

## EFFECT OF TEETH WHITENING LIGHT-ACTIVATED SYSTEM ON MINERAL CONTENT OF HUMAN TOOTH STRUCTURE

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### ABSTRACT

**Aim:** evaluation of the effect of teeth whitening light-activated system on the mineral content of human tooth structure.

**Materials and Methods:** Forty intact extracted human anterior teeth were utilized in this study that were randomly divided into two equal groups according to the tested whitening toothpaste as follows: Group I: Blue covarine-containing whitening toothpaste (CloseUp White Now toothpaste). Group II: Actilux-activated whitening toothpaste (Blanx® White Shock Toothpaste). Each one was applied on the specimen's labial surface following manufacturer instructions. Mineral contents were measured by Energy Dispersive X-ray analysis (EDX) before and after toothpaste application.

**Results:** The mean calcium and phosphorus values in each group I and II before treatment were comparable to those observed after treatment with non-significant differences in each group and between both groups after treatment. In contrast, the fluoride content in each group was increased before and after treatment with a significant difference in each one. However, no significant difference was found between either group after treatments.

**Conclusion:** Both treatments did not affect the calcium or phosphorus content of tooth structure, but they enhanced the fluoride content without any difference between them.

**Clinical significance:** Both treatments can be safely used without harming tooth mineralization. Additionally, they improve fluoride absorption by teeth which is important in increasing tooth resistance to acid demineralization.

**KEYWORDS:** Dental Bleaching, Light-activated tooth whitening systems, Minerals contents, Enamel.

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## INTRODUCTION

Patients' awareness of achieving a beautiful white smile has grown recently in response to wide media exposure.<sup>1</sup> However, teeth discoloration represents an esthetic problem contributing to the patient's loss of smile and interaction with others. It occurs due to either intrinsic or extrinsic reasons. Extrinsic stains are primarily caused by smoking, poor oral hygiene, or chromophore-containing foods such as coffee and tea. These chromophores are directly absorbed into the tooth structure, especially on rough surfaces.

Accordingly, teeth whitening has become essential for improving discolored teeth appearance.<sup>2</sup> It can be achieved by a variety of procedures including whitening toothpastes and bleaching agents.<sup>3</sup> However, teeth bleaching with concentrated peroxides causes a high rate of adverse effects and poses major biological risks. These negative effects have resulted in increasingly restrictive regulations regarding the use of peroxide-containing products and even questions regarding the use of the procedure.<sup>4</sup>

Hence, whitening toothpaste can offer a viable alternative with promising clinical outcomes. These toothpastes provide the same therapeutic benefits of conventional toothpastes but with additional whitening activity from abrasives, adsorbent particles, enzymes, or optical effect ingredients through chemically modifying pigments adhered to the teeth that reduce the intensity and appearance of discoloration.<sup>5,6</sup>

Recently, optical modifying toothpaste has been introduced to the market such as silica-based whitening toothpaste containing Blue Covarine pigment that is uniformly deposited and retained on pellicle-coated tooth surfaces. This can alter the visual perception of tooth color by depositing a thin, semitransparent film of such bluish pigment on the tooth surface that immediately modifies the interaction of incident light shifting the reflected color of teeth from yellow to blue region. Thus, an illusion of higher luminosity and whitening is obtained.<sup>5,7,8</sup>

Moreover, BlanX white shock toothpaste is a revolutionary peroxide-free non-abrasive whitening toothpaste based on Actilux technology that uses all-natural ingredients. This technology is based on Actilux micro-crystal activation to whiten the teeth using light. It is activated by a special LED-light accelerator supplied with toothpaste or natural sunlight. The manufacturer claims that BlanX toothpaste can restore a naturally apparent white smile.<sup>2</sup>

These new whitening formulations with alternative ingredients and additives are supposed to have minimal effects on the mineral component of tooth structure.<sup>9</sup> On the other hand, many studies<sup>3,10,11</sup> reported the deleterious effect of whitening toothpaste on the mineral content of hard tooth tissues resulting in increased enamel surface roughness. These observed variations in the mineral component of the enamel contribute to the need for further research in this field.

