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Whitening Efficacy of Whitening Mouth Rinses Used Alone or in Conjunction With Carbamide Peroxide Home Whitening

JBS Oliveira • RS Sarlo • E Bresciani • TMF Caneppele

Clinical Relevance

Several over-the-counter whitening products, such as whitening mouth rinses, are available to consumers. The bleaching efficacy of some mouth rinses may increase the longevity of carbamide peroxide home whitening outcomes over time.

SUMMARY

Objectives: The aim of this study was to compare the effectiveness of whitening mouth rinses on teeth previously whitened or not, exposed to food dyes.

Methods and Materials: One hundred twenty enamel-dentin specimens, 3 mm in diameter, were obtained from bovine incisors. The specimens were stained for 14 days in staining broth. After staining, the initial color reading was performed via a spectrophotometer CM-2600d (Konica Minolta). Half of specimens were submitted to whitening (10% carbamide peroxide [CP]) for 14 days. They were then divided into three groups and were submitted to cycles of staining (five minutes) and mouth rinses (two minutes) for 12 weeks, with the following: CP-LI, Listerine Whitening; CP-PL, Plax Whitening; CP-BP, bromelain + papain; CP-DW, deionized water. LI, PL, BP, and DW groups were submitted to the same cited cycles but with no prior bleaching. The color measurements were performed after four, eight, and 12 weeks of treatment with mouth rinses. Data were submitted to repeated measures analysis of variance (ANOVA) and Tukey's test for multiple comparisons, with significance level at 5%.

Results: The results showed that the CP-LI, CP-PL, LI, and PL groups had greater color change than did the others. The CP-BP and BP groups were similar to CP-DW and DW.

Conclusions: We therefore conclude that Listerine Whitening mouth rinse presented the

Juliana Boa Sorte de Oliveira, MSc student, Restorative Dentistry, Institute of Science and Technology, São Paulo State University–UNESP, São José dos Campos, Brazil

Renata Silva Sarlo, undergraduate student, Restorative Dentistry, Institute of Science and Technology, São Paulo State University–UNESP, São José dos Campos, Brazil

Eduardo Bresciani, PhD, Department of Restorative Dentistry, Institute of Science and Technology, São Paulo State University–UNESP, São José dos Campos, Brazil

*Taciana Marco Ferraz Caneppele, PhD, Restorative Dentistry, Institute of Science and Technology, São Paulo State University–UNESP, São José dos Campos, Brazil

*Corresponding author: Av. Francisco José longo, 777, São José dos Campos, São Paulo 12245-000, Brazil; e-mail: taciana@fosjc.unesp.br

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highest bleaching effect, followed by Plax Whitening mouth rinse. Both maintained CP bleaching effect after 12 weeks of dye-rinse cycles. However, none of these rinses were able to produce whitening similar to CP. Bromelain- and papain-containing mouth rinses did not show bleaching effect, being similar to the control groups.

INTRODUCTION

Staining of extrinsic origin is an important factor among many others affecting the esthetics of teeth.[1] Staining is the result of extrinsic agents, and it affects the outer surface of teeth or it could be incorporated in the internal structure of teeth. Food dyes, beverages, tobacco, some mouthwashes, and drugs are etiologic agents responsible for chromogenic changes of teeth.[2] Dental prophylaxis eliminates the adsorbed stains on dental surfaces. If the pigments are incorporated in tooth structure, dental whitening techniques might be used.[3,4]

Besides the most common professional techniques for dental whitening (in-office and at-home supervised modalities), some whitening mouth rinses and tooth pastes are also available to consumers as over-the-counter (OTC) products.[5,6]

Mouth rinses were developed to aid controlling dental biofilm, which is mainly responsible for infections and the prevalence of dental caries and periodontal disease.[7] However, to satisfy the requirements of the current consumer society, whitening mouth rinses are freely sold, and advertisements convey the idea of achieving white teeth in a short period of time. They have become a very popular OTC whitening product due to their low cost, easy application, and wide availability in supermarkets and drugstores.[6] In their composition, there may be low concentrations of hydrogen peroxide, usually 1% to 2%[5] and according to Naik and others,[8] such concentrations usually do not cause gingival irritation.

