

# Effect of various tooth-whitening products on enamel microhardness

SADJ November 2009, Vol 64 no 10 p474 - p479

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## ABSTRACT

### Objectives:

The purpose of this *in vitro* study was to evaluate the effect of various tooth-whitening products containing carbamide peroxide (CP) or hydrogen peroxide (HP), on enamel microhardness.

### Methods:

Enamel blocks were exposed to: Nite White® ACP 10% CP (Group 2, n=10); Yotuel® Patient 10% CP (Group 3, n=10); Opalescence® PF 10% CP (Group 4, n=10); Opalescence® PF 20% CP (Group 5, n=10); Opalescence® Treswhite Supreme 10% HP (Group 6, n=10); Yotuel® 10 Minutes 30% CP (Group 7, n=10); Opalescence® Quick 45% CP (Group 8, n=10); Yotuel® Special 35% HP (Group 9, n=10); Opalescence® Boost 38% HP (Group 10, n=10) according to the instructions of the manufacturers. The control (Group 1, n=10) was enamel blocks kept in artificial saliva at 37°C without any treatment. The microhardness values were obtained before exposure and after a 14-day treatment period. Specimens were kept in artificial saliva at 37°C between treatments. Data were analysed using Kruskal-Wallis one-way ANOVA and Tukey-Kramer Multiple Comparison Test. Indent marks on the enamel blocks were also examined under the Scanning Electron Microscope.

### Results:

All whitening products decreased enamel microhardness except group 10 but only Groups 2,3,4,5 and 7 showed significant decrease in enamel microhardness as compared to the control group ( $p < 0.05$ ). Groups 2, 3 and 7 differed significantly from all the other groups ( $p < 0.05$ ). The highest damage was recorded for Group 2 (Nite White® ACP 10% CP), which differed significantly from Groups 3 and 7. SEM images also showed damage to enamel.

### Conclusions:

All products tested in this study decreased enamel microhardness except Opalescence® Boost 38% HP. The products containing carbamide peroxide were more damaging to enamel because of the longer application times. Nite White ACP 10% CP showed the highest reduction in enamel microhardness as compared to other products tested.

### Key words:

Tooth-bleaching, whitening, peroxide, microhardness, enamel

## INTRODUCTION

Tooth-bleaching or tooth-whitening has become an increasingly popular dental procedure to lighten discoloured teeth. Over time, different bleaching techniques have been advocated, such as at-home bleaching<sup>1</sup> and in-office bleaching.<sup>2</sup>

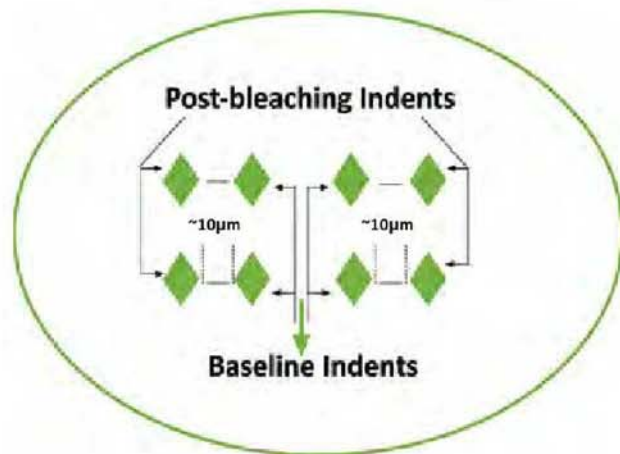


Figure 1: Schematic diagram showing the position of control (baseline) and post-bleaching indentations on polished enamel.

The active ingredient in a bleaching product is mainly hydrogen peroxide (HP) or its precursor carbamide peroxide (CP).<sup>3</sup> Currently a wide range of whitening products containing varying concentrations of CP and /or HP are available on the South African market. It is stated that 10% CP is still the most commonly used at-home bleaching concentration,<sup>4,5</sup> because of its reported safety and effectiveness.<sup>6-9</sup> However, high peroxide concentrations are used in in-office procedures due to their ability to promote faster whitening.<sup>10</sup>

Tooth-whitening involves direct contact of bleaching agent with the enamel surface for an extensive period of time which differs between manufacturers. This fact increased concerns about the possible adverse effects of bleaching products on the enamel/dentine. The available literature is contradictory. Some scanning electron microscope studies reported changes in surface morphology of enamel following bleaching with carbamide peroxide<sup>11-15</sup> and/or hydrogen peroxide products,<sup>11,16</sup> while others reported no alterations in the enamel morphology.<sup>2,17,18</sup> For example, Hegedüs *et al*,<sup>19</sup> in an atomic force microscopy study, demonstrated that carbamide peroxide and hydrogen peroxide were capable of causing alterations in enamel surface.

