

Journal of Biomedical Optics

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Abstract. Pigments of food and beverages could affect dental bleaching efficacy. The aim of this investigation was to evaluate color change and mineral loss of tooth enamel as well as the influence of staining solutions normally used by adolescent patients undergoing home bleaching. Initial hardness and baseline color were measured on enamel blocks. Specimens were divided into five groups ($n = 5$): G1 (control) specimens were kept in artificial saliva throughout the experiment (3 weeks); G2 enamel was exposed to 10% carbamide peroxide for 6 h daily, and after this period, the teeth were cleaned and stored in artificial saliva until the next bleaching session; and G3, G4, and G5 received the same treatments as G2, but after bleaching, they were stored for 1 h in cola soft drink, melted chocolate, or red wine, respectively. Mineral loss was obtained by the percentage of hardness reduction, and color change was determined by the difference between the data obtained before and after treatments. Data were subjected to analysis of variance and Fisher's test ($\alpha = 0.05$). G3 and G5 showed higher mineral loss (92.96 ± 5.50 and 94.46 ± 1.00 , respectively) compared to the other groups ($p \leq 0.05$). G5 showed high-color change (9.34 ± 2.90), whereas G1 presented lower color change (2.22 ± 0.44) ($p \leq 0.05$). Acidic drinks cause mineral loss of the enamel, which could modify the surface and reduce staining resistance after bleaching. © 2013 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.18.10.108004]

Keywords: tooth bleaching; enamel; color; spectrophotometry; hardness; carbamide peroxide.

Paper 130364R received Jun. 4, 2013; revised manuscript received Sep. 19, 2013; accepted for publication Oct. 2, 2013; published online Oct. 28, 2013.

1 Introduction

Aesthetics have propitiated the development and improvement of techniques that satisfy beyond the cosmetic function of tooth appearance. Tooth bleaching has become an increasingly popular procedure; it is minimally invasive and highly effective, if properly used by the patients and supervised by a professional.¹ Bleaching agents are unstable, releasing oxygen free radicals when in contact with dental tissues and oral moisture, therefore promoting oxidation of pigments present in the tooth while making it clearer.²

However, even when obtaining satisfactory clinical results,^{3,4} some clinicians have still expressed concerns about the effects of carbamide peroxide bleaching on the oral tissues, which have motivated studies to evaluate the possible adverse effects produced during and after the whitening procedures.⁵⁻⁷ Scientific evidence has shown that enamel can show changes in its structure when exposed to carbamide peroxide affecting its composition and its morphology⁸⁻¹⁰ and causing surface changes while decreasing the tooth's hardness.^{1,11} Greater changes on the enamel surface are observed with the use of acid bleaching products.⁵

Tooth whitening is an important parameter for dental aesthetics and bleaching clinical success.¹² Thus, it has been reported that the effects of cumulative exposure to food, dyes, and alcohol can result in damage to the tooth's surface,^{13,14} which raises concerns regarding the effect that these habits could generate on the enamel surface when combined with the bleaching agents.

Several studies of the color change after bleaching have used bovine teeth to prepare the specimens.^{7,12,15,16} Regarding the environmental storage, most studies have used artificial saliva to store the specimens between one treatment and another, aiming to simulate clinical situations.¹⁵⁻¹⁷ To eliminate the subjective interpretation of the visual color comparison, spectrophotometers and colorimeters are used to measure color change in the tooth.¹⁸

Therefore, the assessment of mineral and color changes of the bleached teeth submitted to the action of beverages with potential dyes during the home bleaching treatments becomes essential to enable the maintenance of aesthetics that is desired by patients and dentists alike. Thus, the objectives of this *in vitro* study were to evaluate the influence of the home bleaching treatments combined with staining/acid solutions on a tooth's mineral loss and color change. The null hypotheses tested were: (1) that the combination of staining/acid solutions to tooth

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whitening would not influence the mineral content of the tooth enamel and (2) that these solutions tested would not affect the bleaching effectiveness on the color.

