Optical Dental Whitening Efficacy of Blue Covarine Toothpaste in Teeth Stained by Different Colors

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ABSTRACT

Objective: Evaluate the immediate and cumulative optical whitening efficacy of a blue covarine toothpaste.
Materials and Methods: 180 bovine tooth specimens with similar shade ($\Delta E < 3.5$) were staining of different beverage: black tea(BT), green tea (GT), red wine (RW), orange soda (OS), and brazilian ačai juice (AJ), and then submitted to tooth brushing with a blue covarine toothpaste (Op) or a control abrasive toothpaste (Ab). The whitening effect was evaluated at baseline (B), after staining (S), after 1 day (1D) and 7 days of cumulative use of toothpastes (7D). The color shade chances were assessment by Vita Easyshade reflectance spectroscope and the data of CIELab color coordinates (L*, a*, and b*), color difference (ΔE) and the whiteness index optimized (WIO), were analyzed by two-way mixed analysis of variance (ANOVA) for repeated measures and Bonferroni-corrected *t*-tests ($\alpha = 0.05$).
Results: The analysis showed statistically significant differences before and after staining by colored beverages ($p < 0.05$), but no differences were found due to the action of toothpaste ($p > 0.05$), in the CIELab coordinates, ΔE and WIO index.
Conclusions: The use of toothpastes (Op or Ab) reduced the dental staining caused by different colored beverage, but the whitening effect of blue covarine toothpaste could not be confirmed ($p > 0.05$).

CLINICAL SIGNIFICANCE

Many professionals and patients have reported contradictory results after using the blue covarine toothpaste. This article demonstrates the limitations of this optical whitening toothpaste and guides professionals in the correct indication of this alternative to tooth whitening.

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INTRODUCTION

Frequent consumption of beverages containing high concentrations of organic pigments such as tea, coffee, and wine may lead to the severe discoloration of teeth.[1] The levels of tooth discoloration are often related with exposure frequency, oral hygiene technique, enamel quality, presence of restorative materials and, most importantly, with certain physico-chemical characteristics of pigments such as electric charge, molecular weight and size. Depending on the combination of these pigment-related factors, teeth may have their colors altered either by extrinsic discoloration (ED) or by intrinsic discoloration (ID).[2,3]

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Daily oral hygiene with fluoride-containing toothpastes is the most common technique used to control ED.[4] These toothpastes have in their formulation silica, calcium carbonate, and other types of particles that remove pigments from the surfaces of teeth by an abrasive action.[4,5] A novel tooth whitening approach, based on the use of toothpastes containing blue covarine was reported in the literature. More recently, this minimally invasive and "optical" tooth whitening technique has been used in the treatment of both ED and ID as an alternative to conventional peroxide-based tooth bleaching techniques.[6–10]

Studies investigating the efficacy of blue covarine containing toothpastes have reported that significant and immediate tooth whitening results were obtained after a single use of optical toothpastes. According to these studies, the tooth whitening results obtained using this novel technique persisted for periods over 8 hours after its use.[10] In addition, their findings also indicated that the most significant color changes produced by the use of optical toothpastes were observed on the color parameters b* (yellowness) and WIO.[7,10]

The main mechanism of action of these optical whitening toothpastes is based on the deposition of a blue covarine thin-film onto the surfaces of enamel. Depending on the quality and thickness of the film deposited, it will alter the natural colors of teeth from yellowish-brown to a more pleasing and aesthetic white-bluish color. However, limited information is currently available in the literature regarding the efficacy of optical whitening toothpastes in challenging aesthetic situations, where teeth discoloration results from aging, trauma, endodontic treatments or even from the incorporation of large amounts of organic pigments from the host's dietary habits. Therefore, the objective of this study was to assess the immediate and cumulative dental whitening efficacy of a toothpaste containing blue covarine on bovine teeth severely discolored by organic staining solutions. The null hypothesis tested in this study was that the toothpastes investigated would have similar tooth-whitening effects.

MATERIALS AND METHODS

The in vitro characterization of the optical whitening efficacy of a blue covarine toothpaste was performed using a randomized, controlled, double-blind experimental design (the operator responsible for measuring the color and the statistician) of repeated measurements.

