

# Spectrophotometric analysis of the effectiveness of bleaching agents used for non-vital teeth bleaching

## Análise espectrofotométrica da eficácia dos agentes clareadores utilizados no clareamento de dentes não vitais.

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### Abstract

**Objectives:** This study aimed to compare the efficiency of three different substances used for the bleaching of non-vital teeth. **Methods:** Forty bovine teeth were divided into five groups: three test groups (sodium perborate + 20% hydrogen peroxide – SPG; 37% carbamide peroxide – CPG; 35% hydrogen peroxide – HPG) and two control groups (CG1 and CG2). Teeth of the test groups were stained artificially with blood and bleached using the in-office and walking bleach techniques. The efficiency of the bleaching agents was evaluated objectively by comparing the color variables L\*, a\*, and b\* measured spectrophotometrically according to the CIELab system. The data were analyzed by ANOVA and the Tukey test, adopting a 5% level of significance. **Results:** The results showed a reduction in lightness (L\*) after staining and an increase after the bleaching sessions. The values of a\* and b\* increased after staining and decreased after the application of the bleaching substances. **Conclusions:** All substances used for non-vital tooth bleaching exhibited the same bleaching efficiency. No significant differences in this efficiency were observed between the bleaching techniques at the end of the experiment. However, in-office bleaching provided the same bleaching result as the walking bleach technique within a shorter period. **Clinical significance:** Given the increasing demand for esthetics, the use of different bleaching techniques and the variety of whitening agents, oral health professionals should offer evidence-based treatment, more efficient and in less time.

**Keywords:** Tooth bleaching. Non-vital tooth. Hydrogen peroxide.

### Resumo

**Objetivos:** este estudo teve como objetivo comparar a eficiência de três substâncias diferentes utilizadas no clareamento de dentes não vitais. **Métodos:** quarenta dentes bovinos foram divididos em cinco grupos: três grupos-teste (perborato de sódio + 20% de peróxido de hidrogênio - PS; 37% de peróxido de carbamida - PC; 35% de peróxido de hidrogênio - PH) e dois grupos-controle (CG1 e CG2). Os dentes dos grupos-teste foram pigmentados artificialmente com sangue e clareados usando as técnicas de branqueamento em consultório e walking-bleach. A eficiência dos agentes clareadores foi avaliada objetivamente, comparando-se as variáveis de cor L\*, a\* e b\* medidas espectrofotometricamente de acordo com o sistema CIELab. Os dados foram analisados por ANOVA e teste de Tukey, adotando nível de significância de 5%. **Resultados:** os resultados mostraram uma redução da luminosidade (L\*) após a pigmentação e um aumento após as sessões de clareamento. Os valores de a\* e b\* aumentaram após a pigmentação e diminuíram após a aplicação das substâncias clareadoras. **Conclusões:** todas as substâncias utilizadas no clareamento de dente não-vital apresentaram a mesma eficiência. Não foram observadas diferenças significativas nesta eficiência entre as técnicas de branqueamento no final do experimento. No entanto, o branqueamento em consultório forneceu o mesmo resultado de clareamento que a técnica walking-bleach em um período mais curto. **Significado clínico:** Dada a crescente demanda por estética, o uso de diferentes técnicas de clareamento e a variedade de agentes clareadores, os profissionais de saúde bucal devem oferecer tratamento baseado em evidências, mais eficiente e em menos tempo.

**Palavras-chave:** Clareamento dentário. Dente não vital. Peróxido de hidrogênio

### INTRODUCTION

The number of patients seeking a harmonious smile with white teeth has increased in dentistry over recent years. The growing demand for esthetic dental services is guided by both media influence and the beauty standards imposed by the individual's environment. Within this context, the improvement of techniques and the development of materials that permit the transformation of a disharmonious into a beautiful smile are necessary.

