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Influence of pH on the Effectiveness of Hydrogen Peroxide Whitening

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Clinical Relevance

Verification that pH influences the bleaching efficacy will contribute to the development of more efficient bleaching products.

SUMMARY

Objective: To evaluate the influence of pH on the bleaching effect of hydrogen peroxide on chromogen agents.

Method: Hydrogen peroxide 50% was mixed with red wine or with an alcoholic solution of tobacco in glass cuvettes, resulting in final peroxide concentrations of 16.97% and 21.12%, respectively. The pH of this mixture was measured and adjusted with 3.3 M HCl solution or 2.5 M NaOH solution to obtain the final pH values of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. After mixing, the color of these solutions was evaluated in a reflectance spectrophotometer; readings were repeated after 10 minutes for the wine solution and 20 minutes for the tobacco solution. Ten samples were prepared for each solution at each pH. Color changes (Delta E) were calculated. The data were statistically analyzed using analysis of variance one-way and Tukey tests, with a significance level of 5%.

Results: There were significant differences among the different pH values for the wine and tobacco solutions (p=0.0001). The Tukey test showed that for both solutions, pH 9.0 resulted in a significantly greater bleaching effect than the other values tested.

Conclusion: The efficacy of hydrogen peroxide bleaching is directly proportional to the increase in its pH.

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INTRODUCTION

Tooth bleaching is a treatment widely used in the dental clinic to improve the esthetics of discolored teeth. Changes in tooth color may be of intrinsic or extrinsic causes. The intrinsic causes might be from endogenous origin, such as hemorrhage or disorders during odontogenesis caused by metabolic or infectious diseases, or even with the intake of certain medications. Extrinsic changes are from external

sources, such as pigments from tea; beverages containing chromogenic agents, such as coffee and wine; medications such as chlorhexidine or iron compounds; or even habits such as using tobacco. These substances are deposited on the enamel's surface and can be removed by a simple prophylaxis. However, with time, they can penetrate through the pores of the enamel and become intrinsic.[1-3]

Pigments, also called chromogens, have in common a structure shaped like a complex carbon chain, with many double bonds, which absorbs most ambient light affecting the tooth structure. It is known that the color observed in any structure actually corresponds to the light wavelength being reflected by it. For the bleaching to occur, it is necessary that the chromogen agents' carbon chains are broken, turning them into simpler molecules, reducing light absorption and increasing reflection.[3]

Hydrogen peroxide is the most widely used dental bleaching agent, manufactured at low concentration for at-home techniques or high concentration for in-office techniques.[4-6] It is a highly unstable molecule and decomposes according to a sequence of reactions, which can be influenced by incident light, pH, temperature, interactions with transition metals, and other factors.[7-9] Initially, it decomposes into hydrogen cations (H^+) and the perhydroxyl anion (HO_2^-; equation 1). The H^+ release explains their behavior as a weak acid. The perhydroxyl anion interacts with another peroxide molecule and results in the formation of the free radicals hydroxyl $(HO)^\bullet$ and perhydroxyl $(HO_2)^\bullet$, also called active species (equation 2). The hydroxyl radicals react with more peroxide and result in the formation of more perhydroxyl radicals and water (equation 3). With the completion of the reaction, all of the peroxide is converted to water. Obviously, the description above is simplified due to the possible formation of intermediates and other active species.

$$H_2O_2 \rightarrow H^+ + HO_2^- \quad (1)$$

$$HO_2^- + H_2O_2 \rightarrow HO_2^\bullet + HO^\bullet + OH^- \quad (2)$$

$$HO^\bullet + H_2O_2 \rightarrow HO_2^\bullet + H_2O \quad (3)$$

Free radicals are unstable oxidants, which have in their structure an unpaired electron. To become stable, free radicals bind with electrons from other organic molecules that contact with the pigments.[3] The pigment molecules are broken down into simpler chains in a redox reaction, which changes its behavior and decreases the optical absorption of light.[4,5]