The two independent variables were: (1) type of toothpaste: with optical whitening action (Op) versus abrasive action (Ab); and (2) staining solutions: no dye (ND—control), black tea (BT), green tea (GT), red wine (RW), orange soda (OS), and Brazilian açai pulp juice (AJ). The interaction of these variables resulted in the following groups: OpND and AbND comprised the control groups, and OpBT, AbBT, OpGT, AbGT, OpRW, AbRW, OpOS, AbOS, OpAJ, and AbAJ comprised the experimental groups (Figure 1). The effects of the interaction among these variables were studied at four time points (repeated measures): before staining (B), after staining (S), after a single application of toothpaste (1D), and after seven consecutive applications of toothpaste (7D).

The experimental design of this study has enabled us to assess two major effects: (1) Discoloration of teeth by distinct staining solutions, and (2) Immediate and cumulative tooth whitening results obtained with the use of an optical whitening toothpaste. Both effects were assessed by comparing the set of changes observed in each of the following color parameters: L* (lightness), a* (redness), b* (yellowness), ΔE (color difference), and WIO. The sample size ($n = 15$/group) was previously defined using the G* Power 3.1.7 software[11] considering an 80% statistical power and 95% confidence level ($\alpha = 0.05$).

Visual and spectrophotometric (Vita EasyShade Advance 4.0, Vivadent, Brea, USA; hand piece and calibration holder serial number: H25543)[12] analyses were performed to select a total of 180 bovine teeth based on color similarity ($\Delta E < 3.5$). Square-shaped specimens (area = 36 mm^2, thickness = 2 mm) obtained from the middle third of the buccal face of selected bovine teeth were obtained using a low-speed,

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Code	Composition	Manufacturer
Op	Sorbitol, aqua, hydrated silica, PEG32, sodium lauryl sulfate, aroma, cellulose gum, sodium fluoride, trisodium phosphate, sodium saccharin, PVM/MA copolymer, mica, cl 74160, limonene	Closeup White Now Ice Cool Mint Unilever Brazil Industrial Ltda, Pernambuco - Brazil
Ab	water, calcium carbonate, sorbitol, sodium lauryl sulfate, flavor, sodium silicate, cellulose gum, sodium bicarbonate, sodium saccharin, formaldehyde and sodium monofluorophosphate	Smile Super Refreshing, Colgate-Palmolive Company, São Paulo - Brazil
BT	Leaves and stalks of Camellia sinensis (L.) Kuntze	Leão Fuze Chá Preto natural Coca Cola Company, Coca Cola Brazil - Brazil
GT	Leaves of Camellia sinensis (L.) Kuntze	Leão Fuze Chá verde natural Coca Cola Company, Coca Cola Brazil - Brazil
RW	Cabernet Sauvignon grapes with 12% alcoholic graduation	Marcus James Cabernet Sauvignon, Cooperative Winery Aurora, Bento Gonçalves, RS - Brazil
OS	Carbonated water, sugar, orange juice, artificial synthetic flavoring, citric acid, sodium benzoate conservative, stabilizers acetate isobutyrate sucrose and sodium dioctyl sulfosuccinate and yellow artificial coloring twilight FCF	Fanta Orange Coca Cola Company, Coca Cola Brazil - Brazil
AJ	Frozen pulp of the fruit of Euterpe oleraceade palm tree (Brazilian Açaí)	Medium açaí pulp Icefruit Food Ltda -Tatui, SP - Brazil

FIGURE 1. Code, composition, and manufacturer of products used as independents variables in this study.

water-cooled diamond saw (Isomet 4000, Buehler Ltd., Lake Bluff, USA). Then, sectioned specimens were randomly distributed among the experimental groups (previously described) using a random sequence that was electronically generated (https://www.random.org).

Staining of Specimens

All specimens, with the exception of the specimens that comprised our control group (ND), were submitted to four cycles of staining as proposed by Stookey et al.[13], with modifications. Each staining cycle was composed of 4 hours of immersion in a staining solution (black tea [BT], green tea >, red wine [RW], orange soda [OS], and Brazilian açai pulp juice [AJ]) followed by a 1-hour air-drying period (22 ± 1°C). After the completion of the staining process, specimens were washed in running water (5 minutes) before being stored in artificial saliva for 15 hours (36 ± 1°C).

