Original Article

In vitro tooth whitening effectiveness of whitening mouth rinses

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Regardless of the extensive availability of mouth rinses that claim to whiten teeth, evidence of achievement of such effect is still missing. **Aim:** Therefore, this study assessed *in vitro* the whitening effectiveness of whitening mouth rinses. **Methods:** Sixty intact bovine incisors were embedded in acrylic resin and had their buccal surface flattened and polished. Then, the specimens were randomly allocated to three conventional (Colgate Plax, Cepacol and Listerine Cool Mint) and three whitening mouth rinse groups (Colgate Luminous White, Cepacol Whitening and Listerine Whitening Extreme) (n=10). Following, the specimens were immersed twice a day in the mouth rinses for one minute for 28 days. In between each immersion period, the specimens remained in artificial saliva at 37°C. Color was measured at baseline, 7, 14, 21, and 28 days using a portable spectrophotometer (Easyshade, Vita Zahnfabrik, Germany) with a 6 mm of diameter probe. Color change was analyzed considering the parameters of ΔL^*, Δa^*, Δb^* and, ultimately, ΔE^*. The whitening efficacy of the mouth rinses was analyzed using the Whiteness Index for Dentistry (WI_D). Data of Δs was analyzed using two-way ANOVA and Tukey's test ($\alpha=0.05$). **Results:** The type of mouth rinse affected significantly all the Δ parameters ($p<0.05$). A non-whitening (conventional) mouth rinse produced the highest ΔE^*, followed by the three whitening mouth rinses. The application time also affected ΔE^* ($p<0.05$), with emphasis on the third week of treatment. Only the hydrogen peroxide-containing mouth rinse (Listerine Whitening Extreme) presented a whitening effect, with an increasing trend over time. **Conclusion:** Although the overall color change was not different when comparing conventional and whitening mouth rinses, the hydrogen peroxide-containing whitening mouth rinse produces an increasing whitening trend over time. Not every mouth rinse that claims to whiten teeth produces the desired effect.

Keywords: Color. Mouthwashes. Nonprescription drugs. Tooth bleaching. Tooth bleaching agents.

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Introduction

Over-the-counter (OTC) whitening products have been available in the dental market since the 2000s, increasing the number of alternatives to meet the tooth whitening demand. Different from in-office tooth whitening and tray-based home whitening, professional prescription and orientation are not mandatory for applying OTC products[1]. Dentifrices, mouth rinses, whitening strips, dental flosses, and paint-on gels fall into the category of OTC whitening products and eventually contain low concentrations of hydrogen peroxide[1,2].

Chemically-induced tooth whitening derives from the interaction of the whitening agent, which is usually hydrogen peroxide, with the dental structure. When applied, hydrogen peroxide diffuses into the dental structure and releases reactive oxygen molecules that, through oxidation, break the double bonds of organic and inorganic coloring molecules[3]. This process relies on both the concentration of the whitening agent and the contact time with the tooth[4]. We may apply hydrogen peroxide directly to the tooth or it may result from a chemical reaction from sodium perborate or carbamide peroxide. The concentration of the former presentation may vary from 5% to 35%, and the latter, from 10% to 35%[3], but a 10% carbamide peroxide solution produces only 3.35% hydrogen peroxide[5].

Whitening mouth rinses may contain sodium hexametaphosphate, which protects the tooth surface from pigments[2]. They may also contain hydrogen peroxide at low concentrations, usually around 1.5% to 2%[2,6]. Nonetheless, this concentration is close to the hydrogen peroxide concentration of the ADA-recommended at-home whitening technique based on 10% carbamide peroxide gel[3], suggesting a whitening potential for this oral hygiene technology. However, the contact time of mouth rinses with the tooth surface is low, lasting for only a few minutes and leading to questioning about the whitening capacity of these products under the application circunstances.