2 Materials and Methods

Twenty-five extracted bovine teeth were cleaned with periodontal cures (Duflex SS White, Rio de Janeiro, Brazil) and polished with pumice and water along with a Robinson brush (KG Sorensen Ind. e Com, São Paulo, Brazil) at low speed hand piece. The teeth were stored in 0.5% chloramine solution for a week and, after this period, were placed in distilled water. The roots were removed and discarded, and fragments of the flatter region of the crown with dimensions 5 × 5 mm were chosen. Subsequently, the fragments were embedded in acrylic resin self-polymerized Orto-Clas (Artigos Odontológicos Clássico Ltda., São Paulo, Brazil) transparent acrylic resin mixed with Orto Cril black (Vipi Ltda., São Paulo, Brazil) at 3.5 and 1.5 g, respectively, in order that specimens could be standardly positioned in all readings, leaving only the enamel surface exposed. The color black was chosen in order to avoid any interference during the reading of a tooth's color in the spectrophotometer. Enamel surfaces embedded in acrylic resin have been planned in polisher APL-4 (Arotec, Cotia, São Paulo, Brazil) with 400-, 600-, and 1200-grit silicon carbide (SiC) abrasive paper with water cooling and a speed of 300 rpm. The polishing process was performed with a felt disc and diamond pastes Top (6 μm), Ram (3 μm), and Supra (1 and 1/2 μm) cooled with mineral oil to standardize the surface texture and the gloss enamel of all fragments. After obtaining a smooth and polished surface, the samples were cleaned during the course of 10 min with a neutral detergent in an ultrasonic cleaner (model 2210, Branson Corp., Danbury, Connecticut).

Initial superficial microhardness (SMH_i) was performed using a microhardness tester (HMV-2000; Shimadzu Corp., Kyoto, Japan), with a 25-g load applied for 10 s. The specimens were individually fixed in a clamping apparatus and positioned perpendicular to the tester tip. SMH values were evaluated by the C.A.M.S program (New Age Industries, Southampton, Pennsylvania). In each sample, five indentations were recorded, one central and other four at 100 μm away, and the hardness average value was calculated.

Baseline color was measured according to the Commission Internationale de l'Éclairage (CIE) $L^*a^*b^*$ color scale, relative to the standard illuminant C over a white background on a reflection spectrophotometer (UV-2450; Shimadzu Corp.). The CIE $L^*a^*b^*$ color system is a three-dimensional color measurement: L^* refers to the lightness coordinate, and its value ranges from 0 for perfect black to 100 for perfect white. a^* and b^* are chromaticity coordinates on the green-red ($-a^*$ = green; $+a^*$ = red) and blue - yellow ($-b^*$ = blue; $+b^*$ = yellow) axes.¹⁸

After the initial analysis, the specimens were divided into five groups ($n = 5$) and received the following treatments:

G1 (control): the specimens were stored in vials containing artificial saliva (pH 7.09, 50 mmol/l KCl, 1.5 mmol/l Ca, 0.9 mmol/l PO₄, and 0.1 mmol/l Tris buffer)¹⁹ for 3 weeks, and every day, the enamel surfaces were cleaned with a toothbrush (Oral-B Indicator Plus 35, Gillette do Brasil Ltda., Manaus, Amazonas, Brazil) and fluoridated toothpaste (Colgate Total 12, Colgate-Palmolive Ltda., Osasco, São Paulo, Brazil), then returned to be stored in saliva, which was changed daily.

G2: the specimens were submitted to home bleaching technique, remaining in contact with the whitening gel at 10% carbamide peroxide based for 6 h daily (Whiteness Perfect, FGM Produtos Odontológicos, pH 6.07, Joinville, Santa Catarina, Brazil). Then, before and after this period, the enamel surface was cleaned with a toothbrush and fluoridated toothpaste and stored in artificial saliva until the next bleaching session (3 weeks).