Again in terms of microhardness tests, conflicting results have been reported in the literature. Some studies reported that tooth-bleaching decreased enamel<sup>5,11,20,22</sup> and dentine,<sup>22,23</sup> microhardness, while others reported no detrimental effects of bleaching on enamel<sup>24,26</sup> and dentine<sup>26</sup>. Lewinstein *et al*<sup>27</sup> reported that in-office bleaching products i.e. 35% HP and 35% CP, reduced hardness of

**Table 1:** Composition of artificial saliva

Composition	g/l
Sodium carboxymethylcellulose	10.0
Sorbitol	30.0
Potassium chloride	1.2
Sodium chloride	0.844
Magnesium chloride	0.052
Calcium chloride	0.146
Potassium dihydrogen phosphate	0.342
pH	7.0

enamel and dentine significantly more than the home bleaching products with lower concentrations i.e. 10% CP, but stated that the application of 0.05% fluoride solution for five minutes completely restored the softened tooth structure. Faraoni-Romano *et al*<sup>28</sup>, however, reported that bleaching of enamel with varying concentrations of CP and/or HP did not alter the microhardness and surface roughness of enamel. From the literature it becomes clear that different products, different concentrations as well as different bleaching agents will all influence the effect on enamel or dentine differently.

Therefore, the purpose of this in vitro study was to determine the effect of various tooth-whitening products containing carbamide peroxide or hydrogen peroxide on enamel microhardness.

## MATERIALS AND METHODS

### Specimen Preparation

Freshly extracted, non-carious human molar teeth were collected and stored in water with a few thymol crystals. The roots were removed approximately 2-3 millimetres apical to the cemento-enamel junction using a double-sided diamond saw in a low-speed motor. Enamel blocks of 5x5 mm<sup>2</sup> were sectioned longitudinally to the crowns. These enamel blocks were then examined under a stereomicroscope at 25x magnification and those with stains or cracks were discarded. One hundred of the selected enamel blocks were individually embedded in acrylic in PVC rings with a length of 1 cm (cut from a 25mm diameter electrical tubing) with the enamel surface exposed above the acrylic and 90° to the PVC ring. The exposed enamel surfaces of the specimens were polished using water cooled carbide paper up to 1200 grit fineness. The specimens were randomly divided into 10 treatment groups (1-10) with 10 specimens each.

For the treatment of enamel blocks, individual bleaching trays were fabricated (to simulate the in vivo bleaching procedure) for Groups 2 to 5 and 8 using impressions of the enamel blocks. The manufacturers do not suggest the fabrication of trays for other materials. Models were poured in yellow stone and light-cured resin block-out material was used to create a reservoir for bleaching materials, with the exception of Group 2, as suggested by the manufacturer. The trays were fabricated with a 0.035" thick, 5x5" soft tray material in a heat/vacuum tray-forming machine. The trays were trimmed to fit each specimen perfectly.

### Treatments were performed as follows:

#### Group 1 (Control) (n=10):

The enamel blocks were stored in the prepared artificial saliva<sup>29</sup> (Table 1) at 37°C without any whitening treatment.

#### Group 2 (Nite White® ACP 10% CP) (n=10):

The bleaching trays were filled with a layer (approx 1 mm thick)

of this bleaching gel and applied to the enamel surfaces of the blocks for 8 hours/day for 14 days, as suggested by the manufacturer. During the treatment period specimens were kept in 100% relative humidity at 37°C. After each bleaching procedure, the bleaching gel was gently removed from the enamel surfaces using a paper towel and then thoroughly rinsed and stored in the artificial saliva at 37°C until the next treatment. The artificial saliva was replaced on a daily basis.

#### Group 3 (Yotuel® Patient 10% CP) (n=10):

The treatment procedure in this group was exactly as in the Group 2 except for the bleaching agent.