Some studies have evaluated the effectiveness of whitening mouthwashes with hydrogen peroxide.[5,9-11] A study evaluated the efficacy and safety of a whitening mouth rinse (2% hydrogen peroxide) and whitening strips (10% hydrogen peroxide) that were used twice daily for one week. The results showed that, although both products had been well tolerated, the group treated with whitening strips experienced a significantly greater tooth color improvement than the whitening mouth rinse.[11] Torres and others[9] found the use of a 2% hydrogen peroxide mouth rinse resulted in whitening efficacy similar to that obtained with 10% carbamide peroxide (CP) at-home whitening. Jaime and others[10] and Karadas and others[12] reported that mouth rinses containing hydrogen peroxide were able to lighten the darkened human enamel, but to a lesser degree than the whitening produced by 10% CP. Different methodologies, and protocols of use, might have led to different results in those studies.

Due to the small number of studies evaluating the effectiveness of whitening mouthwashes and their association with home whitening, more studies on that issue are needed. Understanding the whitening mouth rinses would help clinicians know their effects and to clarify to patients whether they are needed or not.

Thus, this study aimed to compare the efficacy of whitening mouth rinses on teeth previously whitened or not, and exposed to food dyes. The null hypotheses tested were as follows: 1) the mouth rinses are not able to maintain the outcomes obtained with 10% CP whitening over the studied time period; 2) there is no difference in the whitening efficacy among the tested products; and 3) the mouth rinses are not able to promote a whitening effect similar to that of supervised home whitening.

METHODS AND MATERIALS

Freshly extracted and intact bovine incisors were stored until required in a 0.1% thymol solution and refrigerated at 4°C. Cylindrical enamel samples (3 mm diameter and 2 mm height: 1 mm of enamel and 1 mm of dentin) were prepared from the labial surface of the tooth using a trephine mill.

The specimens were stained in a staining broth (27 g of finely ground instant coffee; 27 g of finely ground instant tea; and 20 g of finely ground gastric mucin dissolved in 8 L of deionized water, 6 mL FD&C [Food, Drug, and Cosmetic] Red 40, 6 mL FD&C Yellow 5, and 750 mL red wine; adapted from Wozniak and others[13]). The specimens were stained for 14 days, under constant agitation.

The specimens were then positioned in a silicone mold with a cavity 6 mm in diameter and 3.1 mm in depth. On the bottom of the mold, there was a second-level cavity (3 mm in diameter and 0.1 mm in depth). The specimens were placed inside the internal cavity with the enamel surface directed toward the bottom of the mold, which was then filled with low-viscosity composite resin (Opallis Flow, FGM, Joinville, SC, Brazil). The specimens were attached to a metal holder, and the enamel surface

Table 1: *Products, Manufacturers, and Their Components*

Product	Manufacturer	Components
Whiteness Perfect 10%	FGM, Joinville, SC, Brazil	Carbamide peroxide, neutralized carbopol, potassium nitrate, sodium fluoride, humectant (glycol), deionized water
Listerine Whitening	KIK Custom Products, Etocicoke, Canada	Water, 8% alcohol, hydrogen peroxide 2%, sodium phosphate, poloxamer 407, sodium lauryl sulfate, sodium citrate, mint aroma, menthol, eucalyptol, sodium saccharin, sucralose
Colgate Plax Whitening	Colgate-Palmolive, São Bernardo do Campo, SP, Brazil	Water, sorbitol, ethanol, hydrogen peroxide 1.5%, poloxamer 338, polysorbate 20, methyl salicylate, menthol, sodium saccharin, CI 42090
Bromelain + papain	Experimental	Bromelain 0.5%, papain 0.25%, methylparaben, Propylparaben