G3: the specimens were bleached and cleaned the same way as the G2, but then they were submitted to aging in the cola soft drink (Coca-Cola, pH 2.60, Co, Ribeirão Preto, São Paulo, Brazil) for 1 h and again cleaned and stored in artificial saliva until the next bleaching session.

The G4 and G5 received the same treatments as the G3, but they were submitted to aging for 1 h in melted chocolate (Chocolates Garoto S/A, pH 6.24, São Paulo, Brazil) and red wine (Concha y Toro Cabernet Sauvignon 2006, pH 3.60, Santiago, Chile), respectively.

The specimens were stored at 37°C throughout the experimental period, simulating oral conditions. The pH of all solutions was measured using a pH meter (290A; Orion Research Inc., Boston, Massachusetts).

After the experimental period (3 weeks), the final microhardness (SMH_f) and color were re-evaluated. Color change (ΔE) was calculated between the color coordinates before (baseline) and after treatments as measured in the reflectance mode applying the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Mineral loss (%SMH_{red}) was obtained indirectly by the percentage of microhardness reduction using the formula %SMH_{red} = $100 \times [(SMH_i - SMH_f) / SMH_i]$. The %SMH_{red} and ΔE values were analyzed by one-way analysis of variance (ANOVA) and Fisher's protected least significant difference test at a preset α of 0.05. Color coordinates L^* , a^* , and b^* were also singly analyzed statistically by ANOVA and Fisher's test ($\alpha = 0.05$).

3 Results

The specimens submitted to the home bleaching combined with the red wine (%SMH_{red} 94.46 ± 1.0) as well as those combined with a cola soft drink (%SMH_{red} 92.96 ± 3.3) have higher mineral loss, which was statistically significant from the other groups ($p \leq 0.05$) (Table 1).

The bleached group combined with red wine (ΔE 9.34 ± 2.90) showed a high-color change, without statistical difference for the groups submitted to bleaching combined with a cola soft drink (ΔE 7.49 ± 2.50) and only bleached

Table 1 Mineral loss (%SMH_{red}) and color change (ΔE) values after treatments.

	%SMH _{red}	ΔE
Control	6.06 (6.70) a	2.22 (0.44) c
Bleached (BL)	9.04 (5.50) a	6.56 (0.82) ab
BL + cola	92.96 (3.30) b	7.49 (2.50) ab
BL + chocolate	9.16 (1.50) a	5.49 (1.80) b
BL + wine	94.46 (1.00) b	9.34 (2.90) a

Note: Means (SD) followed by distinct letters in column are statistically different ($p \leq 0.05$).

Table 2 Means (SD) of the L^* values color coordinate (lightness), baseline and after treatments.

	L^*	
	L^*_0	L^*_f
Control	55.72 (3.60)	56.91 (3.08)
Bleached (BL)	54.07 (2.30)	59.03 (1.13)
BL + cola	53.33 (3.64)	58.79 (2.20)
BL + chocolate	54.24 (5.01)	54.89 (2.35)
BL + wine	58.36 (5.76)	55.13 (3.99)

Note: " L^*_0 " initial value; " L^*_f " final value. There was no statistical difference comparing the initial and final values ($p = 0.0738$) and the experimental groups ($p = 0.7073$).

($\Delta E 6.56 \pm 0.82$). These last two did not differ from the group submitted to bleaching combined with chocolate ($\Delta E 5.49 \pm 1.80$). The group that remained in artificial saliva showed the smallest color change ($\Delta E 2.22 \pm 0.44$), with statistical difference for all other groups ($p \leq 0.05$). These findings are illustrated in Table 1.