#### Group 4 (Opalescence® PF 10% CP) (n=10):

The treatment procedure in this group was exactly as in the Group 2 except for the bleaching agent.

#### Group 5 (Opalescence® PF 20% CP) (n=10):

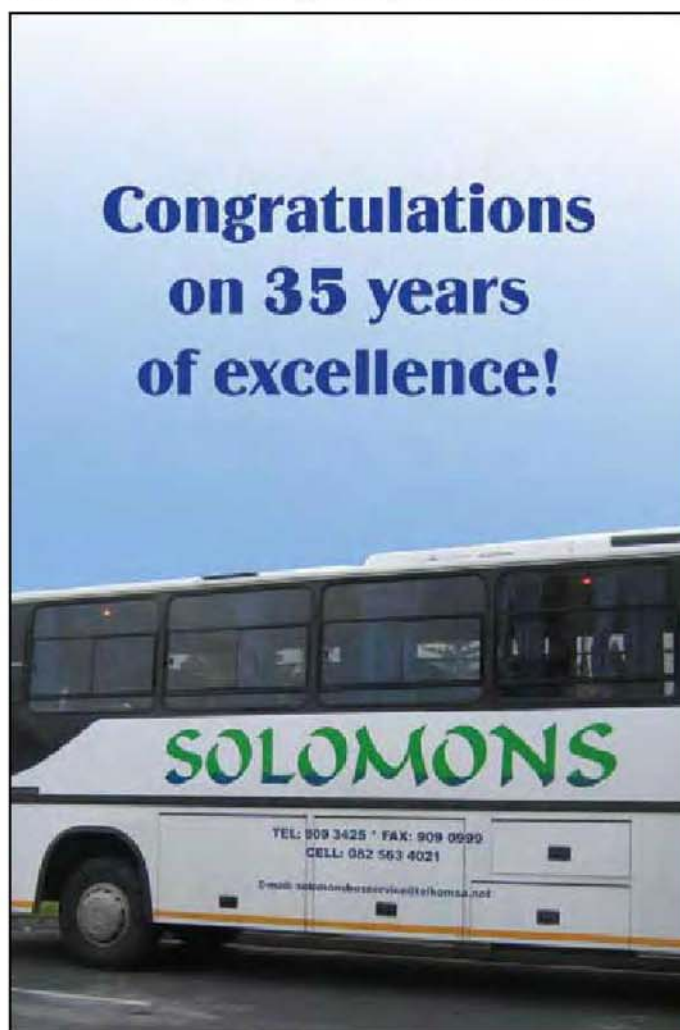
The treatment procedure in this group was exactly as in the previous groups, except that 20% CP was used.

#### Group 6 (Opalescence® Trèswhite Supreme 10% HP) (n=10):

The treatment procedure in this group was also as for Group 2, except that 10% HP gel was used for 30 minutes/day for 14 days, as suggested by the manufacturer.

#### Group 7 (Yotuel® Patient 30% CP) (n=10):

The treatment procedure in this group was also as for Group 2, except that 30% CP gel was used for 10 minutes/day for 14 days without bleaching trays, as suggested by the manufacturer.



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**Table 2:** General information about the bleaching products according to the manufacturers.