In turn, for investigating the structural changes on the prepared specimens in terms of mineral content, various methods of analysis can be applied such as Fourier Transform infrared spectroscopy, Fourier Transform Raman spectroscopy, atomic absorption spectroscopy, surface microhardness, induced plasma mass spectrometry, total reflection X-Ray fluorescence and energy-dispersive X-ray spectroscopy (EDX).<sup>12</sup> EDX is a microanalytical technique that can be employed to estimate quantitatively the amount of minerals in a given tooth specimen.<sup>13</sup>

Despite many studies<sup>14-16</sup> having been done to assess the mineral content changes after bleaching by peroxide-containing systems, few have been conducted to evaluate the tooth mineral content alterations after using peroxide-free whitening toothpastes. Hence, the present study was designed to evaluate the effect of peroxide-free whitening toothpastes (Blue covarine and Actilux-activated containing whitening toothpaste) on the mineral content of human tooth structure. The null hypothesis is that there is no difference between the

tested whitening toothpaste concerning the mineral content of human tooth structure after 6 weeks of use.

## MATERIALS AND METHODS

### Study design and setting

This in-vitro study was conducted as a randomized laboratory study at the laboratories of the Restorative Dentistry Department, Faculty of Dentistry, and Tanta University, Egypt.

### Sample size

The minimum sample size for this study was 40 samples to be distributed in two equal groups which were calculated based on a previous study<sup>17</sup> using a computer program G power version 3.1.9. The significance level was 0.05, the power sample size was more than 80%, the confidence interval was 95%, and the actual power was 96.69. The calculation formula for sample size was:

$$\text{Sample size} = \frac{Z^2 P(1-P)}{C^2}$$

Where:

Z = Z value (1.96 for 95% confidence level).

P = percentage picking a choice, expressed as decimal

C = confidence interval, expressed as decimal.

### Ethical considerations:

The purpose of the present study was explained to the patients and informed consents were obtained to use their extracted teeth in the research according to the guidelines on human research adopted by the Research Ethics Committee, Faculty of Dentistry, Tanta University that was approved under a number (# R-RD-6-24-3118).

### Specimens' selection:

Forty intact human anterior teeth previously extracted for diabetes and/or periodontal reasons at

the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tanta University of patients aged (35-55) years were selected for this study. Immediately after extraction, they were scraped from any residual tissue remnants using periodontal scalers and curettes and washed under tap water. They were then examined with a magnifying glass to be sure they didn't have any structural defects, caries, fractures, or cracks.

The teeth crowns were polished with prophylaxis paste (Alpha prophyl paste, Dental Technologies, Inc.) and a rotating brush (COXO Professional Dental Manufacturer). Then, the shade of the selected teeth was measured by VITA Easy shade advanced 4.0, their shade ranged between (A3.5-A4). After that, they were stored in a refrigerator at 4°C in normal saline according to ISO recommendation that was changed daily until the start of the experiment time.<sup>18</sup>

### Specimens' preparation:

Teeth were de-coronated at the cement enamel junction using a straight handpiece (Apple Dental) and diamond disc (3M Dental Products-US) with water cooling. The teeth crowns were embedded in acrylic blocks (Acrostone, Anglo-Egyptian Company, Cairo, Egypt) leaving the labial surfaces exposed.<sup>19</sup> Samples were kept individually in artificial saliva, at 37°C which was changed daily throughout the study steps.<sup>20</sup> It was prepared at the Department of Analytical Chemistry, Faculty of Pharmacy, Tanta University, Egypt, and consists of 0.33 g KH<sub>2</sub>PO<sub>4</sub>, 0.34 g Na<sub>2</sub>HPO<sub>4</sub>, 1.27 g KCl, 0.16 g NaSCN, 0.58 g NaCl, 0.17 g CaCl<sub>2</sub>, 0.16 g NH<sub>4</sub>Cl, 0.2g urea, 0.03 g glucose, 0.002 g ascorbic acid, 2.7g mucin in 1000 ml distilled water at pH 7.<sup>21</sup>

### Specimens' grouping:

The specimens were labeled and randomly split into two equal groups, each with 20 specimens, based on the tested whitening toothpaste used for brushing as follows:

Group I: Blue covarine-containing whitening toothpaste (CloseUp White Now toothpaste).

