THE JOURNAL OF PROSTHETIC DENTISTRY

RESEARCH AND EDUCATION

Effectiveness and one-year whiteness stability of different in-office bleaching agents and alternative protocols

Bibiana Gabardo Perez Mariano da Rocha, PhD,[a] Javier Ruiz-López, PhD,[b] María M. Pérez, PhD,[c] Bruna Gaidarji, MSc,[d] Giovanna Teixeira Frasson, DDS,[e] and Letícia Brandão Durand, PhD[f]

ABSTRACT

Statement of problem. In-office bleaching has been widely researched. However, few studies have evaluated alternative protocols for this procedure. Moreover, information on the long-term stability of in-office whitening is limited.

Purpose. The purpose of this in vitro study was to evaluate the effectiveness and 1-year stability of in-office bleaching with 35% hydrogen peroxide (35%HP) and 37% carbamide peroxide (37%CP) using traditional and alternative protocols.

Material and methods. Forty human third molars were stained with tea and allocated to groups (n=10). Traditional protocols consisted of 3 applications of 35%HP for 15 minutes and 1 application of 37%CP for 45 minutes. Alternative protocols consisted of 1 application of 35%HP for 45 minutes and 3 applications of 37%CP for 45 minutes. Protocols were applied for 3 weeks. CIELab color coordinates were measured at baseline and weekly during treatment and at 1-week, 6-month, and 1-year follow-ups. Effectiveness and stability of the bleaching treatments were interpreted using 50:50% perceptibility and acceptability thresholds. CIELab, chroma, hue angle, and whiteness index were analyzed using the Wilcoxon signed-rank test (α=.05).

Results. All protocols showed improvement in bleaching after the first week ($P\leq.005$). All bleaching procedures presented excellent whitening outcomes. Alternative protocols showed a larger rebound effect after 1 year, indicating less stability. For the whiteness index, no differences between the completion of the treatments and 1-year follow-up was found for the bleaching treatments and protocols ($P>.05$), except for the 37%CP alternative protocol ($P=.005$).

Conclusions. All tested protocols presented excellent effectiveness in bleaching. Traditional protocols exhibited a greater whiteness stability, while the alternative protocols showed a greater rebound effect after 1 year. (J Prosthet Dent xxxx;xxx:xxx-xxx)

In-office bleaching has been a conservative and popular esthetic treatment,[1–3] typically with hydrogen peroxide (HP)[4–6] or carbamide peroxide (CP)[6–12] in different concentrations and commercial presentations.[6–8,11] New protocols[6] and different bleaching agents have been proposed,[2,13] and bleaching may be performed with HP at concentrations from 15% to 40%[14–18] and CP at concentrations from 10% to 40%.[10,14–16,18]

In-office bleaching is indicated for the rapid improvement of discolored teeth with safe results.[6–8,13,19–21] The 35% HP has been the most widely used formulation for the in-office technique.[2,13,22,23] Although studies have demonstrated adequate whiteness effectiveness[13,23] and patient satisfaction[13] with HP in-office bleaching, this technique may cause dentin sensitivity[5,7,11,13,14,20,22,24] immediately after its application.[7,13,22] Alternatively, to avoid dentin sensitivity, CP in high concentrations may be used for in-office bleaching.[7] However, although the sensitivity is reduced, less whitening effect has been reported compared with HP.[23]

Supported in part by the Government of Andalusia, grant number P20–00200.
Funding for Open Access publication provided by University of Granada (UGR), Consortium of University Libraries of Andalusia (CBUA), Spain.
No conflict of interest.
[a]Assistant Professor, Department of Restorative Dentistry, School of Dentistry, Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil.
[b]Assistant Professor, Department of Optics, Faculty of Science, University of Granada, ibsGranada, Granada, Spain.
[c]Full Professor, Department of Optics, Faculty of Science, University of Granada, ibsGranada, Granada, Spain.
[d]PhD Student, Post-Graduate Program in Oral Science, Division of Restorative Dentistry, Federal University of Santa Maria (UFSM), Rio Grande do Sul, Brazil.
[e]Private Practice, Santa Maria, Rio Grande do Sul, Brazil.
[f]Professor, Department of Restorative Dentistry, School of Dentistry, Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil.

Clinical Implications

Excellent bleaching effectiveness was observed for all tested protocols using 35% hydrogen peroxide and 37% carbamide peroxide. In addition to the effectiveness of the bleaching, the stability and possibility of a rebound effect should be considered while making treatment decisions. The protocols recommended by the manufacturers for both bleaching agents were more stable, while alternative protocols presented a greater rebound effect after 1 year.

The use of high CP concentration has been found to be an effective and safe approach to in-office bleaching.[7,8] CP dissociates into HP and urea at approximately one third of the original concentration.[6,15,25,26] Furthermore, the slower degradation of CP compared with HP[6,17,26] enables its use for a longer time, with reduced risk of tooth sensitivity.[27] Dental whitening effectiveness is related to the concentration of the bleaching agent, the number of applications in each session, the time that it remains in contact with the dental surfaces, and its ability to reach the chromophore molecules.[6,10,28] A recent study reported that the application time is more important for color change than the HP concentration. For this reason, alternative protocols based on HP reactivity have been studied on an ongoing basis.[14]

Although in-office bleaching has been widely researched, a well-established determination regarding the amount of time and the protocol for applying the products to achieve the optimal whitening effect with less sensitivity and reduced structural change is lacking.[8,13,26] The characteristics related to the dissociation, degradation, and release of CP[26] make it possible to apply the product for a longer time[10,26,28] and still maintain reactivity and prevent dentin sensitivity.[29] Considering that over 50% of CP remains active after 2 hours,[26,30] a suitable alternative for patients who request rapid results with reduced sensitivity would be to increase the application time in an effort to enhance the whitening effect. In this respect, evaluating the effectiveness of in-office bleaching by using different protocols may contribute to optimizing treatment and improving esthetic outcomes in both the short and long term. To date, few studies have evaluated alternative protocols for in-office dental bleaching.[29] Moreover, information on how long the whitening effect lasts over time and on the probability of a rebound effect in color after in-office bleaching is limited.[8–10,13,22] Therefore, the purpose of the present in vitro study was to evaluate the effectiveness and 1-year color stability of in-office bleaching with 35% hydrogen peroxide (35%HP) and 37% carbamide peroxide (37%CP) using traditional and alternative protocols. The research hypotheses were that the bleaching protocols tested would promote different whitening effectiveness upon the completion of the bleaching treatment (T3-T0) and different whiteness stability at subsequent follow-up evaluations (T6-T3).

