In vitro colorimetric evaluation of the efficacy of home bleaching and over-the-counter bleaching products

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Nacer Benbachir, Dr Med Dent and Ivo Krejci, Professor Dr Med Dent

Objective: Various bleaching modalities are now offered to patients, either monitored by the dental office or self-directed, for which relative efficiency is unknown. The aim of this in vitro study was to evaluate the ability of bleaching products and protocols to lighten enamel and dentin. Method and Materials: Bovine tooth specimens of standardized thickness (2.5 ± 0.025 mm with similar dentin and enamel thickness) were prepared and stained with whole blood and hemolysate before being submitted to seven supervised or self-directed bleaching regimens: tray-based bleaching using 10% (Opalescence, Ultradent; Nite White, Discus Dental) or light-activated 30% (Metatray, Metatray) carbamide peroxide (CP); 6% (Zoom, Discus Dental) or 9% (TresWhite, Ultradent) hydrogen peroxide (HP); strips (Whitening Strips, Oral B–Rembrandt); and paint-on gel (Paint on Plus, Ivoclar Vivadent) containing 8.1% and 6% HP, respectively. Colorimetric measurements were performed on each specimen side, according to the CIE L*a*b* system, before and after staining, as well as after 5, 10, and the recommended number of bleaching applications. Results: Color change after recommended number of applications (ΔEr) varied from 15.72 (Metatray) to 29.67 (Nite White) at enamel and 14.91 (Paint on Plus) to 41.43 (Nite White) at dentin side; Nite White (10% CP) and TresWhite (9% HP) were more effective than Metatray (30% CP) and Paint on Plus (6% HP) after 5 or the recommended number of applications. Conclusion: In this in vitro study based on bovine teeth, tray-based systems produced the faster and better bleaching effect, regardless of the product and concentration used, at both enamel and dentin sides. (Quintessence Int 2010;41:505–516)

Key words: carbamide peroxide, home bleaching, hydrogen peroxide, over-the-counter bleaching products, tooth bleaching,
Except for a few other products such as oxalic acid, chlorine, and muriatic acid, hydrogen peroxide has been used as the main active ingredient for all kinds of bleaching techniques. Carbamide peroxide, formerly used for topical disinfection following oral surgery, was found to be an interesting alternative source of hydrogen peroxide, providing a slower release of active, oxidizing ions. Today, bleaching products are frequently found in the form of gels containing varying concentrations of carbam ide peroxide or hydrogen peroxide, depending on the application methods, combined with a few other substances such as stabilizers, catalysts, and desensitizing and flavor agents. Lower concentrations of both carbam ide peroxide and hydrogen peroxide are used for home bleaching, while higher concentrations are necessary for in-office treatments. Chairside techniques also make use of intense light sources (halogen, ultraviolet light, laser, plasma-activated curing, light-emitting diode [LED]) or chemicals to activate and accelerate the degradation of the bleaching gel; this approach is known as power bleaching. Despite manufacturers’ claims and some optimistic marketing statements, it is not known whether all bleaching products and techniques are equally effective. Actually, because diffusion within the dental tissues is mostly governed by concentration and the application time of the active substances, the efficiency of the protocols and product concentrations is likely to vary. A large number of over-the-counter systems with original application methods were recently launched and provided patients with various options such as strips, paint-on gels, and preloaded trays.

Clinical reports have demonstrated the clinical efficiency and safety of home bleaching techniques over more than 10 years, while there is only short-term information available about the potency of over-the-counter systems. It is also quite difficult to compare the effect of the numerous products and application protocols because there is no consensus about how the efficiency of bleaching techniques should be assessed. In clinical trials, patients’ self-appreciation, intraoral photographs, comparisons to shade guides, and spectrophotometric/colorimetric measurements have been used so far. In addition to numerous evaluation methods employed, there is a large variation in the posttreatment observation-measurement intervals. Moreover, one has to take into account the limitations of in vitro trials, which naturally vary from clinical conditions, as well as the limited number of products, variables, and parameters that can be simultaneously assessed. While numerous in vitro studies were designed to assess the effect of bleaching techniques on tooth composition and microstructure, little was undertaken to measure their effects on tooth color or specific lightening potential. Staining techniques were developed to assess the lightening effect of bleaching techniques but for mainly nonvital teeth.