The decomposition of hydrogen peroxide can be initiated with or without the presence of a catalyst. The radicals are formed slowly, without the presence of a catalyst in a reaction called self oxidation-reduction.[9] In the presence of metal ions or enzymes, that reaction can be accelerated.[10] The same is observed with increasing temperature.[11]

It is very important to develop and produce dental bleaching gels that are the most efficient and safe as possible, making the technique simpler for the dentist and more comfortable for the patient. In relation to the efficacy of the bleaching procedure, the oxidative activity of the hydrogen peroxide molecule is strongly dependent on several factors. When we think about oxidation of chromogenic molecules, the action of hydrogen peroxide is essentially the same, whether we are bleaching wood pulp, cotton, or cloth in industrial processes; dirty clothes or dishes in the home; or chromogenic molecules inside the intercrystalline spaces of enamel. Therefore, to develop a more efficient dental bleaching gel, we have to improve the activity of the hydrogen peroxide molecule itself, boosting the chemical reaction that promotes the bleaching. For that, we have to understand and control all variables that exert a direct effect on free radical generation and availability.

The direct effect of pH on bleaching effectiveness was previously demonstrated in the industrial bleaching of cotton fibers or wood pulp. Hydrogen peroxide solutions with a higher pH are used to increase the efficacy of the process.[12] In relation to dental bleaching gels, a large number of products present an acidic pH in order to increase the product's shelf life, since hydrogen peroxide is more stable in an acidic environment. However, this low pH can promote enamel demineralization. Several studies have shown that bleaching in acidic pH can produce changes in chemical composition and surface morphology, calcium loss, and reduction in hardness and fracture resistance.[13-17] On the other hand, studies of dental bleaching agents with alkaline pH have shown an increased bleaching efficacy,[18] reducing its deleterious effects on enamel surface properties.[19] However, there is a lack of literature in relation to the influence of pH on bleaching of chromogens commonly found in the oral cavity that are responsible for tooth darkening, such as wine and tobacco. Therefore, determining a pH that is safe and allows for bleaching of chromogenic substances available in the oral environment with maximum efficacy is very important for the development of new formulations and products.

For a preliminary study on the influence of bleaching agent formulation on their efficacy, the use of teeth is laborious. Thus, an *in vitro* method for testing the action of peroxide on chromogens without the use of tooth substrate is simple, and a larger number of combinations can be assessed, avoiding the complexities resulting from chemical diffusion and optical transmission within the tooth.[18] When we use tooth structure, other variables are present, such as tooth age, mineralization status, initial color, diameter and number of dentin tubules, and differences in the numbers of organic and inorganic compounds.

Given the importance of objective parameters for the development of new formulations, the aim of this study was to determine the optimal pH to maximize the efficacy of the bleaching effect of hydrogen peroxide on chromogens. The null hypothesis tested was that the pH does not influence the bleaching efficacy.

METHODS AND MATERIALS

To evaluate the bleaching effect of hydrogen peroxide *in vitro,* its action on the colored dye solutions containing known chromogens was measured by a reflectance spectrophotometer (CM 2600d, Konica Minolta, Osaka, Japan), as proposed by Maiolo and others[20] and Young and others.[18] Wine (Santome, Itupeva, São Paulo, Brazil) and an alcoholic solution of tobacco *(Nicotianatabacum L.)* were used as chromogen agents.[21,22] To prepare the solution, a portion of 80 g of dry tobacco was mixed and chopped into slices of 7 mm (maximum) in 100 mL of alcohol 54°Gay-Lussac. The solution was allowed to rest for 24 hours in a closed container. After this time, the solution was filtered to remove the solid portion of tobacco and then stored in a capped bottle under refrigeration at 5°C until use.