MATERIAL AND METHODS

This in vitro study evaluated the effectiveness and whiteness stability of 2 in-office bleaching agents (35% HP and 37%CP) (Table 1) using different protocols (traditional and alternative) on extracted human teeth stained with black tea. The color measurements were conducted at baseline (T0) and weekly (T1, T2, and T3) until treatment was completed and again at 1-week (T4), 6-month (T5), and 1-year follow-ups (T6). This study was reviewed and approved by the research ethics committee of the participating institution (CAAE 84178418.8.0000.5346, 2.576.646). The study design is presented in Figure 1. Forty human permanent intact third molars with no restorations, carious lesions, fractures, or discolorations such as from tetracycline staining or dentinogenesis imperfecta were selected from a tooth bank. Their surfaces were polished with a rubber cup and pumice and stored in distilled water. The roots were sectioned 2.0 mm apical to the cement-enamel junction with a diamond disk (Extec 7″×. 0.025 × 0.5; Extec Corp) on a cutting machine (Labcut 1010; Extec Corp), and the pulpal tissue was removed.[18]

All the teeth were artificially stained by immersion for 7 days in black tea in accordance with a previously reported protocol.[18] After staining, the specimens were distributed into 4 groups (n=10) (Fig. 1) using an online randomizer (http://www.randomizer.org). The number of specimens was estimated by using the Open-Source Epidemiologic Statistics for Public Health, version 3.01 (free open source software - https://www.openepi.com), with a confidence interval of 95% and power of 90%. The mean values of the CIELab color difference and

Table 1. Composition and information on bleaching agents

Bleaching Agent	Manufacturer	Composition	Batch #
Whiteness 35% (HP)	FGM	35% hydrogen peroxide, thickener, red dye, glycol, water	141217
Power Bleaching 37% (CP)	BM4	37% carbamide peroxide, potassium oxalate, sodium fluorite, thickener, neutralizer, preservative, humectant, purified water	130921

CP, carbamide peroxide; HP, hydrogen peroxide.

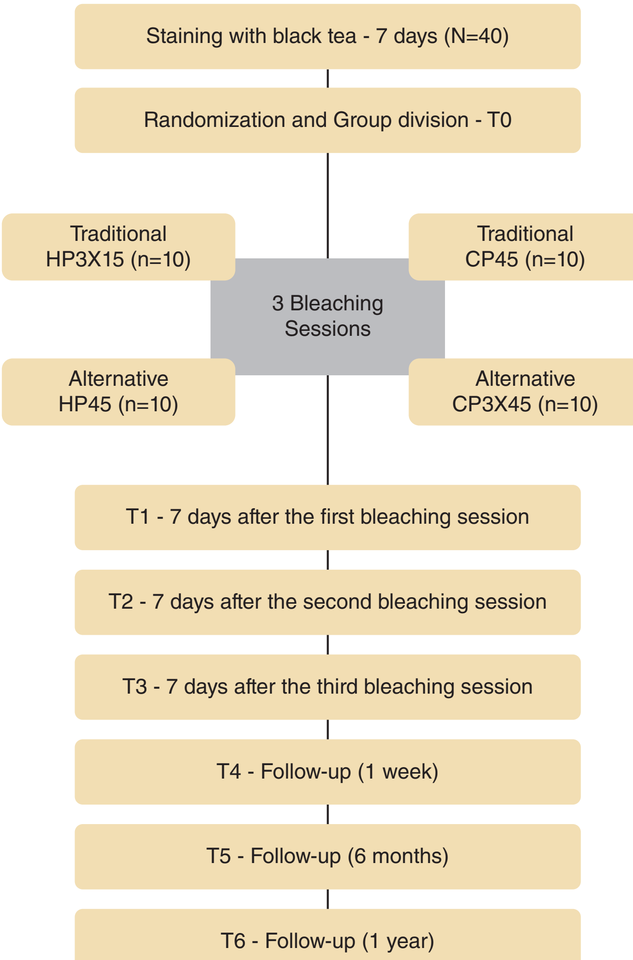

Figure 1. Study flowchart. CP45, carbamide peroxide, 1 application of 45 min; CP3×45, carbamide peroxide, 3 applications of 45 min each; HP3×15, hydrogen peroxide, 3 applications of 15 min; HP45, hydrogen peroxide, 1 application of 45 min; T0, baseline.

standard deviations (35%HP – ΔE^*_{ab} 19.13 [3.28] and 37%CP – ΔE^*_{ab} 13.60 [3.50]) reported by 2 in vitro studies[3,25] conducted on extracted teeth stained with tea were used for sample calculation, resulting in a minimum sample size of n=8 for each group.

