10.5005/jp-journals-10024-2087

ORIGINAL RESEARCH

Effect of Hydrogen and Carbamide Peroxide in Bleaching, Enamel Morphology, and Mineral Composition: *In vitro* Study

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ABSTRACT

Aim: The aim of the study was to evaluate the bleaching effect, morphological changes, and variations in calcium (Ca) and phosphate (P) in the enamel with hydrogen peroxide (HP) and carbamide peroxide (CP) after the use of different application regimens.

Materials and methods: Four groups of five teeth were randomly assigned, according to the treatment protocol: HP 37.5% applied for 30 or 60 minutes (HP30, HP60), CP 16% applied for 14 or 28 hours (CP14, CP28). Changes in dental color were evaluated, according to the following formula: $\Delta E = [(L_a - L_b)^2 + (a_a - a_b)^2 + (b_a - b_b)^2]^{½}$. Enamel morphology and Ca and P compositions were evaluated by confocal laser scanning microscope and environmental scanning electron microscopy.

Results: ΔE HP30 was significantly greater than CP14 (10.37 ± 2.65/8.56 ± 1.40), but not between HP60 and CP28. HP60 shows greater morphological changes than HP30. No morphological changes were observed in the groups treated with CP. The reduction in Ca and P was significantly greater in HP60 than in CP28 ($p < 0.05$).

Conclusion: Both formulations improved tooth color; HP produced morphological changes and Ca and P a gradual decrease, while CP produced no morphological changes, and the decrease in mineral component was smaller.

Clinical significance: CP 16% applied during 2 weeks could be equally effective and safer for tooth whitening than to administer two treatment sessions with HP 37.5%.

Keywords: Carbamide peroxide, Enamel, Hydrogen peroxide, Mineral component, Morphological changes, Tooth color, Tooth whitening.

How to cite this article: Llena C, Esteve I, Forner L. Effect of Hydrogen and Carbamide Peroxide in Bleaching, Enamel Morphology, and Mineral Composition: *In vitro* Study. J Contemp Dent Pract 2017;18(7):576-582.

Source of support: Nil

Conflict of interest: None

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INTRODUCTION

Tooth whitening techniques are commonly used to treat discolored teeth. Most whitening products are based on hydrogen peroxide (HP), which exerts potent oxidizing action and gives rise to the formation of other very effective whitening agents, such as perhydroxyl anions (HO_2^-) and hydroxyl radicals (OH^-).[1,2] The pH of the product, temperature, the activation procedure used, or the presence of certain transition metal elements all modify the type of reaction produced.[3] Carbamide peroxide (CP) in turn decomposes into HP and urea – the latter causing denaturalization of enamelin and amelogenin, which are proteins present in the matrix component found between the enamel prisms. This could increase enamel permeability and thus induce microstructural changes. On the contrary, urea induces alkalinization, which may result in reduced demineralization.[2,4]

High concentrations of HP and CP are used as whitening agents in office, while HP concentrations of up to 6% (or the equivalent in the case of CP) can be applied, under professional supervision, at home.[5]

Although HP is a potent and effective whitening agent, there is controversy regarding its safety and possible adverse effects.[6,7] In this regard, HP has been associated to morphological changes, as well as to variations in microhardness, and in the mineral component of enamel and dentin,[8-12] also changes in the dentin-enamel junction[13] and in the elasticity and mechanical properties of dentin,[14] as well as to alterations of their organic components.[15,16] However, there are also many authors

who have observed no relevant changes in enamel and dentin after such whitening treatment.[17-26]

Many factors can condition such heterogeneous results, including parameters related to the substrate and the preparation and evaluation procedures involved, as well as the origin of the samples (human or bovine), age, and the tooth preservation conditions involved.[27] Regarding the sample preparation and observation procedures used for investigating the morphological changes, it is advisable to use techniques, such as environmental scanning electron microscopy (ESEM) or confocal laser scanning microscopy (CLSM), which do not require drying or other treatments, to avoid interferences with the results obtained.[28,29]

Another factor that may account for the discrepancies in the published results is the pH of the product used. In this regard, some authors consider the pH of the product to be more important than HP concentration in conditioning the changes in morphology and roughness.[30-33]

Regarding the concentration of the products, some authors have only described morphological changes or variations in the mineral component when using high concentration gels,[34-36] while others have recorded changes even with products at low concentrations.[37,38] In turn, some investigators have observed no changes when using HP at a concentration of 7.5% or CP at a concentration of 10 or 16%.[39]

The literature describes the clinical efficacy of different product concentrations and application protocols in relation to changes in tooth color,[40] though strategies must be established that combine clinical efficacy with the minimization of possible side effects.