To evaluate the effect of hydrogen peroxide bleaching on the wine chromogens, 1.27 mL of wine, 1.27 mL of hydrogen peroxide to 50% (Cosmochemistry, Barueri, São Paulo, Brazil), and 1.20 mL of ultra-pure water (type 1) were mixed in a glass cuvette with an optical path of 10 mm and a total capacity of 4.0 mL (G4, Biocell Buckets, Taboao da Serra, São Paulo, Brazil), resulting in 16.97% final peroxide concentration. For the tobacco, 1.0 mL of the tobacco solution, 1.61 mL of hydrogen peroxide 50%, and 1.20 mL of ultra-pure water were mixed, resulting in 21.12% final peroxide concentration. The pH of this mixture was measured with a pH meter (SevenMulti, Mettler Toledo, Schwerzenbach, Switzerland), equipped with an electrode (Inlab Viscous, Mettler Toledo), which was calibrated using solutions with pH 4.01 and 6.86.

For the final adjustment on different pH values, a portion of the ultra-pure water in the mixture was replaced by a 3.3 M HCl or 2.5 M NaOH solution. The final pH of the mixture was set at 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. The final concentration of hydrogen peroxide solution into the wine mixture was 20% (w/v) and of the tobacco mixture was 25% (w/v).

A custom-made device for adjustment in the spectrophotometer was used at the time of color reading, so that three sides of the cuvette were covered with a standard white background, which had the chromaticity coordinates $L^* = 93.10$, $a^* = -1.27$, and $b^* = 4.95$ (Leneta, The Leneta Co, Mahwah, NJ, USA), as shown in Figure 1.

The reading frame of the spectrophotometer was adjusted to contact the front part of the cuvette. The entire set was covered with a dark chamber adapted to the shape of the spectrophotometer so that the reading of the solution's color was held in the dark (Figure 1). The apparatus was adjusted to the D65 standard illuminant, small area view mode with the specular component included. The observer angle was set to 2° and ultraviolet emission at 100%. Three consecutive measurements were carried out, obtaining an average of L* a* b* chromatic coordinates. The L* value is a measure of lightness from 0 (perfect black) to 100 (full white), the a* axis represents red (positive a*) to green (negative a*), ranging from +120 to −120, and the b* axis represents yellow (positive b*) to blue (negative b*), also ranging from +120 to −120.[23] The data were analyzed by the software Spectramagic NX (Konica Minolta Inc, Tokyo, Japan).

An initial reading of the L*a*b* chromatic coordinates was performed immediately after mixing and then after 10 minutes for wine and 20 minutes for the tobacco solution. The waiting time between the first and second readings of the spectrophotometer was determined based on previous tests, performed in order to determine a significant color variation, that is, $\Delta E \geq 3.0$, which is visually perceptible.[24,25] The variation in the color of solutions containing the chromogen agents after bleaching can be seen in Figure 2. During the pilot study, it was observed that it takes more time for a significant lightening of the tobacco solution compared with wine, and because of this, a longer time was set for it.

All procedures were performed at 24°C (±1°C) and a relative humidity between 30% and 36%. After the