While a long-term randomized controlled trial[7] confirmed the whitening efficacy and safety of professionally supervised tooth whitening procedures, studies attesting the efficacy of whitening mouth rinses are scarce. From the best of our knowledge, there are no clinical trials attempting to verify the whitening efficacy and the possible production of adverse effects by whitening mouth rinses. Also, the few existing *in vitro* studies that compare whitening mouth rinses between themselves and with other whitening alternatives vary a lot methodologically. Based on that, discrepancies related to the whitening potential, either in favor of the whitening mouth rinses[6,8,9] or towards no difference[10] may have been caused by different methodological decisions.

This scenario impairs comparisons between products and a sound decision-making process by either consumers or dental professionals responsible for indicating tooth whitening and oral hygiene products. Therefore, the present study aimed to inform about the *in vitro* whitening efficacy of whitening mouth rinses, testing the hypothesis that there is no difference in color change results from the type of mouth rinse.

Materials and Methods

Specimen preparation

For this *in vitro* study, 60 extracted intact bovine incisors were stored in 0.1% thymol solution for seven days and cleaned with pumice and Robinson brush using a low-speed handpiece (KaVo, Joinville, SC, Brazil). Next, the roots were removed using a high-speed cutting saw (Figure 1-A) and the teeth were embedded in chemically cured acrylic resin (VipiFlash, Vipi, Pirassununga, SP, Brazil) (Figure 1-B). Then, the buccal surfaces of the specimens were ground and polished using #400, 600, and 1200 grit sandpapers under water cooling, leaving a flat surface area of at least 6 mm^2 in the center and cervical regions of the tooth (Figure 1-C). Teeth that had their enamel removed during grinding and polishing were excluded. The included specimens were stored in artificial saliva prior to the experiment.
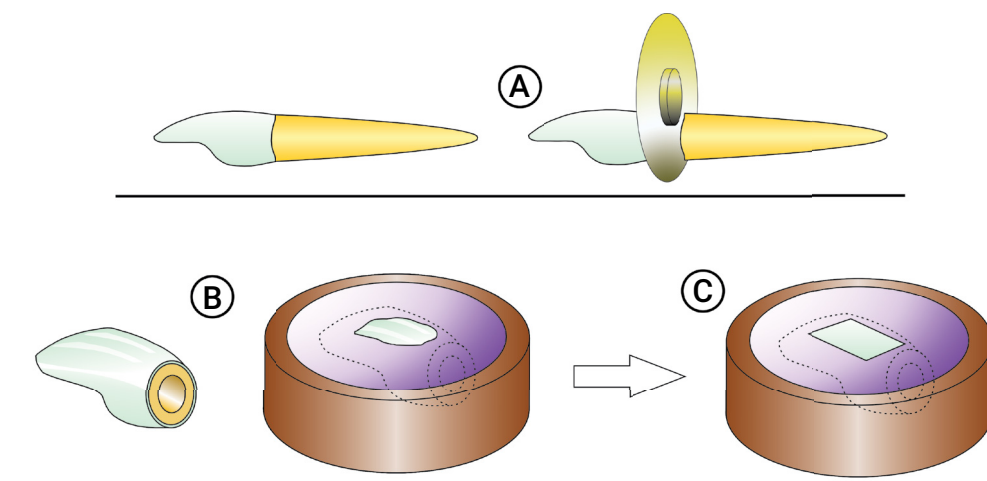

Figure 1. Specimen preparation: A – root removal; B – tooth embedding in chemically cured acrylic resin; C – grinding and polishing of the specimen´s buccal surface

Immersion protocol

An online random sequence generator (www.random.org) randomized the specimens to any of the six mouth rinse groups (n=10) (Table 1). Considering that most manufacturers recommend a one-minute mouthwash, the specimens were immersed in the mouth rinses for one minute, twice a day. The trial period lasted for 28 days. After immersion, the mouth rinses were discarded, and the specimens were returned to the artificial saliva and maintained at 37°C. The artificial saliva was replaced daily.

