potential. Another study reported that CPP-ACP cream was more effective at remineralizing eroded enamel than fluoride varnish, fluoride toothpaste, fTCP varnish.[27] Rani *et al.*[28] reported that varnish containing CPP-ACP has a superior protective potential compared to varnishes containing F and fTCP. These findings are consistent with the results of this study, in which MI varnish applied after bleaching showed the highest remineralization potential. Some studies reported that Clinpro White varnish has more remineralization efficiency than MI varnish.[15, 29] In a study comparing the effects of 5% sodium fluoride varnish with/without fTCP, it was reported that the addition of fTCP significantly increased the remineralizing ability of the varnish.[30] Shen *et al.*[31] and Rani *et al.*[28] evaluated the ability of various varnishes to inhibit enamel demineralization. It was reported that all varnishes containing F significantly inhibit enamel demineralization, but varnishes containing calcium phosphate and F are more effective than varnishes containing only F. In addition, MI varnish reported higher levels of Ca and P release than Clinpro White varnish, consistent with the findings of this study. Therefore, the second hypothesis was also rejected.

Application of these substances after bleaching can promote remineralization and greater surface sealing, as well as reduce the absorption of pigments and provide greater color stability.[32] In this study, there was no significant difference between the amount of color change in the group treated with MI varnish after bleaching and in the unbleached group. The color stability of the groups treated with Clinpro White and Profluoroid varnish after bleaching was higher than the bleached group only, and lower than the unbleached group. Therefore, the third hypothesis was rejected. Total color change (ΔE) in study groups is attributed to the shift of the "L" parameter to the negative direction and the positive direction of the "a" and "b" parameters. In other words, the parameter “L” shifted to darker tones, “a” more red and “b” more yellow. The fact that black tea contains yellow dyes with different polarities can explain this situation. The results are consistent with the results of the studies of Karadaş and Seven[33], Amorim *et al.*[34] The “L” and “b” parameters had the greatest effect on ΔE; is consistent with previous studies.[32, 35, 36] Singh *et al.*[32] showed that tea has a significant effect on the color change of newly bleached enamel, and this effect is manifested by the negative (darker) shift of the “ΔL” parameter. Another study reports that the bleached group had the highest L* means, while the groups treated with CPP-ACP and F had the lowest L* mean after the unbleached group,[37] consistent with the findings of this study.

Chen *et al.*[38] reported that exposure to coffee and tea during the bleaching treatment did not affect the efficacy of the treatment, but exposure after the bleaching adversely affected the efficacy of the treatment. Considering that the changes in enamel after bleaching are responsible for its sensitivity to staining, the use of CPP-ACP, fTCP and F after bleaching can be very beneficial in terms of stability of the treatment result and reduction of tooth hypersensitivity. In addition, administration of these agents can also assist in removing the dietary restrictions that are usually recommended for patients after bleaching treatment.

Conclusions

Within the limitations of the current study, in-office bleaching resulted in reduced Ca and P in enamel. The use of dual-acting agents such as MI, Clinpro White and Profluoroid varnish applied after bleaching was effective in replacing mineral loss from enamel and reducing the susceptibility of tooth enamel to staining with tea. MI varnish provides the most remineralization, followed by Clinpro White varnish and Profluoroid, respectively. MI

varnish is most effective in reducing discoloration with tea. However, there is no difference between Clinpro White and Profluoroid varnishes.

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Conflict of Interest

The authors declare no conflict of interest for the authorship and/or publication of this article.

Table 1. The materials used in this study and their compositions

Product	Manufacturer	Composition (w/w)
MI Varnish	GC, Tokyo, Japan	30-50% polyvinyl acetate, 10-30% hydrogenated rosin, 20-30% ethanol, 1-8% sodium fluoride, 1-5% CPP-ACP, 1-5% silicon dioxide
Clinpro White Varnish	3M ESPE, MN, USA	30-75% pentaerythritol glycerol ester of colophony resin, 10-15% n-hexane, 1-15% ethyl alcohol, 1-5% sodium fluoride, 1-5% flavour enhancer, 1-5% thickener, 1-5% food grade flavour, <5% modified tricalcium phosphate
Profluorid varnish	VOCO GmbH. Cuxhaven, Germany	5%sodium fluoride, ethanolic colophony
Opalescence Boost Office Bleaching Agent	Ultradent, USA	40% hydrogen peroxide, thickeners, 1.1% sodium fluoride, 3% potassium nitrate, pH regulators.

Table 2. Evaluation of the groups in terms of △L, △a, △b and △E.

	△L	△a	△b	△E
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**
No bleaching	-4.05±1.53a	0.20±1.18a	2.40±0.33a	4.91±1.27a
Bleaching	-7.73±1.07b	0.56±1.01a	6.18±2.72b	10.10±2.30b
MI varnish	-4.99±1.10a	0.12±0.24a	1.24±0.76a	5.19±1.12a
Clinpro varnish	-5.82±1.09c	0.74±1.31a	3.64±0.58c	7.04±1.06c
Profluoroid varnish	-5.96±1.10c	0.81±1.00a	3.75±0.61c	7.19±0.89c
p	0.012*	0.349	0.001*	0.001*

Oneway ANOVA Test **p<0.05*
Different letters in the columns indicate the difference between groups.

Table 3. Evaluation of groups in terms of calcium and phosphorus.

	Ca	P
	mean±SD	**mean±SD**
No bleaching	44.76±1.30a	20.26±0.23a
Bleaching	33.52±0.63b	16.36±0.53b
MI Varnish	46.54±2.15a	20.31±0.39a
Clinpro varnish	39.51±1.90c	18.57±0.78c
Profluoroid varnish	35.92±0.83d	17.43±0.34d
p	0.001*	0.001*

Oneway ANOVA Test **p<0.05*
Different letters in the columns indicate the difference between groups.

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