Two in-office bleaching agents, 35%HP and 37%CP (Table 1), and 2 protocols, traditional and alternative, were used in this study (Fig. 1). The traditional protocols consisted of 3 applications of HP for 15 minutes (HP3×15) and 1 application of CP for 45 minutes (CP45). The alternative protocols were 1 application of HP for 45 minutes (HP45) and 3 applications of CP for 45 minutes each (CP3×45). All the groups were bleached weekly for 3 consecutive weeks. The specimens were stored for 1 year in deionized water at 37 °C, changed weekly.[31]

The CIELab coordinates were measured using a clinical spectrophotometer (Easyshade Advance 4.0; Vita Zahnfabrik) with an aperture size of 6 mm.[32] The chroma (C*), and the hue angle (h°) were calculated according to a* and b* coordinates.[33] The clinical spectrophotometers' repeatability intraclass correlation coefficient (ICC) ranges from 0.992 to 0.994, showing accuracy of 93.75%.[34]

After staining the specimens, color measurements were made in triplicate with the clinical spectrophotometer at different evaluation times: at baseline (T0) and weekly (T1, T2, and T3), until completion of the treatments and also at 3 follow-up times: at 1 week (T4), 6-months (T5), and 1-year (T6). Each specimen had an individual silicone guide (Zetalabor; Zhrmack Labordental) with a central orifice to standardize the location of the color measurements.

The new whiteness index for dentistry[35] (WI_D) was calculated from the CIELab color coordinates from $WI_D = 0.511L^* - 2.234a^* - 1.100b^*$. The whitening effectiveness and the stability of the treatments were evaluated by the CIEDE2000 color difference formula (ΔE_{00}) and by ΔWI_D between consecutive times.

The CIEDE2000 color difference (ΔE_{00}) was calculated from[33].

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 \cdot + R_T\left(\frac{\Delta C'}{k_C S_C}\right)\left(\frac{\Delta H'}{k_H S_H}\right)\right]^{\frac{1}{2}}$$

The ΔE_{00} color shift of each protocol was analyzed according to differences in the CIEDE2000 lightness, chroma, and hue values[36–38]:

$$\Delta L_{00} = \frac{\Delta L'}{k_L S_L}; \Delta C_{00} = \frac{\Delta C'}{k_C S_C}; \Delta H_{00} = \frac{\Delta H'}{k_H S_H}.$$

Lastly, whiteness differences between consecutive times (ΔWI_D) were calculated from[39] $\Delta WI_D = WI_D(B) - WI_D(A)$.

The effectiveness and stability of the bleaching treatments and the interpretation of the bleaching-dependent color and whiteness differences were based on 50:50% color and whiteness perceptibility (PT=0.8 and WPT=0.7) and acceptability thresholds (AT=1.8 and WAT=2.6)[39,40] and the interpretation ratings reported by Paravina et al.[41] A whitening outcome with a difference exceeding $\Delta E_{00}>5.4$ or $\Delta WI_D>7.8$ was assumed to have excellent bleaching effectiveness. Very good bleaching effectiveness (VGE) was considered when $3.6<\Delta E_{00}\leq 5.4$ or $5.2<\Delta WI_D \leq 7.8$. Good bleaching effectiveness (GE) was considered when $1.8<\Delta E_{00}\leq 3.6$ or $2.6<\Delta WI_D\leq 5.2$. Bleaching was considered moderately effective (ME) when $0.8<\Delta E_{00}\leq 1.8$ or $0.7<\Delta WI_D\leq 2.6$. Bleaching was considered not effective (NE) when $\Delta E_{00}\leq 0.8$, or $\Delta WI_D\leq 0.7$.[39–41]

Table 2. Mean values ±standard deviation of CIELab color coordinates (L*, a*, b*, C*, h°) and WI_D of each bleaching treatment and protocol at baseline (T0), at different treatment times (T1, T2, T3), and at follow-up evaluation times (T4, T5, T6)