Group II: Actilux-activated whitening toothpaste (Blanx® White Shock Toothpaste).

The details of the tested toothpaste including their brand names, composition, and manufacturers, are presented in Table 1.

### Brushing procedures:

Each specimen in its corresponding group was brushed twice daily (morning and evening) for 2 minutes using a rotatory electric toothbrush machine (Oral-B Pro 1000) with a pressure sensor to simulate normal manual brushing force ( $1.6 \pm 0.3$  N).<sup>22</sup> A soft bristle classic rotary toothbrush head was used and changed weekly throughout the experiment to ensure proper standardization and decrease variables. Each toothpaste was diluted in deionized water at a ratio of 1:3 by weight.<sup>20</sup> This mix was applied onto the labial surface of the specimen by a syringe.<sup>23</sup> This ratio was employed to resemble tooth brushing in the oral cavity<sup>24</sup> and prevent the syringe tip used for toothpaste application from clogging.<sup>25</sup>

After brushing, each specimen in group II was light-activated using Blanx LED Bite according

to manufacturer instructions which were applied directly on its labial surface for 1 minute. Then, all the specimens were rinsed under running tap water and dried with blotting paper to remove any remaining toothpaste<sup>26</sup> and restored in artificial saliva till the next step. This process was repeated daily for a total of six weeks.

### Mineral content measurement

The quantitative evaluation of the Calcium (Ca), phosphorus (P), and fluoride (F) contents in weight percentage (wt. %) of all the specimens was performed before and after toothpaste application using a Scanning Electron Microscope (SEM) (Model FEI Quanta 3D 200i) attached with Energy Dispersive X-ray unit (EDX Analyses/thermos-fisher pathfinder) at Grand Egyptian Museum Conservation Center, Egypt. Each specimen was fixed on aluminum stubs with standard diameter using a carbon double sticky tape and placed in the SEM chamber to obtain the SEM micrographs using a Secondary Electron Detector (SED) and Backscattered Electron Detector (BSED) at 20~30 Kv and  $\times 1000$  magnification<sup>1</sup> to be examined directly using EDX analysis.

TABLE (1) Brand names, composition, and manufacturers of the tested toothpaste used.

Materials	Composition	Manufacturer
CloseUp White Now toothpaste Blue-covarine-containing whitening (toothpaste)	1450ppm fluoride), sorbitol, aqua, HS,) Sodium Fluoride SLS, aroma, CG, SS, TSP, glycerin, polyvinyl methyl ,32-ether, and maleic acid (PVM/MA) copolymer, PEG lecithin, mica, limonene, blue covarine pigments	Unilever - Mashreq Under License from Unilever PLC, England
BlanX® White Shock Toothpaste with special LED accelerator light Actilux-activated, peroxide-free, non-) (abrasive whitening toothpaste)	Aqua, sorbitol, hydrated silica, glycerin, silica, sodium lauryl sulfate, cellulose gum, aroma, hydroxyapatite, Actilux microcrystals, isopropyl alcohol, phenoxyethanol, sodium monofluorophosphate, sodium PVM/MA ,42090 CI ,benzoate, benzyl alcohol, CI77891 .copolymer, cetraria islandica extract, sodium	Coswell, Italian Innovators, Italy www.Coswell.biz

### Statistical analysis

Statistical Package for Social Science (SPSS Inc, Chicago, IL, US) Version 26 was utilized to analyze our data. The normality of the collected data was checked using Shapiro-Wilk. Normal distribution was confirmed; thus, data were summarized mainly using mean, standard deviation (SD), and range. A paired t-test was applied for comparison before treatment (at baseline) and after treatment (after 6 weeks) in each group regarding Ca, P, and F content whereas; an independent t-test was used to compare the two groups concerning Ca, P, and F content before treatment and after treatment. P value <0.05 was considered a significant difference.