Dental chromatic alterations can be of extrinsic origin when

chromogens are deposited on the external surface of the tooth or can be due to intrinsic factors such as substances deposited on the internal surface or changes in internal tooth structure. The main causes of intrinsic chromatic alterations include abnormal development of the tooth germ, pulp calcification, post-traumatic bleeding, and pulp necrosis, or iatrogenic factors during endodontic treatment. The most common iatrogenic causes are related to the blood that remains after hemorrhage caused by pulpotomy and pulpectomy or residues of drugs and/or sealing materials in the pulp chamber<sup>1</sup>. Recently, tooth

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Received: 219 ago 24; Revised: 2020 Abr 17; 2020 Abr 20; Accepted: 2020 Abr 20

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darkening has been reported as an unfavorable outcome associated with regenerative endodontic procedures<sup>2-5</sup>.

Knowledge of the etiology of color changes is of paramount importance for a good prognosis and success of restorative procedures. A range of commercial bleaching agents is available. The most common substances are carbamide peroxide, hydrogen peroxide that can be used alone or in combination, sodium perborate that can be combined with hydrogen peroxide, or deionized water. The whitening effect of these substances is the result of an oxi-reduction. This chemical process allows the oxygen radicals present in hydrogen peroxide to penetrate the dentinal tubules and interact with the unsaturated double bonds of the chromophores deposited on the enamel and dentin, reducing them to practically colorless low molecular weight substances. The theory is that the bleaching process opens the carbon ring of the pigment molecules and converts them into shorter chains that are lighter in color, with carbon dioxide and water as subproducts<sup>6</sup>.

Two possible bleaching techniques exist for non-vital teeth: in-office bleaching, which is entirely performed in the dental office, and the walking bleach technique. The former consists of the application of the bleaching agent to the external surface of the tooth and the pulp chamber for a period of 30-45 minutes. In the walking bleach technique, the bleaching agent is used as a temporary dressing in the pulp chamber, followed by sealing of the access cavity. The medication is left in place for 3 to 4 days. The two techniques can be combined to achieve the desired effect.

Because of the increasing demand for esthetics, the use of different bleaching techniques and the variety of whitening agents, the objective of this study was to evaluate the efficiency of different substances and techniques for non-vital tooth bleaching by spectrophotometric analysis of artificially stained bovine teeth.

## MATERIALS AND METHODS

Forty extracted permanent bovine central incisors obtained from young animals of the same age range were selected. After cleaning and disinfection by immersion in 1.0% sodium hypochlorite (Asfer, São Caetano do Sul, SP, Brazil) for 3 minutes, the specimens were stored in distilled water at room temperature until use.

All specimens had their roots sectioned 3 mm below the cemento-enamel junction using a carborundum disc (American Burrs, Palhoça, SC, Brazil) and micromotor (Beltec Mini, Araraquara, SP, Brazil). A squared area (7 x 7 mm) was delimited on the buccal side of each tooth with adhesive tape (silver tape, Scotch 3M, São Paulo, SP, Brazil). The first color recording of the specimens was then obtained with an Easy-Shade spectrophotometer (VITA, Petrópolis, RJ, Brazil), called Reading 1 (R1). The probe of the spectrophotometer (6 mm in diameter) was placed perpendicular to the middle third of the buccal surface within the previously delimited area. This point was

measured three times and the arithmetic mean of the values found was calculated.

After R1, the specimens were divided into five groups as shown in Table 1. The teeth of the experimental (SPG, HPG e CPG) and control (GC2) groups were artificially stained with blood. Fresh blood (0.1 ml per tooth) was introduced into the pulp chamber through the radicular opening with disposable syringes and needles (30G). The radicular opening was then sealed with temporary cement (Coltosol, Vigodent, Rio de Janeiro, RJ, Brazil). For staining, the specimens were stored in a horizontal position for 15 days in an oven at 37°C and 100% humidity. After this period, the temporary cement of the opening was removed and a 2-mm cervical sealing was fabricated with Natural Look composite resin (Nova DFL, Rio de Janeiro, RJ, Brazil).

