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RESEARCH ARTICLE

Effect of temperature and storage time on dental bleaching effectiveness

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Abstract

Objective: To evaluate the effect of storage time and temperature on the effectiveness of bleaching agents.

Methods: Enamel slabs were randomly allocated according to the concentration of carbamide peroxide (CP): 10% (CP10) and 16% (CP16). Shade evaluations were conducted at baseline and then after 3 and 12 months of storage at 3 different temperatures: 10°C ($\pm 2^\circ\text{C}$), 25°C ($\pm 2^\circ\text{C}$), and 35°C ($\pm 2^\circ\text{C}$). Objective color evaluation was performed (spectrophotometer CIEL*a*b* system and CIEDE2000) to calculate the color change (ΔE_{00}). Subjective evaluation was performed using the VITA classical shade guide followed by shade variation (ΔS).

Results: The bleaching effect of different CP concentrations (10% and 16%) at baseline showed no differences with regard to objective (ΔE_{00}) and subjective parameters (ΔS) ($P > .05$). No differences were observed between CP concentrations stored for the same length of time and at the same temperature for either parameter ($P > .05$). In terms of objective measurement (ΔE_{00}), a reduction in the bleaching effect was shown for both CP concentrations (10% and 16%) when stored for 12 months at 35°C, and compared with the baseline ($P < .05$). As regards the subjective parameters (ΔS), after 12 months, both CP concentrations (10% and 16%) stored at 35°C showed a decrease in bleaching capacity compared to baseline ($P < .05$). CP10 and CP16 stored at 10°C and 25°C did not suffer any loss in bleaching ability, even after 12 months ($P > .05$).

Conclusions: The storage of bleaching agents for long periods at high temperatures can reduce the bleaching effectiveness of CP at 10% and 16%.

Clinical Significance: Clinicians must be made aware that they should store their bleaching products under proper temperature conditions and use them within an appropriate time frame; otherwise, the bleaching effect could be lost.

KEYWORDS

bleaching and heat, carbamide peroxide, tooth bleaching

1 | INTRODUCTION

In recent decades, the desire for a more aesthetic smile has increased the demand for the bleaching of discolored teeth.^{1,2} Tooth discoloration can occur as a consequence of biological, chemical, or mechanical changes, affecting aesthetic appearance.³ Oxidizing chemical agents are used to bleach discolored teeth, and these agents can be

applied by the professional in the dental office, at home by the patient under professional supervision, or by the patient without supervision, using over-the-counter products.^{4,5} In-office and at-home bleaching protocols produce favorable results, with the leaching of chromogenic agents present inside the dentin.³

Different bleaching agents are available in the market, with various concentrations ranging from 3.5% to 35%. The bleaching products

are mostly based on hydrogen peroxide (HP) or one of its precursors, such as carbamide peroxide (CP).^{6,7} Recent meta-analysis has shown that CP is slightly better in terms of whitening effectiveness when compared to HP, demonstrating similar tooth sensitivity and gum irritation.⁸

Temperature is an important factor to be considered for bleaching products, because an increase of 10°C doubles the rate of chemical reaction, accelerating the bleaching treatment.⁹ In this regard, the storage of bleaching agents at high temperatures can induce the dissociation of components, reducing the shelf life of these products.¹⁰ A study evaluated the pH of at-home and in-office bleaching agents stored at room temperature or refrigerated, observing that refrigerated, at-home bleaching gels had the highest pH values and that the storage temperature directly influenced the pH of the evaluated products.^{11,12} Although some manufacturers recommend refrigerating the bleaching products to increase longevity, little information is available concerning the impact of different storage conditions and on the effectiveness of CP-based agents.¹¹

Therefore, the aim of this *in vitro* study was to investigate different storage conditions (time and temperature) on the effectiveness of different CP concentrations, testing the hypothesis that the effectiveness of dental bleaching decreases as storage temperature and time increase.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Two hundred and ten recently extracted bovine teeth without cracks were used in this study, to evaluate the effectiveness of bleaching using different concentrations of CP (10% and 16%) stored for different periods of time (baseline, 3 months, and 12 months) and temperatures (10 ± 2°C, 25 ± 2°C, or 35 ± 2°C). The bleaching agents used in this study, along with their chemical composition, manufacturer, and batch number, are presented in Table 1. The present study was approved by the Ethics Committee (462.122).

2.2 | Specimen preparation

The bovine teeth were cleaned and evaluated under ×40 magnification, and any teeth exhibiting cracks were excluded. The selected teeth were stored in chloramine-T solution for 1 week for the

purposes of disinfection. Tooth roots were removed, and the crowns were sectioned with a water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL) to obtain standard enamel slabs (6 mm × 6 mm × 3 mm), measured with a digital caliper (DC 500, Mitutoyo, Suzano, SP, Brazil). The enamel surface was flattened and polished using sandpapers from #600 to #1200 grit and with water cooling and stored in distilled water until staining.