The observed discrepancies in terms of the morphological changes, variations in mineral component, and clinical efficacy of the different concentrations point to the need for further research in this field. This study evaluates the whitening effects and changes in the morphological characteristics and percentage of calcium (Ca) and phosphate (P) in the enamel of human teeth whitened with HP 37.5% and CP 16%, using different application regimens.

The following null hypotheses are tested: (1) At the end of treatment, both procedures result in similar color improvement, with a color change (ΔE) of <3 units, (2) no differences are observed in the morphological changes in the enamel with the two commercial products used, and (3) the changes in mineral component at the end of treatment do not differ between the two products.

MATERIALS AND METHODS

Human teeth (canines and incisors) extracted for different reasons were used. Approval of the study was obtained from the Research Ethics Committee of the Universitat de València.

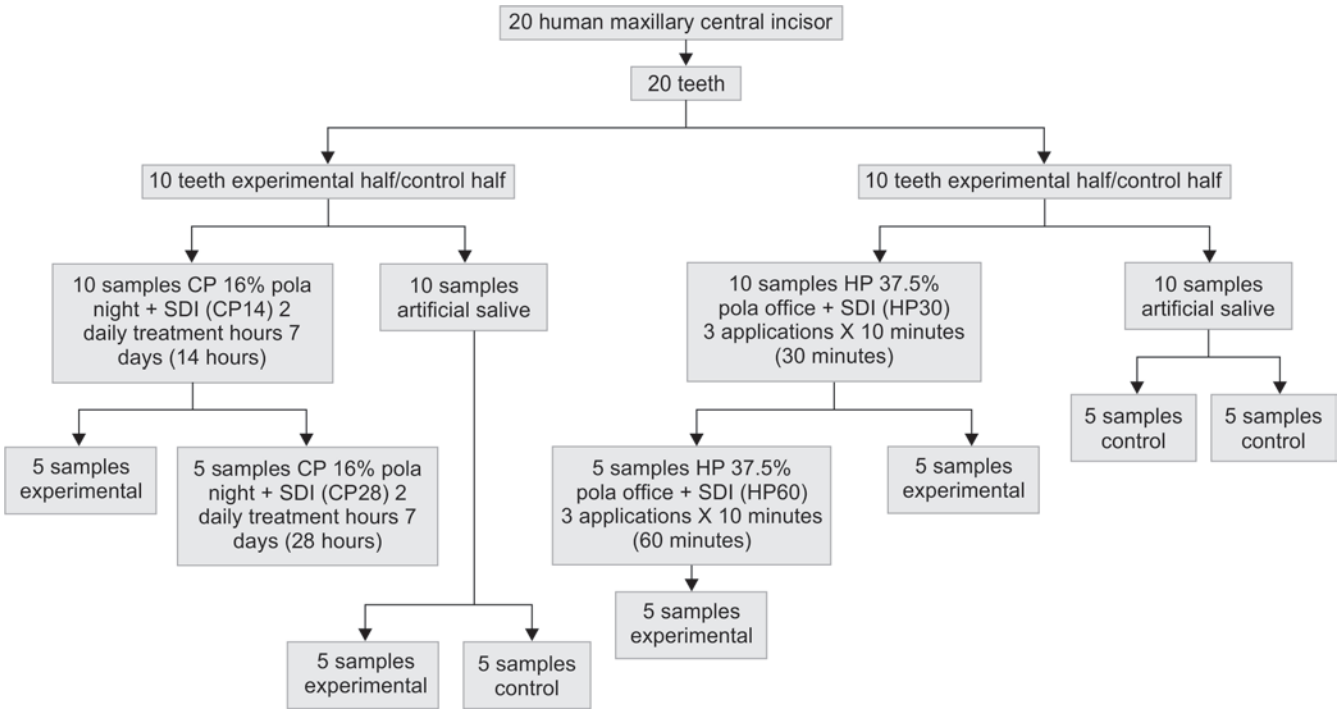
All the teeth were carefully examined under magnification (×10), and those presenting cracks, caries, or structural enamel defects were excluded from the study. A total of 20 teeth were finally included in the study. The teeth were subjected to calculus removal and were kept in thymol 0.1% during 5 days. Crowns were sectioned 2 mm below the cementoenamel junction using a disk (Isomet; Buehler Ltd., Lake Bluff, Illinois, USA) at low speed. The crowns were randomly divided into two groups (n = 10 each). Each tooth was divided into two halves: An experimental half and the corresponding control.[29] Control half was stored in artificial saliva. Experimental halves were divided into two groups according to treatment procedure. One of the groups was treated with HP 37.5% Pola Office + (SDI, Victoria, Australia) and the other with CP 16% Pola Night (SDI, Victoria, Australia), both at neutral pH. The specimens were kept throughout the treatment process in artificial saliva, composed of 0.5% carboxymethylcellulose, 0.290 gm NaCl, 0.085 gm $CaCl_2$, 0.17 gm Na_2HPO_4, 0.08 gm NH_4Cl, 0.635 gm KCl, 0.080 gm NaSCN, 0.165 gm KH_2PO_4, and 0.1 gm urea in 500 mL bidistilled water at 37°C.