Technique	L*	a*	b*	C*	h°	WI_D
HP3×15-T0	68.1 ±7.7^{B}	6.2 ±3.5^{A}	38.0 ±5.3^{A}	38.6 ±5.6^{A}	81.2 ±4.2^{C}	-21.4 ±16.6^{C}
HP3×15-T1	76.2 ±5.6^{AB}	0.5 ±1.9^{A}	27.4 ±5.4^{AB}	27.4 ±5.5^{A}	89.6 ±3.7^{CB}	7.6 ±12.3^{BC}
HP3×15-T2	77.7 ±5.6^{A}	-0.1 ±1.8^{B}	25.8 ±5.7^{B}	25.9 ±5.7^{B}	90.9 ±3.6^{AB}	11.6 ±12.6^{B}
HP3×15-T3	77.0 ±5.8	0.4 ±1.9^{B}	26.6 ±5.3^{C}	26.7 ±5.3^{B}	89.9 ±3.5^{A}	9.2 ±12.6^{A}
HP3×15-T4	77.7 ±4.9	-0.7 ±1.2	23.5 ±3.6^{C}	23.5 ±3.6	91.9 ±2.7	15.5 ±8.0^{A}
HP3×15-T5	76.6 ±5.1	-0.4 ±1.6	23.7 ±5.0	23.8 ±4.9	91.8 ±4.5	14.0 ±10.7
HP3×15-T6	76.7 ±3.9	-0.5 ±2.1	25.1 ±5.1	25.3 ±5.0	92.1 ±5.2	12.7 ±11.2
HP45-T0	70.7 ±3.9^{B}	4.4 ±1.9^{A}	36.8 ±4.8^{A}	37.0 ±5.0^{A}	83.4 ±2.0^{A}	-14.5 ±11.7^{A}
HP45-T1	76.2 ±3.9^{B}	0.7 ±1.4^{A}	27.8 ±4.9^{A}	27.9 ±5.0^{A}	88.9 ±2.6^{A}	6.7 ±9.8^{A}
HP45-T2	77.7 ±4.6^{A}	0.0 ±1.7	26.0 ±5.9	26.1 ±5.9	90.7 ±3.8	11.1 ±12.1
HP45-T3	76.7 ±4.8^{A}	0.3 ±1.4	26.3 ±4.8	26.3 ±4.8	89.8 ±3.0	9.6 ±10.3
HP45-T4	76.8 ±4.7	-0.1 ±1.7	24.6 ±5.4	24.7 ±5.4	90.9 ±4.0	12.3 ±11.5
HP45-T5	77.3 ±5.4	0.0 ±1.1	24.1 ±3.9	24.1 ±3.9	90.3 ±2.4	13.1 ±7.6
HP45-T6	73.6 ±5.3	2.0 ±2.2	30.9 ±6.1	31.0 ±6.2	86.8 ±3.5	-1.1 ±13.0
CP45-T0	71.3 ±3.3^{B}	3.7 ±1.4^{A}	33.8 ±3.8^{A}	34.0 ±3.8^{A}	83.9 ±2.2^{B}	-9.4 ±8.0^{B}
CP45-T1	76.5 ±4.3^{AB}	0.7 ±1.0^{AB}	25.1 ±3.0^{A}	25.2 ±3.0^{AB}	88.7 ±2.3^{AB}	9.9 ±7.0^{AB}
CP45-T2	79.3 ±4.3^{A}	-0.3 ±1.0^{B}	22.8 ±3.5	22.8 ±3.4^{B}	91.2 ±2.9^{A}	16.3 ±7.1^{A}
CP45-T3	79.3 ±5.0	0.1 ±0.7^{C}	23.8 ±3.2	23.8 ±3.2^{C}	89.9 ±1.6	14.1 ±5.7
CP45-T4	75.8 ±8.4	-0.4 ±0.8^{C}	21.9 ±3.3	21.9 ±3.3^{C}	91.4 ±2.4	15.6 ±8.0
CP45-T5	77.8 ±5.2	-0.1 ±1.1	23.1 ±4.3	23.2 ±4.3	90.6 ±3.1	14.5 ±8.1
CP45-T6	79.0 ±4.3	-0.2 ±1.8	24.8 ±6.2	24.8 ±6.2	91.7 ±5.0	13.6 ±12.3
CP3×45-T0	67.6 ±4.2^{A}	6.0 ±3.0^{A}	38.2 ±6.5^{A}	38.7 ±7.0^{A}	81.4 ±2.5^{B}	-21.5 ±14.4^{B}
CP3×45-T1	75.2 ±4.1^{A}	1.1 ±1.1^{AB}	28.8 ±5.6^{A}	28.8 ±5.6^{AB}	87.9 ±2.0^{AB}	4.1 ±7.8^{B}
CP3×45-T2	76.8 ±3.8	0.1 ±1.1^{BC}	27.0 ±7.1	27.0 ±7.1^{BC}	90.3 ±2.4^{A}	9.3 ±8.8
CP3×45-T3	76.5 ±4.6	1.1 ±1.0^{C}	28.6 ±5.7	28.6 ±5.7^{C}	88.0 ±1.8	5.2 ±7.2
CP3×45-T4	75.3 ±2.8	0.6 ±1.5	27.8 ±7.0	27.8 ±7.0	89.3 ±3.4	6.4 ±10.2
CP3×45-T5	75.5 ±3.8	1.3 ±1.4	27.1 ±7.2^{B}	27.2 ±7.3	87.6 ±2.4^{C}	5.7 ±10.5^{A}
CP3×45-T6	73.9 ±4.3	1.8 ±1.7	31.2 ±5.5^{B}	31.3 ±5.5	87.0 ±2.7^{C}	-0.7 ±9.5^{A}

CP45, carbamide peroxide, 1 application of 45 min; CP3×45, carbamide peroxide, 3 applications of 45 min each; HP3×15, hydrogen peroxide, 3 applications of 15 min; HP45, hydrogen peroxide, 1 application of 45 min; T0, baseline; T1, 1 week after the first bleaching session; T2, 1 week after the second bleaching session; T3, 1 week after the third bleaching session; T4, 1-week follow-up; T5, 6-month follow-up; T6, 1-year follow-up; WI_D, whiteness index for dentistry.

Same uppercase letters in same column and treatment and protocol indicate statistically significant difference ($P<.05$).

To analyze statistically the changes of the color coordinates L*, a*, b*, C*, h°, and WI_D for the different agents and protocols during time, the related samples Wilcoxon signed-rank test was used ($\alpha=.05$), since equal variances and normal distribution of the data could not be assumed for all color coordinates and WI_D groups after performing the Levene test of homogeneity of variance and Shapiro-Wilk test, respectively. The Bonferroni correction was applied considering the multiple comparisons performed. Statistical analysis was performed using a standard statistical software package (IBM SPSS Statistics, v20.0; IBM Corp).

RESULTS

Table 2 presents the CIELab color coordinates and the WI_D values for HP3×15, HP45, CP45, and CP3×45 at baseline (T0), at each different treatment time point (T1, T2, and T3) and at the follow-up evaluations (T4, T5, and T6). All bleaching treatments and protocols showed an improvement in the bleaching effectiveness after the first bleaching session (T1), considering the mean values observed in Table 2. The traditional protocols (HP3×15 and CP45) remained stable at the T6 follow-up evaluation in comparison with the mean values reported at the 1-week follow-up (T4), ($P\geq.066$). The alternative protocol HP45 did not demonstrate the same behavior, since the mean of all color parameters values was, in general, statistically different only at the first treatment time (T1) ($P\leq.005$). As for the alternative protocol CP3×45, the b*, h°, and WI_D values were statistically different from T5 at the T6 follow-up evaluation ($P\leq.005$). Regarding the WI_D, no statistically significant differences were found between T3 and T6 for the bleaching treatments or protocols ($P\geq.093$), except for CP3×45 ($P=.005$).

