BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-979020201000X32e19501

Article

Current status of whitening agents and enzymes in Dentistry

Maria Cibelle Pauli[1], Michel Yudi Shinkai Kanemaru[2], Waldemir Francisco Vieira-Junior[3], Débora Alves Nunes Leite Lima[4], Juliano Lemos Bicas[2], Gislaine Ricci Leonardi[5*]

[1]Department Translational Medicine, Federal University of São Paulo, São Paulo, SP, Brazil, [2]Department of Food Science, School of Food Engineering, University of Campinas, Campinas, Brazil, [3]Department of Restorative Dentistry, São Leopoldo Mandic Institute and Dental Research Center, Campinas, São Paulo, Brazil, [4]Department of Restorative Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, São Paulo, Brazil, [5]Faculty of Pharmaceutical Sciences, University of Campinas, Campinas, Brazil

This study reviews the knowledge on the use of conventional dental whitening and the use of enzymes as a new approach in bleaching. A review of the literature was based on academic articles and on patents related to the use of enzymes in dental bleaching. Tooth whitening techniques used nowadays are well reported in the literature, and its mechanism of action consists of an oxidoreduction reaction with the release of free radicals. The great instability of radicals, when in contact with the tissues, promotes oxidation and reduction in the size of the pigment chains incorporated into them. These pigments are eventually broken down into smaller and smaller molecular chains and end up being diffused from the dental structure. In turn, the use of enzymes aimed at tooth whitening can be a less harmful alternative to the tooth because their specificity regarding the substrate makes them of great interest to perform specific reactions, reducing collateral effects. The use of proteolytic enzymes and oxidoreductases paired with the application of peroxides, can be a promising alternative for obtaining even better results in the dental bleaching process.

Keywords: Dental bleaching. Biotechnology. Oxidoreductase enzymes.

Clinical Relevance: The combined application of several enzymes paired with the application of peroxides for tooth whitening can be a less harmful alternative for the teeth, reducing undesirable side effects and is therefore a promising alternative for dental bleaching.

Clinical Relevance: The combined application of several enzymes together with the application of peroxides for tooth whitening can be a less harmful alternative to the tooth, reducing unwanted collateral effects and, thus, becoming a promising alternative for dental bleaching.

INTRODUCTION

The search for aesthetics and cosmetic care is highly valued, especially in dental offices. Teeth appearance has never been so important as today: a smile with pearly whites, well outlined and aligned, is commonly associated to a beauty standard in current society (Van der Geld *et al.,* 2007). Chromatic alterations, whether of a single tooth or a group of teeth, cause discomfort related to the individual's aesthetics, negatively interfering in their social and professional life (Filho Menezes *et al*., 2006; Viegas *et al.,* 2014).

The search for aesthetics and cosmetic care is highly valued, especially in dental offices. Teeth appearance has never been so important as today: a smile with pearly whites, well outlined and aligned, is commonly associated

***Correspondence:** G. R. Leonardi. Faculdade dDe Ciências Farmacêuticas - FCF. Rua Cândido Portinari, 200 - Cidade Universitária. CEP: 13083-871 - Campinas/SP/Brasil. E-mail: gislaine.leonardi@fcf.unicamp.br
Waldemir Francisco Vieira-Junior Orcid: https://orcid.org/0000-0001-8226-3100

to a beauty standard in current society (Van der Geld *et al.,* 2007). Chromatic alterations, whether of a single tooth or a group of teeth, cause discomfort related to the individual's aesthetics, negatively interfering in their social and professional life (Filho Menezes *et al.*, 2006; Viegas *et al.,* 2014).

Teeth are polychromatic structures arising from the overlapping of tissues with different characteristics (Joiner, 2004; Watts, Addy, 2001) and optical properties such as translucency, fluorescence, and opalescence (Priest, Lindke, 1999). Tooth color is associated with light reflection, dispersion, and absorption by the enamel (Vieira-Junior *et al.,* 2018), dentin and pulp; however, the general chromatic appearance of the teeth is mainly related to the color of the dentin (Ten Bosch, Coops, 1995).