Color assessment

A single operator measured color with the help of an Easyshade portable spectrophotometer (Vita Zahnfabrik, Bad Säckingen, Germany), at baseline and after 7,

Table 1. Substances used in the study

Product	Manufacturer	Components
Artificial saliva (Santos[11] 2008)	Unochapecó Pharmacology Laboratory	Potassium chloride, sodium chloride, magnesium chloride, potassium phosphate, calcium chloride, nipagin, nipasol, carboxymethylcellulose, sorbitol, distilled water
Colgate Plax	Colgate-Palmolive, São Bernardo do Campo, SP, Brazil	Water, glycerin, propylene glycol, sorbitol, poloxamer 338, poloxamer 407, aroma, PEG-40 hydrogenated castor oil, cetylpyridinium chloride, potassium sorbate, sodium fluoride, sodium saccharin, citric acid, sucralose, CI 42053
Cepacol	Sanofi-Aventis Farmacêutica Ltda., Suzano, SP, Brazil	Alcohol, water, cetylpyridinium chloride, disodium EDTA, sodium saccharin, polysorbate 80, glycerin, sodium phosphate, disodium phosphate, eucalyptol, menthol, methyl salicylate, aroma, parfum (benzyl alcohol, cinnamal), CI 19140
Listerine Cool Mint	Johnson & Johnson Industrial Ltda., Yumbo, Valle, Colombia	Water, sorbitol, alcohol, poloxamer 407, benzoic acid, sodium saccharin, eucalyptol, aroma (d-limonene), thymol, methyl salicylate, sodium benzoate, menthol, CI 42053
Colgate Luminous White	Colgate-Palmolive, São Bernardo do Campo, SP, Brazil	Water, glycerin, propylene glycol, sorbitol, tetrapotassium pyrophosphate, polysorbate 20, tetrasodium pyrophosphate, zinc citrate, PVM/MA copolymer, aroma, benzyl alcohol, sodium fluoride, sodium saccharin, CI 42051
Cepacol Whitening	Sanofi-Aventis Farmacêutica Ltda., Suzano, SP, Brazil	Water, sorbitol, glycerin, sodium benzoate, sodium saccharin, sodium cyclamate, poloxamer 407, PEG-40 hydrogenated castor oil, PVP, methylparaben, aroma (eugenol, d-limonene and linalool), citric acid, propylene glycol, cetylpyridinium chloride and sodium fluoride
Listerine Whitening Extreme	Johnson & Johnson Industrial Ltda., Yumbo, Valle, Colombia	Water, alcohol, 2.5% hydrogen peroxide, aroma, poloxamer 407, sodium saccharin, menthol, phosphoric acid, disodium phosphate, sodium fluoride, sucralose

14, 21, and 28 days of immersion. Color was read after a minimum interval of two hours after the last immersion in the mouth rinses, by positioning the 6-mm tip of the spectrophotometer between the middle and cervical thirds of the specimen, perpendicular to the specimen's flat surface. A standard white background was used for all measurements, under the same lightning. Color expression was based on the three-dimensional color space of the CIEL*a*b* system consisting of three distinct axes. The L* axis varies from 0 to 100 and represents the degree of lightness in color, considering 0 totally black and 100 totally white; a* represents the variation between green (a-) and red (a+); and b* represents the variation between blue (b-) and yellow (b+). The L*, a*, and b* values in each assessment interval allowed determining a Δ for each parameter and ultimately calculating ΔE*, which is a non-directional summary of color change based on the Δs of each directional parameter, as follows:

$$\Delta E^*_{ab} = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

Analysis of whitening efficacy

The whitening efficacy of the mouth rinses was assessed using the Whitening Index for Dentistry (WI_D)[12], which is calculated as follows:

$$WI_D = 0.511L* - 2.324a* - 1.100b*$$

According to the authors[12], high positive values of the index indicate high whiteness in the specimen; low values, and even negative values indicate low values of whiteness.