Figure 2 shows the color difference values for bleaching treatments and protocols between each consecutive application and follow-up evaluation time point in comparison with the PT and AT. In addition, the magnitude of the lightness, chroma, and hue components in the total CIEDE2000 color difference are depicted by the height of the fragments in the bars. In general, the color differences achieved by the bleaching protocols continued to progress at the evaluation time points from T0 to T4, except for T3 to T2, which remained in the range of ME when $0.8<\Delta E_{00}\leq1.8$. The ΔE_{00} values were predominantly higher at T1-T0, demonstrating excellent effectiveness ($\Delta E_{00}>5.4$) for all the bleaching treatments and protocols. In addition, all the treatments and protocols presented ΔE_{00} values above the visual PT between consecutive applications after T1.

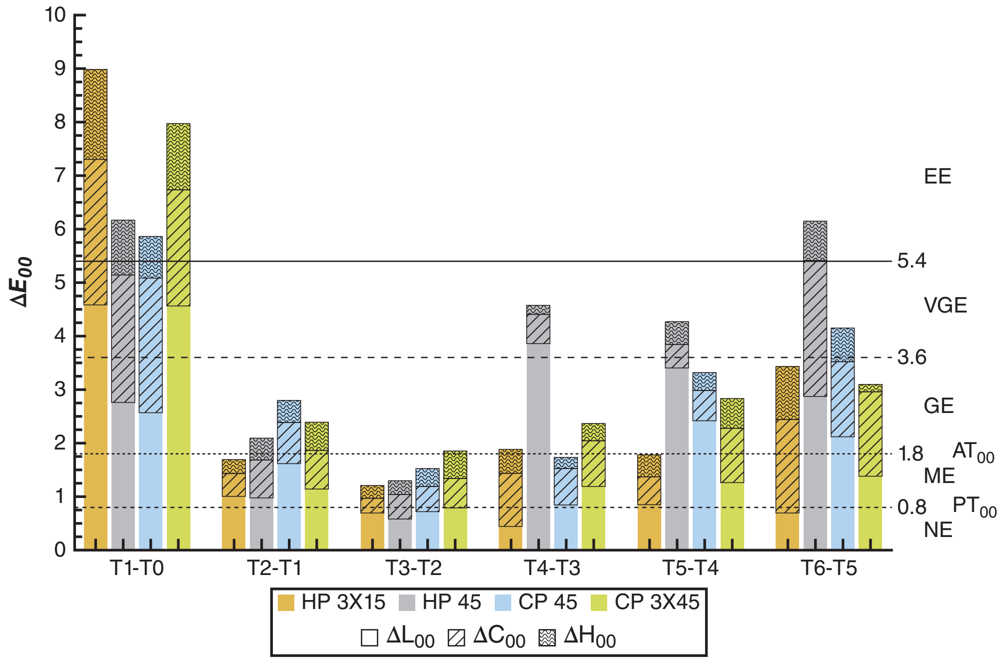

Figure 2. CIEDE2000 (ΔE_{00}) color shift for bleaching treatments and protocols among each consecutive application and follow-up evaluation times, with threshold interpretation ratings and influence of lightness, chroma, and hue differences on total color shifts. CP45, carbamide peroxide, 1 application of 45 min; CP3×45, carbamide peroxide, 3 applications of 45 min each; EE, excellent effectiveness (ΔE00>5.4); GE, good effectiveness (ΔE00>1.8≤3.6); HP3×15, hydrogen peroxide, 3 applications of 15 min; HP45, hydrogen peroxide, 1 application of 45 min; ME, moderately effective (ΔE00>0.8≤1.8); NE, not effective (ΔE00≤0.8); T0, baseline; T1, 1 week after first bleaching session; T2, 1 week after second bleaching session; T3, 1 week after third bleaching session; T4, 1-week follow-up; T5, 6-month follow-up; T6, 1-year follow-up. VGE, very good effectiveness (ΔE00>3.6≤5.4); ΔE00, CIEDE2000 color difference.

Mostly, the color changes continued to progress between the evaluation time points but with different values for each consecutive application and with a color rebound effect at the follow-up time points of T3, T5, and T6. Changes in color were mainly due to changes in lightness and chroma for all the bleaching treatments and protocols, albeit with different values for each consecutive application and follow-up evaluation time point (Fig. 2).

Figure 3 shows the difference in whiteness values for bleaching treatments and protocols between each consecutive application and follow-up evaluation time point in comparison with the WPT and WAT. All the bleaching treatments and protocols presented ΔWI_D>7.8 between T1-T0, demonstrating excellent effectiveness. Regarding the effectiveness among all the treatments, HP3×15 achieved the highest ΔWI_D value. At time point T2, a progress in whiteness was observed with differences higher than the WAT. However, at time point T3, negative differences in the WI_D were found, indicating a rebound effect in whiteness with perceptible whiteness differences for all strategies and protocols. At time point T4, ΔWI_D became positive again, showing a progression for WI_D, and HP3×15 presented the highest whiteness difference with ΔWI_D>5.2, demonstrating very good bleaching effectiveness. After the 6-month and 1-year follow-ups (T5 and T6), a rebound effect again was found. The HP45 and CP3×45 alternative protocols showed the largest rebound effect in whiteness with ΔWI_D>5.2, indicating less whiteness stability for these strategies after 1 year.

DISCUSSION

The research hypothesis that the bleaching protocols produce different whitening effectiveness was rejected. However, the research hypothesis that the different whiteness stability at follow-up evaluations was not rejected. The present in vitro study was designed to evaluate the effectiveness and 1-year whiteness stability of 2 different bleaching agents and protocols used for the in-office technique on extracted teeth stained with black tea.[20] All treatments presented excellent effectiveness according to the interpretation ratings for whitening-dependent color differences.[39–41] However, although all the protocols presented a rebound effect, the intensity of this effect was different between traditional and alternative protocols.