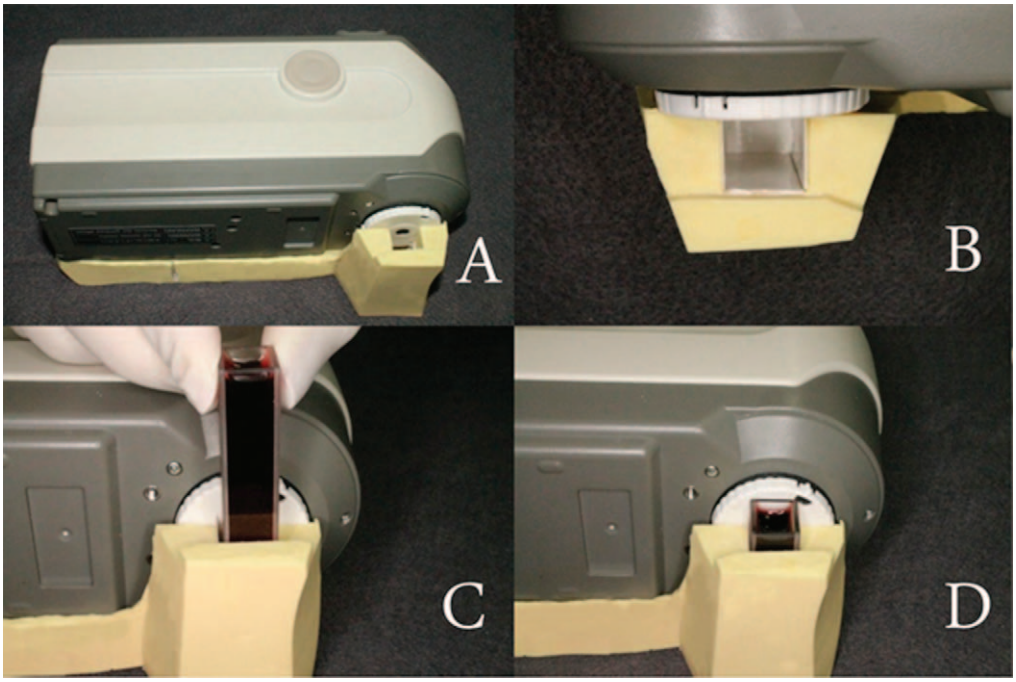

Figure 1. *(A): Support for the spectrophotometer with the device positioned. (B): View of the approximate area where the cuvette holder was placed. The walls are lined with white standardized background (Leneta). (C): Cuvette with stained solution being positioned in support. (D): Cuvette in position and in contact with the spectrophotometer's frame reading.*

respective periods, the solutions became clearer, indicating that hydrogen peroxide promoted the oxidation of chromogens (Figure 2). Then, a final color reading was performed, and the software calculated the values of the general color variation (ΔE) using the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, according to the instructions of the Commission Internationale de l'Éclairage.[23]

The data were analyzed using statistical parametric tests of one-way analysis of variance (ANOVA) and Tukey, with a significance level of 5%.

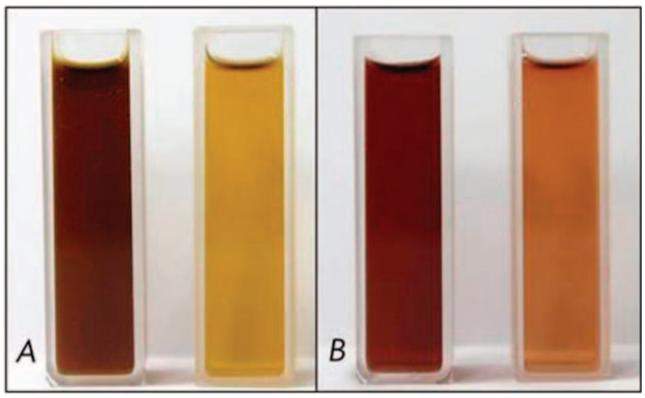

Figure 2. *(A): Tobacco solution at baseline and after 20 minutes of bleaching. (B): Wine solution at baseline and after 10 minutes of bleaching.*

RESULTS

The ANOVA test showed significant differences for color variation considering the different pH of wine (p=0.0001, F=37.61, df=6) and tobacco solutions (p=0.0001, F=281.22, df=6).

Table 1 presents the results of the Tukey test for the wine and tobacco solutions. There were no significant differences in the bleaching effect, comparing the wine solutions with pH between 3 and 5. For solutions with pH between 6 and 8, the whitening effect was significantly higher, and the solution with pH 9 presented the greatest color change. The mean ΔE values for the wine solutions are shown in Figure 3. Among the tobacco solutions with pH values between 3 and 5, no significant variation was observed in the bleaching effect. From pH 6 onward, the alkalinity significantly increased the bleaching effect of hydrogen peroxide on the colored solution. Figure 4 shows the mean values of ΔE for the different pH values of tobacco solution.