Specimen Treatment

The 10 experimental specimens in the CP 16% group (Pola Night SDI, Victoria, Australia) were treated with 5 µL of the whitening product, applied to the surface of the enamel using a brush, and were kept in a humid environment for 2 hours a day, until completing 7 days of treatment. After this 2-hour period, the specimens were washed with distilled water and stored in artificial saliva at 37°C until the next application. After the 7 days of treatment, 5 of the 10 experimental specimens and their respective controls were placed in individual containers with distilled water for study after 24 hours (14 hours of treatment: Group CP14). The remaining five specimens were treated for a further week using the same procedure (28 hours of treatment: Group CP28). After the 14 days of treatment, the experimental specimens and controls were placed in distilled water for study after 24 hours.

In the HP 37.5% group, three 10-minute applications of the product were made using the same procedure as described above. After the third application (total treatment time: 30 minutes; group HP30), the specimens were divided into two subgroups in the same way as those treated with CP: Five experimental specimens and their respective controls were placed in distilled water for study after 24 hours, while the remaining five were kept in artificial saliva for a further 7 days, after which a second treatment comprising three successive 10-minute applications was carried out (total treatment time: 60 minutes; group HP60). After treatment, the

Flow Chart 1: Schematic representation of the treatment process in each group



experimental specimens and their controls were placed in distilled water for study after 24 hours. Flow Chart 1 provides a schematic representation of the treatment process in each group.

Color Recording

A Vita Easy Shade spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany) was used for color recording. To make sure that the reading tip was always placed in the same position, a series of positioning splints were prepared using a heat-adaptable plastic lamina measuring 0.4 mm in thickness (SDI, Victoria, Australia). Perforations were made coinciding with the center of each specimen, using a punch (OMNIA SpA, Fidenza, Parma, Italy). The color was recorded with the hydrated specimens before treatment and 24 hours after the end of treatment, in each of the experimental groups. The color was established from the CIEL*a*b* spatial coordinates, always in the same zone. The ΔE for each specimen was calculated from the variables L, a* and b*.

Confocal Microscopy Evaluation

A FV1000 confocal microscope (Olympus, Melville, USA) at ×40 magnification, with immersion oil, in reflection mode, and with illumination provided by a He-Ne laser (λ = 633 nm) was used for morphological evaluation. After selecting an image, a general view was recorded, and a ×3 zoom image of a clear zone of the main view was obtained. The specimens were subsequently stored in distilled water.

Study of the Proportions of Calcium and Phosphate

The ESEM (XL30, Olympus, Melville, USA) combined with a microanalysis system (EDX-Genesis) was used to study the proportions of Ca and P of each sample. The operating parameters were: Working distance of 10 mm, acceleration voltage 20 kV, acquisition time 50 seconds, temperature 10°C, and magnification ×100.