was polished using sequential aluminum oxide abrasive papers (1200, 2400, and 4000 grit x96 FEPA-P, Struers, Ballerup, Denmark) in a polishing device (DP-10, Panambra, Sao Paulo, SP, Brazil). The specimens were immersed in an ultrasonic bath with deionized water for five minutes (Ultrasonic Cleaner, Odontobras, Ribeirão Preto, Brazil) for the removal of all waste. The prepared specimens were examined under a stereomicroscope (Stemi 2000-20×, Carl Zeiss, Tokyo, Japan) to certify the absence of cracks or other surface defects and then stored in deionized water to avoid dehydration.

Prior to treatment, the baseline L* values of each specimen were assessed under standardized ambient conditions, according to the Commission Internationale de l'Eclariage (CIE) L*a*b* system, using a spectrophotometer (CM2600d, Konica Minolta, Osaka, Japan) and an integrating sphere. The device was adjusted to use the D65 standard light source with 100% ultraviolet (UV) light and specular component included (SCI). The observer angle was set at 2°, and the device was adjusted to a small reading area (SAV). The color of each sample was measured three times and averaged. The results of the color measurements were quantified in terms of three coordinate values (L*, a*, b*), as established by the CIE, which locates the color of an object in a three-dimensional (3D) color space. The L* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). Axis a* represents the degree of green/red color, whereas b* axis represents the degree of blue/yellow color within the sample. L* value of each specimen was used for stratified allocation among eight groups (n=15). The color was measured over white (L*: 84.95; a*: −0.38; b*: 2.93) standard background. Values alterations in L* (ΔL*), a* (Δa*), and b* (Δb*) were calculated from color measurements at baseline and after the whitening procedures. Total color change or variation in color perception of each specimen was calculated and designated by the abbreviation ΔE*. This parameter was calculated according to the following formula: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

Table 1 shows all products used in this study, including manufacturers and their components. Half of the specimens were submitted to a whitening treatment with CP.

A 10% CP gel was used (Whiteness Perfect 10%, FGM, Joinville, SC, Brazil). The bleaching gel was applied over the surface of specimens in a 2-mm-thick layer (approximately 0.1 g) for eight hours. The gel was removed using a vacuum aspirator, and the specimens were stored in artificial saliva for 16 hours. The whitening procedure was repeated for 14 days. Artificial saliva was prepared according to Gohring and others[14]: 22.1 mmol/L $NaHCO_3$; 16.1 mmol/L KCl; 14.5 mmol/L NaCl; 2.6 mmol/L KH_2PO_4; 0.8 mmol/L H_3BO_3; 0.7 mmol/L $CaCl_2 \cdot 2 H_2O$; 0.4 mmol/L KSCN; 0.2 mmol/L $MgCl_2 \cdot 6 H_2O$; and mucin and adjusted to a pH of 7.0 with an HCl solution.

Twenty-four hours after the last application of bleaching gel, the color reading was performed. Then, they were submitted to dye-rinse cycles, according to the following groups (n=15): CP-LI, Listerine Whitening mouth rinse, 2% hydrogen peroxide; CP-PL, Colgate Plax Whitening mouth rinse, 1.5% hydrogen peroxide; CP-BP, experimental mouth rinse prepared using 40 g bromelain (Sigma-Aldrich, St. Louis, MO, USA), 20 g of papain (Vetec, Duque de Caxias, RJ, Brazil), 2 g of methylparaben, and 1.2 g of propylparaben, diluted in 8 L deionized water (0.5% bromelin and 0.25% papain); CP-DW, deionized water.