When the color parameters were analyzed separately (L*, a*, b* C*, and h°), all the bleaching protocols presented an increase in L* mean values and a reduction

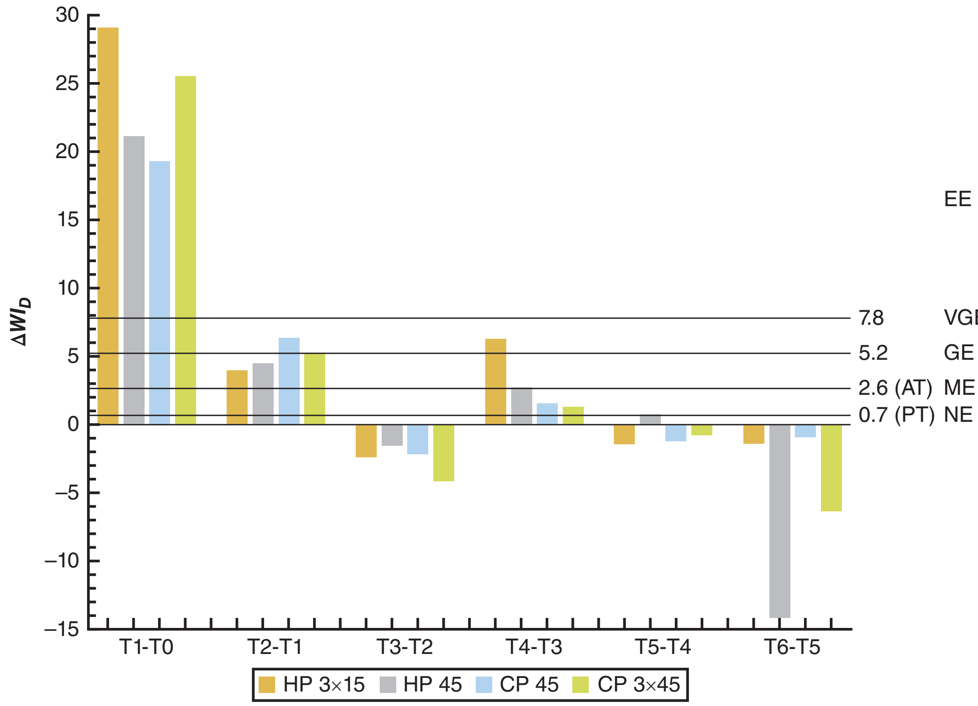

Figure 3. Whiteness differences (ΔWI_D) for bleaching treatments and protocols among each consecutive application and follow-up evaluation times, with threshold interpretation ratings. CP45, carbamide peroxide, 1 application of 45 min; CP3×45, carbamide peroxide, 3 applications of 45 min each; EE, excellent effectiveness (ΔWID>7.8); GE, good effectiveness (ΔWID > 2.6≤5.2); HP3×15, hydrogen peroxide 3 applications of 15 min; HP45, hydrogen peroxide 1 application of 45 min; ME, moderately effective (ΔWID > 0.7≤2.6); NE, not effective (ΔWID≤0.7); T0, baseline; T1, 1 week after first bleaching session; T2, 1 week after second bleaching session; T3, 1 week after third bleaching session; T4, 1-week follow-up; T5, 6-month follow-up; T6, 1-year follow-up. VGE, very good effectiveness (ΔWID > 5.2≤7.8); ΔWID, Whiteness index for dentistry difference.

in a*, b*, and C* mean values during treatment, resulting in more achromatic and lighter teeth with reduced redness and yellowness.[21] This result was expected and also consistent with previous studies that evaluated bleaching effectiveness.[4,6,7,21] However, at the T6 follow-up evaluation, the alternative protocols presented a decrease in L* and WI_D (CP3×45), an increase in b*, h° (CP3×45), a*, and C* (HP45), indicating a rebound regarding these color parameters after 1 year in comparison with T3.

The measurement of whiteness is an important evaluation for the outcome of bleaching agents and to determine stability after bleaching.[35] The present study used the new WI_D to evaluate the whiteness changes during bleaching and after bleaching. This index has been recommended because it performs better when assessing whiteness, and correlates best to visual judgements.[35] High positive WI_D values indicate increased whitening outcomes, while low (even negative) values indicate less whiteness.[35] In addition, the use of whiteness index visual thresholds is important when interpreting instrumental whiteness differences, as the index adequately correlates with visual perception.[39] Furthermore, WI_D is recommended to assess color discrimination and thus provide clinical relevance to the instrumental data.[40]

All tested protocols showed ΔWI_D>7.8 at T1, demonstrating excellent whitening. Negative whiteness differences revealed a rebound effect at T3. However, the mean whiteness difference values became positive again at T4, indicating a residual whitening effect 1 week after the completion of the treatments. Negative whiteness differences were observed again at the T5 and T6 evaluations, with lower negative values for HP45 and CP3×45 at T6, indicating less stability and a more pronounced rebound effect for both protocols after 1 year.

Alternative protocols were as effective as traditional ones when observing the whiteness-dependent color differences. However, a greater trend towards the rebound effect in the alternative protocols was reported in the long term. For HP45, increasing the application without successive changes in the bleaching agent may have decreased the stability and increased the rebound effect. Moreover, increasing the application time and the number of applications of CP3×45 in the same session did not ensure how long whiteness stability would last or prevent rebound. Furthermore, touch-up applications at follow-up evaluations to reestablish the whitening effect after completing the bleaching treatment were not tested. Future studies should investigate the effect of reapplications on reestablishing the initially achieved whitening outcome.

The strengths of the study include follow-up evaluations for 1 week, 6 months, and 12 months after bleaching, since few studies[4,9,21] evaluate the long-term maintenance of bleaching outcomes. Furthermore, the color was measured at various stages throughout the treatment. In addition, the mean L*, b*, and C* after tea staining were compatible with a clinical situation of severely discolored teeth,[21] thus simulating a challenging baseline scenario.