Microanalysis involved location of a smooth zone of the specimen with the obtainment of three measurements: One in the central zone and two at points equidistant from the first point, along with the diagonal of the specimen. The mean percentages of Ca and P for each specimen were calculated.

Statistical Analysis

The color variations at the different study times in each group were calculated as: $\Delta E = [(L_a - L_b)^2 + (a_a - a_b)^2 + (b_a - b_b)^2]^{½}$.

The nonparametric Wilcoxon test was used to compare the changes in percentage Ca and P, and the color variations between the experimental specimens and their respective controls. The comparisons between groups were carried out using the Mann–Whitney U-test. Statistical significance was considered for $p < 0.05$ in all cases.

RESULTS

Color Changes

The color change (ΔE) in group HP30 was 10.37 ± 2.65, which was significantly greater than in group CP14

(8.56 ± 1.40), p = 0.031 (Mann–Whitney U-test). In group HP60, the ΔE was 15.28 ± 3.12 *vs* 14.06 ± 4.56 in group CP28 without significant differences between these groups (p = 0.512).

Analysis of Enamel Morphology (CLSM)

The areas of lighter color reflect more light, while the darker areas are translucent. Correctly conformed enamel prisms are translucent to laser light, while the less structured spaces between the enamel prisms reflect CLSM light.

In the control specimens, the enamel prisms showed a rounded and homogeneous contour, producing the typical keyhole-like appearance of the crystalline organization of hydroxyapatite. The specimens treated with HP 37.5% for 30 minutes (group HP30) showed a loss of prism homogeneity, which was intensified when treatment was prolonged for 60 minutes (group HP60) (Fig. 1).

The specimens treated with CP 16% (groups CP14 and CP28) showed no variations in prism morphology when compared with the control and other experimental specimens. All of the images were similar, with repetition of the normal keyhole-like hydroxyapatite pattern and regular and lobulated enamel prism contour (Figs 1A to E).

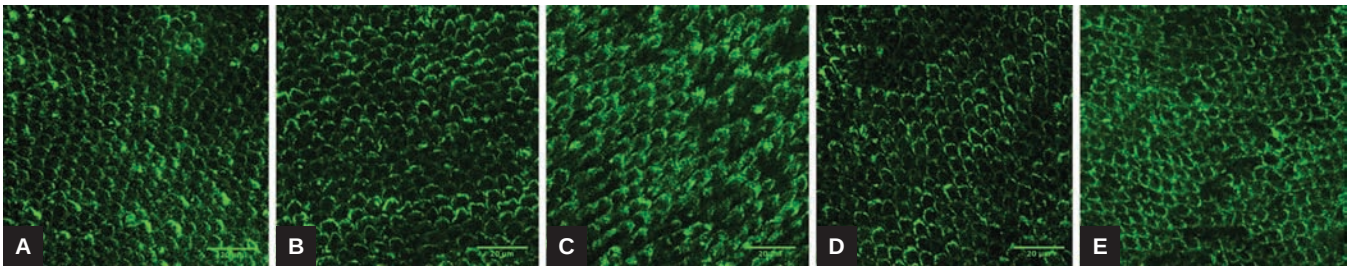
Changes in the Proportion of Calcium and Phosphate (EDX)

The proportions of Ca and P in group HP30 and its corresponding controls were very similar, while group HP60 showed a decrease in percentage Ca and P *vs* the control specimens. Statistical significance between case and control groups was not reached (p > 0.05) (Graph 1).