Product	Group	Composition	Treatment Time	Treatment (Total hours)	Equivalent % H <sub>2</sub> O <sup>2</sup>
Nite White® ACP 10% CP	2	Propylene Glycol, Glycerin, Water, Dicetyl Phosphate, Cetearyl Alcohol, Ceteth-10 Phosphate, Silica, Carbamide Peroxide, Hydrogen Peroxide, Hydroxypropylcellulose, Potassium Nitrate, Flavor, Sodium Phosphate, Calcium Nitrate, Calcium Carbonate, Potassium Hydroxide	8 hrs/day	112	3.35
Yotuel® Patient 10% CP	3	Glycerin, Urea Peroxide, Xylitol, Potassium Citrate, Carbomer, Aroma, Potassium Fluoride, Sodium Saccharin	8 hrs/day	112	3.35
Opalescence® PF 10% CP	4	Carbamide peroxide, Potassium nitrate, 0.11% ion fluoride, carbopol, glycerin, flavour.	8 hrs/day	112	3.35
Opalescence® PF 20% CP	5	Carbamide peroxide, potassium nitrate, 0.11% ion fluoride, carbopol, glycerin, flavour.	8 hrs/day	112	6.7
Opalescence® Trèswhite Supreme	6	Carbamide peroxide, hydrogen peroxide, sodium fluoride, potassium nitrate, fillers, flavour.	30 min/day	7	10
Yotuel® 10 Minutes 30% CP	7	Gel: Glycerin, Aqua, Urea Peroxide, Triethanolamine, Xylitol, Carbomer, Aroma, Potassium Fluoride, Diazolidinyl Urea. Activator: Potassium Fluoride, Xylitol.	10 min/day	2h 20min	11
Opalescence® PF Quick 45% CP	8	Carbamide peroxide, potassium nitrate, 0.11% ion fluoride, carbopol, glycerin.	30 min/day	7	15
Yotuel® Special 35% HP	9	Gel: Aqua, Hydrogen Peroxide, Carbomer, Triethanolamine, Xylitol, Sodium Hydroxide, Potassium Fluoride, Diazolidinyl Urea. Activator: Potassium Fluoride, Xylitol.	60 min/session	2	35
Opalescence® Boost 38% HP	10	Gel: Hydrogen peroxide. Activator: Potassium hydroxide, 1.1% fluoride and 3% potassium nitrate.	80 min/session	2h 40min	38

**Group 8 (Opalescence® Quick PF 45% CP) (n=10):**

The treatment procedure in this group was also as for Group 2, except that 45% CP gel was used for 30 minutes/day for 14 days, as suggested by the manufacturer.

**Group 9 (Yotuel® Special 35% HP) (n=10):**

The bleaching material was freshly prepared as suggested by the manufacturer and applied onto the polished enamel surfaces of all specimens in a 0.5 to 1.0 mm thick layer for 20 minutes. The material was agitated after 10 minutes with a brush soaked in activator. After 20 minutes the bleaching gel was removed gently from the enamel surfaces using a paper towel. The specimens were thoroughly rinsed with distilled water and air dried. The bleaching procedure was performed three times in one session. The specimens were stored in artificial saliva at 37°C until the next treatment. The artificial saliva was replaced on a daily basis. After 7 days, the same bleaching procedure was repeated.

**Group 10 (Opalescence® Boost 38% HP) (n=10):**

The bleaching material was freshly mixed according to the manufacturer's instructions and applied onto the polished enamel surfaces of all specimens in a 0.5 to 1.0 mm thick layer for 20 minutes. The material was agitated every 5 minutes. After 20 minutes, the bleaching gel was removed gently using a paper towel. The specimens were thoroughly rinsed with distilled water and air dried. The bleaching material was applied four times in one session. The specimens were stored in artificial saliva at 37°C until the next treatment. The artificial saliva was replaced on a daily basis. After 7 days, the same bleaching procedure was repeated.

**Microhardness Measurements**

Surface microhardness of the enamel blocks were measured using a digital hardness tester with a Vicker's diamond indenter. The saliva soaked specimens were wiped gently with a tissue paper, rinsed with distilled water and tissue blot dried before each microhardness measurement. Before any treatment, 4 indentations were made (base-line hardness values) on the polished enamel surface of each enamel block (10 blocks, Figure 1) with a 300g

load applied for 15 seconds. The indents were repeated after 14 days of active bleaching treatment close to the already men-