2.3 | Staining procedures

In an attempt to standardize tooth shade, artificial staining of the specimens was carried out following the method proposed by Sulie-man.³ Enamel surfaces were etched with 35% phosphoric acid gel for 60 seconds and rinsed in distilled water for 30 seconds. Specimens were immersed in a standardized coffee solution at room temperature (25 ± 2°C) 24 hours a day for 7 days. The coffee solution was produced each day by boiling 12 g of coffee (Melitta, Melitta Ind. e Co. LTDA, Avaré, SP, Brazil) in 200 mL of distilled water in a coffee maker (Melitta). After staining, the specimens were washed and stored in distilled water at room temperature. To be included in the study, the specimens had to darken to a color of A3.5, determined by spectrophotometer (Vita Easys shade; Vita Zahnfabrik, Bad Säckingen, Germany).

2.4 | Bleaching procedures

After staining, 45 specimens were randomly selected to evaluate the effectiveness of bleaching agents without storage (baseline). These specimens were divided into 2 groups (n = 15) according to the CP concentration: 10% and 16%. The remaining 210 specimens were divided into 14 groups (n = 15) according to the following factors: storage period (3 months and 12 months), storage temperature (10 ± 2°C; 25 ± 2°C; and 35 ± 2°C), and CP concentration (10% and 16%; Table 2). The CP agents were kept in a dark environment at a constant temperature (10 ± 2°C; 25 ± 2°C; and 35 ± 2°C), for 3 and 12 months. At the end of these periods of time, the respective experiments were carried out.

The specimens treated with 10% and 16% CP were covered with the respective bleaching agents (0.2 mL) for 4 hours a day for 2 weeks (14 days). This bleaching procedure was repeated 2 times (three 20-minute applications per session) at intervals of 5 days, and the specimens were stored in distilled water at 37°C until the next session.

2.5 | Tooth shade measurement

Objective and subjective methods were used to evaluate tooth color. The tooth color coordinates, based on the CIEL*a*b* system, were objectively measured using a pre-calibrated digital spectrophotometer (Vita Easys shade; Vita Zahnfabrik), under CIE standard illuminant D65 (diffuse/0° illuminating/measuring geometry) inside a viewing cabinet (CAC 60, VeriVide Limited, Leicester, United Kingdom) using a gray background (Flexipalette Color Match). The color difference (ΔE_{00}) between any 2 measurements was calculated using the CIEDE2000 metric:

TABLE 1 Description of the bleaching agents used in this study

Brand names and lots	Manufacturer	Chemical composition
Whiteness perfect 10% (batch #110107)	FGM, Joinville, SC, Brazil	10% carbamide peroxide, carbopol, potassium nitrate, sodium fluoride, glycol, and deionized water
Whiteness perfect 16% (batch #150307)	FGM, Joinville, SC, Brazil	16% carbamide peroxide, carbopol, potassium nitrate, sodium fluoride, glycol, and deionized water

TABLE 2 Description of the experimental groups

Storage time	Storage temperature (°C)	Bleaching agent concentration	Experimental group (n = 15)
No storage	...	CP 10%	Ws _{10%}
		CP 16%	Ws _{16%}
3 mo	10	CP 10%	3m _{10°C10%}
		CP 16%	3m _{10°C16%}
	25	CP 10%	3m _{25°C10%}
		CP 16%	3m _{25°C16%}
	35	CP 10%	3m _{35°C10%}
		CP 16%	3m _{35°C16%}
12 mo	10	CP 10%	12m _{10°C10%}
		CP 16%	12m _{10°C16%}
	25	CP 10%	12m _{25°C10%}
		CP 16%	12m _{25°C16%}
	35	CP 10%	12m _{35°C10%}
		CP 16%	12m _{35°C16%}

Abbreviation: CP, carbamide peroxide.

$$\Delta E_{00} = \left[\left(\Delta L' / K_L S_L \right)^2 + \left(\Delta C' / K_C S_C \right)^2 + \left(\Delta H' / K_H S_H \right)^2 + R_T (\Delta C' / K_C S_C) (\Delta H' / K_H S_H)^2 \right]^{1/2}$$

All shade readings were conducted 3 times, by a calibrated examiner, with the active point of the spectrophotometer positioned at the center of the specimen. The average value was calculated and recorded.