### RESULTS

The mineral contents data were collected from specimens before treatment (at baseline) and after treatment with either blue covarine-containing whitening toothpaste (group I) or actilux-activated whitening toothpaste (group II). Our results were divided into two parts: the first refers to the statistical comparison of Ca, P, and F obtained from EDX in each group separately by Paired t-test whereas; the second part presents the comparison between the two groups regarding Ca, P, and F after 6 weeks by independent t-test.

Ca content in each group was illustrated in Table 2 and Figure 1a. As shown, the mean Ca values in each group I and II were 25.06 and 25.05 respectively before treatment (at baseline) which were nearly similar to those observed after treatment at 25.26 and 25.21. A non-significant difference by a paired t-test between all the recorded values in each group with p-values of 0.950 and 0.981 respectively means that both treatments did not affect the Ca content of tooth structure.

A non-significant difference was also found between both groups by an independent t-test after treatment with blue covarine-containing whitening toothpaste (group I) and actilux-activated whitening toothpaste (group II) as presented in Table 2 and Figure 1b. This finding denotes that the Ca content of tooth structure was similar in both groups after treatments.

Similarly, the phosphorus content in each group, I and II, were comparable before treatment with values of 14.37 and 14.34 respectively, and after treatment with values of 14.23 and 14.22. A paired t-test revealed non-significant differences between all recorded values in each group, with p-values of 0.950 and 0.981, respectively. This indicates that both treatments did not affect the phosphorus content of the tooth structure. This is presented in Table 3 and Figure 2a.

An independent t-test revealed no significant difference between the two groups, who used blue covarine-containing whitening toothpaste and actilux-activated whitening toothpaste, as shown in Table 3 and Figure 2b. This finding indicates that the phosphorus content of the tooth structure was almost identical in both groups after the treatments.

In contrast, the fluoride content in each group, I and II, was initially measured at 0.18 before treatment. After treatment, the fluoride content increased to 0.75 in group I and 1.04 in group II. A paired t-test revealed significant differences between all recorded values in each group, with p-values of 0.044 and 0.011 as shown in Table 4 and Figure 3a. This indicates that both treatments significantly enhance the fluoride content in tooth structure but with no significant difference between them (P-value = 0.897 and 0.387), as shown in Table 4 and Figure 3b. This indicates that both treatments have similar impacts on the fluoride content of tooth structure.

TABLE (2) Statistical analysis of the mean (wt. %) values of Ca content between both groups at baseline and after 6 weeks.

Groups	Ca content	At baseline	After 6 weeks	Paired t-test	P-value
<b>Group I</b>	Mean $\pm$ SD.	25.06 $\pm$ 0.35	25.26 $\pm$ 4.34	0.065	0.950
	Min. – Max.	24.70 – 25.75	19.63 – 30.27		
<b>Group II</b>	Mean $\pm$ SD.	25.05 $\pm$ 0.50	25.21 $\pm$ 5.86	0.025	0.981
	Min. – Max.	24.35 – 25.97	20.39 – 38.07		
Independent t-test		0.043	0.019		
P-value		0.966	0.985		