Toothpaste Application

Slurries of each one of the toothpastes investigated in this study were fabricated by mixing artificial saliva (Reativa—Homeopathy and Manipulation Pharmacy), water and the selected toothpaste (either optical or abrasive) in a 1:1:1 ratio. Then, slurry aliquots (10 mL at 36 ± 1°C) were individually applied over the surfaces of the specimens immediately before subjecting the specimens to tooth brushing procedures in a semi-automated tooth brushing machine (MEV 2T—ODEME, Biotechnology, Joaçaba, Brazil) for 3,150 brushing cycles (150 strokes/minute, 3 minutes/day, 7 days, 200 g load).

Operator Calibration

The calibration of the operator was accomplished in a preliminary pilot study (data not shown). In that study, the operator assessed the natural colors of bovine teeth ($n = 20$) employing a subjective (visual analysis) and an objective method (reflectance spectroscopy) in a metamerism box (MAKO Industry and Commerce of Photographic Equipment Ltd., Rio Negro, Brazil) equipped with standardized light sources (DIN 6173, Mako CVB-D65 Daylight). The operator's calibration in the accurate determination of the natural colors of bovine teeth was confirmed by the interclass correlation coefficient test (ICC = 0.82).[14]

Spectrophotometer Calibration and Color Measurements

As described in the Vita EasyShade Advance spectrophotometer operation manual (page 9, Vivadent, Brea, USA), the unit requires an initial calibration step every time that the unit is turned on.

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TABLE 1. Statistical results of two-way ANOVA of repeated measurement for variables interaction and main effects

Effect	Variables	Statistic results	Color parameter				
			L*	a*	b*	ΔE	WIO
Interaction	TP*SB*TA	p value	0.07	0.02*	0.78	0.00*	0.00*
		CI 95%	(70.41–76.64)	(4.25–7.14)	(33.29–37.50)	(14.67–18.72)	(50.57–60.70)
		η^2_p	0.05	0.07	0.02	0.04	0.36
	TP*SB	p value	0.23	0.52	0.83	0.03*	0.00*
		CI 95%	(71.02–76.03)	(4.44–6.95)	(33.70–37.07)	(15.06–18.32)	(51.81–59.47)
		η^2_p	0.04	0.02	0.01	0.04	0.33
	TP*TA	p value	0.08	0.14	0.18	0.01*	0.00*
		CI 95%	(72.26–74.80)	(5.11–6.28)	(33.70–37.07)	(15.87–17.52)	(53.57–57.70)
		η^2_p	0.01	0.01	0.01	0.01	0.13
	SB*TA	p value	0.00*	0.00*	0.00*	0.00*	0.00*
		CI 95%	(71.33–75.73)	(4.67–6.72)	(33.90–36.88)	(15.26–18.13)	(52.05–59.22)
		η^2_p	0.65	0.75	0.76	0.48	0.51
Main effect	TP	p value	0.16	0.43	0.05	0.03*	0.38
		CI 95%	(72.50–74.60)	(5.18–6.20)	(34.71–36.08)	(16.02–17.36)	(54.07–57.20)
		η^2_p	0.01	0	0.02	0.01	0.01
	SB	p value	0.00*	0.00*	0.00*	0.00*	0.00*
		CI 95%	(71.76–75.30)	(4.81–6.58)	(34.20–36.58)	(15.54–17.85)	(52.93–58.34)
		η^2_p	0.75	0.74	0.79	0.48	0.62
	TA	p value	0.00*	0.00*	0.00*	0.00*	0.00*
		CI 95%	(72.63–74.43)	(5.28–6.11)	(34.78–36.00)	(16.11–17.28)	(54.18–57.10)
		η^2_p	0.79	0.83	0.65	0.4	0.73

TP = toothpaste; SB = staining beverage; TA = time point of analysis.
*Statistically significant at $p < 0.05$; η^2_p = percentage of variance determined by the action of the variable under study (effect size).