Tables 2–4 show the values of L^* , a^* , and b^* color coordinates, respectively. It was observed that no significant difference was found between the L^* values ($p > 0.05$). No significant change was detected between a^* coordinate before and after the treatments ($p > 0.05$), except for G5, which observed an increase after the red wine storage ($p \leq 0.05$). The data revealed a decrease in the b^* values for all groups ($p \leq 0.05$).

4 Discussion

This study evaluated combinations that may occur daily in the lives of patients who undergo home bleaching. Even with the recommendations of the nonuse of solutions that act potentially as dyes/acids during the bleaching, and often without the supervision of the dentist, the patients generally do not abandon their feeding habits at the risk of jeopardizing the outcome of the bleaching during the treatment.

Surface microhardness is a simple method to determine the mechanical properties of the enamel and dentin surfaces, and it

Table 3 Means (SD) of the a^* values color coordinate (green–red axis), baseline and after treatments.

	a^*	
	a^*_0	a^*_f
Control	-1.36 (0.48) A	-1.54 (0.26) Ab
Bleached (BL)	-1.64 (0.55) A	-1.58 (0.43) Ab
BL + cola	-1.27 (0.56) A	-1.55 (0.48) Ab
BL + chocolate	-1.46 (0.22) A	-1.47 (0.31) Ab
BL + wine	-1.31 (0.24) B	1.11 (0.63) Aa

Note: " a^*_0 " initial value; " a^*_f " final value. Distinct letters (capital in the row and lower in the column) are statistically different ($p \leq 0.05$).

Table 4 Means (SD) of the b^* values color coordinate (blue–yellow axis), baseline and after treatments.

	b^*	
	b^*_0	b^*_f
Control	2.10 (0.35) A	-0.90 (0.81) Ba
BL	0.83 (2.06) A	-2.99 (0.81) Bb
BL + cola	1.09 (1.61) A	-2.21 (1.30) Bab
BL + chocolate	1.92 (2.28) A	-2.57 (1.36) Bab
BL + wine	2.95 (1.27) A	-1.53 (1.18) Ba

Note: " b^*_0 " initial value; " b^*_f " final value. Distinct letters (capital in the row and lower in the column) are statistically different ($p \leq 0.05$).

is related to the loss or gain of minerals into the tooth structure. It has demonstrated to be an adequate method in determining the small changes that occur with these tissues.²⁰ The results of this investigation showed that the dental fragments submitted to bleaching combined with red wine (%SMH_{red} 94.46) and with cola soft drink (%SMH_{red} 92.96) showed higher mineral loss compared to the other groups (Table 1). Higher enamel microhardness values were reported for unbleached teeth compared to the bleached teeth similar to other studies,^{7,11} and these were more susceptible to demineralization after contact with acid substances.⁷ Thus, the first null hypothesis was rejected.

Teeth exposed to a pH < 5.5 for enamel and dentin for 6.0 for an extended period of time can lead to demineralization²¹ and erosion of enamel.²² The cola soft drink (pH 2.60) and red wine (pH 3.60) used in this study are highly acidic solutions compared to artificial saliva (pH 7.09) and melted chocolate (pH 6.24), showing that the low pH of those solutions may have had a major effect on the structure of the teeth bleached. The erosive loss of enamel is higher with the cola soft drink, besides having a low pH that is constituted of phosphoric acid that has a high erosive power.¹³ The red wine has a low pH and is also an alcoholic beverage, which may have contributed to the higher enamel demineralization of this solution.

Demineralization studies have demonstrated surface defects and degradation of dental enamel exposed to bleaching.⁸ In a previous investigation,²³ it was observed that bleaching systems with more acids reduced enamel's hardness, whereas bleaching agents with a neutral pH caused increases in the hardness; thus, the pH of the bleaching agents can influence the mineral loss. To minimize such damage, bleaching agents should have a pH around 7.0.²⁴ The pH of the bleaching agent used in this study was 6.07, slightly lower than the value desired; consequently, this product could cause demineralization of the tooth substrate.²¹