Limitations of the study included that the color difference was assessed exclusively using an instrumental method. Although the objective data were interpreted based on the perceptibility and acceptability thresholds and their respective rating,[39–41] visual analysis could complement the results and offer a greater relevance to the study. In addition, the teeth were stained exclusively with tea. Other staining methods may lead to different results. Furthermore, this study evaluated only 2 in-office bleaching agents, and the results should not be extrapolated for all commercially available bleaching agents.

Both bleaching agents and protocols were considered effective. However, the treatment choice should consider the chair time[21] and the stability of the whitening effect, since long-lasting results are expected.[4,9] In addition, adverse effects and the mechanism of action of the bleaching agents should be taken into consideration when recommending bleaching treatments.[5,6,15,16,25,26]

The present study found no advantages in using alternative protocols. Even if they were to demonstrate better performance compared with traditional protocols, it is unlikely that they would be feasible clinically, particularly when considering patient acceptability and clinical outcomes. Additionally, alongside increased chair time and a lack of whitening stability, the alternative options may lead to increased tooth sensitivity and microstructural changes associated with prolonged contact time and the acidity of the bleaching agents.[16,20] Future clinical studies comparing both in-office bleaching agents, following the manufacturer's protocol, are recommended to investigate long-term whitening, dentin and gingival sensitivity, as well as potential adverse effects.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. All tested protocols presented excellent effectiveness of the bleaching treatments for tea-stained teeth.
2. The whitening effect was more stable for traditional protocols, while a greater rebound effect was observed for alternative protocols.

REFERENCES

1. Haywood VB. History, safety, and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique. *Quintessence Int*. 1992;23:471–488.
2. Greenwall-Cohen J, Greenwall LH. The single discoloured tooth: Vital and non-vital bleaching techniques. *Br Dent J*. 2019;226:839–849.
3. Sulieman M, Addy M, MacDonald E, Rees JS. The effect of hydrogen peroxide concentration on the outcome of tooth whitening: An in vitro study. *J Dent*. 2004;32:295–299.
4. Kothari S, Jum'ah AA, Gray AR, M Lyons K, Yap M, Brunton PA. A randomized clinical trial investigating three vital tooth bleaching protocols and associated efficacy, effectiveness and participants' satisfaction. *J Dent*. 2020;95:103322.
5. Lima SNL, Ribeiro IS, Grisotto MA, et al. Evaluation of several clinical parameters after bleaching with hydrogen peroxide at different concentrations: A randomized clinical trial. *J Dent*. 2018;68:91–97.
6. Knezović Zlatarić D, Žagar M, Illeš D. A clinical study assessing the short-term efficacy of combined in-office/at-home whitening treatment. *J Esthet Restor Dent*. 2019;31:140–146.
7. Basting RT, Amaral FL, França FM, Flório FM. Clinical comparative study of the effectiveness of and tooth sensitivity to 10% and 20% carbamide peroxide home-use and 35% and 38% hydrogen peroxide in-office bleaching materials containing desensitizing agents. *Oper Dent*. 2012;37:464–473.
8. Bernardon JK, Ferrari P, Baratieri LN, Rauber GB. Comparison of treatment time versus patient satisfaction in at-home and in-office tooth bleaching therapy. *J Prosthet Dent*. 2015;114:826–830.
9. Bersezio C, Martín J, Mayer C, et al. Quality of life and stability of tooth color change at three months after dental bleaching. *Qual Life Res*. 2018;27:3199–3207.
10. Cvikl B, Lussi A, Moritz A, Flury S. Enamel surface changes after exposure to bleaching gels containing carbamide peroxide or hydrogen peroxide. *Oper Dent*. 2016;41:E39–E47.
11. Goettems ML, Fernandez MDS, Donassollo TA, Henn Donassollo S, Demarco FF. Impact of tooth bleaching on oral health-related quality of life in adults: A triple-blind randomized clinical trial. *J Dent*. 2021;105:103564.
12. Peixoto AC, Vaez SC, Pereira NAR, et al. High-concentration carbamide peroxide can reduce the sensitivity caused by in-office tooth bleaching: A single-blinded randomized controlled trial. *J Appl Oral Sci*. 2018;26:e20170573.
13. Bernardon JK, Sartori N, Ballarin A, Perdigão J, Lopes GC, Baratieri LN. Clinical performance of vital bleaching techniques. *Oper Dent*. 2010;35:3–10.
14. Altınışık H, Akgül S, Nezir M, Özcan S, Özyurt E. The effect of in-office bleaching with different concentrations of hydrogen peroxide on enamel color, roughness, and color stability. *Materials*. 2023;16:1389.
15. Kwon SR, Wertz PW. Review of the mechanism of tooth whitening. *J Esthet Restor Dent*. 2015;27:240–257.
16. Maran BM, Matos TP, de Castro ADS, et al. In-office bleaching with low/medium vs. high concentrate hydrogen peroxide: A systematic review and meta-analysis. *J Dent*. 2020;103:103499.
17. Monteiro RV, Monteiro S, Jr, Caldeira de Andrada MA. Clinical evaluation of two in-office dental bleaching agents. *Am J Dent*. 2018;31:239–242.
18. Sulieman M, Addy M, Rees JS. Development and evaluation of a method in vitro to study the effectiveness of tooth bleaching. *J Dent*. 2003;31:415–422.
19. de Paula EA, Nava JA, Rosso C, et al. In-office bleaching with a two- and seven-day intervals between clinical sessions: A randomized clinical trial on tooth sensitivity. *J Dent*. 2015;43:424–429.
20. Loguercio AD, Servat F, Stanislawczuk R, et al. Effect of acidity of in-office bleaching gels on tooth sensitivity and whitening: a two-center double-blind randomized clinical trial. *Clin Oral Investig*. 2017;21:2811–2818.
21. Gaidarji B, Perez BG, Ruiz-López J, Pérez MM, Durand LB. Effectiveness and color stability of bleaching techniques on blood-stained teeth: An in vitro study. *J Esthet Restor Dent*. 2022;34:342–350.
22. Donassollo SH, Donassollo TA, Coser S, et al. Triple-blinded randomized clinical trial comparing efficacy and tooth sensitivity of in-office and at-home bleaching techniques. *J Appl Oral Sci*. 2021;29:e20200794.
23. Borges AB, Zanatta RF, Barros AC, Silva LC, Pucci CR, Torres CR. Effect of hydrogen peroxide concentration on enamel color and microhardness. *Oper Dent*. 2015;40:96–101.
24. Acuña ED, Parreiras SO, Favoreto MW, et al. In-office bleaching with a commercial 40% hydrogen peroxide gel modified to have different pHs: Color change, surface morphology, and penetration of hydrogen peroxide into the pulp chamber. *J Esthet Restor Dent*. 2022;34:322–327.
25. Meireles SS, Fontes ST, Coimbra LA, Della Bona Á, Demarco FF. Effectiveness of different carbamide peroxide concentrations used for tooth bleaching: An in vitro study. *J Appl Oral Sci*. 2012;20:186–191.
26. Mailart MC, Ferracioli CD, Torres CR, Palo RM, Borges AB. Hydrogen peroxide degradation of bleaching systems with different trays: Randomized clinical trial. *Am J Dent*. 2020;33:89–94.