The unit's calibration is performed automatically after the operator places the tip of the spectrophotometer (hand piece) in contact with the ceramic calibration block (L* = 87.5, a* = 0, and b* = 4.7) located at the unit's base. All subsequent color measurements performed in this study, were carried out perpendicular to the specimens' surfaces and in contact mode. Unless otherwise specified, the data reported from the color measurements in this study are a true comparison between the color of the specimens, at a given time point, and the color of the ceramic calibration block. For this reason, independently of the step of color measurement considered, all data reported in this study will be different than zero.

Dental Bleaching Efficacy

The specimens' total color variation (ΔE),[15] in each experimental step investigated (B, S, 1D, and 7D), was the main parameter for determination of the tooth whitening efficacy attained with the use of traditional (Smile Super Refreshing, Colgate-Palmolive Company, São Paulo, Brazil) or optical (Closeup White Now Ice Cool Mint Unilever Brazil Industrial Ltd., Pernambuco, Brazil) toothpastes on severely discolored bovine teeth. The values of luminosity (L*), color variation in the green-red axis (a*), and in the yellow–blue axis (b*) recorded were utilized to calculate ΔE (equation (1)).[15] Then, L*, a*, and b* values were transformed into Y, x, and y values using the color calculator resource available online (www.easyrgb.com/index.php?X=CALC). Thereafter, the whitening index optimized (WIO)[16] was calculated using equation (2).

$$\Delta E = \sqrt{(L_i - L)^2 + (a_i - a)^2 + (b_i - b)^2} \quad (1)$$

$$\text{WIO} = Y + 1075.012(x_n - x) + 145.516(-y) \quad (2)$$

where (L_i, a_i, b_i) are the color coordinates of the initial reference, (x_n, y_n) are the chromaticity coordinates of the white reference, and (L, a, b, x, y) are the specimens' colors in each experimental step investigated.

Statistical Analysis

The interactions and main effects of the variables "*type of toothpaste*" and "*staining solution*" at four time points (B, S, 1D, and 7D) were analyzed using two-way mixed ANOVA for repeated measures and Bonferroni-corrected *t*-tests for the pairwise comparisons of interest. The dimension of the dental bleaching effect (η^2p) was also determined and the statistical significance adopted for all analyzes was 5%. All statistical analyzes were performed using SPSS Statistics V19 (SPSS Inc., Chicago, USA).

RESULTS

The results of the two-way repeated measures ANOVA demonstrated statistically significant differences for the interaction between staining solution and time-point of color assessment (SB*TA) of all color parameters investigated in this study (Table 1). The main effects analysis indicated that the color changes observed are mainly due to the effect of the staining solutions (ND, BT, GT, RW, OS, and AJ) and the time-point (B, S, 1D, and 7D) color assessments. The whitening effects promoted by the use of the toothpaste containing blue-covarine was only observed when the ΔE index was considered, but the whitening effects observed had low practical value ($\eta^2_p < 0.01$). This finding was corroborated by the analysis of the remaining color parameters investigated in which the whitening effect promoted by the use of the blue covarine toothpaste was similar ($p > 0.05$) to the whitening effect attained by the use of a conventional toothpaste.

In addition, the results of the color assessments performed on the specimens in the control group (ND) have demonstrated that independent of the color parameter considered (ΔE or WIO), type of toothpaste used (Op or Ab) or toothpaste application (1D or 7D), neither of the toothpastes investigated in this study were able to promote observable or measurable whitening effects due to the deposition of a blue covarine thin-film on the specimens' surfaces nor by an abrasive action ($p > 0.05$). The color variation observed in the specimens that were submitted to the staining process (BT, GT, RW, OS, and AJ) reveals that mechanical abrasion was the main effects of the toothpastes investigated (Figures 3 and 4).