**Table 1.** Bleaching technique and agent used in the different groups.

Group	Substances used	Commercial brand
Control group 1 (CG1) (n=5)	No staining and no bleaching	
Control group 2 (CG2) (n=5)	Staining and no bleaching	
SP group (SPG) (n=10)	Stained and bleached with sodium perborate + 20% hydrogen peroxide	Whiteness Perborato, FGM, Joinville – SC, Brazil
HP group (HPG) (n=10)	Stained and bleached with 35% hydrogen peroxide	Whitegold, Dentsply Sirona, Petrópolis – RJ, Brazil
CP group (CPG) (n=10)	Stained and bleached with 37% carbamide peroxide	Whiteness Super-endo, FGM, Joinville – SC, Brazil

The pulp chamber was accessed with No. 8 and 702 carbide burs (Angelus Prima Dental, Londrina, PR, Brazil) coupled to a micromotor (Beltec Mini, Beltec, Araraquara, SP, Brazil). Reading 2 (R2) was obtained after endodontic access.

The walking bleach technique was used in groups SPG and CPG for internal bleaching. For this purpose, the pulp chamber was etched with 37% phosphoric acid (Alpha Etch Gel, Nova DFL, Rio de Janeiro, RJ, Brazil) for 15 seconds, followed immediately by washing with distilled water and drying with a cotton pellet. The pulp chamber was then filled with the bleaching agent. Sodium perborate was manipulated as recommended by the manufacturer (2 tablespoons of powder for 1 drop of peroxide solution). In SPG and CPG, the bleaching agent was protected with filter paper and the crown opening was sealed with composite resin (Natural Shade, Nova DFL, Rio de Janeiro, RJ, Brazil). The bleaching agent was changed every 4 days over a period of 3 weeks when readings 3, 4 and 5 (R3, R4, and R5) were obtained.

In HPG, bleaching consisted of etching the pulp chamber with

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37% phosphoric acid (Alpha Etch Gel, Nova DFL, Rio de Janeiro, RJ, Brazil) for 15 seconds, washing with distilled water, and drying with a cotton pellet. Next, the bleaching agent was applied to the pulp chamber and the buccal side of each tooth for 45 minutes. The bleaching agent was then removed by washing with distilled water and the specimens were dried with cotton pellets. Reading 3 (R3) was obtained from this group.

After the bleaching procedure, all specimens of the experimental groups were submitted to the washing of the pulp chamber with distilled water and sealing of endodontic access with cotton and temporary cement (Coltosol, Vigodent, Rio de Janeiro, RJ, Brazil). The specimens were stored in an oven at 37°C and 100% humidity throughout the experiment. The final reading (R6 for SPG and CPG and R4 for HPG) was obtained one week after the last bleaching session.

The following equation was used to evaluate the color difference ( $\Delta E$ ):  $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$ , where  $\Delta L$  ( $L_{final} - L_{initial}$ ),

$\Delta a$  ( $a_{final} - a_{initial}$ ) and  $\Delta b$  ( $b_{final} - b_{initial}$ ) for lightness ( $L^*$ ) and shades ( $a^*$  and  $b^*$ ). The values inserted in this equation were obtained from the baseline reading (no staining), reading after staining, and reading after each bleaching session. The spectrophotometrically measured values of  $L^*$ ,  $a^*$  and  $b^*$  were submitted to analysis of variance (ANOVA) and the Tukey test using the Minitab 18 package (Minitab, Inc., State College, PA, USA). A level of significance of 5% was adopted.

### RESULTS

Table 2 shows the mean and standard deviation of the  $L^*$ ,  $a^*$ , and  $b^*$  parameters at baseline (R1) and after staining (R2) in CG2, SPG, HPG, and CPG; after the first bleaching session (R3) in SPG and CPG and after the single bleaching session (R3) in HPG; after the second bleaching session (R4) in SPG and CPG and after 1 week of storage without the bleaching agent (R4) in HPG; after the third bleaching session (R5) in SPG and CPG; after 1 week of storage without the bleaching agent (R6) in SPG and CPG.