This approach, however, presents an interesting potential for evaluating vital bleaching techniques, as well. Because of the tremendous interest of patients in cosmetic dental procedures and bleaching in particular, the industry has tried to broaden the choice of application methods. It first happened with modifications or improvements of classic home and chairside techniques, and it continued with several over-the-counter bleaching systems for which relative efficacy was never tested in a controlled in vitro environment.

Today, clinicians and patients are faced with a large selection of bleaching systems but know very little about their relative effect. The aim of the present study was to test the hypothesis that different bleaching products and protocols have the potential to lighten the color of enamel and dentin fragments of discolored bovine teeth at various degrees. For this purpose, a standardized staining technique of the specimens was used, and all color changes were assessed in vitro with a colorimeter.

**METHOD AND MATERIALS**

**Preparation and staining of specimens**

Thirty-five permanent bovine maxillary incisors, collected from calves sacrificed at the age of 18 months (± 1 month) and randomly
allocated to seven groups, were used for this study. The preparation, coloration, and measurement of the specimens were performed by the same operator. After careful cleaning with pumice, the buccal side was flattened with a model trimmer to obtain an even surface of 14 × 8 mm (± 1 mm). Roots were cut about 1 mm below the cementoenamel junction (CEJ). Each tooth was then embedded in a self-curing acrylic resin (Technovit 4071, Heraeus-Kulzer), the flattened area facing the mold base. Embedded specimens were trimmed again until a section about 2.6 mm thick was obtained; specimens were further polished on both sides with sandpaper of decreasing grit (250, 500, and 2,400) (LaboPol-II, Struers), providing a 2.5-mm (± 0.05 mm) section (about half enamel and half dentin in thickness) with aforementioned width and length dimensions (Fig 1).

Colorimetric measurements (initial measurements [I]) of specimens were performed on each side (dentin and enamel) using a reflectance colorimetric device (Minolta CR-21, Minolta). Each specimen was taken from its humid environment just before the measurement and covered with a moist towel to avoid any false score due to the dryness of the specimen. Then, for the measurement, the specimen was placed on a white opaque ceramic plate. This device was set to produce color parameters based on average daylight (D65: 6,504 K). The optical geometry of this system consisted of a 45-degree illumination angle and a 0-degree (normal) observation angle. The measuring window is a 19.6 mm² circle positioned in the middle of the specimen. The color parameters were recorded in the L*a*b* color space, as established by the Commission Internationale de l’Eclairage (CIE) in 1976.33

Specimens were stained with human blood, using the technique described by Freccia and Peters,27 with slight modifications related to the use of tooth fragments rather than whole tooth crowns. Enamel and dentin surfaces were first etched with a 35% phosphoric acid gel (Ultraetch, Ultradent) for 30 and 15 seconds, respectively, to remove smear layers. Specimens (five per group) were then randomly immersed in test tubes (n = 7) containing whole blood and centrifuged at 4,000 rpm for 30 minutes three times a day for 3 consecutive days. At completion of this first step, only the hemolysate was recuperated for further hemolysis of the red blood cells. The hemolysate was mixed with the same amount of distilled water and centrifuged at 4,000 rpm twice for 30 minutes. Thereafter, the specimens were immersed again in the new hemolysate and centrifuged at 4,000 rpm for 30 minutes three times a day for 3 consecutive days. Specimens were stored in a humid environment for 7 days before proceeding with new L*a*b* colorimetric measurements on each specimen side (postcoloration measurements [PC]).

**Bleaching procedures**

The bleaching effect on blood pigments of the selected bleaching products and techniques was assessed. Bleaching products were applied as an approximately 1-mm layer, on only enamel surfaces. Table 1 describes the composition of all bleaching products under evaluation and details their respective application protocols.
• **Home bleaching for use with an individualized tray:** Opalescence 10, Nite White Excel III 10, Zoom (Discus Dental)

• **Home bleaching for use with a prefabricated tray:** TresWhite, Metatray (with LED activation)

• **Self-applied bleaching varnish:** Paint On Plus

• **Over-the-counter bleaching strips:** Whitening Strips

### Comparison of colorimetric measurements

Color differences for each specimen were calculated between the initial measurement and postcoloration situation and then between postcoloration and 5 applications of the bleaching product, between 5 and 10 applications, and finally between postcoloration and the recommended number of applications, following the equation $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$.