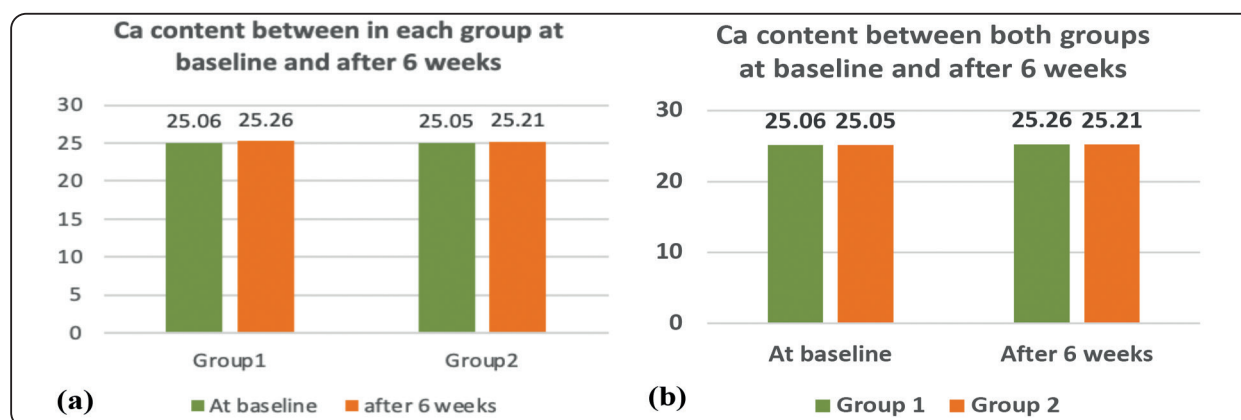


Fig. (1) Bar chart showing the mean values of Ca content in each group (a) and between both groups (b) at baseline and after 6 weeks

TABLE (3) Statistical analysis of the mean (wt. %) values of P content between both groups at baseline and after 6 weeks.

Groups	P content	At baseline	After 6 weeks	Paired t-test	P-value
<b>Group I</b>	Mean $\pm$ SD.	14.37 $\pm$ 0.18	14.23 $\pm$ 2.14	0.279	0.790
	Min. – Max.	14.14 – 14.63	10.58 – 16.86		
<b>Group II</b>	Mean $\pm$ SD.	14.34 $\pm$ 0.14	14.22 $\pm$ 1.51	0.424	0.686
	Min. – Max.	14.22 – 14.62	12.26 – 17.1		
Independent t-test		0.39	0.016		
P-value		0.733	0.988		

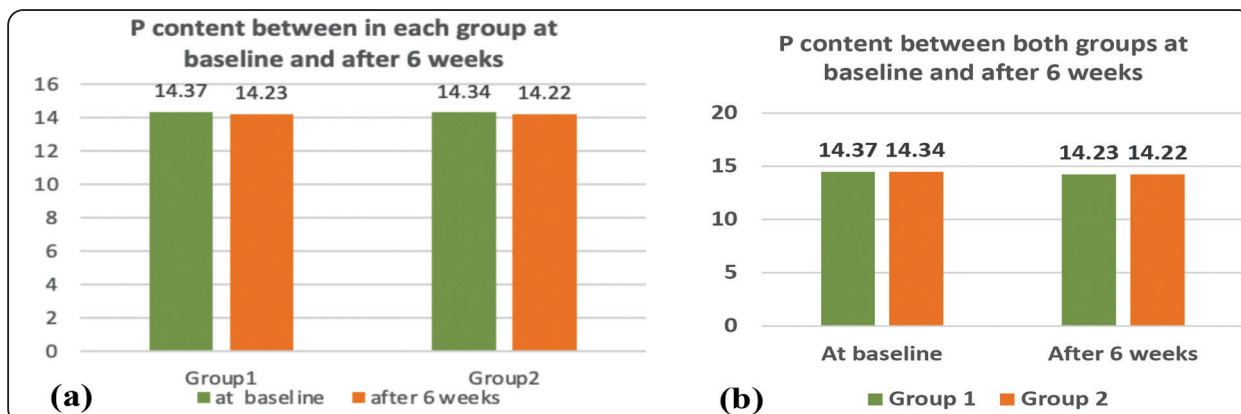


Fig. (2) Bar chart showing the mean values of P content in each group (a) and between both groups (b) at baseline and after 6 weeks

TABLE (4) Statistical analysis of the mean (wt. %) values of F content between both groups at baseline and after 6 weeks.