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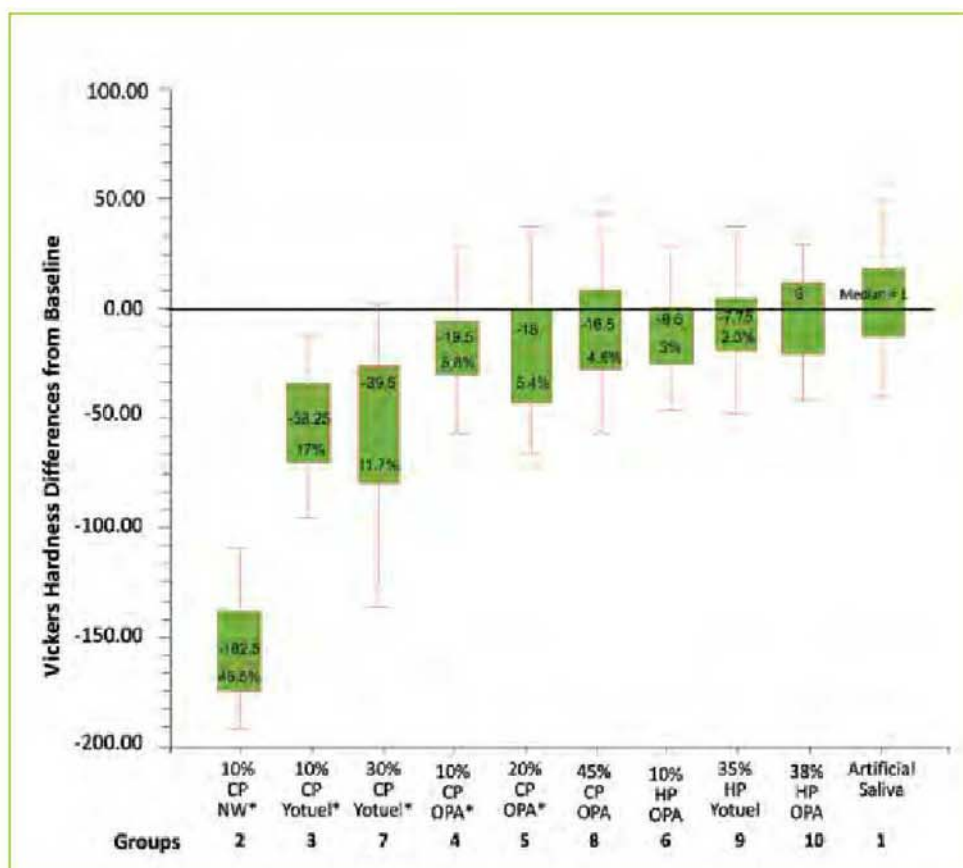


Figure 2: Box-and-Whisker plots of the Vickers microhardness differences for the control and treated groups. Groups that differ significantly from the control group (artificial saliva) are marked with an asterisk.

tioned baseline indents (about 10  $\mu\text{m}$  away from where the base-line indent was made, Figure 1). All data were saved as Vickers Hardness Values (VHN) for statistical analysis. The microhardness data were analysed using Kruskal-Wallis one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test for differences amongst the different groups (significance level was 5%).

Three enamel blocks of each test group were also polished, as already described. One hardness indent was then made on each block and the position of the indent was marked. The blocks were now subjected to the full 14-day treatment as described, thoroughly rinsed under tap water, blot dried and another indent made next to the previous one (10  $\mu\text{m}$  away). This gave a treated as well as untreated indent. Scanning Electron Microscope images of the base-line and post-bleaching indents were taken to compare the demineralization effect on the indents. General information about the composition, treatment period and hydrogen peroxide concentration of the different bleaching products are given in Table 2, according to the manufacturers<sup>30-32</sup>.

## RESULTS

Figure 2 depicts the Box-and-Whisker plots of the median Vickers microhardness differences between the base-line and the post-treatment

hardness values for the control and treatment groups. In each diagram, the top line shows the maximum and the bottom line the minimum hardness values, while the box part shows the location of 50% of the values and the line in the box the median hardness value of the difference for the specific group.

All whitening products tested in this study damaged the enamel except Opalescence Boost 38% HP. Only Groups 2, 3, 4, 5 & 7 (Figure 2 and Table 2) showed statistically significant decrease in enamel microhardness when compared to the control group ( $p < 0.05$ ). Groups 2, 3 & 7 also showed a statistically significant decrease in enamel microhardness compared to all the other groups ( $p < 0.05$ ). Group 2 (Nite White<sup>®</sup> ACP 10% CP) showed the highest damage and also differed significantly from Groups 3 & 7 ( $p < 0.05$ ).

Representative SEM images of indent marks on enamel are illustrated in Figures 3–6. Figure 3 represents an indent mark on enamel which was not exposed to any bleaching treatment. Figures 4–6 represent indent marks exposed to Yotuel<sup>®</sup> Patient 10% CP for 112 hours, Opalescence<sup>®</sup> TrèswHITE Supreme 10% HP for 7 hours, and Yotuel<sup>®</sup> Special 35% HP for 2 hours, respectively. Comparing these images, it can be clearly seen that the indent marks in Figures 4 and 5 faded because of demineralization of the enamel for 14

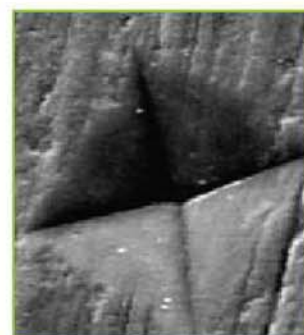


Figure 3: Scanning Electron Micrograph of indentation on enamel that was not exposed to any bleaching treatment. Final magnification 500X.