DISCUSSION

Recently, the use of toothpastes with optical bleaching properties has been proposed as an alternative approach in the treatment of minor dental discolorations on vital teeth.[7,8,10] Its mechanism of action is mainly based on the deposition of a blue covarine thin-film that is theoretically capable of altering the visual perception of the colors of teeth. Some manufacturers claim that the main advantages of optical toothpastes over traditional dental bleaching techniques is the achievement of immediate and significant tooth whitening results without the use of hydrogen peroxide, which has been previously

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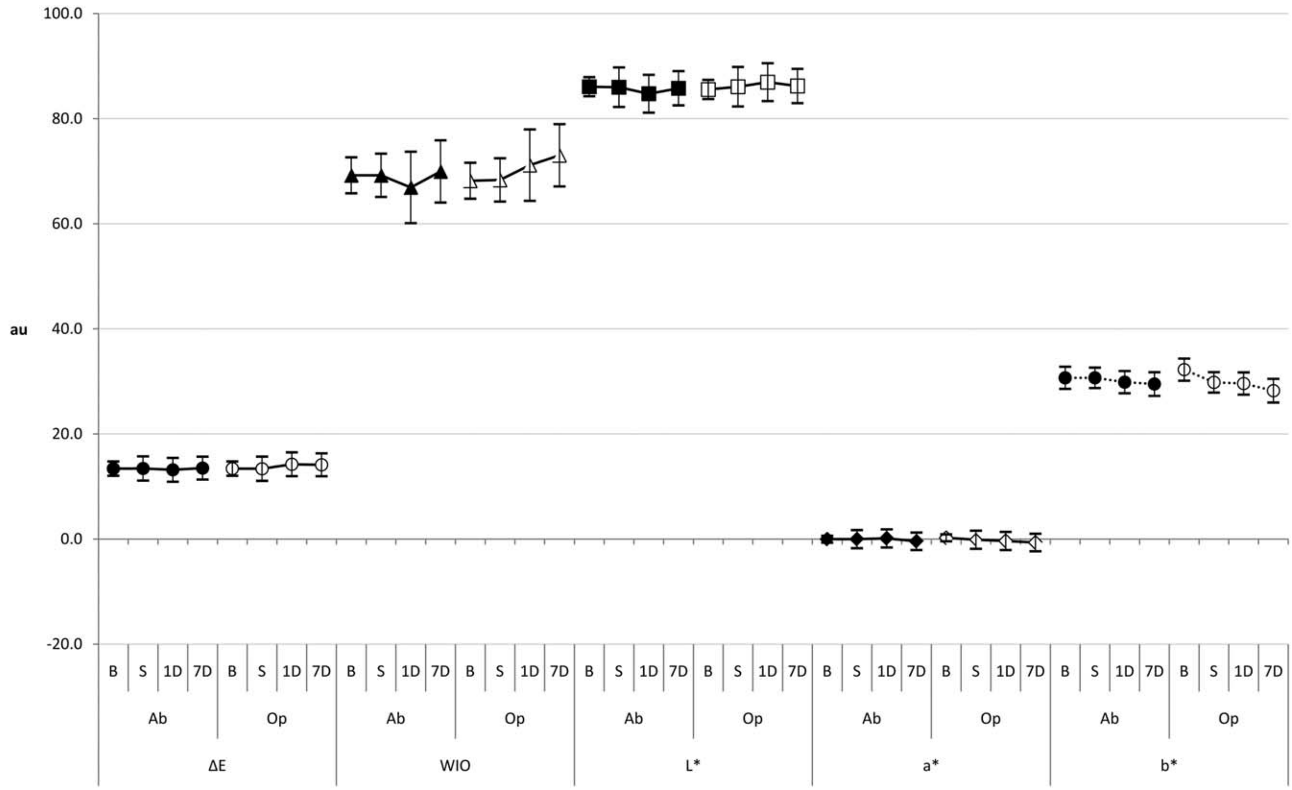

FIGURE 2. Comparison of the mean values and confidence interval (CI 95%) of the total color variation (ΔE), whiteness index optimezed (WIO), luminosity (L*), red–green (a*) and yellow–blue (b*) color coordenate registered before staining (B), after staining (S) and after use of abrasive toothpaste (Ab) or whitening toothpaste containing blue covarine (Op) for one day (1D) and seven consecutive days (7D), for the control groups (ND) expressed in arbitrary unit (au).

demonstrated to cause biological damage to dental tissues.[17–20]

Previous papers have shown that the optical teeth whitening effects attained by the use of blue-covarine containing toothpastes were instantaneous, long lasting (over 8 hours), perceivable (visual analysis) and measurable (colorimetric analysis).[8,10] However, there is no evidence in the literature to support the use of optical whitening toothpastes in cases of severe dental discolorations. Therefore, the objective of the present randomized, controlled, double blind in vitro study with repeated measures was the assessment of the tooth whitening efficacy of a toothpaste containing blue covarine.

