ORIGINAL ARTICLE

Effect of hydrogen peroxide in different concentrations on the degree of bleaching and susceptibility to staining

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Abstract

Objective: The aim of this study was to evaluate the efficacy of a bleaching material (hydrogen peroxide [HP]) in different concentrations (20% and 35%) as well as the susceptibility to staining after the bleaching procedure.

Materials and Methods: The root portions of 20 healthy bovine incisors were removed, and the crowns were sectioned, obtaining mesial and distal halves, totaling 40 specimens. The specimens were identified as 1-20 (G1) for the mesial half and 21-40 (G2) for the distal half. The specimens of G1 were subjected to staining with coffee and subsequently subjected to bleaching with HP 35% (specimens 1-10) and HP 20% (specimens 11-20). The specimens of G2 were stained with acai berry and later bleached with HP 35% (specimens 21-30) and HP 20% (specimens 31-40). After bleaching, the specimens were again subjected to staining with the same substances. The colorimetry system (CIELab) was carried out with a spectrophotometer at five different moments: Before the first staining, after the first staining and previously to the first session of bleaching, previously to the second bleaching session, previous to the second staining, and finally after the second staining.

Results: No difference was found between HP 35% and 20%, and both materials significantly promoted bleaching.

Conclusions: The acai solution resulted in more staining compared to the coffee solution, before and after bleaching. No increase was observed in susceptibility to staining after bleaching of the bovine teeth.

Introduction

Currently, the esthetics of the smile is very important for patients, and the color of the teeth is one of the factors of greatest dissatisfaction. This quest for whiter smiles has made the dental bleaching one of the most popular treatments sought at the dental clinics, leading to increase in the number of products and techniques as well as the researches in this field.[1-5]

The color changes can be classified into two groups: Intrinsic and extrinsic. Extrinsic stains are usually acquired after tooth eruption and are related to food and products with potential dye, such as coffee, tea, tobacco, red wine, acai berry, associated with the accumulation of plaque, the surface roughness of restorations and presence of cracks and crevices.[6-11] On the other hand, intrinsic stains can be congenital, related to tooth formation or acquired, pre- or post-eruptive.

According to Heymann,[12] there are different bleaching techniques including the home bleaching technique supervised by the dental surgeon, in-office technique, and technique combining home and in-office techniques. The homemade technique employs gels of carbamide peroxide (6-25%) or hydrogen peroxide (HP) (3-7.5%) which should be kept in contact with the teeth by means of a customized mouthpiece. The in-office techniques usually use HP-based gels at higher concentrations (15-38%) with or without light activation.[13-19]

Several studies have described the possibility of alterations in the enamel surface as well as staining by substances and

food dyes such as tea, coffee, cola, and red wine.[6,9,10,13,20] The color changes that occur in the enamel surface after bleaching have been described and studied,[8,21] and it has been assumed that the susceptibility of enamel to staining should not be attributed solely to surface roughness, but also the composition of the enamel, the rate of absorption of water due to changes in permeability and surface irregularities left on bleached that can facilitate the accumulation of pigments and dyes.[22]

The aim of this study was to evaluate the degree of bleaching obtained with HP at different concentrations (20% and 35%) as well as the susceptibility to staining of bleached teeth using coffee and acai berry as coloring agents.

Materials and Methods

20 healthy incisors bovine teeth were used in this experiment. After cleaning with a periodontal curette, all the teeth were fixed with impression sticks on a wooden plate and the root portion removed by cross-section perpendicular to the axis of the tooth using a carborundum disk. The remaining crowns were sectioned, separating the mesial and distal halves, under intense cooling using a cutting machine Isomet® 1000 (Buehler Ltd. Lake Bluff, Illinois, USA), totaling 40 specimens.