Dental color is determined by a combination of intrinsic chromatic characteristics and the presence of any extrinsic stain that may form on the tooth surface (Watts, Addy, 2001). Intrinsic staining is a result of problems and defects that occurred during odontogenesis due to some systemic disorders such as measles and scarlet fever, or due to dental trauma that may lead to internal bleeding, in addition to the darkening caused by the natural aging process (Alqahtani, 2014; Bernardon *et al.,* 2010; Bizhang *et al.,* 2009; Lo Giudice *et al.,* 2016). Extrinsic color is related to the adsorption of chromogens, especially in the acquired pellicle of the enamel surface, which may later cause pigmentation (Alqahtani, 2014; Bernardon *et al.,* 2010), Extrinsic chromatic changes may result from exaggerated or continuous exposure to some foods and beverages such as coffee, tea, soft drinks and dyes, in addition to smoking (Alqahtani, 2014; Bernardon *et al.,* 2010; Bizhang *et al.,* 2009; Lo Giudice *et al.,* 2016).

Historically, dental bleaching techniques exist since ancient Egypt, where the population used vinegar to whiten teeth (Kihn, 2007; Lima *et al.,* 2012). The Romans tried to use urine for the same purpose (Kihn, 2007). However, only in the late 19th century with the advent of the "organic chemistry revolution" these methods started to be further explored.

In the 19th century, the first reports on the use of peroxides arose, associated with an electric current to accelerate the reaction of oxygen release, thus promoting whitening in vital and non-vital teeth. In the 20th century, there are literature reports on the use of hydrogen peroxide together with other bleaching agents such as sodium perborate and carbamide peroxide associated with the application of electric current, heat source, and high-intensity lamp, aimed at better results in tooth whitening (Fasanaro, 1992).

Dental bleaching is a non-invasive technique in which chemical agents are able to diffuse through the tooth surface, releasing free radicals that oxidize organic pigments of the teeth structure and, thus, cause whitening (Carey, 2014; Féliz-Matos, Hernández, Abreu, 2014; Ontiveros, Paravina, 2009; Torres *et al.,* 2011;). Categorically, there are different approaches to the bleaching treatment (Joiner, 2006): I) At-home whitening, performed by the patient under supervision of a dental surgeon, using low-concentration bleaching agents in a high-frequency regime (Haywood, Heymann, 1989; Yey, Su, Lu, 2005); II) clinical bleaching, using high-concentration agents that are applied by a dental surgeon in a clinical environment; III) over-the-counter products (OTC), which have bleaching, abrasive or optical active principles added to the composition of toothpastes, rinses and strips, and are sold directly to the patient. Sometimes, OTC products do not have sufficient concentrations of bleaching agents for an in-depth tooth whitening, nor an ability to propagate to the dentin or potentiate the bleaching treatment performed by the dentist (Joiner, 2010; Vieira-Junior *et al.,* 2019). In this category there are compounds capable of optically changing the perception of light or contributing to the removal of extrinsic pigments (Lima *et al.,* 2008; Kugel *et al.,* 2007), including those associated to the acquired pellicle of the enamel (Joiner, 2010).

Regardless of the technique and bleaching agent used, some adverse effects are reported, such as oral mucosa irritation, sensitivity, among others, which may be caused by both the whitening agents as well as by the device used during homemade treatment, transient sensitivity and changes in the physicochemical properties of hard dental tissues (Alqahtani, 2014; Kihn, 2007; Kugel *et al.,* 2007; Vieira-Junior *et al.,* 2006; Yey, Su, Lu, 2005).

The search for satisfactory whitening results with less adverse effects has been increasing, arising from a population highly driven by aesthetic standards and appearance (Kwon, Wertz, 2015). Thus, considering

the growing demand for these cosmetic procedures and the need to minimize the adverse reactions associated, the search for alternative whitening methods, more conservative and effective, has attracted commercial attention (Kwon, Wertz, 2015). Given this context, biotechnology offers promising tools to meet such demand. This review describes whitening agents while focusing on the potential use of enzymes for this purpose, which provides advantages such as the reduction of adverse effects caused by conventional treatments.