$\Delta E$ depicts the global color change including the three dimensions of the CIE L*a*b* system and was therefore used to compare the efficacy of the bleaching protocols, as well as the difference among application times within each group.

### Statistics

The results for both dentin and enamel sides were submitted to a parametric statistical analysis. Differences in initial and postcoloration L*a*b* values were tested with a repeated-measures analysis of variance (ANOVA) followed by a multiple-comparison Scheffe test. Differences in $\Delta E$ values among the seven bleaching groups after each experimental phase were tested with a factorial ANOVA followed by the Scheffe test. Differences between $\Delta E_{5}$ and $\Delta E_{10}$ values within each group were tested with a paired t test. All tests were carried out at a 5% level of significance.

### RESULTS

L*a*b* values for both enamel and dentin sides, before and after coloration, as well as after each treatment phase, are presented in Tables 2a and 2b. Table 2c presents statistical comparison of pre- and postcoloration L*a*b* values. Color differences for both enamel and dentin sides represented by $\Delta E$ values are presented in Table 3a, together with statistical differences. Table 3b presents
statistical comparison of $\Delta E$ values at each step. Figures 2 and 3 depict color values ($L^*a^*b^*$) and changes ($\Delta E$) at each experimental step for both enamel and dentin sides. Figure 4 depicts visually the overall results obtained with tested bleaching systems; surface and in-depth effects can be appreciated for brands with high (Nite White), intermediate (Zoom), and limited (Paint on Plus) efficacy.

There was no statistical difference in initial $L^*a^*b^*$ values between groups at either the enamel or dentin side. The staining of specimens did produce a clear drop in $L^*$ enamel and dentin values and an increase in $a^*$ and $b^*$ values on both specimen sides (see Tables 2a and 2b and Figs 2 and 3). After staining, no difference in $L^*$ or $a^*$ values was reported at either enamel or dentin side. A few significant differences in $b^*$ values were found at dentin side (Zoom specimens were the darkest ones). $\Delta E_{PC}$ did present only one difference for enamel (Zoom against Nite White).

### Table 2a

<table>
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<th>Product</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
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<td>0.5</td>
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### Table 2c

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<td>1.711</td>
<td>2.816</td>
<td>3.153</td>
<td>2.373</td>
<td>1.702</td>
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<td>Dentin</td>
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<td>2.932</td>
<td>0.583</td>
<td>1.097</td>
<td>1.017</td>
<td>6.885</td>
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(NS) No significant difference among groups; (S) significant differences among groups.
In contrast, all bleaching treatments produced an increase in L* values (from 48.5 to 82.9 for enamel and from 35.4 to 80.8 for dentin) and a decrease in a* values (from 3.2 to –2.2 for enamel and from 7.9 to –3.9 for dentin), but of varying amplitude, especially on the dentin side (see Figs 2a, 2b, 3a, and 3b). The b* dentin values did decrease or increase following the first 5 applications or after 10 applications and recommended regimen (see Figs 2c and 3c). On the enamel side, variations in b* values were of minimal amplitude with no clear trend. Overall, bleaching induced a reduction of specimen chroma at either the dentin or enamel side.

The bleaching effect on enamel proved to vary according to the product used after 5 and 10 applications, as well as for the recommended treatment regimen (ΔE5, ΔE10, and ΔEr) (see Table 3a and Fig 2d). After 5 applications, Nite White was the most potent bleaching product (ΔE 23.67), significantly better than Metatray (ΔE 10.41); after 10 applications, Whitening Strips (ΔE 11.55) and TresWhite (ΔE 8.91) became the most potent products, superior to Nite White (ΔE 1.45), which had developed most of its effect formerly. When the bleaching effect was compared following the recommended number of applications, Nite Wite (ΔE 29.67) and TresWhite (ΔE 29.35) were more effective than Paint on Plus (ΔE 16.07) or Metatray (ΔE 15.72).