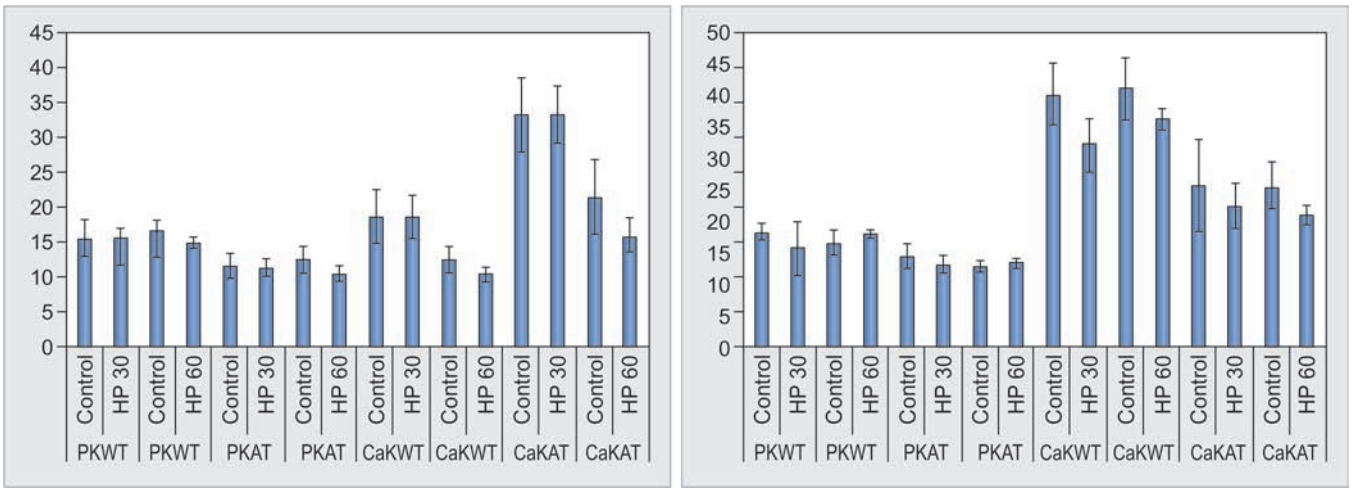
The values corresponding to groups CP14 and CP28 are reported in Graph 1. Both groups showed a greater decrease in percentage Ca than in percentage P *vs* the corresponding controls, though the differences were not statistically significant (p > 0.05).

The comparisons between the HP 37.5% and CP 16% groups are shown in Table 1. The decrease in percentage Ca and P was significantly greater in group HP60 than in group CP28.

There were no significant differences in the composition of Ca and P between groups HP30 and HP60, though the percentages of both minerals were lower in group HP60 (p > 0.05). In turn, the values corresponding to Ca and P were greater in group CP28 than in group CP14, though the differences were not statistically significant (p > 0.05).



Figs 1A to E: Appearance of the enamel: (A) Untreated enamel; (B) hydrogen peroxide 37.5% applied during 30 minutes (HP30); (C) hydrogen peroxide 37.5% applied during 60 minutes (HP60); (D) carbamide peroxide 16% applied during 14 hours (CP14); (E) carbamide peroxide 16% applied during 28 hours (CP28)



Graph 1: Variations in calcium and phosphate composition in the enamel of groups HP30 and HP60; HP30: Hydrogen peroxide 37.5% applied during 30 minutes; HP60: Hydrogen peroxide 37.5% applied during 60 minutes; CP14: Carbamide peroxide 16% applied during 14 hours: CP28: Carbamide peroxide 16% applied during 28 hours. PKWT: Percentage weight of phosphate; PKAT: Percentage atoms of phosphate; CaKWt: Percentage weight of calcium; CaKAT: Percentage atoms of calcium

Table 1: Differences in percentage of weight and atoms of Ca and P after treatment between groups

Parameter	*n = 10 in each group*	*Mean*	*CI*	*p-value*	*n = 10 in each group*	*Mean*	*CI*	*p-value*
PKWT	HP30	15.8	10.38–21.20	0.423	HP60	15.02	13.83–16.02	0.008
	CP14	15.79	4.35		CP28	18	17.16–18.85	
PKAT	HP30	11.41	9.65–13.08	0.052	HP60	10.46	9.15–11.77	0.008
	CP14	13.29	11.45–15.14		CP28	13.48	12.50–14.47	
CaKWT	HP30	33.45	28.17–38.72	1	HP60	29.79	25.64–33.95	0.032
	CP14	32.38	22.12–42.64		CP28	36.41	34.16–38.67	
CaKAT	HP30	18.72	14.83–22.60	0.152	HP60	16.06	13.04–19.08	0.032
	CP14	22.58	18.17–26.87		CP28	21.1	19.20–23.00	