Data analysis

For statistical data analysis of the Δs, the adherence of data to the normal distribution was verified using the Anderson-Darling test. Levene's test was used to check for equal variances and Grubb's test for the presence of outliers. When identified, outliers were removed from the dataset. Two-way ANOVA and Tukey's test verified the influence of time and the type of mouthwash on color and color change (Δ), at a 5% significance level (Minitab 17.0, Minitab LLC, State College, PA, USA). Data of WI_D were analyzed descriptively considering each mouth rinse and the application time period, based on the parameters set by Perez et al.[12] (2016).

Results

Figure 2 shows the changes in L*, a*, and b* by immersion in the mouth rinses.

Table 2 presents the results of ΔL*, Δa*, Δb*, and ΔE*. The mouth rinse affected ΔL* significantly (p=0.017), while time (p=0.107) and the mouth rinse x time interaction (p=0.971) were not significant. Listerine Whitening Extreme produced the lowest reduction in L* (-3.60), while Listerine Cool Mint and Cepacol Whitening generated the greatest reduction (-10.29 and -9.91, respectively). Similarly, the mouth rinse significantly affected Δb* (p<0.0001), while time (p=0.844) and the mouth rinse x time interaction (p=0.229) were not significant. Listerine Whitening Extreme produced the greatest reduction in b* values (-5.78), followed by Colgate Luminous White (-2.94). The mouth rinse affected Δa* significantly (p=0.02), which did not occur for either time (p=0.142) or the mouth rinse x time interaction (p=0.791). Listerine Whitening Extreme provided the greatest reduction in a* values (-0.88), while Cepacol Whitening increased a* (0.91). Mouth rinse (p<0.0001) and time (p=0.002) affected ΔE* significantly, which did not occur for their interaction (p=0.739). Listerine Cool Mint produced the highest ΔE* (13.72), while Cepacol showed the lowest ΔE* (7.99). The three whitening mouth rinses produced intermediate ΔE* values. The period of 21 days of mouth rinse application resulted in the highest ΔE* (14.26).