27. Mena-Serrano AP, Parreiras SO, do Nascimento EM, et al. Effects of the concentration and composition of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber. *Oper Dent*. 2015;40:E76–E82.
28. Kury M, Lins RBE, Resende BA, Picolo MZD, André CB, Cavalli V. The influence of the renewal or the single application of the peroxide gel on the efficacy and tooth sensitivity outcomes of in-office bleaching-A systematic review and meta-analysis. *J Esthet Restor Dent*. 2022;34:490–502.
29. Kury M, Wada EE, Silva DPD, Tabchoury CPM, Giannini M, Cavalli V. Effect of violet LED light on in-office bleaching protocols: a randomized controlled clinical trial. *J Appl Oral Sci*. 2020;28:e20190720.
30. Matis BA, Gaiao U, Blackman D, Schultz FA, Eckert GJ. In vivo degradation of bleaching gel used in whitening teeth. *J Am Dent Assoc*. 1999;130:227–235.
31. de Oliveira DC, Ayres AP, Rocha MG, et al. Effect of different in vitro aging methods on color stability of a dental resin-based composite using cielab and ciede2000 color-difference formulas. *J Esthet Restor Dent*. 2015;27:322–330.
32. AlGhazali N, Burnside G, Smith RW, Preston AJ, Jarad FD. Performance assessment of Vita Easy Shade spectrophotometer on colour measurement of aesthetic dental materials. *Eur J Prosthodont Restor Dent*. 2011;19:168–174.
33. The International Commission on Illumination. CIE 015:2018 Colorimetry. 4th ed.. Vienna, Austria: CIE Central Bureau; 2018.
34. Knezović D, Zlatarić D, Illeš IŽ, Alajbeg M, Žagar. In vivo and in vitro evaluations of repeatability and accuracy of VITA Easyshade Advance 4.0 Dental Shade-Matching Device. *Acta Stomatol Croat*. 2015;49:112–118.
35. Pérez MM, Ghinea R, Rivas MJ, et al. Development of a customized whiteness index for dentistry based on CIELAB color space. *Dent Mater*. 2016;32:461–467.
36. Nobbs JH. A lightness, chroma and hue splitting approach to CIEDE2000 colour differences. *Adv Colours Sci Technol*. 2002;5:46–53.
37. Perez BG, Pérez MM, Ruiz-López J, Gaidarji B, Durand LB. Effect of layering strategy and prolonged water aging on masking ability of composite resins. *J Prosthet Dent* 2023.
38. Melgosa M, Ruiz-López J, Li C, García PA, Della Bona A, Pérez MM. Color inconstancy of natural teeth measured under white light-emitting diode illuminants. *Dent Mater*. 2020;36:1680–1690.
39. Pérez MM, Herrera LJ, Carrillo F, et al. Whiteness difference thresholds in dentistry. *Dent Mater*. 2019;35:292–297.
40. Paravina RD, Ghinea R, Herrera LJ, et al. Color difference thresholds in dentistry. *J Esthet Restor Dent*. 2015;27:S1–S9.
41. Paravina RD, Pérez MM, Ghinea R. Acceptability and perceptibility thresholds in dentistry: A comprehensive review of clinical and research applications. *J Esthet Restor Dent*. 2019;31:103–112.

Corresponding author:
Dr Javier Ruiz-López
Department of Optics
University of Granada
Faculty of Science
Campus Fuentenueva, Edificio Mecenas, s/n
18071 Granada
SPAIN
Email: jruizlo@ugr.es

Acknowledgments
The authors acknowledge funding support from the Spanish Ministry of Science and Innovation (PID2022.142151OB.I00).

CRediT authorship contribution statement
Bibiana Gabardo Perez Mariano da Rocha: Conceptualization, Methodology, Investigation, Visualization, Writing - original draft. **Javier Ruiz-López:** Data curation, Visualization, Formal analysis, Writing - review and editing. **María M. Pérez:** Methodology, Visualization, Supervision, Project administration, Formal analysis, Writing - review and editing, Funding acquisition. **Bruna Gaidarji:** Conceptualization, Methodology, Writing - original draft, Visualization. **Giovanna Teixeira Frasson:** Methodology, Investigation, Data curation. **Letícia Brandão Durand:** Conceptualization, Methodology, Investigation, Visualization, Writing - review and editing, Project administration.

https://doi.org/10.1016/j.prosdent.2024.06.011