DISCUSSION

Wine is produced from grapes and contains anthocyanin, which is responsible for the reddish and purple pigmentation in fruits and vegetables.[26,27] The carbon chain of this chromophore is shown in Figure 5A.[28] The polyphenols contained in tobacco

Table 1: *Mean Values and Standard Deviation (SD) of the Parameter ΔE for Wine and Tobacco Solutions*

Group	Wine[a]	Tobacco[b]
pH 3.0	9.09 (2.40)a	1.25 (0.91)A
pH 4.0	9.07 (0.75)a	1.07 (0.12)A
pH 5.0	8.95 (0.55)a	1.84 (0.27)A
pH 6.0	12.20 (1.17)b	3.33 (0.24)B
pH 7.0	4.12 (0.54)b	6.22 (0.90)C
pH 8.0	12.82 (1.22)b	8.57 (0.84)D
pH 9.0	18.50 (3.41)c	11.23 (1.18)E

[a] *Different lowercase letters mean significant differences among the groups for wine solutions ($p<0.05$).*
[b] *Different capital letters mean significant differences among the groups for tobacco solutions ($p<0.05$).*

plants play an important role in physiological processes of the plant metabolism. They are considered vital components of tobacco, especially because of their contribution to the sensory properties of taste, color, bitterness, and antioxidant properties. One of the main polyphenol components of tobacco is rutin,[29-31] whose structure is shown in Figure 5B.[31] According to the measured values of ΔE, it is evident that the chromogen solution present in tobacco is more resistant to oxidation by hydrogen peroxide than the one present in wine. Such a difference may be due to the chemical structure of these molecules.

The factors that change the decomposition of hydrogen peroxide include impurities, temperature, pH, and metal ions from solutions.[8,10,32] As it concerns pH, the results reported in our study, directly measuring the color of the solution containing the chromogen, clearly show that the efficacy of

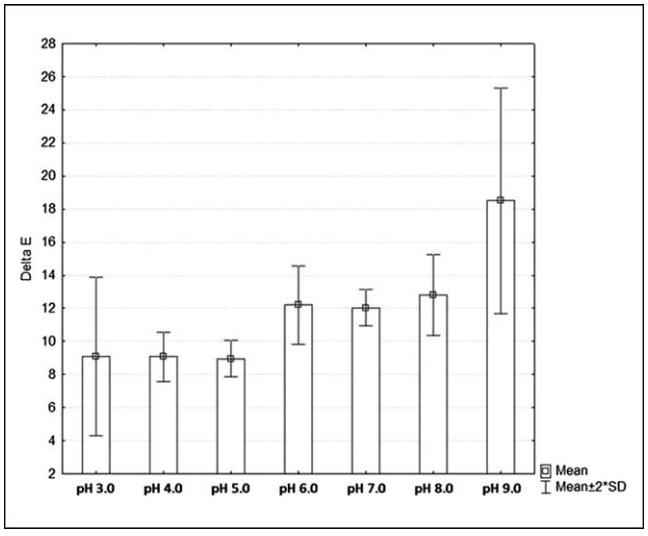

Figure 3. *Mean ΔE values in relation to the increase in the pH of wine.*

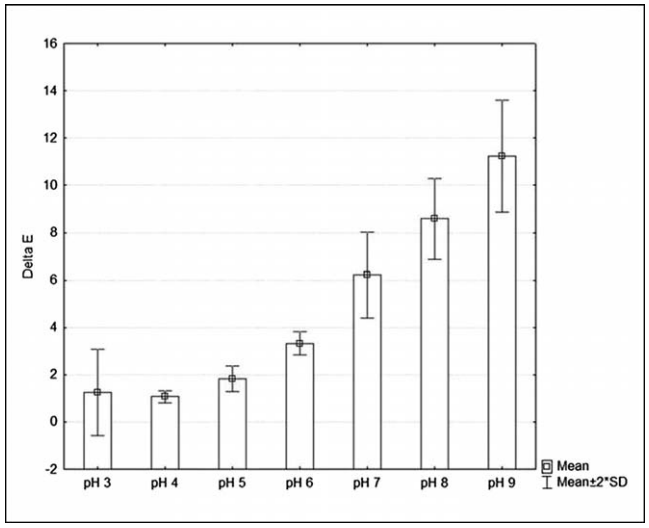

Figure 4. *Mean ΔE values in relation to the increase in the pH of the tobacco.*

hydrogen peroxide bleaching is directly proportional to the pH of the solution. The significant increase in bleaching outcomes occurs from pH 6.0, with maximum effectiveness achieved at pH 9.0, both for wine and tobacco solutions; thus, the null hypothesis was rejected.