The assessment of color change performed in this study has shown that independently of the beverage used to stain the specimens, significant color changes were observed in all experimental groups. The assessment of color change after one single application (1D), and after seven consecutive applications (7D), of the toothpaste containing blue covarine has shown that regardless of the color index considered, all experimental groups had significant color changes. Even though our findings demonstrate that significant changes were observed on all color parameters after one (1D) and seven (7D) applications of the toothpastes investigated (Figures 2–4), the tooth whitening effects achieved were not sufficiently strong to overcome severe discoloration. Further analysis of the evolution of color using the ΔE color parameter, has demonstrated that under the testing conditions and limitations of this study, the optical tooth whitening effects were very small or insignificant, and were not able to restore the specimens' initial colors. Our findings demonstrated that the blue covarine toothpaste (Op) had a whitening efficacy that was

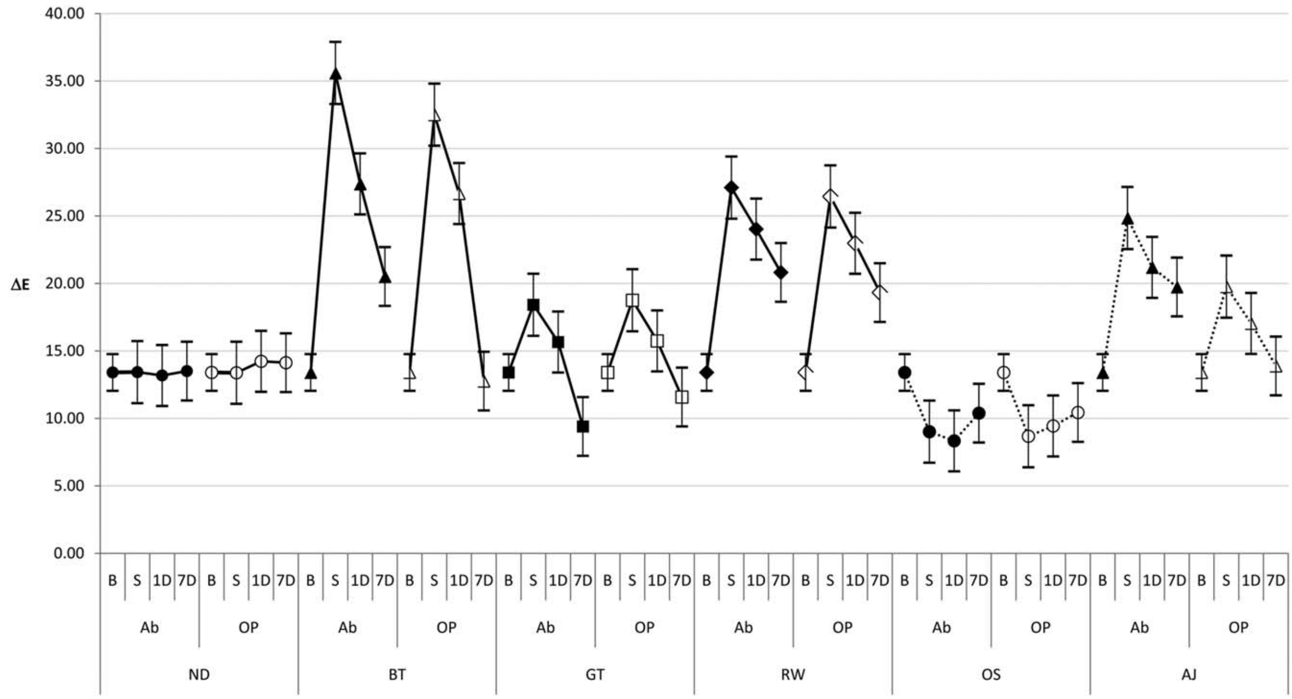

FIGURE 3. Comparison of the mean values and confidence interval (CI 95%) of the total color variation (ΔE), registered before staining (B), after staining (S) and after use of abrasive toothpaste (Ab) or whitening toothpaste containing blue covarine (Op) for one day (1D) and seven consecutive days (7D) in function of the staining beverage used.

comparable to the efficacy of traditional toothpastes (Ab; $p > 0.05$).