METHODS AND MATERIAL

A narrative review of the literature was performed with the following inclusion criteria: original articles of quantitative and qualitative research, in English, to get information on the subject under scrutiny. The search was based on scientific articles using the keywords: *Tooth Whitening, Tooth Bleaching Agent, Biotechnology, Enzymes*, using as criterion articles published in the last 12 years. In addition, classical references pertaining to the history of dental bleaching use were included. The search for patents related to the use of enzymes in dental bleaching was carried out using the Software Orbit Intelligence of Questel with the following research words: *Dental AND Bleaching AND Enzyme*, *Toothpaste AND Enzyme, Laccase AND Tooth, Catalase AND Tooth OR Dental, Peroxidase AND Tooth.*

Conventional approaches use for dental bleaching

The most commonly used active ingredient in various dental bleaching techniques is hydrogen peroxide. Whitening agents can be made available in various forms, such as gels, stripes, tubes of toothpaste, among others (Joiner, 2010; Lima, Araújo, 2006), under the presentation of hydrogen peroxide, carbamide peroxide or sodium perborate.

The action mechanism suggested for dental bleaching consists of an oxidoreduction reaction with release of free radicals (Kwon, Wertz, 2015). The high instability of radicals when in contact with the intended tissues oxidizes and reduces the size of pigments chains incorporated to them (Kwon, Wertz, 2015). Such pigments are cleaved in increasingly smaller molecular chains and end up being spread from the tooth structure (Eimar *et al.*, 2012; Kwon, Wertz, 2015; Marson *et al.*, 2008) or, due to the reduced size of their chains, they indirectly promote the absorption of light, making the tooth apparently whiter (Sulieman *et al.*, 2004). A description regarding conventional bleaching techniques will be presented with the aim of presenting a broad discussion and providing a critical comparison of biotechnological compounds.

Dental bleaching with Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a naturally colorless liquid, slightly more viscous than water, and has a molar mass of 34.01 $g.mol^{-1}$. Due to its low molecular weight, it has a high capacity of penetration in the enamel and dentin (Ubaldini *et al.*, 2003). It is typically used at a 35-38% concentration in clinical applications or 1.5-7.5% in at-home applications using individual devices (Bernardon *et al.*, 2010; Kihn, 2007; Sulieman *et al.*, 2006).

The H_2O_2 dissociates into free radicals of oxygen (•O•), peroxyl (HO•) and perhydroxyl (HOO•), which are highly unstable and reactive since they have unpaired electrons in the valence band (Bernardon *et al.*, 2010; Kihn, 2007; Sulieman *et al.*, 2006). These free radicals diffuse in enamel and dentin and react by attacking chemical bonds present in high-molecular-weight pigments, fragmenting them into smaller molecules without the original coloration (Bernardon *et al.*, 2010; Kihn, 2007; Sulieman *et al.*, 2006). The reaction of H_2O_2 with pigments is presented in Figure 1.