**Table 2.** Mean and standard deviation of the  $L^*$ ,  $a^*$  and  $b^*$  parameters in the groups studied.

Reading/Variable		Group							
		CG2		SPG		HPG		CPG	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
R1	$L^*$	81.7	6.08	77.66	6.04	76.70	7.33	79,96	7,94
	$a^*$	1.15	2.095	1.120	1.519	2.670	2.915	1,540	2,210
	$b^*$	26.28	4.84	27.34	4.16	30.89	2.973	29,79	4,71
R2	$L^*$	83.45	5.58	75.74	7.09	68.14	9.19	74,39	6,81
	$a^*$	0.680	2.219	2.53	3.45	4.580	2.726	2,740	2,211
	$b^*$	23.56	6.74	22.75	4.47	24.55	4.78	25,76	5,86
R3	$L^*$			89.03	6.35	72.09	7.87	82.33	7,38
	$a^*$			-2.23	1.711	3.760	2.359	-0.05	1,976
	$b^*$			22.79	5.70	23.56	3.92	24.78	5,35
R4	$L^*$			90.20	5.80	80.97	7.89	84.66	7,39
	$a^*$			-2.50	1.897	0.523	2.224	-0.61	1,842
	$b^*$			20.37	5.97	22.63	4.79	24.86	5,71
R5	$L^*$			90.54	5.87			86.46	6,85
	$a^*$			-2.57	1.717			-1.18	1,592
	$b^*$			18.55	4.96			23.99	5,11
R6	$L^*$			89.04	5.96			87.94	5,75
	$a^*$			-1.78	1.754			-1.03	1,588
	$b^*$			20.03	4.91			23.86	5,61

SD: standard deviation.

#### Variable $L^*$

No statistically significant difference was observed between the experimental groups at R1 ( $p > 0.05$ ). The mean  $L^*$  values were reduced at R2 ( $p < 0.05$ ) in SPG, HPG and CPG, demonstrating the

efficiency of the staining process.

The mean  $L^*$  values were significantly higher ( $p < 0.05$ ) at R3 in SPG and CPG after the first bleaching session and in HPG after the single bleaching session when compared with R1.

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Considering that values close to 100 indicate a white color, the results showed whitening in all test groups, with the highest value in SPG.

An increase in mean L\* values was observed at R4. The comparison showed a significant difference ( $p < 0.05$ ) between groups, with the highest value in SPG. However, when R3 and R4 were compared, greater variation in the L\* parameter was observed in HPG, although the specimens were without the bleaching agent for one week. No significant difference between SPG and CPG was found at R5 ( $p > 0.05$ ). At R6, the mean L\* value had decreased in SPG and increased in CPG, but the difference was not statistically significant ( $p > 0.05$ ).

##### Variable a\*

No significant differences in the mean a\* values were observed between groups at R1 ( $p > 0.05$ ). At R2, the mean a\* values were higher, indicating greater redness, but there was no difference between groups ( $p > 0.05$ ). A reduction in a\* values was found at R3, demonstrating a trend towards greenness. A comparison of the groups showed a lower value in SPG compared to HPG and CPG ( $p < 0.05$ ).

The mean a\* values were lower at R4 and this reduction was greater in HPG than in SPG and CPG ( $p < 0.05$ ). No statistically significant differences were found between SPG and CPG at R5 and R6 ( $p > 0.05$ ).

##### Variable b\*

There were no significant differences at R1, R2, R3, R4 or R6. The mean b\* values were lower at R5, indicating a less yellow color of the specimens evaluated.

##### ΔE values

The L\*, a\* and b\* values obtained by spectrophotometric analysis permitted to measure the color difference of the specimens using ΔE. The values shown in Table 3 were obtained as the difference between the baseline reading and the readings obtained after artificial staining, after the bleaching sessions, and after 1 week without the bleaching agent.

