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# IN VITRO STUDY ON THE EFFECTIVENESS OF A GROUP OF EXPERIMENTAL HYDROGEN PEROXIDE AND CARBAMIDE PEROXIDE BLEACHING GELS UPON DENTAL ENAMEL

**DIANA DUDEA[a], MARIOARA MOLDOVAN[b], LAURA SILAGHI DUMITRESCU[b], HORATIU COLOSI[c], ALEXANDRA BOTOS[a], ADA IRIMIE[a], CAMELIA ALB[a]**

**ABSTRACT.** Chemical dental bleaching products are mainly based on hydrogen peroxide (HP) or it's organic derivate carbamide peroxide (CP). This study aimed to demonstrate the bleaching effect of five experimental bleaching agents formulas (EBA), containing either CP or HP, when compared with five commercial bleaching agents (CBA), with similar concentration in active ingredients. The bleaching effect was tested on five groups of extracted human permanent teeth and the CIEL*a*b* color parameters were recorded with a dental spectrophotometer (Vita Easyshade). In each case, on the facial surface EBA was applied, while on the lingual part CBA with similar concentration in active ingredients was used. Increasing of lightness (L*) and a tendency of reducing the yellowness (b*) and redness (a*) were recorded, for both EBA and CBA. The most important variations were obtained for the formulas based on 15% CP and the lowest for the 35% HP, following the indicated clinical protocols. Overall significant differences regarding lightness (ΔL*) and color (ΔE*) between the 5 groups of bleaching agents with various formulas, for both EBA and CBA were recorded.

***Key words:*** *carbamide peroxide, hydrogen peroxide, bleaching*

## INTRODUCTION

One of the most important factors related to the dental appearance is the color, which is considered as a complex parameter, being influenced by reflection, absorption, and dispersion of the incident light through tissues with different optical properties (enamel, dentine, dental pulp) [1,2]. According to the origin of the coloring agent and it's location related to the dental structure, either extrinsic or intrinsic discoloration are generated [1,3].

[a] *University of Medicine and Pharmacy "Iuliu Haţieganu", Dept. of Dental Propaedeutics, Faculty of Dentistry, Clinicilor Str.32, RO- 40006 Cluj-Napoca, Romania, ddudea@umfcluj.ro*

[b] *University Babeş-Bolyai, Institute of Chemical Research "Raluca Ripan", Str. Fântânele Nr. 30, RO-400294, Cluj-Napoca,Romania*

[c] *University of Medicine and Pharmacy "Iuliu Haţieganu" Dept. of Medical Informatics and Biostatistics, Faculty of Medicine, Pasteur str.6, RO-400349 Cluj-Napoca, Romania*

In the case of extrinsic discoloration, which affect the enamel surface, the chromophores, represented mainly by polyphenolic compounds, originate more often from dietary sources. Polyphenols range from small flavonoids, such as the green tea catechines and grape-skin antocyanins (molecular weight-300), to highly polymerized structures containing more than 50 flavanol molecules, such as the black tea teaflavins (1kDa) and thearubigins (>1kDa). Acidic proline –rich proteins contained in saliva are included into the pellicle which coats the enamel; they are believed to mediate the staining of the enamel by dietary polyphenols [4].

Indirect extrinsic tooth staining is mainly associated with cationic antiseptics, such as bis- biguanides (chlorhexidine), pyrimidine derivatives (hexetidin), oral iron salts in liquid form, essential oils, and amoxicillin [2, 5, 6]. In these circumstances, the agent is without color or with a different color from the stain produced on the tooth surface.

Internalized stains are the result of extrinsic stains entering the dentine via dental structure's defects [7]. In these circumstances, the chromophores may become attached to the organic tissue contained in interprismatic sites, by chemical binding of their hydroxyl and amino groups. The pigmented substances may also form with the calcium ions new molecules varying in size and optical properties [8].

In the case of intrinsic discoloration, the pigmentation, which affects mainly the dentine, has pulpal or systemic origin [9]. The chromogens enter the tooth by an endogenous route, in case of metabolic disorders with or without genetic involvement. In the same group are included discoloration caused by medication (staining caused by tetracycline or dental fluorosis) or local pigmentation due to pulpal hemorrhage or generated by substances used during endodontic treatment [8,10].