For the dye-rinse cycles, the specimens were immersed for five minutes in the staining broth;

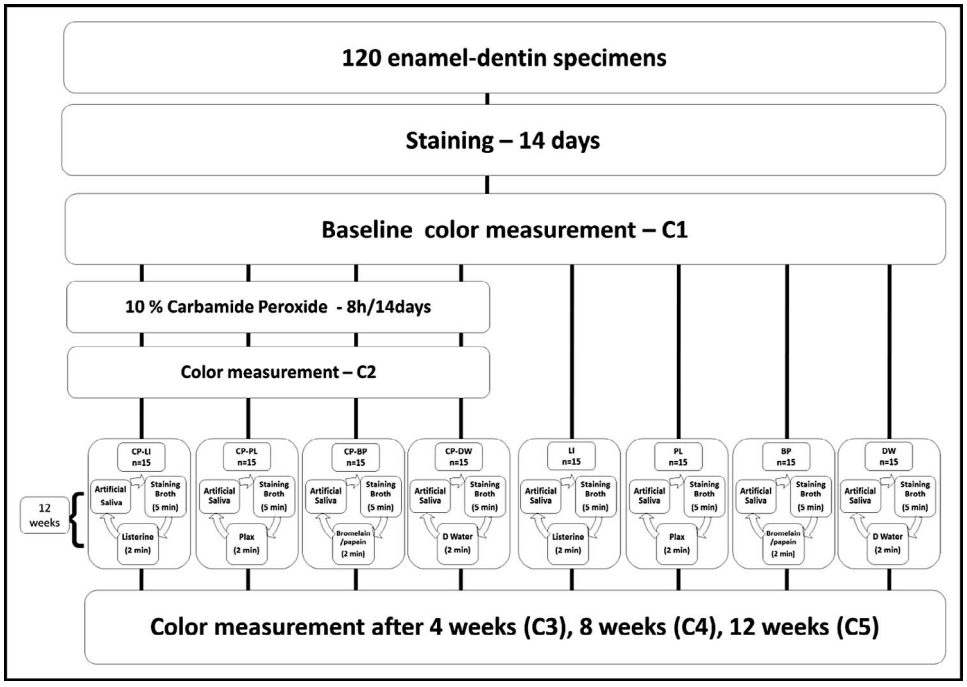

Figure 1. *Experimental design.*

subsequently, they were immersed for two minutes in the mouth rinses and then remained in artificial saliva. This cycle was repeated for 12 weeks. While immersed in the broth and mouthwash, the specimens were kept in constant agitation. Artificial saliva was replaced daily. Color reading was performed after four, eight, and 12 weeks.

The five-minute period in staining broth was recommended to simulate an extreme situation of daily consumption of food dyes, whereas two minutes in the mouth rinses represented the time recommended by the manufacturers.

Groups LI, PL, BP, and DW were submitted to the same cycles as groups CP-LI, CP-PL, CP-BP, and CP-DW were submitted, except for the submission to previous CP whitening. Figure 1 shows the study design.

During the whitening procedure and storage time in artificial saliva, all specimens were kept in a bacteriologic oven (ECB 11 Digital, Odontobrás, Ribeirão Preto, SP, Brazil) at 37°C. The staining and mouth rinse cycles were performed at room temperature (25°C).

With L*a*b* values after the teeth staining (C1, baseline values) and values obtained after different periods of tested treatments, it was possible to calculate ΔE* and then determine the efficacy after simulated home bleaching (ΔE^*_1), as well as treatments at four (ΔE^*_2), eight, (ΔE^*_3), and 12 weeks (ΔE^*_4).

The data obtained were statistically analyzed using Statistica for Windows (Statsoft, Tulsa, USA). Repeated measures analysis of variance (ANOVA) and the Tukey's test were applied at a significance level of 5%.

RESULTS

A descriptive table with ΔL*, Δb*, Δa*, and ΔE* values of all groups is presented as Table 2. At baseline, all color coordinate (L*, a*, and b*) means for each group were not statistically different.

The overall color change of specimens after whitening with 10% CP and after four, eight, and 12 weeks of dye-rinse cycles is shown in Table 3. The cross-product treatment versus time and the factors were statistically significant (p=0.001).