From the L\*, a\* and b\* values found after artificial staining (R2) and at baseline in all teeth (R1), it was possible to measure the color difference (ΔEa), which showed that the teeth were indeed stained ( $p < 0.05$ ). The ΔEb values found showed a significant color difference ( $p < 0.05$ ) between groups. The highest degree of whitening was obtained for the group treated with sodium perborate combined with 20% hydrogen peroxide (SPG). Analysis of the ΔEc values showed a difference between SPG, HPG and CPG ( $p < 0.05$ ). The highest degree of whitening was observed in the group treated with 35% hydrogen peroxide (HPG). The values of ΔEd, ΔEe, ΔEf or ΔEg did not differ significantly between groups ( $p > 0.05$ ).

**Table 3.** Mean and standard deviation of ΔE values in the in the groups studied.

Group	Delta values (ΔE)**	Mean	Standard deviation
CG2	ΔE a	7.97	7.13
	ΔE a	10.219	3.080
	ΔE b	14.42	5.88
	ΔE c	6	3.99
SPG	ΔE d	3.427	1.891
	ΔE e	2.656	1.355
	ΔE f	17.15	7.76
	ΔE g	15.16	8.14
HPG	ΔE a	11.71	4.83
	ΔE b	5.47	2.283
	ΔE c	9.88	4.77
	ΔE h	13.96	5.70
CPG	ΔE a	8.63	3.37
	ΔE b	9.81	5.35
	ΔE c	4.47	3.38
	ΔE d	2.960	1.611
	ΔE e	5.32	5.99
	ΔE f	13.15	5.71
	ΔE g	14.61	9.22

\*\*ΔEa: color change between R2 and R1; ΔEb: color change between R3 and R2; ΔEc: color change between R4 and R3; ΔEd: color change between R5 and R4; ΔEe: color change between R6 and R5; ΔEf: color change between R5 and R2; ΔEg: color change between R6 and R2 and between R4 and R2.

## DISCUSSION

The main causes of intrinsic dental chromatic alterations related to endodontic treatment are the decomposition of necrotic tissue, pulp bleeding in the chamber, drugs and filling materials left in the pulp chamber, and, recently, regenerative endodontic procedures of immature permanent teeth. Regardless of the etiological factor, the discoloration of non-vital teeth can eventually have esthetic and psychological implications for the patients. Studies investigating bleaching agents and techniques are therefore important.

Pulp bleeding is one of the leading causes of internal tooth discoloration. Once blood vessels rupture, blood accumulates in the pulp chamber and red blood cells penetrate the dentinal tubules and undergo hemolysis, releasing hemoglobin. The degradation of hemoglobin releases iron which, in turn, reacts with hydrogen sulfide to form iron sulfide (Fe<sub>2</sub>S<sub>3</sub>). The latter is responsible for the staining of teeth<sup>7</sup>.

The results showed a reduction in lightness (L\*) after staining of the teeth with blood. The closer the value to 0, the darker the specimen. Analysis of the  $\Delta E_a$  values showed a color difference between specimens before and after artificial staining, demonstrating the efficiency of the staining technique used. For this study, the teeth were stained artificially by injecting blood into the pulp chamber, similar to the study of Marin et al<sup>8</sup>. Freccia and Peters<sup>9</sup> used immersion of the tooth in blood as an artificial staining technique, which permits staining of the external part of the tooth. However, the method used by these authors does not reflect the clinical reality of non-vital darkened teeth.

The  $\Delta E_b$  values demonstrated a statistically significant and visually noticeable color change ( $p < 0.05$ ). After the first bleaching session (SPG and CPG) and after the single bleaching session (HPG), the group in which sodium perborate was combined with hydrogen peroxide obtained a better bleaching result than the groups in which carbamide peroxide and hydrogen peroxide were used alone. Specimens of SPG had a lighter color. The lower lightness value obtained with carbamide peroxide does not indicate lower bleaching efficiency since the values found exceeded the measures of the original color of the teeth. Carbamide peroxide must remain in contact with the dental tissue for a longer period to release the same amount of hydrogen peroxide as sodium perborate combined with any vehicle<sup>10</sup>, which could be observed in the present study.