Similar results were obtained in a previous study investigating the chemical activity of hydrogen peroxide on chromogens of a tea solution, by measuring the absorbance of the solution as a

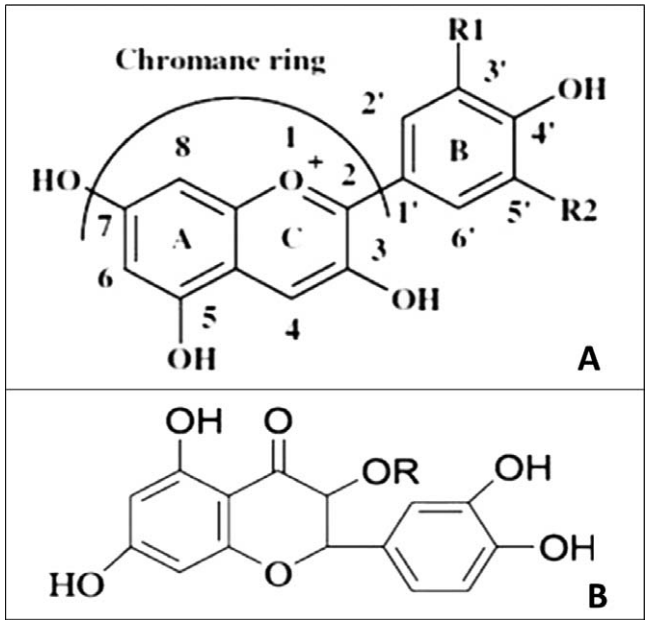

Figure 5. *(A) Chemical structure of the basic anthocyanin, wine chromogen. (B): Chemical formula of rutin, tobacco chromogen.*

function of time. The authors found an increase in the speed of the reaction between pH 8 and 9.[18] In studies with human teeth, an increase in the whitening efficacy of bleaching gels with alkaline pH was also observed, when compared with acid gels.[19,33] In addition, alterations in enamel surface properties were investigated, and there was no evidence of erosion when gels with neutral or alkaline pH were used,[19] different from what occurred in studies on dental tissues in acidic environments.[8,34]

Chen and others[8] investigated hydrogen peroxide decomposition using a system in which two bottles were connected and sealed. They showed that the decomposition was faster and more violent when the peroxide was mixed with 20% sodium hydroxide than when mixed with hydrochloric acid and ether. Adbel-Halim and Al-Deyab[11] evaluated the bleaching in cotton fabrics using hydrogen peroxide and concluded that the rate of H_2O_2 decomposition elevates significantly with the increase of the pH from 5 to 11 and that the pH exhibits a large effect on the time required for the complete decomposition of the hydrogen peroxide and for the bleaching effect.

These positive results obtained with the increase in pH values can be explained based on the chemical reactions involved in the process. According to Brooks and Moore,[35] hydrogen peroxide decomposes into H^+ and perhydroxyl (equation 1). The latter leads to the formation of free radicals (equation 2), which are the active species of oxidizing chromogens. Stoichiometric experiments showed that the formation of perhydroxyl ion is influenced by pH; thus, the higher the pH, the more ions are formed, leading to more free radical production. The perhydroxyl anion is the primary key species responsible for the bleaching outcomes. In one experiment with cotton fabric, the degree of bleaching obtained was directly related to the increase in pH and the concentration of perhydroxyl anion in the whitening solution.[35] An increase of oxygen release as a result of the increased pH has also been observed.[36-38]

Another theory is that the traces of heavy metals such as iron, in contact with the hydrogen peroxide, form unstable peroxides or complex per-ions, which result in its decomposition. However, when the pH is high, there is the formation of insoluble iron hydroxide (colloidal hydroxide), which exerts higher catalytic activity for the peroxide decomposition than the complex peroxide or per-ions. With a further increase of pH and excess of alkalinity, these hydroxides are redissolved and release the metal ions, which increase the catalytic activity even more, therefore increasing the bleaching effect.[39]

On the other hand, other studies have shown similar efficacy comparing whitening gels with acid and neutral pH.[6,32] These results might be due to the use of manufactured bleaching gels with different formulations, which may contain other ingredients in the formula, such as thickeners, fluoride, potassium nitrate, and others (depending on the manufacturer), which could interfere with the final result of bleaching in a more expressive way than just the pH.