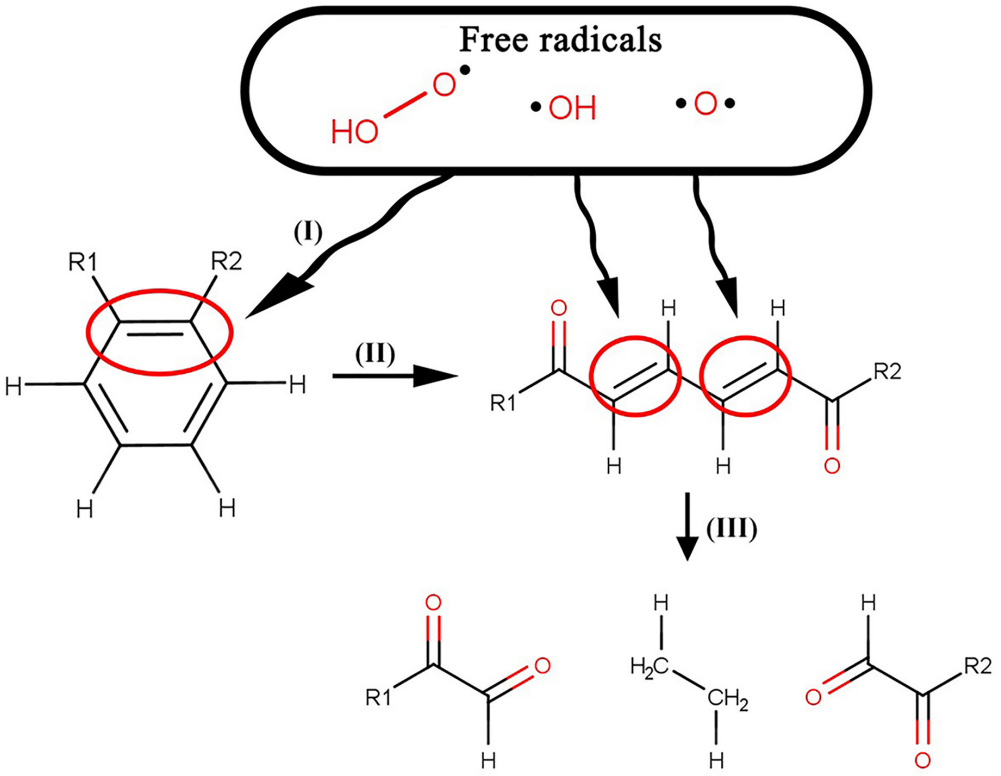

FIGURE 1

Carbamide Peroxide

Carbamide peroxide is the most widely used bleaching agent in at-home whitening, at concentrations between 10 and 22% applied in previously-made devices, although it is also used in office at a 35% concentration (Kihn, 2007; Soares *et al.*, 2013). Carbamide peroxide ($CH_6N_2O_3$), when in contact with the oral environment and saliva, dissociates into hydrogen peroxide (H_2O_2) and urea ($(NH_2)_2CO$). Urea, in turn, decomposes into carbon dioxide (CO_2) and ammonia (NH_3), acting as a stabilizer, responsible for prolonging the life of the bleaching agent and maintaining the pH of the gel close to neutral (Navarra *et al.*, 2014).

The formation of ammonia causes pH to rise, thus increasing the efficiency of the dissociation reaction of the hydrogen peroxide and, consequently, the production of free radicals, responsible for the breakdown of pigmented molecules, as explained in the previous section (Bernardon *et al.*, 2010; Kihn, 2007).

Undesirable effects of conventional bleaching

Effects of bleaching agents on dental tissues have not been completely understood, their intensity and range of cases vary according to different methods and study designs (Attin, 2009; Joiner, 2007; Zeczkowski *et al.*, 2015). However, it is worth noting that the human saliva plays an essential role in promoting the remineralization or reduction of demineralization and deleterious effects on teeth subjected to bleaching treatment (Zeczkowski *et al.*, 2015).

Among the adverse effects reported, the following are noteworthy: I) increased permeability and changes in the roughness and topography of the enamel (Camargo *et al.*, 2007; Hosoya *et al.*, 2003; Llena, Esteve, Forner, 2017; Markovic *et al.*, 2007; Vieira-Junior *et al.*, 2018);

II) decrease surface and cross-sectional microhardness (Al-Salehi, Wood, Hatton, 2007; Berger *et al.*, 2010; Cavalli *et al.*, 2018); III) surface changes visualized in a Scanning Electron Microscopy (D'Amario *et al.*, 2012; Grazioli *et al.*, 2018; Vieira-Junior *et al.*, 2018); IV) dissolution of calcium, phosphorus, and fluorine of the dental hydroxyapatite crystal for the whitening gel (Al-Salehi, Wood, Hatton, 2007; Cavalli *et al.*, 2018; Vieira-Junior *et al.*, 2018); V) changes in properties of resin-based restorative materials (Gouveia *et al.*, 2016; Gouveia *et al.*, 2006; Telang *et al.*, 2018); VI) alteration in the bond strength of dental substrates by restorative materials (Lima *et al.*, 2011; Miranda *et al.*, 2013).