The teeth received prophylaxis with Robinson brush, pumice stone, and water at the low rotation, and they were stored in black bottles with distilled water at 4°C. The bottles were numbered from 1 to 20 (G1) for the mesial halves and 21-40 (G2) for the distal halves, and a staining solution was established for each group: G1 - Coffee (Café Brasileiro® Super Forte, São Paulo, Brazil) prepared according to the manufacturer's instructions and G2 - Açaí Sport® (DeMarchi, Jundiaí, São Paulo, Brazil) ready for consumption, and subdivided randomly according to the whitening gel: G1a - specimens 1-10 stained with coffee and bleached with HP 35% (Whiteness HP Blue, FGM Dental Products, Joinville, Brazil), G1b - Specimens 11-20 stained with coffee and bleached with HP 20% (Whiteness HP Blue, FGM Dental Products, Joinville, Brazil), G2a - Specimens 21-30 stained with acai berry and bleached with HP 35%, and G2b - 31-40 stained with acai berry and bleached with HP 20%.

For the evaluation and registration of color, the spectrophotometer VITA Easyshade (Vivadent, Brea, CA, USA) was used, which provided the coordinate values a*, b*, and L*. The L* axis describes the value of variation from white to black (0-100), the a* axis measures the chroma hue toward the red-green region (from −120 to +120) axis, and b* represents the chroma hue toward the blue-yellow region (from −120 to +120). The ΔE (color difference) is found from the equation: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)\ 1/2$, where: $\Delta L = L_{final} - L_{initial}$, $\Delta a = a_{final} - a_{inicial}$ and $\Delta b = b_{final} - b_{inicial}$. The reading was carried out in a pre-determined area, the flatter surface of the specimens. After the initial registration of color (L0), the teeth were randomly divided and immersed in staining solutions. The solutions were changed daily and kept at 37°C. The teeth were then removed from the solution after 1 week, washed in water for 5 s, and then dried with paper towels before the second color evaluation with the spectrophotometer (L1). Afterward, the specimens were submitted to the first session using HP 20% and 35%, according to the group and following the manufacturer's directions.

The specimens were fixed on a utility wax plate to facilitate handling and application of the whitening gel which comes in two phases: A thickener and HP, each material placed in a syringe. The phases were then mixed, connecting the syringe, pushing the piston alternately for 8 times, and then all the mixed content was pushed to one of the syringes for use. The gel remained on the tooth surface for 45 min and was removed with water. The teeth were stored again in dark bottles with distilled water at 37°C for 7 days. The third color reading was performed (L2) at the end of this period, and the new whitening session was carried out following the steps described above. A week after the second session of bleaching, the fourth color reading (L3) was performed, and the specimens were again subjected to the means of immersion for 7 days, and the final reading (L4) was done after this period.

Statistical analyzes

Analyzes of variance of repeated measurements over time were employed in the assessment of color changes defined by ΔE, ΔL, Δa, and Δb. These analyzes were complemented by multiple comparisons of means using the Tukey test, all at the significance level of 5%.

Results

No evidence of a significant difference was found in all the analyzes of variance between the two bleaching agents in terms of color variation. However, significant differences were found between the mean values of color variation related to the treatments as well as between the mean values of the interaction of these variations with the type of staining solution ($P < 0.001$). The Tukey's test at a significance level of 5% was applied to study these interactions. The results are summarized in Table 1.

Table 2 shows mean values and standard deviations of color variation according to staining solution. Graph 1 shows variation values of ΔE, as well as confidence intervals of 95% for the mean population. These intervals indicate the precision of the mean values of ΔE.

The analyzes of variance did not indicate significant differences in relation to the mean values of the color components and among the three experimental groups ($P > 0.05$). These mean values provide an "initial color" which is representative of the samples to be subjected to staining and whitening in a row.

The a component presented a small variation for coffee, less than the previous one, toward the green, while the variation for the acai berry was equivalent to the previous one, toward the red, thus intensifying the color. The b component suffered the highest mean variation, but in opposite direction. With coffee, the change was toward the