As to WI_D results, all groups presented negative mean values, except Listerine Whitening Extreme. Also, the index values per application time were always negative in all groups. Again, the exception was the hydrogen peroxide-containing Listerine Whitening Extreme, which raised the index values from baseline and became positive only after 14 days-application time (Figure 3). The mean, minimum and maximum WI_D values of the groups are as follows: Cepacol (Mean=-10.9; minimum=-28.5; maximum=5.31); Cepacol Whitening (Mean=-15.4; minimum=-27.6; maximum=0.03); Colgate Plax (Mean=-8.3; minimum=-23.6; maximum=8.9); Colgate Luminous White (Mean=-10.7; minimum=-25.1; maximum=5.2); Listerine Cool Mint (Mean=-13.4; minimum=-33.4; maximum=8.4); Listerine Whitening Extreme (Mean=0.11; minimum=-21.5; maximum=25.8).
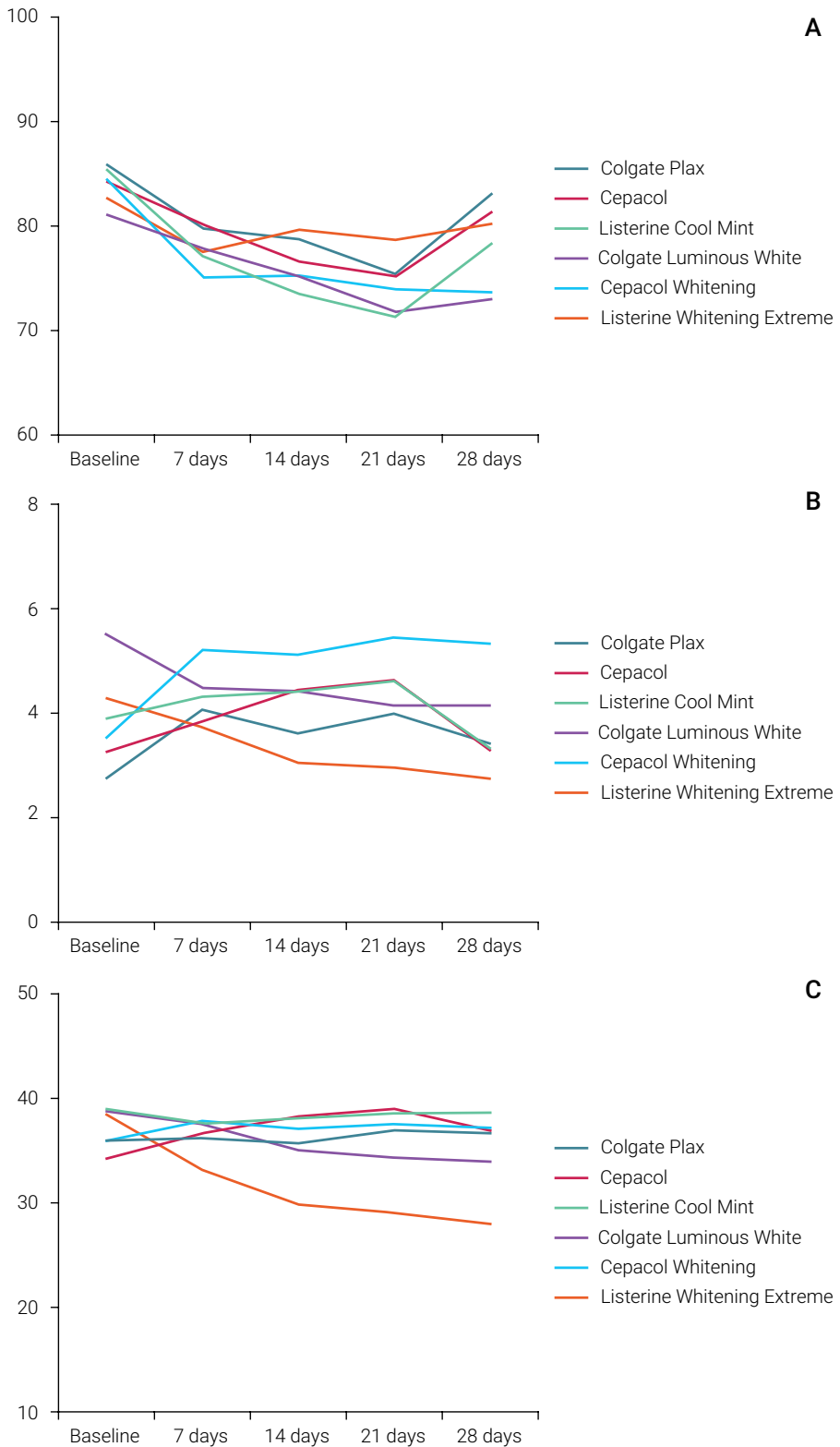
Figure 2. Behavior of L* (A), a* (B) and b* (C) parameters, respectively, throughout the experiment

Table 2. Results of ΔL*, Δa*, Δb* and ΔE* (mean and SD) for each assessment interval