Concerning b* values, negative changes in Δb* values were observed, except for the CP-BP group at 12 weeks and BP and DW at all assessed periods. b* values decreased during the course of the experiment, reflecting a reduced yellowness in specimens.

Figure 2 shows reflectance curves of the mean values of the CP groups at baseline, after whitening, and after 12 weeks of dye-rinse cycles. The major reflectance was observed after bleaching; however, after 12 weeks, CP-LI still presented a level of reflectance similar to that after whitening. CP-BP showed decreased reflectance after 12 weeks of dye-rinse cycles. The major changes could be observed

Table 2: *Mean and Standard Deviation (SD) of ΔL*, Δa*, Δb*, and ΔE* for All Groups at All Measurement Times*

Group	Time	ΔL*		Δa*		Δb*		ΔE*	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
PC-LI	Bleaching	3.09	2.42	0.15	0.58	−5.05	1.45	6.41	1.34
	Four weeks	2.04	3.38	−0.19	0.52	−5.59	1.11	6.55	2.23
	Eight weeks	0.62	4.60	−0.08	0.60	−5.78	1.85	6.88	3.22
	12 weeks	0.27	3.98	0.01	0.58	−5.31	1.92	6.53	2.11
PC-PL	Bleaching	2.04	3.86	0.45	0.66	−5.30	1.49	6.84	1.52
	Four weeks	2.28	2.60	−0.12	0.56	−5.23	1.40	6.20	1.67
	Eight weeks	1.03	4.63	0.02	0.63	−4.71	1.62	6.24	2.76
	12 weeks	0.75	4.55	0.03	0.62	−3.32	1.62	5.09	2.89
PC-BP	Bleaching	4.70	1.33	0.44	0.43	−4.17	1.32	6.51	0.92
	Four weeks	2.96	1.27	−0.13	0.37	−2.24	1.65	4.03	1.35
	Eight weeks	0.22	1.96	0.00	0.46	−2.33	1.29	3.08	1.18
	12 weeks	2.95	2.20	0.52	0.51	1.52	1.80	3.90	2.02
PC-DW	Bleaching	3.63	2.28	0.23	0.46	−4.78	1.61	6.41	1.64
	Four weeks	3.11	1.50	0.04	0.54	−3.55	1.52	4.99	1.44
	Eight weeks	0.54	1.77	−0.12	0.55	−3.63	2.06	4.23	1.72
	12 weeks	2.31	2.73	0.26	0.59	−1.11	1.68	3.73	1.69
LI	Four weeks	0.42	1.02	−0.14	0.25	−1.67	1.37	2.33	0.62
	Eight weeks	2.70	1.60	−0.13	0.35	−1.20	1.97	3.63	1.37
	12 weeks	3.78	2.03	0.22	0.42	−0.96	2.15	4.46	1.99
PL	Four weeks	−1.51	2.05	0.56	0.56	−0.55	1.37	2.74	1.20
	Eight weeks	0.26	1.92	0.24	0.35	−0.27	0.87	1.89	0.99
	12 weeks	0.75	1.54	0.34	0.42	−0.64	0.95	1.89	0.90
BP	Four weeks	−2.70	1.93	0.28	0.38	3.53	1.91	4.91	1.73
	Eight weeks	−2.78	2.17	−0.31	0.46	1.82	1.40	4.02	1.21
	12 weeks	−1.35	1.86	0.66	0.60	3.92	2.05	4.75	1.63
DW	Four weeks	−1.64	1.24	0.42	0.37	0.99	0.80	2.32	0.83
	Eight weeks	−0.70	2.35	0.22	0.31	1.77	1.56	3.25	0.85
	12 weeks	−1.81	2.12	0.40	0.48	2.90	1.11	3.99	1.27

near 400-450 nm, which is the spectrum region corresponding to blue reflection.