Table 1: Distribution of groups according to the procedure undertaken

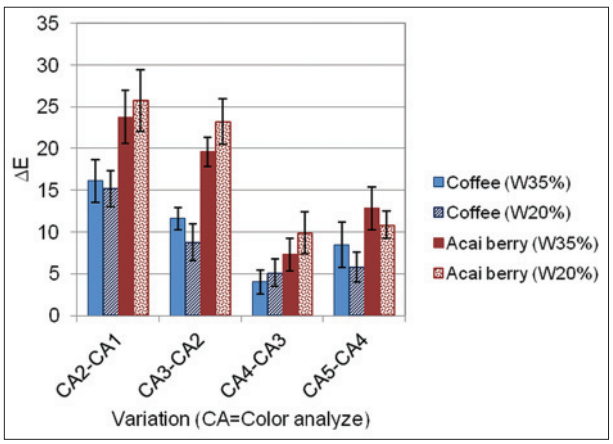
Component	Variables	Coffee (%)		Açaí (%)	
		HP 35	HP 20	HP 35	HP 20
ΔE	L2-L1	16.1 (3.5)	15.2 (2.9)^{d}	23.8 (4.4)	25.8 (5.2)^{e}
	L3-L2	11.6 (1.9)	8.8 (3.1)^{bc}	19.6 (2.5)	23.2 (3.8)^{e}
	L4-L3	4.0 (2.0)	5.1 (2.3)^{a}	7.3 (2.7)	9.9 (3.5)^{bc}
	L5-L4	8.4 (3.8)	5.8 (2.5)^{ab}	12.8 (3.5)	10.8 (2.3)^{c}
ΔL	L2-L1	−12.7 (5.1)	−13.2 (2.6)^{c}	−21.7 (3.7)	−23.7 (4.9)^{d}
	L3-L2	−2.2 (6.0)	−1.3 (3.5)^{a}	−2.7 (6.6)	−3.2 (5.9)^{a}
	L4-L3	0.1 (1.8)	−0.7 (3.0)^{a}	3.7 (3.9)	6.2 (2.7)^{b}
	L5-L4	−2.4 (3.9)	−0.8 (1.7)^{a}	−3.0 (6.5)	−3.4 (3.1)^{a}

Mean variation accompanied by the same letter are not significantly different (Tukey's test: $P<0.05$), HP: Hydrogen peroxide

Table 2: Mean values and SD of color variation according to staining solution

Component	Variables	Coffee (%)		Açaí %	
		HP 35	HP 20	HP 35	HP 20
Δa	L2-L1	5.7 (2.3)	4.0 (1.3)^{c}	7.8 (2.4)	5.8 (2.4)^{c}
	L3-L2	−1.2 (1.3)	−0.9 (1.0)^{a}	5.6 (1.4)	7.4 (2.4)^{c}
	L4-L3	−0.5 (1.4)	−0.6 (1.7)^{a}	−3.9 (2.1)	−4.8 (2.4)^{b}
	L5-L4	0.4 (1.7)	−0.3 (0.8)^{a}	0.2 (2.5)	−0.5 (2.2)^{a}
Δb	L2-L1	6.6 (2.7)	5.2 (4.1)^{b}	−5.2 (3.1)	−7.2 (4.2)^{ac}
	L3-L2	−9.8 (1.0)	−8.0 (2.9)^{cd}	17.4 (3.0)	20.9 (3.7)^{e}
	L4-L3	−3.1 (2.5)	−3.1 (3.3)^{a}	−1.7 (3.6)	−3.7 (4.9)^{a}
	L5-L4	7.3 (3.2)	5.3 (2.9)^{b}	−10.7 (3.2)	−9.7 (2.0)^{d}

Mean variation accompanied by the same letter are not significantly different (Tukey's test: $P<0.05$). SD: Standard deviations, HP: Hydrogen peroxide



Graph 1: The sample means of color variation ΔE, as well as confidence intervals of 95% for the mean population. These intervals indicate the precision of the mean values of ΔE

blue, but with acai berry there was a greater change toward the red.

After the second staining, the mean changes ΔE were found to be greater for the acai berry as compared to those for the coffee, but both were smaller than the changes observed after the first staining. The fact that the whitening procedure did not produce extraordinary effects should be taken into consideration, and besides, the samples did not return to their initial color conditions. At this point, the mean values of both ΔL and Δa were found to be among the lowest, thus suggesting a little variation. The variation of b was the most important and was greater for the acai berry. However, while the variation occurred toward the blue for the acai berry, the variation observed for the coffee occurred in the opposite direction specifically toward the yellow.