	Time	Colgate Plax	Cepacol	Listerine Cool Mint	Colgate Luminous White	Cepacol Whitening	Listerine Whitening Extreme
ΔL*	B-7	-6.11 (5.68)[a]	-4.12 (6.40)[a]	-8.26 (6.99)[a]	-3.21 (14.14)[a]	-9.30 (7.33)[a]	-5.10 (9.16)[a]
	B-14	-7.14 (9.69)[a]	-7.65 (5.43)[a]	-11.83 (12.38)[a]	-5.90 (11.78)[a]	-9.17 (9.51)[a]	-3.00 (10.41)[a]
	B-21	-10.45 (8.58)[a]	-9.06 (5.47)[a]	-14.05 (13.62)[a]	-9.27 (9.99)[a]	-10.45 (8.14)[a]	-3.92 (10.63)[a]
	B-28	-2.78 (7.84)[a]	-2.93 (4.30)[a]	-7.02 (9.25)[a]	-8.07 (14.47)[a]	-10.71 (7.57)[a]	-2.39 (9.92)[a]
Δa*	B-7	1.31 (2.60)[a]	0.58 (2.63)[a]	0.42 (2.17)[a]	-1.02 (3.77)[a]	1.68 (3.02)[a]	-0.55 (2.01)[a]
	B-14	-0.45 (1.97)[a]	0.60 (0.83)[a]	0.10 (3.59)[a]	-0.07 (2.62)[a]	-0.08 (1.39)[a]	-0.67 (2.01)[a]
	B-21	1.23 (2.16)[a]	1.36 (2.67)[a]	0.72 (4.60)[a]	-1.36 (3.70)[a]	1.92 (3.32)[a]	-1.31 (2.92)[a]
	B-28	-0.64 (1.97)[a]	-0.55 (1.64)[a]	-0.99 (3.60)[a]	-0.34 (2.18)[a]	0.13 (1.53)[a]	-0.98 (2.04)[a]
Δb*	B-7	0.23 (6.23)[ab]	2.37 (2.82)[ab]	-1.31 (2.38)[ab]	-1.28 (5.20)[ab]	1.90 (2.55)[ab]	-5.29 (3.60)[bc]
	B-14	-0.45 (3.54)[ab]	1.61 (2.92)[ab]	0.47 (6.51)[ab]	-2.44 (2.63)[abc]	-0.74 (2.28)[ab]	-3.29 (5.89)[bc]
	B-21	1.01 (4.80)[ab]	4.68 (5.12)[a]	-0.38 (7.29)[ab]	-4.45 (6.67)[bc]	1.61 (3.84)[ab]	-9.38 (8.15)[c]
	B-28	0.50 (3.05)[ab]	0.26 (2.93)[ab]	1.00 (7.21)[ab]	-3.59 (3.88)[bc]	-0.69 (2.39)[ab]	-5.16 (5.73)[bc]
ΔE*	B-7	9.08 (5.46)[ab]	7.17 (4.91)[b]	9.04 (6.81)[ab]	12.41 (9.19)[ab]	11.15 (5.87)[ab]	11.17 (4.76)[ab]
	B-14	9.62 (8.04)[ab]	8.18 (5.70)[ab]	15.38 (10.07)[ab]	10.10 (4.11)[ab]	12.09 (5.41)[ab]	11.06 (5.79)[ab]
	B-21	11.69 (8.58)[ab]	11.50 (5.82)[ab]	18.47 (10.05)[a]	15.46 (3.44)[ab]	12.95 (5.84)[ab]	15.49 (6.18)[ab]
	B-28	7.35 (4.88)[b]	5.12 (3.25)[b]	11.99 (6.90)[ab]	15.07 (7.97)[ab]	11.89 (6.03)[ab]	11.15 (5.86)[ab]

* Different letters indicate statistically significant differences between groups within each Δ
** B means Baseline − each value represents the Δ between the baseline and each mouth rinse application time
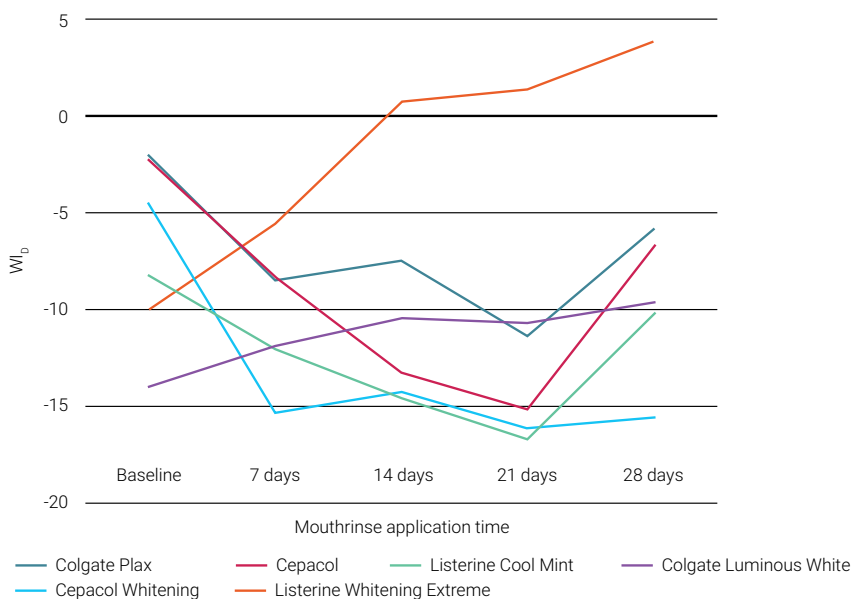
Figure 3. WI_D results of each mouth rinse over time