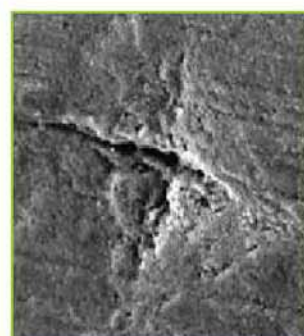


Figure 4: Scanning Electron Micrograph of indentation on enamel that was exposed to Yotuel Patient 10% CP for 112 hours. Final magnification 500X.

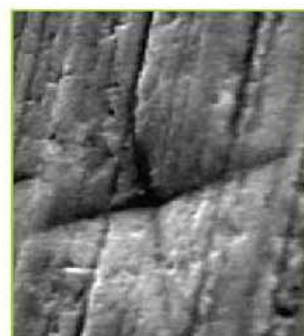


Figure 5: Scanning Electron Micrograph of indentation on enamel that was exposed to Opalescence TrèswHITE Supreme for 7 hours. Final magnification 500X.

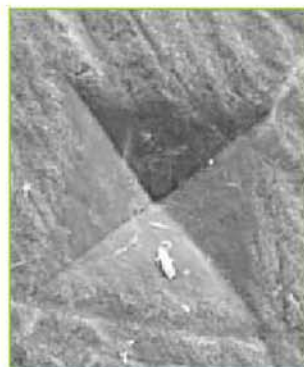


Figure 6: Scanning Electron Micrograph of indentation on enamel that was exposed to Yotuel Special 35% HP for 2 hours. Final magnification 500X.

days when compared to the indent mark in Figure 3, which was not subjected to any bleaching process. Indent mark in Figure 6 showed relatively less damage. These images correspond to the results found in their hardness values (Figure 2).

## DISCUSSION

Tooth-bleaching agents may damage dental enamel even under prescribed conditions. The guidelines by the American Dental Association for any bleaching agent recommended that enamel hardness should be evaluated to ensure that no substantial changes in the morphology and/or properties of enamel would occur during tooth-bleaching treatment according to the product's usage instructions.<sup>33</sup> Following bleaching treatment, a decrease in surface hardness/softening of enamel has been reported.<sup>5,11,20</sup> Surface-softening lesions have been identified as the initial stage of caries lesion formation and dental erosion can occur easily in softened enamel.<sup>21</sup> Furthermore, it is now generally accepted that microhardness determinations give a reliable indication of the demineralization (damage) of enamel or dentine and are commonly used for this purpose.<sup>15,34-36</sup>

One of the main reasons for the controversial results in microhardness studies might be due to the differences in the study design. Human enamel exhibits large regional variations in structure related to the differences in local chemistry (varying levels of mineralization, organic matter and water) and microstructure (fractions of inorganic crystals and organic matrix).<sup>37,38</sup> Enamel microhardness may consequently vary from area to area.<sup>37</sup> From the results (Figure 2), it can be seen that the damaging effect on enamel could be relatively small as far as the decrease in the hardness value is concerned. Therefore, good planning as to the exact position of the indentations to be compared was necessary to keep the site variations of the hardness values small. The solution to this problem was to have the base-line indent and the test indent thereafter done as close as possible to each other without the one interfering with the other. This is even more important when the hardness variation is very small and could consequently be easily masked when different areas on enamel were used.

The control group (Figure 2) was enamel blocks stored in artificial saliva for the whole period of the experiment (14 days) and showed almost no hardness change over 14 days (median difference between the start and the end was only 1.0). This finding showed that the artificial saliva solution did not alter the hardness of sound enamel either positively or negatively and could therefore be rightfully used as a soaking medium in the experiment.