Figure 3 presents ΔL* and ΔE* results of all groups submitted to dye-rinse cycles with no previous 10% CP whitening treatment. For ΔE*, the cross-product treatment versus time and the factors were statistically significant ($p=0.001$).

LI presented a positive ΔL* for the entire time period, showing color change direction toward the white region of L* axis and therefore whitening of the specimens. For PL, from eight weeks of rinse treatment, a whitening effect could be noted (positive ΔL*). However, for BP and DW, a darkening of the specimens could be observed (negative ΔL*).

One-way ANOVA and Dunnet's test were performed to compare the whitening effect of rinse treatment after 12 weeks with 10% CP. No rinse was able to produce the same whitening effect as that of 10% CP.

Table 3: *Mean and Standard Deviation of Color Changes After Bleaching and Four, Eight, and 12 Weeks of Dye-rinse Cycles*[a]

Group	Bleaching (ΔE^*_1)	4 weeks (ΔE^*_2)	8 weeks (ΔE^*_3)	12 weeks (ΔE^*_4)
CP-LI	6.41 (1.34) Aa	6.55 (2.23) Aa	6.88 (3.22) Aa	6.53 (2.11) Aa
CP-PL	6.84 (1.52) Aa	6.20 (1.67) ABab	6.24 (2.76) ABab	5.09 (2.89) Ab
CP-BP	6.51 (0.92) Aa	4.03 (1.35) Bb	3.08 (1.18) BCb	3.90 (2.02) Bb
CP-DW	6.41 (1.64) Aa	4.99 (1.44) ABab	4.23 (1.72) Cb	3.73 (1.69) Bb

[a] *Different capital letters mean significant differences among rows ($p<0.05$). Different lowercase letters mean significant differences among lines ($p<0.05$).*

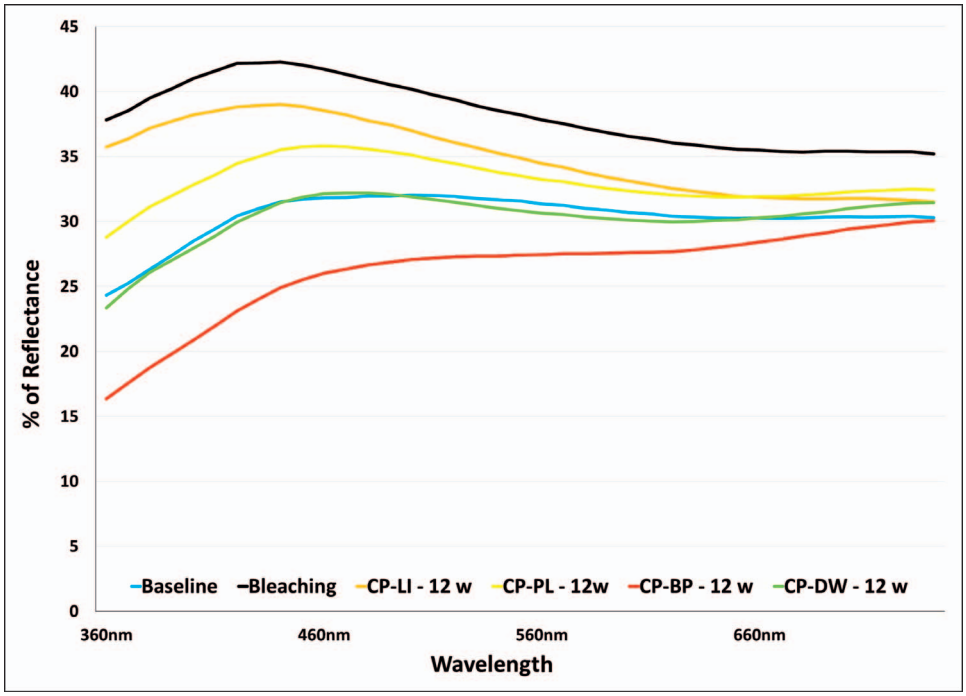

Figure 2. *Reflectance curves of CP subgroups.*

DISCUSSION

The null hypotheses tested in this study were that evaluated mouth rinses are not able to maintain the whitening outcome after whitening with 10% CP; there is no difference in whitening efficacy among the tested products; and they are not able to promote a whitening effect similar to that of supervised home whitening. According to the results, the first hypothesis was partially accepted, the second one was rejected, and the third hypothesis was accepted.