Discussion

The limited evidence on the whitening effect of whitening mouth rinses associated with the extensive availability of these over-the-counter (OTC) products motivated this study. The influence of the type of mouth rinse on the overall color change (ΔE*) led to the rejection of the study hypothesis. Interestingly, a conventional (non-whitening) mouth rinse (Listerine Cool Mint) produced the highest ΔE*, followed by whitening mouth rinses, which did not show statistical differences. On the other hand, WI_D results showed a growing whitening effect over time from the hydrogen peroxide-containing mouth rinse Listerine Whitening Extreme (Figure 3), suggesting that the presence of this active ingredient is key for a mouth rinse that claims to whiten teeth to, in fact, provide this effect.

The whitening potential of whitening substances has been assessed, traditionally, by changes in CIEL*a*b* coordinates, expressed as Δs (ΔL*, Δa*, Δb* and ΔE*)[6-8]. Still, most recently, the Whiteness Index for Dentistry (WI_D) was developed aiding at determining more precisely the amount of whiteness produced by tooth whitening technologies[12]. According to Perez et al.[12] (2016), the index has a very straightforward interpretation: the higher the positive index value, the whiter the tooth. Lower values, and even negative values are considered as poorly associated to whiteness[12]. In this study, all WI_D values at baseline were negative. The only ascending tendency by immersion in mouth rinse over time was observed with the whitening mouth rinse containing hydrogen-peroxide, which only achieved positive values after 14 days of immersion.

As to CIEL*a*b* measures, the increase of lightness (increasing L*) and the reduction of yellowness (decreasing b*) are the main inducers of tooth whitening. The reduction of redness (decreasing a*) affects whitening to a lesser extent[8,13]. Our results showed a reduction of L* (Figure 2 and Table 2), irrespective of the treatment group or application time, characterizing darkening of the tooth structure throughout the experiment. Figure 2 also shows a tendency of lightness recovery by some mouth rinses from day 21 to day 28, although not fully restoring it. The presence of organic substances in the artificial saliva[10] and the demineralizing effect of some low pH mouth rinses[8] supposedly explain the lowering effect of lightness for *in vitro* settings.

A significant yellowness (Δb*) reduction was observed only for the 2.5% hydrogen peroxide Listerine Whitening Extreme mouth rinse. This mouth rinse also presented the lowest reduction of L*, meaning a reduced darkening of the tooth, although not statistically significant. Finally, this mouth rinse, along with Colgate Luminous White, produced negative a* (again, not statistically significant), which means a reduced redness that tends to manifest after longer periods of whitening and to a lesser extent, representing improved tooth whitening[8].