The strong whitening effects observed on the specimens in the black tea group (BT) are isolated results and are not corroborated by the behavior of the remaining groups or by the overall statistical analysis performed in this study. However, it is possible that these results may demonstrate the impact of intrinsic characteristics of the pigments' molecules over the ability of an organic molecule to penetrate deeply into the specimens' crystalline structure. Assuming that this hypothesis holds valid, the results obtained for the specimens pertaining to BT clearly demonstrate that the presence of an optimized abrasive system composed of hydrated silica particles may help to efficiently remove ED from the surfaces of teeth, which in Figure 3 can be translated into ΔE values that were comparable to the baseline values.

Significant positive increases were observed for the color parameters L* and WIO, whereas significant reductions were observed in the color parameters a*, b*, and ΔE. The combination of these results can be interpreted in terms of a significant reduction of the amount of pigments in the crystalline structure. Our statistical analysis indicated that "type of staining solution" and "time-point" were the main factors associated with significant color changes of the specimens. When ΔE is considered, 48% of the total color change can be attributed to the type of staining solution and 40% can be attributed to the time-point at which the color assessment was carried out.

These values increased to 62 and 73%, respectively, when the index WIO was the color parameter of choice (Table 1). In addition, our findings indicate that both traditional and optical toothpastes had similar tooth-whitening efficacies ($p > 0.05$). However, a statistically significant difference of 1% was observed between the ΔE values of both toothpastes investigated ($p < 0.05$), which from the clinical perspective is an insignificant or unperceivable color difference and therefore contradict previous findings.[8,10] Our results are corroborated by the recent paper published by

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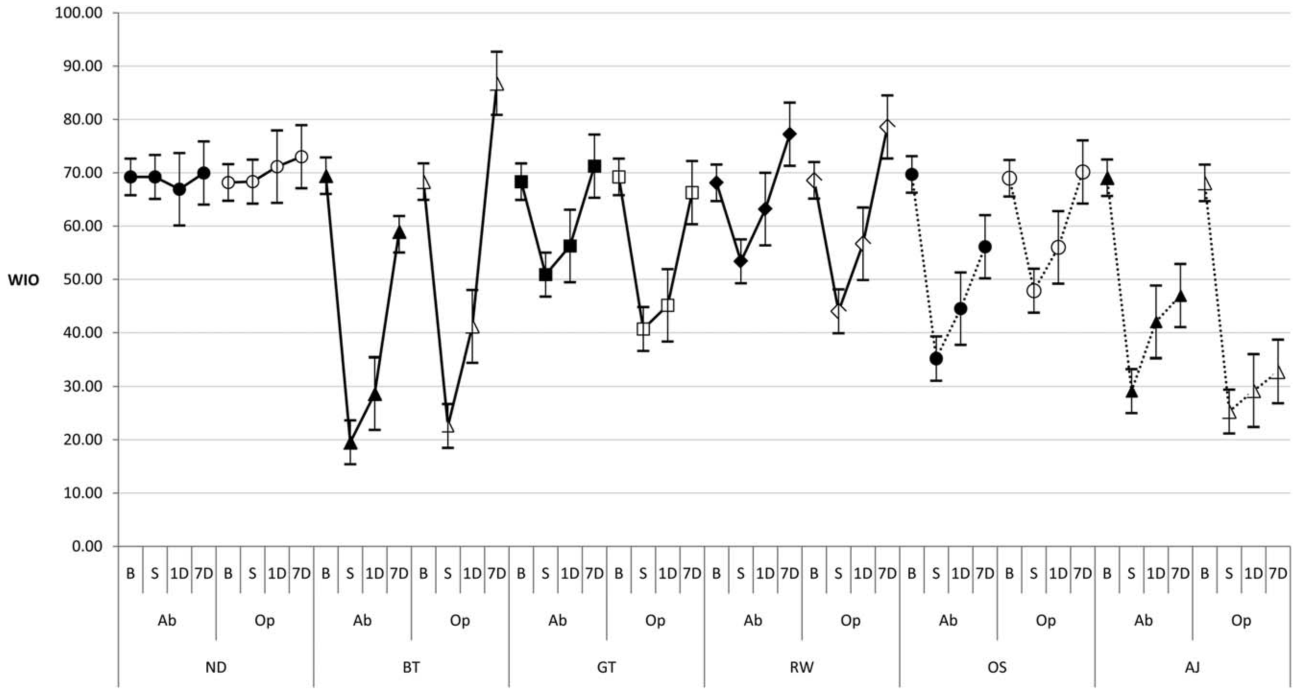