The bleaching effect on dentin proved to vary according to the product used after 5 and 10 applications, as well as for the recommended treatment regimen (ΔE5, ΔE10, and ΔEr) (see Table 3a and Fig 2d). After 5 applications, Nite White was the most potent bleaching product (ΔE 23.67), significantly better than Metatray (ΔE 10.41); after 10 applications, Whitening Strips (ΔE 11.55) and TresWhite (ΔE 8.91) became the most potent products, superior to Nite White (ΔE 1.45), which had developed most of its effect formerly. When the bleaching effect was compared following the recommended number of applications, Nite Wite (ΔE 29.67) and TresWhite (ΔE 29.35) were more effective than Paint on Plus (ΔE 16.07) or Metatray (ΔE 15.72).

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The bleaching effect on enamel proved to vary according to the product used after 5 and 10 applications, as well as for the recommended treatment regimen (ΔE5, ΔE10, and ΔEr) (see Table 3a and Fig 2d). After 5 applications, Nite White was the most potent bleaching product (ΔE 23.67), significantly better than Metatray (ΔE 10.41); after 10 applications, Whitening Strips (ΔE 11.55) and TresWhite (ΔE 8.91) became the most potent products, superior to Nite White (ΔE 1.45), which had developed most of its effect formerly. When the bleaching effect was compared following the recommended number of applications, Nite Wite (ΔE 29.67) and TresWhite (ΔE 29.35) were more effective than Paint on Plus (ΔE 16.07) or Metatray (ΔE 15.72).

The bleaching effect on dentin proved to vary according to the product used after 5 and 10 applications, as well as for the recommended treatment regimen (ΔE5, ΔE10, and ΔEr) (see Table 3a and Fig 2d). After 5 applications, TresWhite (ΔE 32.61), Nite White (ΔE 32.30), and Zoom (ΔE 29.91) were the most efficient bleaching products, significantly superior to Paint on Plus (ΔE 9.44); after 10 applications, the Whitening Strips (ΔE

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### Table 3a

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<th>Product</th>
<th>ΔEPC</th>
<th>ΔE5</th>
<th>ΔE10</th>
<th>ΔEr</th>
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<th>ΔE5</th>
<th>ΔE10</th>
<th>ΔEr</th>
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See Table 1 for explanation of product codes. (ΔEPC) Postcoloration; (ΔE5) after the first 5 applications of bleaching agent; (ΔE10) after 5 more applications of bleaching agent (total of 10 applications); (ΔEr) after the application time recommended by the manufacturer (depends on the bleaching agent).

*Color differences relative to untreated specimens. Groups with same lowercase letter are not statistically different (Scheffe).

Columns without letter showed no statistical difference between groups.

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### Table 3b

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<th>ΔEPC</th>
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</tr>
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<td>F</td>
<td>.067*</td>
<td>.000*</td>
<td>.000*</td>
<td>.04*</td>
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<tr>
<td>Differences</td>
<td>PONP6 vs TRW, Zo6, N10</td>
<td>WIS8.1 vs all</td>
<td>N10 and TRW vs PONP6</td>
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* Significant differences among groups.
† No significant difference among groups.
19.70) became the most potent product, statistically superior to all other products during this experimental phase. When the bleaching effect was compared after the recommended number of applications, Nite White (ΔE 41.43) and TresWhite (ΔE 38.12) were more effective than Paint on Plus (ΔE 14.91). L* values did confirm the aforementioned findings and the good performance of TresWhite and Nite White (see Figs 2a and 3a).

In regard to the evolution of bleaching effect within each group, Nite White, Zoom, TresWhite, and Paint on Plus showed the most pronounced action after the first 5 applications on both enamel and dentin sides. Opalescence had the most important bleaching effect on enamel after 5 applications and between 5 and 10 applications on dentin.