A difference between groups was observed when the  $\Delta E_c$  values were compared ( $p < 0.05$ ). A higher degree of whitening was found in HPG one week after bleaching and in the absence of the bleaching agent compared to the second bleaching session in SPG and CPG, which did not differ from one another. The greater bleaching effect achieved with 35% hydrogen peroxide during this period might be associated with the amount of oxygen free radicals that remained in the dentinal tubules even after irrigation of the specimens without losing their

effectiveness.

An increase in the L\* values was observed after the single bleaching session in HPG (R3), but this increase was not sufficient to reestablish the initial color of the specimens. However, the reading obtained one week after bleaching (R4) showed an increase in L\* values that exceeded the baseline readings. This finding might be associated with the residual effect of the substance used. The higher the concentration of hydrogen peroxide in the bleaching substances, the larger the amount of oxygen free radicals to be released<sup>11</sup>. Even after washing the teeth, the residual oxygen that remains in the dentinal tubules continues to act, losing its effectiveness within 7 to 14 days<sup>12, 13</sup>.

Analysis of  $\Delta E_d$ ,  $\Delta E_e$  and  $\Delta E_f$  showed no significant difference between SPG and CPG. Both sodium perborate and carbamide peroxide had the same bleaching efficiency after the second and third sessions and one week after bleaching. Similar results have been reported by Pinto de Oliveira et al<sup>14</sup> who found no differences between these substances when the bleaching agents were replaced every 7 days over a period of 3 weeks. In a comparative study of sodium perborate combined with carbamide peroxide and sodium perborate mixed with distilled water, Souza-Zaroni et al<sup>15</sup> obtained similar bleaching results, suggesting that the combination of two bleaching agents can cause excess active ingredients that diffuse through the dentinal tubules without reacting. However, a better bleaching performance was observed in the present study for the combination of sodium perborate and hydrogen peroxide.

Carbamide peroxide (CPG) exerted a gradual bleaching effect over the period studied. This result may be related to the presence of Carbopol in the composition of carbamide peroxide, which delays the release of hydrogen peroxide, prolonging the action of this substance<sup>16</sup>.

After the three bleaching sessions in SPG and CPG, a gradual decrease in a\* values was identified, which tended towards negative values (green color). The shift of tooth color to a greenish tone indicates the whitening of the teeth as they tend to have more red in their composition after artificial staining. In HPG, the lowest a\* value was detected in the reading obtained one week after bleaching.

Concerning the b\* parameter, a significant difference was observed between SPG and CPG after the third bleaching session. Although this parameter decreased in both groups, sodium perborate combined with 20% hydrogen peroxide provided a better result.

A comparison of  $\Delta E_g$  showed no significant difference between the experimental groups. The  $\Delta E$  values found reveal that all bleaching agents had the same efficiency after staining and after the bleaching sessions, but in-office bleaching with 35%

hydrogen peroxide exhibited this performance within one week after the single bleaching session. The use of sodium perborate or carbamide peroxide resulted in the same bleaching efficiency as 35% hydrogen peroxide, but three sessions were necessary to achieve the same degree of whitening, in agreement with the studies of Pinto de Oliveira et al<sup>14</sup> and Monteiro et al<sup>17</sup>.

Taken together, the present results suggest that a single session using the in-office bleaching technique is sufficient to bleach non-vital teeth. Clinically, this result is important as a larger number of sessions increase the costs of treatment.

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## CONCLUSIONS

Spectrophotometric analysis of artificially stained bovine teeth showed that all substances used for non-vital tooth bleaching exhibited the same efficacy. No significant differences in bleaching efficiency were observed between the bleaching techniques at the end of the experiment. However, in-office bleaching provided the same bleaching result as the walking bleach technique within a shorter period of time.

### How to cite this article/Como citar este artigo:

Santos MR, Carvalho FB, Cangussu MCT, Barros LR, Trindade RF. Spectrophotometric analysis of the effectiveness of bleaching agents used for non-vital teeth bleaching. *J Health Biol Sci.* 2020 J; 8(1):1-6.