Some substances with whitening potential are common to whitening toothpastes and whitening mouth rinses. Phosphate-derived substances such as pyrophosphate, tripolyphosphate, and hexametaphosphate have prevented superficial stains[14]. Tetrapotassium pyrophosphate and tetrasodium pyrophosphate are present in Colgate Luminous White, which also tended to reduce a* and b* values. Moreover, considering the general results, it seems that effective tooth whitening relates somewhat to the

presence of hydrogen peroxide[15]. Among the three whitening mouth rinses, Listerine Whitening Extreme was the only one to contain hydrogen peroxide, to reduce b* substantially (Figure 2) and to present a true whitening effect (Figure 3). Torres et al.[6] (2013) observed a similar *in vitro* whitening effect for a mouth rinse containing 2% hydrogen peroxide and 10% carbamide peroxide gel. Considering that the 10% carbamide peroxide decomposes into 3.35% hydrogen peroxide[3], the concentrations of the active whitening substance are similar.

Methodological disparities hindered the comparison of results of previous and the present *in vitro* study. They involve the type of tooth (human[8] or bovine[6,9,10]), pre-staining (yes[6,9,15]/no[8,10]), varying application protocols (number of immersions – 1x[6], 2x[8,9], 3x[10]; and immersion times – 1 minute[6,8,9] or 2 minutes[10]), assessment times (30 days[10], 45 days[8], up to 8 weeks[9], and up to 12 weeks[6,15]), and color assessment procedures (standard digitized photographs[8] or spectrophotometers[6,9,10]). Regarding the choices made in this study, bovine teeth have long been a reasonable alternative to mimic the characteristics of human teeth[10]. We did not pre-stain the teeth in this study, because artificial staining creates conditions to confirm a whitening effect in the presence of chromogenic molecules that may not be very intense in the teeth of a person demanding tooth whitening. Most mouth rinse manufacturers recommend a one-minute immersion. Although there is no specific recommendation on the frequency of daily applications, twice a day[8,9] would be a clinically feasible frequency and it would improve the whitening effect as compared with a single daily application. The total application time of 28 days almost doubled some enhanced protocol times[7,16] for tooth whitening with carbamide or hydrogen peroxide, which rarely takes one month in present days. The authors understand that this application time was sufficient to confirm any whitening effect from whitening mouth rinses. Finally, the digital spectrophotometer has long been reported as a reliable method for measuring the color change in whitening studies[17] and, together with the WI_D[12], it presents a good scenario of the whitening effect of whitening substances.

We assessed color weekly within a 28-day application period, generating four assessment intervals. The application time only affected ΔE*, with emphasis on the third week of whitening, which presented the highest ΔE* values from the baseline (Table 2). Nonetheless, the yellowing reduction with Listerine Whitening Extreme was evident from day 7 (Figure 2). Chemically-induced peroxide tooth whitening products showed accumulation over time, enhancing the total contact time with the tooth structure[4]. Therefore, we may speculate that a longer experiment period would show clearer results from the hydrogen peroxide mouth rinse and that it could even affect the overall color change.

The color of the mouth rinses varied. Products from Cepacol had a yellow color, while those from Colgate were blue and those from Listerine presented a blueish/greenish color. Regardless of that, one believes that the product color may not have influenced the color results of teeth, since they remained in contact with the substances for a short period. Also, following the immersion protocols, the artificial saliva with some residual pigment was exchanged daily. One limitation of the study was that the pH of the mouth rinses was not assessed.

Whitening mouth rinses are easy to acquire and represent low-cost alternatives for tooth whitening[10]. However, *in vitro* studies using different methods showed controversial results[6,8,10], which are difficult to pool and compare. This study, for instance, used the conventional non-whitening counterparts as controls and revealed that some whitening mouth rinses do not achieve the expected whitening results. Dental researchers and dental clinicians could incorporate this information to design comprehensive independent clinical studies and advise potential consumers of whitening products. As for therapeutic technology, a sound decision-making process and the indication of whitening mouth rinses for tooth whitening depend on the scrutiny of proven effects resulting from randomized controlled trials attesting clinical efficacy and safety.

The overall color change produced by whitening mouth rinses in one month is not different from that produced by conventional mouth rinses. The whitening mouth rinse containing hydrogen peroxide reduced yellowing significantly during such application period and was the only whitening mouth rinse to present a true whitening effect.

Acknowledgements

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