In addition to these, whitening-related tooth sensitivity is also a pretty common adverse effect (Goldberg, Grootveld, Lynch, 2010; He *et al.*, 2012; Li, 2011; Pretty, Edgar, Higham, 2003), Such sensitivity possibly comes from the diffusion of peroxides through dental substrates (Li, 2011), as the hydrogen peroxide is able to reach the dental pulp and have a cytotoxic effect, even when this gel is used at low concentrations (Kwon, Wertz, 2015).

Use of enzymes as whitening agents

Industrial biotechnology is an essential tool for developing new drugs and cosmetic assets (Fox, 2005; Orladelli *et al.*, 2012). After the discovery of antibiotics, the use of enzymes is considered to be the most promising tool for the pharmaceutical industry and may bring economic and environmental benefits. They have paramount importance in the production of new medicines and products, in addition to having been increasingly popular in the cosmetics industry—also called Enzymes-cosmetics (Fox, 2005).

Among these cosmetic products, enzymes are mainly used in personal care products, skin exfoliation and anti-aging creams aimed at protecting the skin against external agents, promoting biological peeling, fighting free radicals, among others (Fox, 2005; Orladelli *et al.*, 2012).

The use of enzymes in the treatment of stained teeth is possible due to the fact extrinsic tooth pigmentation derives mainly from the incorporation of organic compounds containing chromogens (the part of the organic molecule which generates color) in the acquired pellicle (Joiner, 2010; Watts, Addy, 2001). These compounds with color present in dark foods and beverages such as coffee or wine are usually derived from polyphenols (Joiner, 2010; Watts, Addy, 2001). Two different strategies may be applied: the first by removing the pellicle using proteases, and the second using enzymes to chemically change the color compounds, with specific enzymes (Wiliams, Prencipe, Masters, 2004). One of the main classes of polyphenols that cause stains in teeth are tannins, which are soluble in water and present a molecular weight from 500 to 3000 kDa, being present in different plant parts, such as bark, wood, leaves, fruits, roots, and seeds (Govindarajan *et al.*, 2016). In dentistry, wine is a relevant tannin source, responsible for intensifying teeth pigmentation (Côrtes *et al.*, 2013).

One of its most decisive biological characteristics is its ability to establish a complex with different minerals and macromolecules, such as proteins, cellulose, starch and others, affecting the degradation and absorption of these nutrients (Govindarajan *et al.*, 2016). In the case of teeth, such complexation can be associated with the appearance of stains resulting from the association between food tannins and tooth components.

The ability of tannins regarding the complex is due to its structure constituted by multiple hydroxyl groups in their phenolic rings, capable of binding to proteins and chelating metals. Due to this, they can easily bind to tooth proteins (Chavez-González *et al.*, 2012). However, tannin compounds present in foods feature an intense color, ranging from yellow to brown, being one of the classes of compounds responsible for tooth pigmentation; hence, one of the compounds that must be removed during the whitening process (FAO, 2019; Hertel *et al.*, 2017).

The use of enzymes in dental bleaching is a less aggressive alternative for the body, especially compared to conventional methods (Patil *et al.*, 2015). Enzymes can effectively accelerate the reactions it catalyzes (up to more than 10^{12} times in comparison with the spontaneous non-catalyzed reaction) in mild conditions of pH and temperature. In addition, they usually present high specificity, which reduces unwanted side effects (Whitaker, Voragen Wong, 2003).

The use of enzymes in dental bleaching was firstly suggested in the 1960s, with the potential application of fungal proteolytic enzymes (Harrisson *et al.*, 1963). Since then, several patents were filed, claiming the use of several enzymes in the dental bleaching process. Nonetheless, few articles on the application of enzymes as dental bleaching agents can be found (Joiner, 2010). Following, the main enzymes discussed in the literature are described, showing the current state of the knowledge on the topic. The mechanism of action of enzymes in bleaching is shown in Figure 2.