**DISCUSSION**

**Staining technique**

The staining technique applied to the specimens of this study has been used in several studies.\textsuperscript{28–30} This staining technique is based on the penetration of red blood cells and hemoglobin pigments inside the tooth structure from the dentin side of the specimens. The rationale for staining the specimens is to allow for a more discriminative comparison of the bleaching methods and products and in particular the evaluation of their superficial (bleaching of enamel) or in-depth (bleaching of dentin) action. Stains deposit in dentinal tubules, and the protocol stimulates intrinsic organic discolorations. The absence of significant difference in dentin/enamel L* pre- and
postcoloration values, as well as the few differences in $a^*$ and $b^*$ values among groups, proves that a reasonably uniform specimen quality and staining were achieved.

The use of bovine teeth for this study allowed the preparation of specimens having standardized size and quality and, in addition, anatomically relevant tissue thickness. Likewise, it made possible the fabrication of specimens for which dimensions are best adapted to the size of the colorimeter measuring window. A final advantage of using this in vitro protocol with staining technique was the absence of clinical variables. The color parameters were recorded in the $L^*a^*b^*$ color space, as established by the CIE in 1976. The CIELab system is adequately related to human eye color perception in all three dimensions or directions of color space. The Minolta CR-121 has been used in several dental research studies, including those for detection of color differences between metal-ceramic restorations and composite resin restorations and also to evaluate the effects of bleaching. A $\Delta E$ depicts the global color change including the three dimensions of the CIE $L^*a^*b^*$ system and was therefore used to compare the efficacy of the bleaching protocols, as well as the difference among application times within each group. A $\Delta E$ below 1.0 is considered visually nondetectable, while values up to 3.3 are considered moderate visual differences. When the three color dimensions are analyzed separately, $L^*$ values, which depict the specimen lightness, appeared to be the most relevant parameter to make comparisons between products or experimental conditions.
The rationale of the present study was to compare in vitro the effect of bleaching gels, varnishes, and strips containing various concentrations of hydrogen peroxide (HP) or carbamide peroxide (CP). All products under evaluation were based on a home application regimen, supervised by the dental office or self-directed (OTC).

The bleaching effect (increase in L* values) increased in proportion to the number of applications on both dentin and enamel sides for all the products under evaluation. In regard to dentin and enamel a* values, a rapid drop was observed for most of the products (Nite White, Tres White, Zoom, Opalescence, and Metatray) following the first five applications; variations then decreased. For the less efficient products (Paint on Plus and Whitening Strips) the decrease continued until the final set of applications to approach the a* values of other products (see Figs 2a and 2b; Figs 3a and 3b). The change in b* values of stained specimens reflects two chemical phenomena related to bleaching efficiency, which are first the transformation of blood pigments (from reddish to yellowish or brownish tint) and thereafter their bleaching. Then, for the less effective products, there was an initial increase in b* values and then a decrease, while for the most potent products, there was only a decrease of b* values. This observation is particularly evident for the dentin side (see Fig 3c). Following bleaching, a* values tended to approach zero at dentin or enamel side for all products, as did b* enamel values. In contrast, b* dentin values decreased to about 5.0 for the most efficient product (Nite White), while it remained above 10.0 for all other products, showing a less efficient in-depth bleaching effect.

The continuation of color change following additional applications of the bleaching product is in agreement with other in vitro studies using blood or tea for specimen staining. Actually, Sulieman showed that the bleaching effect was highly dependent on CP concentration and duration of exposure; they

Fig 4 (a) Pretreatment specimen (enamel and dentin sides) showing typical aspect after staining (statistics proved the homogeneity of the staining process). Posttreatment specimens representing typical bleaching effect obtained on enamel (b) or dentin (c) following recommended application regimens: from left, 18 applications of Nite White 10% CP (N10), 8 applications of Zoom 6% HP (ZOO), and 14 applications of Paint on Plus 6% HP. While differences in enamel bleaching proved visually discrete among groups, the dentin side of specimens showed marked differences in in-depth product efficacy.
also found that a maximal bleaching effect would be attained, occurring earlier with high CP concentrations. This might be explained by the faster penetration and larger amount of oxidizing ions migrating through hard tissues when HP or high concentrations of CP are used.\textsuperscript{13} The same type of observation was made in vivo; actually, at 2 weeks, a gel containing 15% CP showed a superior bleaching effect compared to a 10% gel,\textsuperscript{45,46} while at 4 weeks posttreatment, no difference could be observed.\textsuperscript{46} However, the present study and a report by Dietschi\textsuperscript{43} did not confirm the phenomenon of reaching a plateau or maximal bleaching effect in the tissue depth. With the most efficient products (TresWhite, Zoom, and Nite White) $\Delta E$ dropped nearly to zero on the enamel side but remained above 5 on the dentin side.