When the chromophore is incorporated into the structure of enamel or dentine, prophylactic methods are not enough, and chemical or prosthetic procedures are indicated [11]. Chemical whitening of discolored teeth was performed in dentistry using various agents: some have an oxidant effect, some act through erosion or abrasive potential, others use a combination of these methods [12]. Except some instances of internal bleaching, when sodium perborate is indicated, most treatments commonly used are based on hydrogen peroxide (HP) or it's organic derivate carbamide peroxide (CP) (peroxid-ureea) [13]. Hydrogen peroxide acts as a powerful oxidizing agent and generates monoanion ($HO_2^-$) and hydroxyl radical (OH). Subsequently, CP releases urea, which is decomposed into carbon dioxide and ammonia. Chemical reaction of the two reagents with the organic extracelullar matrix components, including pigments or chromophores, constitues the chemical basis of tooth whitening [13,14]. On the other hand, the oxidative reaction is unspecific and the peroxides can also affect the organic matrix of the enamel and dentine [15,16].

There are two groups of commercial products: based on HP 5.5% or 35% or CP 10%, 15%, 30% or 35 %. The clinical protocols are carried out either as “in-office” methods, performed by dental surgeons or as “at-home” treatments. Each method needs a different protocol, which varies according to the exposure time to bleaching gel, concentration in active substance and individual characteristics of the bleached substrate [13].

The present study aims to demonstrate the bleaching effect of a group of experimental bleaching agents, based on CP and HP, when compared with a group of commercial bleaching products, with similar concentration in active ingredients.

## RESULTS AND DISCUSSION

A group of 15 permanent human lateral extracted teeth were experimentally stained in coffee infusion and, in order to obtain a buccal part and a lingual portion, the anatomical crown was sectioned mesio-distally (using a low speed diamond saw -Isomet, Buehler LTD, Lake Bluff, USA-, under water lubrication). The teeth were divided into 5 groups $G_1$-$G_5$ (n=3), each group was treated with a different formula of bleaching gel; in each case, similar concentration of active ingredient was used for buccal and lingual surface; on the buccal surface experimental bleaching agent (EBA) was applied, while on the lingual portion commercial bleaching agent (CBA) was used.

Five different formulas of EBA, containing, as active ingredients, either CP 10% ($EBA_1$), CP 15 %, ($EBA_2$ and $EBA_3$), CP 35% ($EBA_4$) or HP 35% ($EBA_5$) were tested in this study (section Experimental, table 5)

The number of applications and the duration of each treatment were selected according to the concentration of the active ingredient, in order to simulate the clinical protocol, indicated for each chemical formula (section Experimental, table 6)

Dental color measurement was performed by an instrumental method, using a dental spectrophotometer- Vita Easyshade.

The CIEL*a*b* color parameters $L_1$*, $a_1$*, $b_1$* and $L_2$*, $a_2$*, $b_2$* evaluated before and after the bleaching treatment for the EBA and CBA are included in tables 1 and 2, respectively.

In each case an increasing of lightness (L*) is noticed, as a result of the bleaching process. Related to the chromatic parameters, a tendency of reducing the yellowness (b*) and redness (a*) is associated with the decreasing of staining. Since the differences in lightness (ΔL*) are best perceived vissualy when the dental shade is evaluated [17], we indicated the mean values of variation in this parameter for each group. The results are included in table 3.

**Table 1.** CIEL*a*b* color parameters evaluated for the EBA before and after the bleaching treatment

| Group of teeth | Tooth | Before bleaching | | | After bleaching | | |
|---|---|---|---|---|---|---|---|
| | | $L^*_1$ | $a^*_1$ | $b^*_1$ | $L^*_2$ | $a^*_2$ | $b^*_2$ |
| G1 | 1 | 77,2 | 0,3 | 24,9 | 88 | -2,1 | 24,6 |
| | 2 | 75 | 4,3 | 26,1 | 85,5 | 2,3 | 27,4 |
| | 3 | 71,7 | 0,4 | 25,1 | 88,1 | 0,4 | 34,5 |
| G2 | 4 | 74,7 | 4,1 | 35,1 | 87,1 | 2,8 | 38,9 |
| | 5 | 71,6 | 6,1 | 39,4 | 86,3 | 2 | 38,5 |
| | 6 | 67,9 | 3,5 | 37,3 | 83,8 | 3,4 | 37,4 |
| G3 | 7 | 67,9 | 7,3 | 43 | 82,2 | 0,8 | 33,9 |
| | 8 | 76,6 | 1,8 | 33,3 | 82,9 | -1 | 23,5 |
| | 9 | 74,8 | 4,2 | 37,7 | 86 | 4,5 | 38,6 |
| G4 | 10 | 67,5 | 6,4 | 42,3 | 71,6 | 3,6 | 38,7 |
| | 11 | 67,4 | 5 | 33,1 | 77,1 | 1,9 | 28,4 |
| | 12 | 70,3 | 6,1 | 39,2 | 75,2 | 2,2 | 33,4 |
| G5 | 13 | 71,7 | 5,4 | 36,7 | 77,2 | 2,6 | 34,7 |
| | 14 | 76,1 | 6,2 | 44,2 | 77,1 | 3 | 38,9 |
| | 15 | 70,3 | 4,4 | 31,8 | 74,3 | 2,8 | 29,1 |