Discussion

The theory of the process of color change is based on the permeability of dental tissues. The dental discolorations, as well as structural changes, are a challenge for the dentist. The diagnosis of the staining is very important to solve an esthetic problem since the successful indication of a product depends on the correct diagnosis of the discolorations.[11,23] Several clinical methods are used to measure the color of the teeth. One of these methods involves the use of a spectrophotometer,[7-13] which was used in this study, and allows us to evaluate the effectiveness of bleaching treatment or the degree of staining of the teeth after the immersion period in the dye solution.

There is a huge consumption of cola, coffee, wine, tea, and more recently acai berry in Brazil.[7,13] Acai berry is a purple drink obtained from the fruit of the acai plant, and it is an important source of lipids, proteins, fibers, and mineral elements, Vitamins B1 and E and anthocyanins, which are the natural dyes. Acai berry was chosen for the study because it is very consumed in some regions of Brazil, and currently it is one of the products with huge demand on the Brazilian market. Coffee is also widely consumed, and it was used as a standard for comparison since many studies have shown its capability of staining teeth and resin.[6,8-10]

The results of the present study showed that acai berry caused a greater change in the enamel surface color; both at the first staining and after the teeth were subjected to bleaching. The difference between staining solutions was statistically significant. The first staining, taking the variation ΔE as the basis, had a decisive role over the color of the samples. This variation is explained by the variations in the color components. Accentuated decreases of L occurred in terms of darkness, being more intense for acai berry compared to coffee. There was an increase in the component "a" toward the red for the two coloring agents. The component "b" experienced an increase toward the yellow for coffee and in the opposite direction (blue) for acai berry.

The low pH values such as that of coffee, tea, and red wine were found to increase dental staining. This was found to occur because the teeth immersion in solutions with low pH may have led to a demineralization of the enamel surface, causing irregularities and dyes retention, and reinforcing the process

of staining.[13] Nevertheless, other aspects that may favor the staining should be taken into account, including surface roughness, porosity, presence of cracks, grooves and depressions, and also the composition of the enamel.[13,15,21,23]

Two different concentrations of HP from the same manufacturer (FGM) were selected for this study. Whiteness HP Blue is an HP-based bleaching material at 20% and 35% of concentration, with calcium in it composition in an attempt to minimize the reduction in the enamel microhardness, according to the manufacturer's information. In this study, no significant difference was found between the two bleaching materials. Both concentrations promoted the teeth whitening in a similar way.

One of the aims of this study was to evaluate the susceptibility of bovine teeth to staining after being subjected to bleaching. Our results showed low final mean values of ΔE ([Table 1] - L4) for all the specimens. This may have occurred because reading was carried out 1 week after the completion of the second session of bleaching. It was only after this period that the specimens were immersed into the dye solutions. Furthermore, the presence of calcium in its composition intended to contribute toward the maintenance of the integrity of the dental enamel, and it may have contributed to the remineralization of the teeth, thereby minimizing the potential for staining.

Most of the *in vitro* studies use human teeth as substrates. The teeth that are most commonly used are the third molars or premolars. Currently, there has been an increased difficulty in obtaining human teeth both for the improvement of the oral health of the population and the development of techniques and dental materials. The growing role of the ethical research committees has also become a trend that should not be underestimated. For these reasons, alternative substrates have been evaluated, including the use of bovine teeth.[20,24-30]

The limitations of *in vitro* studies should be considered to extrapolate the results in clinical experience. Further *in vitro* and *in vivo* studies should be performed, taking into account other factors of the oral environment such as the action of salivary proteins, intermittent exposure to dyes, effect of regular brushing as well as temperature variation.

Conclusion

Under the tested conditions, the following conclusions can be drawn:

1. Acai berry solution resulted in greater staining of bovine teeth compared to the coffee solution, prior to and after bleaching
2. The bleaching materials led to the whitening of the surface of the bovine teeth, significantly reducing its color. Even after the teeth have been whitened, no greater susceptibility to staining was observed for the bovine teeth
3. No significant difference was observed between the different concentrations of HP regardless of the type of staining agent.

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