The difference in the hardness has a negative value when the bleaching treatment resulted in a softer enamel surface (Figure 2). In general, it can be seen that all bleaching agent treatments (except Group 10) resulted in lower hardness values which indicated damage to enamel. In general, carbamide peroxide whitening products (Groups 2-5,7,8) showed more damage than hydrogen peroxide products (Groups 6,9,10) but the treatment periods also influenced the hardness values. It seemed that combination of a short treatment period with a high peroxide concentration (groups 6,8-10) gave rise to a lower degree of damage to enamel when compared to a combination of a low concentration treatment (groups 2-5; 3.35% and 6.7%) for a long period (112 hours). Group 7 (Yotuel® 30% CP) is an exception with a relatively short treatment time period (140 minutes) showed a significant decrease in enamel microhardness. In contrast to our study (8 hours/day), no change in enamel microhardness was found when

10% CP was used for a shorter treatment period of 2 or 3 hours per day for 14 days.<sup>24,39</sup> These results underline the negative effect of longer treatment periods.

However, differences amongst different groups with similar peroxide concentration (10% CP, Groups 2,3 and 4) and treatment times (112 hours) indicated that such negative effects are not only related to the peroxide concentration and application time. Therefore, changes in enamel microhardness following tooth-bleaching could also depend on the composition of the product.<sup>2</sup> The composition of the bleaching products investigated in this study is given in Table 2. Groups 2 to 6 are at-home bleaching products and groups 7 to 10 are in-office bleaching products with high peroxide concentrations. Nite White® ACP (Group 2) 10% CP gel contained amorphous calcium phosphate (ACP), potassium nitrate but no fluoride. While Yotuel® 10% CP (Group 3), 30% CP (Group 7) and 35% HP (Group 9) gels contained potassium fluoride. Opalescence® 10% CP (Group 4), 20% CP (Group 5), 45% CP (Group 8), 10% HP (Group 6) and 38% HP (Group 10) gels contained fluoride and potassium nitrate. Thus, the absence of fluoride in Nite White ACP might have had a negative influence on the results obtained for this product in the present study. Pinheiro *et al*,<sup>45</sup> also reported that Opalescence CP bleaching agent showed significantly less reduction in enamel microhardness than Nite White 10% CP.

Fluoride, potassium nitrate and ACP have been introduced in recent bleaching products to prevent either hypersensitivity or demineralization effects.<sup>40</sup> Fluoride is believed to act as a remineralising agent by forming a calcium fluoride layer on enamel which inhibits demineralization or decrease in microhardness values<sup>41</sup> whereas ACP undergoes rapid hydrolyses to form apatite similar to the carbonated apatite of tooth mineral.<sup>42</sup> However, in this study, a decrease in enamel microhardness was observed for the products containing fluoride, potassium nitrate or ACP. The findings are in agreement with other previous studies.<sup>27,43,44</sup>

Although some studies have reported no significant changes in enamel microhardness after bleaching with Opalescence® 10%,<sup>24,46-48</sup> 20% CP,<sup>49</sup> Xtra Boost 38% HP,<sup>50</sup> Nite White® Excel 22 10% CP,<sup>51</sup> Yotuel® 10% CP,<sup>52</sup> other studies have found a decrease in enamel microhardness after treatment with Opalescence® 10%,<sup>5,6,44,53</sup> 20%<sup>5</sup> and 35% CP,<sup>11</sup> Xtra Boost 38% HP,<sup>53</sup> and Nite White® ACP 10% CP.<sup>44,45</sup>

The damaging effect was also confirmed by SEM images taken of the indents made with the hardness tester before and after the treatment process (Figures 3–6). SEM images were done for all the products but only few images were shown because they are representative of different degrees of damage by the various bleaching products.

## CONCLUSIONS

1. All products tested in this study decreased enamel microhardness except Opalescence Boost 38% HP.
2. The products containing carbamide peroxide were more damaging to enamel because of the longer application times of the bleaching agents.
3. Longer treatment periods influenced the enamel microhardness values negatively.
4. Nite White ACP 10% CP showed the highest reduction in enamel microhardness as compared to other products tested.

## RECOMMENDATION

It seems that it might be safer to use whitening products with

higher peroxide concentrations for shorter periods to prevent damage to enamel.

### ACKNOWLEDGEMENTS

The authors thank SADA (Dentistry Development Foundation) for their financial support.

This project forms part of a PhD study at UWC.

**Declaration:** No conflict of interest was declared

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