**Table 2.** CIEL*a*b* color parameters evaluated for the CBA before and after the bleaching treatment

| Group of teeth | Tooth | Before bleaching | | | After bleaching | | |
|---|---|---|---|---|---|---|---|
| | | $L^*_1$ | $a^*_1$ | $b^*_1$ | $L^*_2$ | $a^*_2$ | $b^*_2$ |
| G1 | 1 | 66,2 | 2,3 | 30,5 | 95,8 | -2,5 | 28,5 |
| | 2 | 72,7 | 4,4 | 29,6 | 83 | 1,9 | 28,5 |
| | 3 | 77,6 | -0,4 | 32,8 | 92,9 | -2,1 | 35,4 |
| G2 | 4 | 69,1 | 4,1 | 32 | 84,4 | 0,4 | 29,3 |
| | 5 | 69,8 | 4,6 | 33,5 | 85,9 | 1,4 | 35,3 |
| | 6 | 71,6 | 4,4 | 34,4 | 86 | 1,2 | 28,4 |
| G3 | 7 | 74,5 | 4,3 | 39,9 | 89,8 | -0,3 | 37 |
| | 8 | 76,9 | 2,7 | 36 | 84,5 | -0,2 | 28,4 |
| | 9 | 71,1 | 5,4 | 40,9 | 89,2 | 3,5 | 42,8 |
| G4 | 10 | 66,3 | 6,2 | 37,4 | 74,1 | 3,7 | 38,2 |
| | 11 | 70 | 4,6 | 34,6 | 75,3 | 0,7 | 24,4 |
| | 12 | 62,3 | 10,4 | 43,2 | 72,3 | 4 | 37,2 |
| G5 | 13 | 72,9 | 5 | 37,5 | 79,8 | 2,5 | 34,1 |
| | 14 | 73,6 | 6,8 | 42,4 | 78,4 | 3,2 | 36,7 |
| | 15 | 72,6 | 4,7 | 29,1 | 75,3 | 0,8 | 23,5 |

The $\Delta L^*$ values ranged between 14.33 - 3.5 for the groups treated with EBA and 18.4 - 4.8 for the groups treated with CBA. The highest results were obtained for the 15% CP formulas and the lowest for the 35% HP following the indicated protocols. In each case, the increasing of the lightness was higher for the groups treated with CBA than for the groups treated with EBA, at similar concentration of the active ingredients.

The results of the variation in the dental color (ΔE*) for the groups treated with EBA and CBA are indicated in table 4. The mean value of ΔE* for each group is also presented.

**Table 3.** Variation of lightness (ΔL*) due to the bleaching treatment with EBA and CBA

| Group of teeth | Tooth nr | ΔL * for EBA | Mean ΔL * for EBA | Std. Dev. | ΔL* for CBA | Mean ΔL* for CBA | Std. Dev. |
|---|---|---|---|---|---|---|---|
| $G_1$ | 1 | 10.8 | 12.567 | 3.323 | 29,6 | 18.400 | 10.016 |
| | 2 | 10.5 | | | 10,3 | | |
| | 3 | 16.4 | | | 15,3 | | |
| $G_2$ | 4 | 12.4 | 14.333 | 1.778 | 15,3 | 15.267 | 0.850 |
| | 5 | 14.7 | | | 16,1 | | |
| | 6 | 15.9 | | | 14,4 | | |
| $G_3$ | 7 | 14.3 | 10.600 | 4.033 | 15,3 | 13.667 | 5.437 |
| | 8 | 6.3 | | | 7,6 | | |
| | 9 | 11.2 | | | 18,1 | | |
| $G_4$ | 10 | 4.1 | 6.233 | 3.028 | 7,8 | 7.700 | 2.351 |
| | 11 | 9.7 | | | 5,3 | | |
| | 12 | 4.9 | | | 10 | | |
| $G_5$ | 13 | 5.5 | 3.500 | 2.291 | 6,9 | 4.800 | 2.100 |
| | 14 | 1 | | | 4,8 | | |
| | 15 | 4 | | | 2,7 | | |