In the present study, NiteWhite proved to bleach faster than Opalescence, even though both brands contain 10% CP, which confirms previous observations.\textsuperscript{43} In fact, NiteWhite contains a mixture of CP and HP that is equivalent in total to 10% pure CP. Because HP is known to penetrate tissues faster than CP,\textsuperscript{13} these results seem logical. In vivo, however, no difference was found between the two products at either 2 or 4 weeks.\textsuperscript{47}

**Comparison among bleaching protocols**

The safety and short- and long-term clinical efficiency of home bleaching using CP gel placed in nightguards have been well-documented\textsuperscript{16,18,48–51}; the effectiveness of this protocol was also demonstrated in vitro.\textsuperscript{43,52} The bleaching effect of CP applied in trays proved satisfactory also in the present in vitro study. In regard to new, self-prescribed bleaching systems (product deposited on teeth with a strip or paint-on gel), numerous clinical studies have documented their efficiency.\textsuperscript{10,11,14,53–55} On the other hand, there is no long-term evidence of safety and bleaching stability with new OTC products; actually, the rather abundant clinical reports on OTC systems mainly describe their immediate surface effect, while it would be mandatory to monitor also long-term results.\textsuperscript{14} There is also a large deviation in the efficiency of OTC systems in relation to the evaluation protocol, concentration of the bleaching agent (mainly HP or CP), and application method. Bleaching strips using 5.3% HP proved to have an effect comparable to a tray-based system using 10% or 20% CP\textsuperscript{30}; by increasing the concentration of HP from 5.3% to 6.5%, a greater bleaching effect was found.\textsuperscript{56–58} Paint-on bleaching gels containing 18% CP proved less efficient than strips with 6% HP.\textsuperscript{59,60} When comparing two paint-on gels, the one containing 18% sodium percarbonate proved more efficient than a similar product with 8.7% HP.\textsuperscript{59} On the contrary, in a controlled in vitro environment, the bleaching effect of strips containing 5.3% HP proved largely inferior to tray-based 10% CP or 7.5% HP products.\textsuperscript{43} In the present study, the tray-based products demonstrated globally a more pronounced bleaching effect in comparison to paint-on gels or strips. Since the concentration of the bleaching agent contained in Whitening Strips (8.1% HP) can be considered appropriate, the possible reasons for their reduced efficiency are a relatively dry surface, strip stiffness, and reduced gel impregnation. In regard to the Paint on Plus (6% HP), the possible reason for its lesser bleaching effect is a shorter contact duration with the tissues; this appears to be the most likely explanation of this finding.

**CONCLUSION**

An in vitro measurement of $L^*a^*b^*$ values of stained bovine teeth specimens after bleaching with different products, concentrations, and protocols allowed for a discriminative comparison of the treatment efficiency on enamel surface and deeper dentin structure. The results of this in vitro trial suggest the following conclusions:

- The study hypothesis was confirmed, namely, that bleaching effect on enamel or dentin was dependent on the mode of application, product composition and concentration, and application time.
- Nite White (10% carbamide peroxide) and Tres White (9% hydrogen peroxide), two tray-based systems, had the most potent
bleaching effect on both enamel (surface) and dentin (depth) specimen sides; the selected over-the-counter strips (Whitening Strips) and paint-on (Paint on Plus) bleaching products demonstrated limited in-depth effect compared to tray-based systems.

- In the tray-based group, the most significant effect was attained after the first five applications for all products containing only or a proportion of hydrogen peroxide; the bleaching effect did slow down but never reached a plateau.

- After the recommended number of applications, hydrogen peroxide gels did not prove significantly more efficient than gels containing carbamide peroxide.

REFERENCES