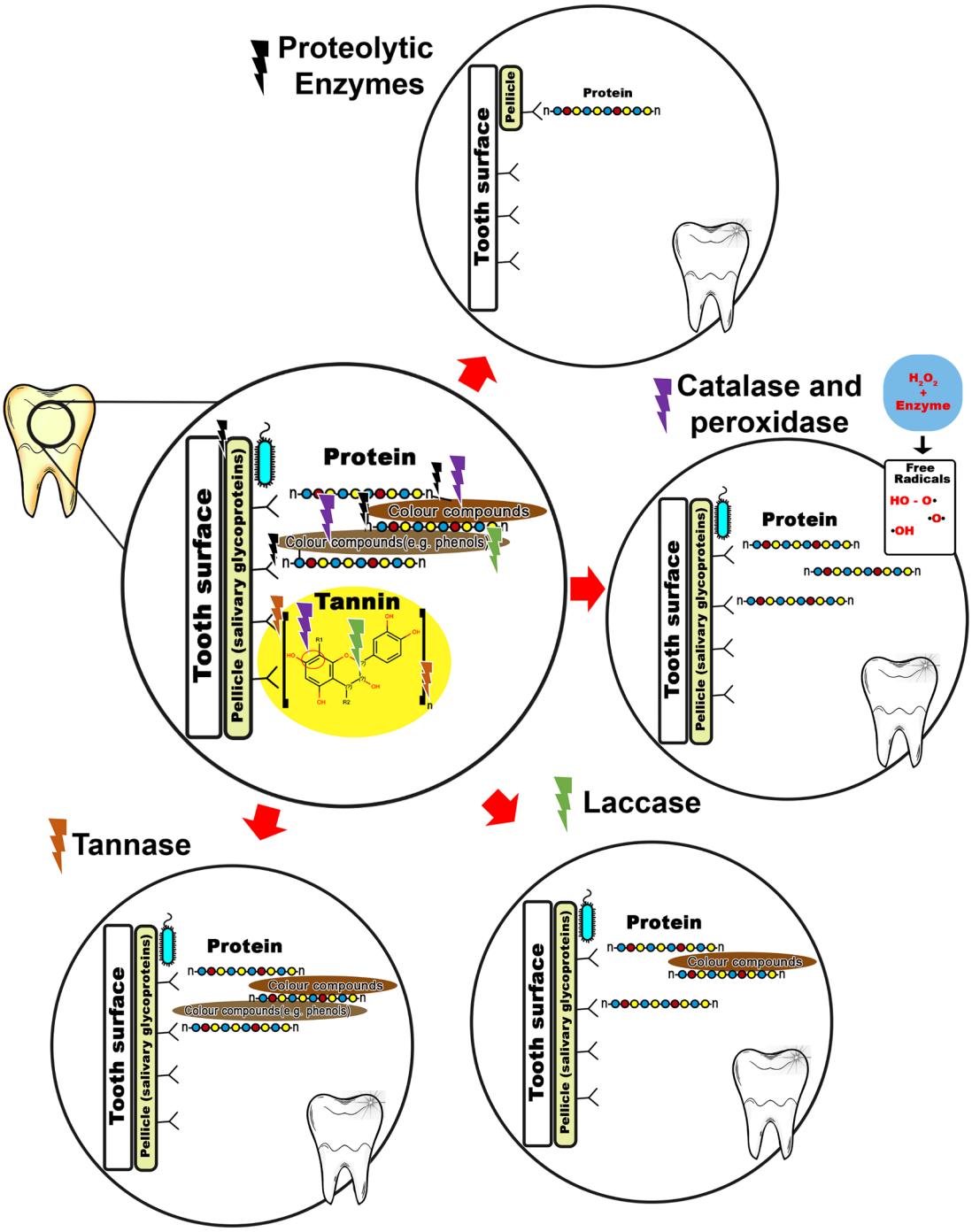

FIGURE 2

Proteolytic enzymes

Proteolytic enzymes were the first to be described as having potential to be applied in dental bleaching based on the fact the external stains are initially incorporated to the teeth surface in the form of a pellicle comprised by saliva proteins (Harrisson *et al.*, 1963). Bleaching using proteolytic enzymes occurs due to the hydrolysis and removal of the biofilm formed on the teeth surface over time, in addition to the breakdown of bonds between proteins and the compounds responsible for the color (Joiner, 2010; Kalyana *et al.*, 2011). Furthermore, the use of proteolytic enzymes has other advantages, such as its potential to prevent bacterial growth, ability to act in pH close to neutral, and biocompatibility of many (such as papain), which do not present cytotoxicity nor secondary effects (Patil *et al.*, 2015).