**Table 4.** Variation of color (ΔE*) for each group, due to the bleaching treatment with EBA and CBA

| Gr. of teeth | Tooth nr. | ΔE * for EBA | Mean ΔE * for EBA | Std. Dev. | ΔE* for CBA | Mean ΔE * for CBA | Std. Dev. |
|---|---|---|---|---|---|---|---|
| $G_1$ | 1 | 11.06 | 13.573 | 4.615 | 30.05 | 18.770 | 10.078 |
| | 2 | 10.76 | | | 10.65 | | |
| | 3 | 18.9 | | | 15.61 | | |
| $G_2$ | 4 | 13.03 | 14.736 | 1.510 | 15.97 | 16.133 | 0.327 |
| | 5 | 15.28 | | | 16.51 | | |
| | 6 | 15.9 | | | 15.92 | | |
| $G_3$ | 7 | 18.15 | 13.790 | 3.793 | 16.23 | 15.216 | 3.685 |
| | 8 | 11.98 | | | 11.13 | | |
| | 9 | 11.24 | | | 18.29 | | |
| $G_4$ | 10 | 6.13 | 8.623 | 2.541 | 8.22 | 11.216 | 2.660 |
| | 11 | 11.21 | | | 12.13 | | |
| | 12 | 8.53 | | | 13.3 | | |
| $G_5$ | 13 | 6.48 | 5.9433 | 0.755 | 8.08 | 7.893 | 0.497 |
| | 14 | 6.27 | | | 8.27 | | |
| | 15 | 5.08 | | | 7.33 | | |

The results of mean ΔE* range between 5.943 and 14.736 for the groups treated with EBA and 7.893 and 18.77 for the groups treated with CBA. Like in the case of ΔL*, the highest ΔE* was obtained for the group treated with CP 15% and the lowest for the groups treated with HP 35%, using the indicated protocols (regarding the number of applications and the duration of each session). The variation of ΔE* was higher for the groups treated with CBA than for the groups treated with EBA, in each case.

Considering the differences between ΔL* and ΔE* corresponding to the EBA versus CBA, no significantly statistic differences were obtained, for all tested formulas ($G_1$-$G_5$) (p=1, exact significance of Wilcoxon signed ranks test for Group 1, p>0.05, exact significance of Wilcoxon signed ranks for groups 2-5).

When the bleaching effects of the various formulas and treatment protocols were compared using an analysis of variation, an overall significant difference has been observed between the 5 groups of different formulas regarding ΔL* and ΔE* values only for the EBA (p<0.05, ANOVA). For the investigated CBA, no overall significant difference between the 5 groups regarding ΔL* and ΔE* values has been observed using ANOVA (p>0.05). Given the reduced size of the evaluated groups, an exact significance of these differences has also been investigated using a non-parametric Kruskal-Wallis test, which rendered overall significant differences regarding ΔL* and ΔE* values (p<0.05) between the 5 groups for both EBA and CBA (p=0.007 for ΔL* in EBA groups, p=0.018 for ΔL* in CBA groups, p=0.017 for ΔE* in EBA groups, p=0.042 for ΔE* in CBA groups).

In our study, the extracted teeth were first experimental stained using coffee infusion, since it was stated that it is difficult to reveal differences in the effect of various bleaching techniques on nonstained samples [18]. This might explain the high values of ΔL* and ΔE* obtained in all instances, for both EBA and CBA.

Hydrogen peroxide was first used in dentistry in periodontal treatment, since it was demonstrated that it is effective against colonization and replication of anaerobic bacteria [19].

Due to the oxidizing process, highly pigmented carbon-ring compounds of the chromophores attached into the dental structure, are opened and converted into chains which are lighter in color. Existing carbon double-bond compounds are further converted into hydroxilic groups, which are usually colorless [14,20]. However, this reaction is unspecific and the organic and inorganic matrix of the enamel and dentine might be affected by the bleaching agents [15].