However, it is noteworthy that the dissociation of carbamide peroxide resulted in ammonia and carbon dioxide,²⁵ raising the pH of the bleaching agent over a period of 15 min and making the oral cavity a more basic environment.²⁶ In addition, clinically, the urea secreted by the parotid gland could help to increase the salivary flow, making the oral cavity even more basic, preventing the enamel demineralization.²⁶

The mineral loss was not influenced by the pH of the bleaching product because there was no statistic difference between the group not bleached and the group that received only bleaching

treatment (Table 1). Furthermore, after receiving bleaching treatment and/or being immersed in staining solutions, dental fragments were immersed in artificial saliva seeking to replicate the *in vivo* condition. Storage in artificial saliva may lead to the remineralization process and offer protection against enamel erosion.¹⁴ In addition, this study simulated home bleaching overnight, in which the patient would perform brushing with dental brush and fluoride toothpaste, which can promote remineralization¹⁷ associated to the artificial saliva.¹⁴

Color change of the teeth submitted to bleaching and aging in staining solutions was evaluated by the CIE $L^*a^*b^*$ method in this investigation. In principle, if the color of a material is completely stable, no difference will be detected after exposure to the environment tested ($\Delta E = 0$). In addition, ΔE values between 3 and 8 will already be moderately visible, and values >8 would be extremely perceptible.¹⁸ There was not a statistical difference between the group that was only bleached and the groups submitted to bleaching in combination with staining solutions. After bleaching, the specimens in the aging groups were stored for an hour in a cola soft drink, melted chocolate, or red wine, simulating, therefore, a 1-h meal (average time) with the ingestion of beverages. The ΔE values of the bleached groups were considered moderately (G2, G3, and G4) and extremely (G5) perceptible.¹⁸

The peroxide can diffuse through the enamel and dentin due to its low molecular weight, reacting with organic materials through the oxidation of macromolecules and stains, thereby making the tooth clearer.²⁵ Thus, the control group was immersed in saliva throughout the experiment, without tooth whitening, and was only cleaned with a toothbrush and fluoridated toothpaste every day, which showed the lower color difference (Table 1).

The L^* color coordinate showed no change after the bleaching treatment (Table 2). An increase in the a^* value was observed for the group that received bleaching combined with red wine (Table 3). Pinto et al.²⁷ reported, in their study, a reduction in hardness and an increase in surface roughness after bleaching with 10% carbamide peroxide. It is known that the larger the surface roughness, the greater the absorption of more pigments, which helps to explain the result obtained for the wine group.²⁷ Additionally, the low pH combined with alcohol molecules of the wine could provide a surface change by the mineral loss, thus leaving the enamel surface more susceptible to staining by reddish grape pigments of the red wine.

The bleached groups presented a decrease in the b^* coordinate values (Table 4), which are considered the most important variable in bleaching studies.²⁸ Thus, the negative values of the b^* color coordinate are in agreement with those reported by Gerlach et al.,²⁹ confirming that the yellowish color of the tooth fragments after the bleaching treatment tended to blue, indicating that there was tooth whitening independent of the combination with the dye solutions,²⁸ and the second null hypothesis was validated.

This study showed that the carbamide peroxide application on the enamel was the determining factor for better efficacy of the tooth whitening, once the highest color change (ΔE) and the negative values of the b^* coordinate were observed for the bleached groups. On the other hand, there was a trend of potential dye substances, such as red wine, to exert influence in the final color; therefore, the consumption of highly colored food and beverages should be decreased. Moreover, high mineral loss was observed when the enamel was exposed to acidic drinks

that could decrease the staining resistance by superficial changes of the dental enamel, making extremely important the guidance to avoid intake of acidic substances, especially during bleaching treatment.

5 Conclusion

In all conditions tested, the bleaching treatment enabled the teeth to become clearer; nevertheless, the intake of food and beverages containing colorants or an acidic pH can negatively affect the effectiveness of tooth whitening.

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