The subjective evaluation was performed using the VITA classical shade guide, under the same conditions used for the objective evaluation. A single calibrated (intra Kappa: 0.9) examiner performed all shade evaluations. The shade difference was calculated for the same specimen before (S_1) and after (S_2) a bleaching protocol ($\Delta S = S_2 - S_1$). The 16 shade tabs were numbered from 1 (highest value, B1) to 16 (lowest value, C4; Table 3).^{13–20}

2.6 | Statistical analysis

The STATA software version 12.0 (Stata Corporation, College Station, TX) was used for statistical analysis. Objective analysis (ΔE_{00}) showed normal distribution; therefore, 3-way analysis of variance and Bonferroni post hoc tests were conducted to verify the association between CP concentration, storage temperature, and storage time ($\alpha = 0.05$). The subjective analysis (ΔS) did not show normal distribution, thus, the data were analyzed using Wilcoxon tests ($\alpha = 0.05$).

3 | RESULTS

The bleaching effect of different CP concentrations (10% and 16%) at baseline did not show any differences for objective (ΔE_{00}) or subjective parameters (ΔS) ($P > .05$). Similarly, no differences were observed

between CP concentrations stored for the same length of time and at the same temperature for either parameter ($P > .05$).

Table 4 displays the results of ΔE_{00} values for the different groups subjected to bleaching agents. The bleaching ability of products stored for 3 months was not reduced for either CP concentration ($P > .05$) at all storage temperatures ($P > .05$) when compared to baseline. However, a reduction in the bleaching effect was demonstrated for both CP concentrations (10% and 16%) when stored for 12 months at 35°C in relation to baseline ($P < .05$).

Considering ΔS parameters, at 3 months, all concentrations stored at all temperatures (10°C, 25°C, and 35°C) showed a similar bleaching ability compared to baseline ($P > .05$). However, after 12 months, all CP concentrations (10% and 16%) stored at 35°C showed a decrease in bleaching capacity compared to baseline (Table 5; $P < .05$). CP 10% and CP 16% stored at 10°C and 25°C did not suffer any loss of bleaching ability, even after 12 months ($P > .05$).

4 | DISCUSSION

The results of this study have great clinical significance for professionals who perform at-home bleaching with CP10 and CP16, because a reduction in bleaching effectiveness was observed in products stored under suitable temperature conditions. The findings showed that the combination of time and temperature can have a negative influence on the effectiveness of bleaching agents, evaluated by objective and subjective parameters, when stored for long periods (12 months) and under unfavorable temperature conditions (35°C).

Storage at high temperatures considerably reduced the ΔE_{00} and ΔS values measured after 12 months in all groups. This outcome demonstrates that storage of at-home CP products for long periods and at high temperatures affects tooth bleaching capacity. Previous reports have shown that increasing storage temperatures can double the speed of the chemical reactions of peroxides, leading to bleaching agent degradation.⁹ This may be because of the chemical equilibrium related to bleaching agent concentration, which increases at the start of the reaction.¹¹

However, in the 3-month evaluation, ΔS and ΔE_{00} values were not reduced in at-home products containing CP10 or CP16, demonstrating that a period of 12-months is required for the degradation of CP present in the products. These findings could be very important for alerting clinicians about the products' shelf life, avoiding storage for long periods at high temperatures. This is also a relevant item of information for clinicians working in hot climates where conditions could accelerate peroxide degradation. Under these circumstances, bleaching products must be refrigerated to reduce such reactions. CP degradation results in oxygen and water, which consequently decreases the pH.^{10,11} This can directly affect the material's properties, leading to a significant reduction in bleaching effectiveness. Decreases in pH values can also change the chemical properties of

TABLE 3 Color scores from lighter to darker colors in the shade guide

Vitapan shade guide	B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
Scores	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

TABLE 4 Mean ΔE_{00} (\pm SD) for the groups submitted to bleaching agents according to factors storage temperature, storage time, and carbamide peroxide concentration ($n = 15$) (ANOVA 3-way test, $P \leq .05$)

Storage time	Storage temperature ($^{\circ}$ C)	Mean ΔE (\pm SD)	
		Carbamide peroxide concentration (%)	
		10%	16%
Baseline	...	9.6 (\pm 3.5)	10.9 (\pm 2.0)
3 mo	10	9.6 (\pm 3.0)	9.6 (\pm 2.6)
	25	9.0 (\pm 3.4)	9.8 (\pm 1.9)
	35	9.3 (\pm 3.2)	9.7 (\pm 2.9)
12 mo	10	9.5 (\pm 3.3)	9.8 (\pm 2.9)
	25	8.2 (\pm 2.3)	8.6 (\pm 1.6)
	35	4.9 (\pm 2.5) ^a	6.2 (\pm 3.0) ^a

Abbreviations: ΔE_{00} , color variation after bleaching; ANOVA, analysis of variance.