When CP gels are used for dental bleaching, upon contact with moisture, CP releases about 33% of it's content as HP [13, 21]. In addition, CP also releases urea, which is decomposed into ammonia and carbon dioxide. It is documented that urea and ammonium ions $(NH_4)$+ interfere

with the organic matrix of the enamel, initiating the degradation of the proteins, which are split into peptides and finally eliminated. By this, the diffusion of the hydrogen peroxide throughout the whole thickness of the enamel is increased [13].

CP [$CO(NH_2)\cdot H_2O_2$] [22] gels contain also stabilizers, catalysts, desensitizing and flavor agents [18]. The base vehicles are mainly represented by anhydrous glycerin, a type of glycol or a dentifrice [21].

Carbopol, a frequently used vehicle, enhances the contact between the CP and the tooth and slows the release of HP [13,15]. However, as an acidic polymer, it is believed to induce further adverse effects in the normal structure of the enamel, like demineralization and a consequent reduction of the enamel microhardness [15,23]; it also exhibits a high calcium binding capacity, that can inhibit hydroxiapatite crystal growth [15,24].

The variations of the lightness ($\Delta L^*$) and color ($\Delta E^*$) obtained in this study as a result of the bleaching process were more important than the results presented by other authors in similar protocols performed on bovine extracted teeth [18,25] or in clinical studies, in vivo [26]. This higher variation might be due to differences in the experimental protocols or to the instrumental methods used for measuring the color parameters.

Some manufacturers claim that an HP bleaching agent is faster in generating the whitening effect, as compared to a CP bleaching agent of similar concentration, since CP has to break down into HP and urea in order to be effective [27]. Our results suggests that the home-bleaching protocol, using lower concentration of peroxide carbamide, during a prolonged period of time is more efficient than the in-office methods, based on more powerful 35% hydoxide peroxide. Other studies reported similar results [18,27] and suggest that a higher concentration of hydrogen peroxide cannot compensate the reduced contact time between the bleaching gel and the tissues [18], mainly in the dentine, where the effectiveness of the procedure is dependent on an increased penetration of the active ions. From a clinical point of view, the “in office’ methods are often followed by several sessions of lower concentration peroxide carbamide-based gels, during ”at home” treatment [28]. Finally, there are studies that indicate no differences between the results obtained when the two methods are used, concluding that “at home” and “in office” methods indicated the same effectiveness [26].

We performed the immersion of the extracted teeth in artificial saliva during the experimental bleaching session, in order to simulate the oral environment; the content in calcium and phosphate might promote a remineralization similar to that produced in human saliva [15].

In order to characterize the dental color, several systems have been used. In this study we used the data derived from CIEL*a*b* system, with three linear coordinates: lightness (L*) green-red chromatic coordinate (a*),

and blue-yellow chromatic coordinate (b*). Two other coordinates are also characteristic for this system- chroma (C*- the strength of color) and hue (h*- the name of the color itself). Color difference, which was calculated in this study reflects the sum of either L*a*b* or L*C*h *color coordinate differences, between the initial situation (before bleaching) and the final one (after bleaching) [29].

Dental color can be assessed either by visual or instrumental methods, which use spectrophotometers, colorimeters or computer analysis of digital images. All these methods have been used in order to assess the effectiveness of bleaching materials, with the usual recording of ΔL* and ΔE*, when the instrumental methods are used; delta shade guide is evaluated in the case of visual recording of the dental color, with a shade guide [30].

In the present study, we used a handheld intraoral spectrophotometer, Vita Easyshade, which is one of the most commonly used instrumental color measuring system in dentistry. These instruments measure one wavelength at a time from the reflectance or transmittance of an object [1,3] and have been used in dentistry not only for clinical reason but also in experimental studies performed on vital or extracted teeth. Though, two major disadvantages influence the predictability of it's results: edge –loss error, generated by an important fraction of the light which enter the tooth to be lost) and the difficulties in reproduction the position on the tooth's surface, since it is a free hand instrument [29] The edge-loss phenomenon and the difficulties in positioning the optical device are increased in the case of an irregular dental surface.

## CONCLUSION

1. As a result of the bleaching treatment, the increase of value (L*) and a tendency of reducing the yellowness (b*) and redness (a*) were recorded using spectrophotometric measurements, for both experimental and commercial bleaching gels.

2.The most important variation was obtained for the formulas based on 15% CP and the lowest for the 35% HP, following the indicated protocols.