This study was performed according to the ADA Recommendations for Laboratory Testing Methods of Whitening agents.[15] The intention of staining was to standardize tooth discoloration to compare different bleaching treatments.

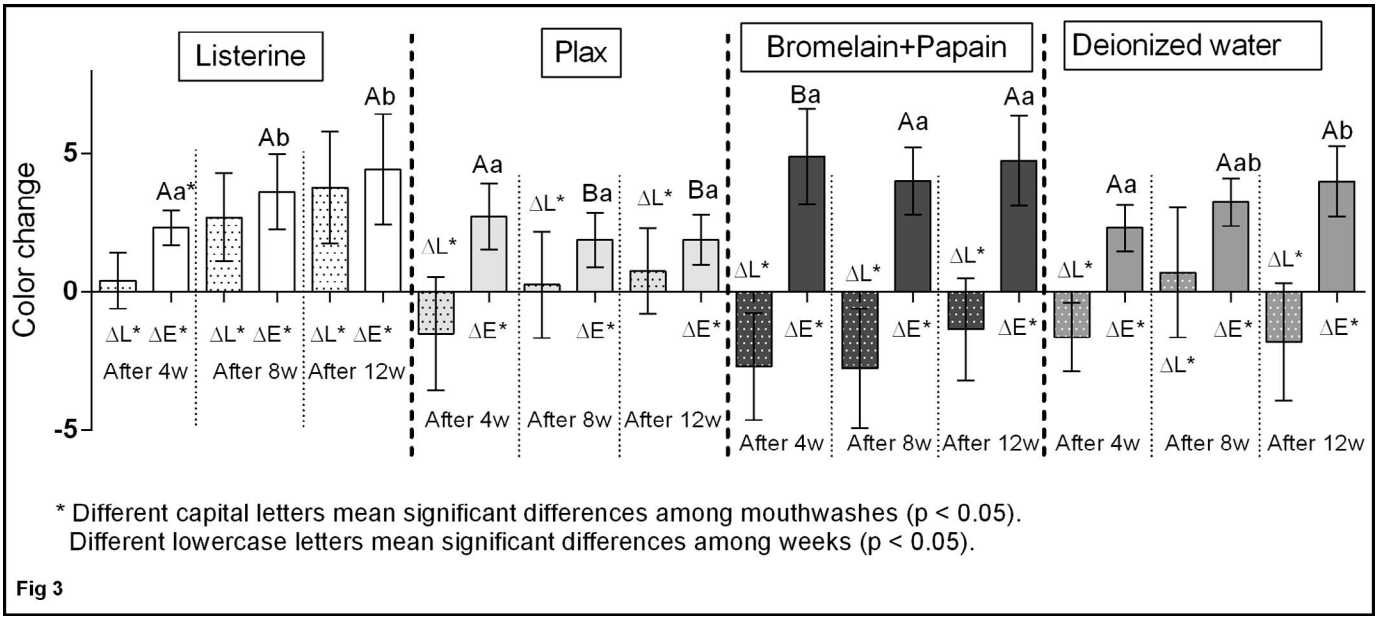

Figure 3. *Results of ΔL and ΔE for subgroups LI, PL, BP, and DW.*

After 14 days of 10% CP bleaching, the results showed that ΔL*, Δa*, and Δb* values confirmed the whitening effect for all groups (Table 2). The effectiveness of this whitening protocol is very well established in the literature; and the protocol has been successfully used since its introduction by Haywood and Heymann.[16] Some studies have noted the longevity of whitening obtained with 10% CP.[17-19] However, after one to two years of clinical follow-up, it was observed that the color of the tooth did not return to the baseline; a mild to moderate relapse might be observed. To provide the maintenance of the result obtained by conventional whitening over time, we tested the use of whitening mouth rinses after 10% CP treatment, associated to *in vitro* simulation of daily exposure to food dyes.