PKWT: Percentage weight of phosphate; PKAT: Percentage atoms of phosphate; CaKWt: Percentage weight of calcium; CaKAT: Percentage atoms of calcium; HP30: Hydrogen peroxide 37.5% applied during 30 minutes; HP60: Hydrogen peroxide 37.5% applied during 60 minutes; CP14: Carbamide peroxide 16% applied during 14 days; CP28: Carbamide peroxide 16% applied during 28 days; CI: Confidence interval

DISCUSSION

Different procedures can be used to evaluate the efficacy of tooth whitening procedures. In this regard, photographs or comparative guides are commonly used as subjective methods, though colorimetric or spectrophotometric techniques, such as that used in our study are much more objective, precise, and reproducible.[27,41,42]

The CIEL*a*b* spatial coordinates are widely used for assessing color variations and can be obtained from the information generated by the Vita EasyShade spectrophotometer used in this study. This technique analyzes the distance between two points of the chromatic space (by calculating the ΔE index) and objectively determines the color variations at different time points during whitening treatment. In this regard, values of $\Delta E \geq 3.3$ are considered to be visually perceptible.[43,44]

In our study, we recorded the ΔE values of 15.28 ± 3.12 for HP60 and 14.06 ± 4.56 for CP28, at the end of treatment. The first null hypothesis is therefore, accepted. In the case of the HP-treated specimens, the most important color changes occurred after the first application (ΔE = 10.37), with modification of ΔE by an additional five points with the second application. These findings are consistent with the data found in the literature.[40,45,46] In all cases, the color recordings were obtained 24 hours after the end of each treatment session, to avoid possible bias in color change due to enamel dehydration during the treatment.

The oxide-reduction effects of whitening agents are known to cause the dissolution of both the organic component and the mineral component of teeth, and overwhitening can leave only carbon dioxide and water.[47] In this study, we used the whitening protocols recommended by the manufacturers, preserving the specimens between treatment sessions in artificial saliva with Ca and P, at a temperature of 37°C. This medium, in turn, was replaced on a daily basis to simulate the clinical conditions as far as possible. Although remineralization produced by saliva is expected to occur in clinical practice,[47-49] some *in situ* and *in vivo* studies have reported a decrease in microhardness produced by loss of the mineral component secondary to demineralization;[50] however, other *in vivo* studies have evidenced no changes in either enamel surface morphology or roughness with HP or CP treatment at different concentrations.[51,52]

In vitro studies have described changes in enamel morphology and in the mineral component when using high concentrations of HP or CP,[21,24,25] though the effects are not as notorious as when orthophosphoric acid is employed.[53] In this study, CLSM revealed changes in enamel morphology consistent with those described in the literature. Progressive enamel alterations were recorded in the specimens exposed to HP 37.5% between the first (HP30) and second application (HP60). These morphological changes were accompanied by a gradual decrease in Ca and P between the two sessions. The second null hypothesis is therefore, rejected.

Structural changes in the enamel have also been described with CP at low concentrations.[54-58] However, in this study, we observed no morphological changes, though a nonsignificant decrease in Ca and P was recorded with respect to the corresponding controls after both 14 hours of application (group CP14) and after 28 hours of treatment (group CP28). Some authors have obtained results similar to our own.[8,59,60]

Attention is drawn to the fact that there was a lesser decrease in Ca and P in group CP28 *vs* group CP14. The only explanation we can offer for this result is the remineralization effect caused by longer immersion (2 weeks) of the specimens in group CP28 in the daily replaced artificial saliva solution.

Ren et al[61] reported similar enamel changes produced by at home dental bleaching and acid beverages, without significant differences *vs* the controls.

It is important to mention that the changes in Ca and P composition did not differ between groups HP30 and CP14. However, the loss of mineral component was significantly greater in group HP60 than in group CP28. The third null hypothesis is therefore, rejected.

Based on the results obtained in our study, it can be concluded that both treatments were equally effective in improving tooth color. HP 37.5% induced morphological changes in the enamel that intensified with the duration of the treatment, and these changes were moreover accompanied by progressive changes in Ca and P composition. We observed no morphological variations in the teeth treated with CP 16%, and the decrease in mineral component was less accentuated than in the case of HP.

Thus, from the clinical perspective, it would be equally effective and safer for tooth whitening to use products based on CP 16% during 2 weeks than to administer two treatment sessions with HP 37.5%.

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