Many bleaching solutions and gels manufactured in a single bottle, ready for use, are acidic or neutral, in order to make the chemical stabilization of hydrogen peroxide easier, preventing their decomposition. This aims to prevent the formation of oxygen and water inside the bottle, thus enhancing the validity of the product, although its whitening efficacy is suppressed.[8,9,40,41] The high level of hydrogen peroxide stability and its reduced catalytic activity in acid systems were attributed to the lack of an initiator necessary to decompose hydrogen peroxide into free radicals.[41] The perhydroxyl ion, which is supposed to start the decomposition of hydrogen peroxide in alkaline solutions, is present in insignificant amounts in acidic solutions since the ionization of hydrogen peroxide is not favored.

There are also gels on the market in which an alkalizing agent should be mixed with hydrogen peroxide during clinical use, so that it is used with a higher pH,[30] thereby improving the bleaching efficacy and preventing dental surface demineralization.[18] The effects of alkaline pH in soft tissues range from mild irritation to severe ulcers. Thus, during the bleaching treatment, the direct contact between gel and soft tissues must be avoided. The possibility of burns is another reason for increasing attention to the total isolation from soft tissues to the teeth using a gingival barrier or a rubber dam.

These results show the importance of the formulation of bleaching products with a pH higher than 6, to achieve better bleaching outcomes and reduce damage to dental tissue, which would make the treatment more effective and safe. Products that are stored in separate bottles and mixed at time of use seem to be the best option, keeping the peroxide at an acidic pH for stability and immediate alkalinization during clinical use. This can be achieved by a self-mixing syringe, in which the gel is prepared only at the time of use.

The hydrogen peroxide molecule has strong oxidation activity. When in contact with oral tissues, the molecule is decomposed, producing highly reactive free radicals. For dental bleaching procedures, two main techniques are available. The at-home bleaching technique uses hydrogen peroxide gel in low concentrations, up to 10%, in trays or strips applied over the patient's teeth with no special isolation, without major irritative effects on the soft tissues. However, for the in-office technique, highly concentrated hydrogen peroxide gels, up to 38%, are applied over the enamel but with some kind of previous gingival isolation, preventing the gel from contacting the soft tissues. If this contact occurs, a chemical burning is always expected by the oxidative effect of hydrogen peroxide, even in a neutral pH. According to the OECD Guidelines for Testing of Chemicals, related to acute dermal irritation/corrosion, substances exhibiting pH extremes such as <2.0 and >11.5 may have strong local effects, identifying those substances as corrosives to skin or mucosa.[42] If the pH is >2.0 and <11.5, it is assumed as not corrosive or irritative in relation to pH. In our study, the pH range tested was 3-9. Therefore, no irritative effects are expected for a bleaching compound in this range, with the possible aggressive effects related to the oxidative action of hydrogen peroxide by itself.

Clinical application of whitening products with an alkaline pH needs further study since other variables inherent to *in vivo* treatment may represent changes in the bleaching efficacy. This *in vitro* study aimed to investigate the influence of pH as an isolate factor, but it is known that other factors as mentioned above also influence the efficacy of the bleaching solutions. The next step after confirmation of the importance of alkalinity to whitening solutions is to perform tests in extracted teeth and subsequently in clinical applications. Factors such as the influence of high pH on the stability of other components of the formulation, the issue of potential pulp irritation caused by alkalinity, and product stability require further analysis.

CONCLUSIONS

The efficacy of hydrogen peroxide bleaching is directly proportional to the increase of its pH. The significant increase in bleaching outcomes occurs from pH 6.0, with maximum effectiveness achieved with pH 9.0.

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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