The whitening mouth rinses used in this study include hydrogen peroxide at different concentrations (Listerine Whitening, 2%; Plax Whitening, 1.5%) or bromelain and papain. Hydrogen peroxide is an effective oxidizer, with a known whitening effect at high concentration.[20,21] Bromelain and papain are proteolytic enzymes derived from pineapple (*Ananas comosus*) and papaya (*Carica papaya*), respectively.[22] Currently, toothpastes and mouth rinses that contain bromelain and papain in their composition are available on the market, but research about these products is still scarce.[22-24]

From the results, we found that Listerine Whitening and Plax Whitening were able to maintain the result obtained initially with 10% CP, for a simulated three-month period of dye-rinse challenge. The dye cycling represents a very intense challenge, and control group specimens, who were challenged by the dye but not exposed to mouth rinses had a significant relapse of color.

Specimens submitted solely to whitening mouth rinse treatments (LI, PL, BP, and DW groups) revealed a whitening effect for groups LI and PL after 12 weeks of treatment. The fact that Listerine Whitening and Plax Whitening mouth rinses have shown the best results, respectively, is probably due to the fact they contain hydrogen peroxide in their composition. Lima and others[5] and Torres and others[9] also performed studies with those rinses and obtained positive results. Joiner[25] stated that the effectiveness of a whitening technique depends on the concentration and exposure time to hydrogen peroxide.

CP-DW and DW control groups were expected not to show a whitening effect and in fact they did not. On the other hand, CP-BP and BP were expected to demonstrate such an effect, because previous studies with toothpastes containing the proteolytic enzymes bromelain and papain in their composition emphasized their whitening effectiveness.[22-24] Such studies, however, did not reveal the chemical composition, including the percentage of enzymes of the tested toothpastes. This may raise the hypothesis that there is another bleaching agent in these toothpastes or that bromelain and papain require a specific base for stability. We prepared an experimental 0.5% bromelain and 0.25% papain containing mouth rinse. That particular concentration was determined in a pilot study, with a lower concentration (0.1% for both substances) showing no whitening effect. Furthermore, when we searched for commercial products with bromelain and papain in their composition, the percentage of 0.5% bromelain and 0.125% papain was found for the toothpaste Janina Liquid Toothpaste Spray (Janina Ultra-White, London, England). The methylparaben and propylparaben were used as antimicrobial preservative agents. The whitening result obtained with LI and PL was lower than the result obtained by 10% CP. There is no consensus in the literature on this outcome.[9,10] Perhaps the methodology used in those studies and the dyes used could promote different results.

The use of whitening mouth rinses is recent, and few studies on this topic are available. Most of the studies, similar to the present one, are *in vitro* studies and aim to simulate a clinical situation. However, more clinical trials are needed so that we would ensure the effectiveness of these products and the best protocol to use them.

CONCLUSIONS

Within the limitations of this study, it is concluded that 1) the mouth rinses LI and PL were able to maintain the result obtained with 10% CP after 12 weeks of a dye-rinse challenge; 2) the mouth rinse Listerine Whitening presented the highest bleaching effect, followed by the mouth rinse Plax Whitening; 3) the bromelain and papain containing mouth rinse did not show a whitening effect, thus resembling the control group; and 4) the mouth rinses were unable to produce the same bleaching effect as 10% CP.

Acknowledgement

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of UNESP - São Paulo State University, São José dos Campos, Brazil.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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