^a Differences in relation to baseline. No difference was observed between the CP concentration groups.

the bleaching agents, which can induce greater mineral loss on the dental surface promoted by the elevation of the H^{+} ion.^{11,21,22} Therefore, it is possible to hypothesize that the application of bleaching agents stored under unfavorable conditions produces a decrease in pH, which could induce greater pulp damage.

Recent studies, evaluating the CP gel stability of manipulated formulations and commercial products, showed an elevated decomposition of CP (in ammonia [urea], oxygen and water [HP]) stored at 32 $^{\circ}$ C, which was more significant in manipulated formulations.²³ These results may be explained by the addition of chemical stabilizers (phosphoric acid and an anhydrous base) in commercially available products. With regard to the commercial product, after 45 days of storage at 32 $^{\circ}$ C, CP16 presented only 89% of CP compared to baseline. This decrease in available CP in the products after storage at high temperatures²³ corroborates the results observed in the present study where storage for 12 months reduces bleaching effectiveness. Besides the change in chemical equilibrium promoted by the increase in temperature, the initial degradation of CP which results in water formation is able to dissolve more CP.²⁴ Consequently, significant alterations can

TABLE 5 Median (25%/75%) values for ΔS for the experimental groups submitted to bleaching agents according to factors storage temperature, storage time, and carbamide peroxide concentration ($n = 15$; Wilcoxon test, $P \leq .05$)

Storage time	Storage temperature ($^{\circ}$ C)	Median ΔS (25%/75%)	
		Carbamide peroxide concentration (%)	
		10%	16%
Baseline	...	10 (12/9)	10 (12/9)
3 mo	10	10 (11/9)	10 (11/9)
	25	9 (11/7)	10 (11/9)
	35	9 (11/8)	9 (11/9)
12 mo	10	7 (10/6)	10 (10/7)
	25	9 (9/7)	9 (10/7)
	35	3 (7/0) ^a	3 (\pm 3/0) ^a

Abbreviation: ΔS , shade variation after bleaching treatment.

^a Differences in relation to baseline. No difference was observed between the CP concentration groups.

be observed in the bleaching agents, leading to a reduction in effectiveness.²⁴ This reaction explains why the peroxides stored with their lids off (and water evaporation) showed a decrease in the peroxide decomposition when compared to those kept in closed flasks.²⁴

Several studies have investigated the bleaching effectivity of in-office and at-home bleaching agents.^{17,25–29} A clinical trial evaluating the bleaching effectiveness of CP10 and CP16 observed no statistical difference between concentrations after 2 years.³⁰ Corroborating this study, no differences in the degree of bleaching were observed between 10% and 15% CP products in other randomized clinical trials.¹⁷ Similarly, in the present study, when comparing the bleaching agents at baseline and other evaluation times, ΔE_{00} and ΔS values were similar in the 10% and 16% groups.

To maintain the effectiveness of bleaching agents, the results of this study recommend the storage of products at low temperatures (10 $^{\circ}$ C and 25 $^{\circ}$ C) for up to 12 months. Further studies, evaluating the chemical properties and degradation rate of bleaching agents exposed to different temperatures and lengths of storage, should be conducted to establish a definitive storage protocol for bleaching products. In addition, professionals, manufacturers, and distributors should be advised about the storage conditions of these products, to maintain their clinical effectiveness.

It is important to highlight that both measurements (objective [ΔE_{00}] and subjective [ΔE_{00}]) presented results along similar lines. This is a significant consideration because it indicates the compatibility and robustness of the present study. The evaluation of color difference (variation in shade) used in the present study has been widely reported.^{13–20} The subjective evaluation is important for color research and can be represented by perceptibility and acceptability thresholds. Although the subjective visual scale is the method most frequently employed by dentists to reproduce specific shades, this method still presents a challenge for clinical dentistry.^{31,32} Studies have reported that visual measurement can be influenced by the different characteristics of the evaluators, such as experience, eye fatigue, and sex.^{33,34} With the aim of reducing the imperfections and inconsistencies of visual shade matching, we also evaluated the samples using an objective method. The use of spectrophotometers using the CIEDE2000 color-difference is a more precise method because the formula incorporates specific corrections for nonuniformity of CIELAB color space.³⁵ Therefore, CIEDE2000 was performed to add an objective assessment, as reported in previous studies.^{35–41} In addition, as perceptibility and acceptability for whiteness are still undefined, the whiteness indices have been reported and could be used for color research in bleached structures.^{42,43}

5 | CONCLUSION

The storage of carbamide peroxide products for 12 months at 35 $^{\circ}$ C decreases the effectiveness of bleaching at both 10% and 16% concentrations.

DISCLOSURE OF INTERESTS

The authors do not have any financial interest in the companies whose materials are included in this article.

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