3. Overall significant differences regarding ΔL* and ΔE* values ($p<0.05$) between the 5 groups for both EBA and CBA were obtained ($p=0.007$ for ΔL* in EBA groups, $p=0.018$ for ΔL* in CBA groups, $p=0.017$ for ΔE* in EBA groups, $p=0.042$ for ΔE* in CBA groups)

4.Even if the variation of ΔL* and ΔE* was higher for the groups treated with CBA than for the groups treated with EBA, for all groups $G_1$-$G_5$, there was no significantly statistic difference between the variation of these parameters in EBA versus CBA groups. ($p=1$, exact significance of Wilcoxon signed ranks test for Group 1, $p>0.05$, exact significance of Wilcoxon signed ranks for groups 2-5).

## EXPERIMENTAL

**Dental staining** was performed by immersion of the extracted teeth in a coffee infusion for 14 days. The solution (7 grams coffee, 300 ml boiled water) was daily renewed.

### Dental bleaching

The bleaching gels covered the dental surfaces in a uniform layer of 1 mm thickness.

The protocols used are presented in table 5 and the formulas of the EBA, as well as their composition and properties are included in table 6.

Between bleaching intervals, the teeth fragments were maintained in artificial saliva (50mmol/l KCl, 1.5 mmol/l Ca, 0.9mmol/l $PO_4$, 20 mmol/l trihydroxymethyl-aminomethane buffer at pH 7.0) [15, 32]

**Tabel 5.** Clinical protocols indicated for each group of bleaching gels

| Group of teeth | Experimental bleaching agents | Commercial bleaching agents | Experimental protocol |
|---|---|---|---|
| G1<br>Samples corresponding to teeth 1-3 | EBA 1 – CP 10% | CBA 1 – CP 10% | 14 days,<br>8 hours/day |
| G2<br>Samples corresponding to teeth 4-6 | EBA 2 – CP 15% | CBA 2 – CP 15% | 14 days,<br>8 hours/day |
| G3<br>Samples corresponding to teeth 7-9 | EBA 3 – CP 15% | CBA 3 – CP 15% | 14 days,<br>8 hours/day |
| Samples corresponding to teeth 10-12 | EBA 4 – CP 35% | CBA 4 – CP 35% | 7 days,<br>1 hour/day |
| Samples corresponding to teeth 13-15 | EBA 5 – HP 35% | CBA 5 – HP 35% | 2 sessions,<br>10 min each. |

**Table 6.** Composition and properties of EBA, used on the buccal surfaces of the extracted teeth

| EBA | pH [33] | Viscosity [33] | ingredients |
|---|---|---|---|
| EBA 1 | 6.5 | 700 CP | Carbamide peroxide 10%,glycerine, water, polyvinilpirolidone (PVP) |
| EBA 2 | 6 | 700 CP | Carbamide peroxide 15%,PVP, polyetilenglycol 200, $SiO_2$, Water |
| EBA 3 | 6 | 700 CP | Carbamide peroxide 15%, glycerine, water, Ethanol, PVP |
| EBA 4 | 4 | 660 CP | Carbamide peroxide 35%,PVP, polyetilenglycol 200 $SiO_2$ Water |
| EBA 5 | 4 | 660 CP | Hydrogen peroxide 35%, PVP, SiO2 |

### Dental color measurement

Vita Easyshade is a dental spot measurement spectrophotometer, it's handpiece ends in a 5 mm fiber optic tip, containing 19 - 1 mm diameter fiber optic fibers [29,31]. The light source of the instrument is represented by a halogen- stabilized lamp, located in the base unit. This is monitored by several spectrophotometers, which also aim to measure the scattered light at 2 different distances from the tooth surface. These readings are combined in order to produce a „ principal spectrum" for the tooth [29]. The dental spectrophotometer was used in „global mode", which indicate a basic color for the evaluated surface. In this mode, the tip of the instrument was located in the middle of the buccal or lingual surface of each tooth and the CIEL*a*b* color parameters: L*, a*, b*, were recorded.

For each tooth, the dental shade was measured on the buccal and lingual surface, before and after the bleaching treatment. As a result, two pairs of CIEL*a*b* color parameters were evaluated: $L_1^*$, $a_1^*$, $b_1^*$ (before bleaching) and $L_2^*$, $a_2^*$, $b_2^*$. Using these parameters, two other values were calculated: $\Delta L^*$ ($L_2$-$L_1$), and $\Delta E^*$ – using the following formula:

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

$$\Delta L^* = L_2^* - L_1^*,$$

$$\Delta a^* = a_2^* - a_1^*,$$

$$\Delta b^* = b_2^* - b_1^*.$$

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