Groups	F content	At baseline	After 6 weeks	Paired t-test	P-value
<b>Group I</b>	Mean $\pm$ SD.	0.18 $\pm$ 0.03	0.75 $\pm$ 0.59	2.548	0.044*
	Min. – Max.	0.15 – 0.22	0.27 – 1.9		
<b>Group II</b>	Mean $\pm$ SD.	0.18 $\pm$ 0.04	1.04 $\pm$ 0.63	3.601	0.011*
	Min. – Max.	0.12 – 0.24	0.08 – 1.64		
Independent t-test		0.053	0.897		
P-value		0.606	0.387		

\*Statistically significant difference at P value < 0.05.

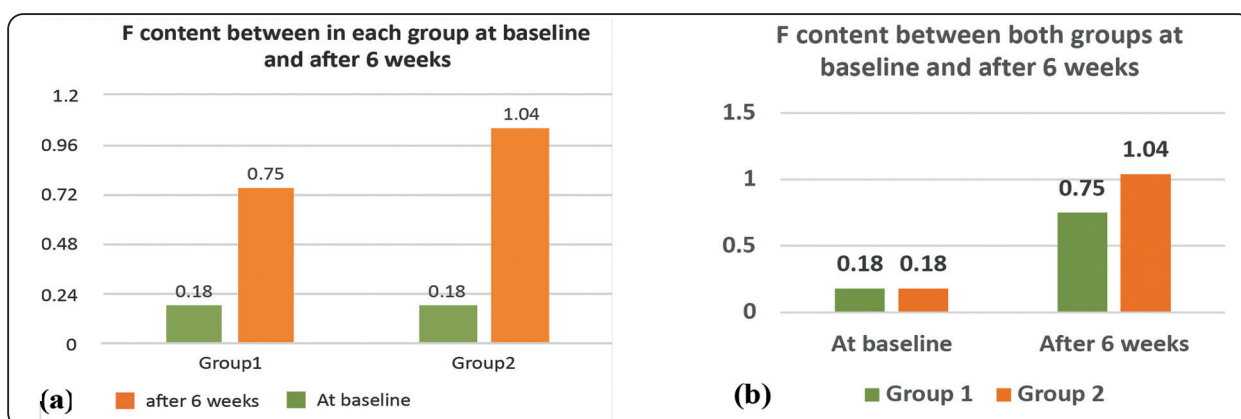


Fig. (3) Bar chart showing the mean values of F content in each group (a) and between both groups (b) at baseline and after 6 weeks

## DISCUSSION

People are constantly striving for whiter, brighter teeth as they enhance the aesthetic appeal of their smiles. As a result, teeth whitening has become an essential component to enhance facial aesthetics.<sup>27</sup> Although teeth bleaching is a frequent conservative procedure, there is still debate over its potential side effects and problems, which are frequently being researched.<sup>28</sup>

Despite, whitening toothpastes are considered a conservative non-destructive treatment for discolored teeth, they are widely available over the counter without a prescription and previous studies<sup>29-32</sup> have shown that they might hurt surface morphology, chemical composition, and microhardness of the whitened enamel surface.

Several factors have been evaluated to analyze the effect of whitening on tooth structure; the most addressed parameter includes the mineral content of tooth structure.<sup>33</sup> For these reasons, this study was carried out to evaluate the effect of peroxide-free whitening toothpastes (Blue covarine-and Actilux-activated containing whitening toothpaste) on the mineral content of extracted human anterior teeth.

In vitro, models were used in this study, for their significance in primary prototype evaluation and treatment conditions optimization. Additionally, to gain information on the effects of contemporary whitening toothpastes on the mineral content of tooth structure.<sup>34</sup>

Currently, CloseUp White Now toothpaste was chosen as it contains Blue Covarine pigment; an optical brightener capable of causing a measurably whitening effect resulting in a color shift that instantly induces an increase in the detection and perception of tooth whiteness.<sup>35</sup> BlanX white shock toothpaste was also selected owing to its whitening effect which is attributed to a patented blend of titanium dioxide and hydroxyapatite Actilux micro-crystals. Titanium dioxide is a bright white pigment

incorporated in toothpaste formulations due to its high refractive index. Furthermore, the LED light accelerator activates Actilux's photocatalytic active ingredient destroying stain molecules and naturally whitening teeth.<sup>2</sup>