FIGURE 4. Comparison of the mean values and confidence interval (CI 95%) of the whiteness index optimized (WIO), registered before staining (B), after staining (S) and after use of abrasive toothpaste (Ab) or whitening toothpaste containing blue covarine (Op) for one day (1D) and seven consecutive days (7D) in function of the staining beverage used.

Torres et al.[21] who also evaluated the tooth-whitening efficacies of traditional and blue covarine containing toothpastes using bovine teeth and objective color measurements (spectrophotometer CM2600d, Minolta, Japan). Their results clearly demonstrated that the use of tooth-whitening toothpastes with exposure times two-times longer than the exposure times investigated in this study did not result in an increased tooth-whitening efficacy.

The results of this study contradict the findings of Joiner et al. (2006) and Collins, Maeeni, and Platten (2008), who demonstrated in vitro and in vivo the obtainment of instantaneous dental bleaching results with the use of a toothpaste containing hydrated silica and blue covarine. We strongly believe that the difference in results could be attributed to methodological differences among the studies cited and this study. The optical whiteness effect from the use of toothpastes containing blue covarine was observed when the evaluation was performed by colorimetric or photographic method, using the WIO index as the color parameter.

Other methodologies have failed to prove the optical whitening effects of blue covarine toothpastes. Among the limitations of this study we can highlight the absence of a control group where specimens would be subjected to the action of the bristles of the tooth-brushing machine alone to demonstrate the effect of the use of toothpaste. In addition, the degradation of the pigments within the crystalline structure over the total assessment period (7 days) could have also overestimated the tooth whitening effects of the toothpastes investigated, and therefore could have masked their differences. The use of bovine teeth can also be considered as one of the limitations of this study because these teeth have optical, physical, and chemical properties that are different from those observed for human teeth.[22]

Considering the limitations and findings of this study, the use of blue covarine containing toothpastes should not be prescribed as the primary whitening agent, or as a substitute to traditional dental bleaching techniques in challenging chromatic cases, such as aging, trauma, endodontic treatments, etc. In addition,

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we believe that additional studies should be conducted to investigate the utility of toothpastes containing blue covarine as maintenance agents after the completion of traditional dental bleaching procedures.

CONCLUSIONS

All staining solutions used promoted significant changes in the color of bovine specimens. These color alterations were more pronounced in the specimens subjected to staining with black tea (BT). The use of traditional (Ab) and blue covarine containing toothpastes (Op) for one (1D) or seven cumulative days (7D) progressively and significantly reduced the specimens' discoloration levels achieved by the in vitro staining solutions investigated. The tooth-whitening effects promoted with the toothpastes investigated were not statistically significant. Based on the results of the statistical analysis performed we accepted the null hypothesis proposed for this study.

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STATEMENT OF AUTHORS' RESPONSIBILITIES

Morgana Oliveira was responsible for the design of the experiment, preparation of specimens, discussion of results, and conclusion.

Eduardo Fernández was responsible for the introduction, experimental design, and discussion of results.

Janaína Bortolatto was responsible for statistical analysis and description of the results.

Osmir Oliveira Junior was responsible for statistical analysis and description of the results and conclusions.

Matheus Bandeca was responsible for the introduction, experimental design, and discussion of results.

Sharukh Khajotia was responsible for the analysis and discussion of results and translation.

Fernando Florez was responsible for the analysis and discussion of results and translation.

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