To standardize the brushing process regarding technique, force, duration, and frequency of brushing, an electric toothbrush simulator was utilized. This was a strong point in the current study simulating clinical conditions while minimizing variations. Moreover, it was considered that the hardness of toothbrush hair determined enamel surface abrasion by toothpaste. Thus, a soft hair toothbrush was preferred and used by one operator to reduce its abrasiveness and to ensure uniform brushing to all the specimens. In this case, the toothbrush's abrasiveness on the tooth surface was almost negligible.<sup>32,36</sup>

EDX was used to detect tooth mineral content as it is considered the most relevant technique to study elemental composition in confined small areas of mineralized enamel in a non-destructive way and to analyze the changes in calcium, phosphorus, and fluoride in the chemical profile following the application of whitening toothpaste giving dependent data that allows us to apply these results clinically.<sup>1,37</sup>

Based on the results obtained, the chemical profile in both groups I and II revealed non-significant changes in the Ca and P content following 6 weeks of the whitening procedure, both exhibited similar behavior. This might be explained by the lack of properly formed acquired pellicles in specimens that received CloseUp White Now toothpaste (group I) since they were stored in the artificial saliva in this study whereas in the other studies<sup>8,38</sup>, human saliva was used indicating a strong affinity of the Blue Covarine components of the acquired pellicle. Concerning BlanX toothpaste representing group II this result was due to the absence of an abrasive component as well as the inclusion of hydroxyapatite micro-crystals in its composition

which have a similar structure to the main mineral component of teeth.<sup>2</sup>

These results contradict previous studies' findings<sup>39,40</sup> which revealed that the whitening process is associated with changes in the surface topography of whitened teeth. Moreover, De Abreu<sup>41</sup> reported variations in the enamel calcium and phosphate contents that were clarified both during and after bleaching. This difference may be attributed to the compositional characteristics of each brand of whitening product that provide changes in the mineral concentration of enamel. Moreover, the difference in brushing technique, brush bristle type and stiffness might play an effective role in changing enamel mineral content.<sup>42</sup>

On the contrary, the fluoride mineral content was changed in both groups. Both studied toothpastes increased significantly the fluoride mineral content of tooth structure but with no significant difference between them. This finding could be explained by the presence of fluoride in the ingredients of both tested toothpaste. CloseUp White Now toothpaste contains sodium fluoride while BlanX toothpaste contains sodium monofluorophosphate in composition that was deposited onto the dental substrate contributing to the maintenance of the fluoride mineral content or even enamel remineralization.<sup>12,43</sup>

In this perspective, fluoride remains the most essential agent to promote mineral balance, by minimizing demineralization and activating remineralization.<sup>44</sup> According to this scenario, our results could signalize that both tested whitening toothpastes could promote remineralization, as well as, fluoride could remineralize enamel surface following the whitening procedure.<sup>45</sup> This finding agreed with other studies<sup>46,47</sup> which confirmed that the fluoride associated with bleaching decreases mineral loss of the enamel surface. Furthermore, previous findings<sup>48,49</sup> observed that F could control the mineral loss. However, no other studies have quantified the enamel mineral content throughout

the whitening procedure using the same pastes as those presented in the current study, thus preventing some comparisons.

No difference was observed between the two tested whitening toothpastes concerning the mineral content of human tooth structure after six weeks of use. Hence, the null hypothesis was accepted. Due to the limitations of this in-vitro study, in-vivo investigations are necessary to clinically explore the results obtained and to evaluate the potential reversibility of the morphological and chemical changes caused by both types of toothpaste studied.

## CONCLUSIONS

It can be assumed that neither blue covarine nor actilux-activated whitening toothpastes affected the calcium or phosphorus content in tooth structure; however, both can enhance fluoride content without any difference between them.

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