Ha*rrisso*n *et al.* (1963) tested proteases, amylases, cellulases and lipases in toothpaste compositions, showing that those containing a mixture rich in proteolytic enzymes of fungal origin were effective in reducing external stains after six months of use. According to the authors, a vessel compatible with high concentrations of enzymes and that releases them in a slower rate, such as chewing gums and tablets, could lead to even better results (Harrisson *et al.*, 1963).

In 2011, Kalyana *et al.*, studied the efficacy of the removal of stains *in vitro* using a toothpaste containing vegetal proteolytic enzymes (bromelain and papain). To evaluate whitening effectiveness, the authors photographed the teeth under standardized lighting conditions and calculated their luminosity. The authors observed that, on average, the luminosity of teeth brushed with the toothpaste containing enzymes was significantly higher than in the control group, showing a reduction in teeth stains. In a similar study, Patil *et al.*, 2015**,** compared commercial toothpastes with chemical abrasives (perlite and calcium carbonate) with toothpastes containing enzymes (bromelain and papain) and found no statistical differences between both products. Thus, the whitening effect of the product with enzymes was considered similar to that of those containing abrasives, preventing the occurrence of new stains.

Oxidoreductase (EC 1)

Oxidoreductases are enzymes that catalyze the electron transfer of a molecule, known as electron giver (reducer), to another to be oxidized (oxidant), also called electron acceptor (Whitaker, Voragen Wong, 2003). This group of enzymes usually employ NAD(P) as co-factor. As these enzymes can catalyze oxidation reactions involving pigments, they have been used in the whitening (color removal) in various industry sectors, such as paper, fabrics, foods, and wastewater treatment (Chauhan, Goradia, Saxena, 2017; Kang *et al.*, 2010; Shraddha *et al.*, 2011; Whitaker, Voragen Wong, 2003). Some patent requests applying oxidases as agents in the dental bleaching process were filed, mainly describing the use of catalase (EC 1.11.1.6), laccase (EC 1.10.3.2) and peroxidase (EC 1.11.1.9), as detailed below.

Catalase (EC 1.11.1.6)

Catalase is an essential enzyme for aerobic organisms and it catalyzes the decomposition of hydrogen peroxide into water and hydrogen. Additionally, catalase is able to oxidize other hydrogen-releasing compounds, such as methanol, ethanol, formic acid, and phenols, using hydrogen peroxide as electron acceptor (Whitaker, Voragen Wong, 2003). Thus, catalase is not used to generate a direct whitening effect: its function on the bleaching process is to degrade peroxides during or after treatment with bleaching agents (hydrogen or carbamide peroxide).

It is well known that these peroxides can be degraded by the catalase and peroxidase of the dental pulp. Because of this, some authors evaluated the potential use of exogenous catalase as a way to neutralize residual peroxide from this process. Rotstein (1993), for example, demonstrated the enzyme was able to eliminate the peroxide in the first rinse, which did not occur with conventional rinsing (only water). In another study, Rotstein, Wesselink, Bab, 1993 show the use of catalase could protect the oral mucosa of rats with induced injuries, observing that, when applied in the tongue before treatments with hydrogen peroxide, the enzyme protects the tissue against adverse reactions

Based on such properties, a patent request (EP1224925) was filed for a tooth-whitening formulation containing hydrogen peroxide as the main active ingredient and catalase as one of the possible activating compounds. The patent claims that the use of the catalase enzyme quickly increases the release rate of O_2 and H_2O and the number of free hydroxyl radicals, which causes a fast whitening action and reduces the time required for the peroxide to act (Banerjee, Friedman, 2002). Similarly, another patent request (BR 102014010685-5 A2) claims that a 5-mg quantity of the enzyme applied for 3 minutes would be enough to break down all the H_2O_2 (35 to 37%), with the advantages of not requiring the use of LEDs, not causing pH changes, and reducing the time of dental whitening (Silva *et al.*, 2014).

Laccase (EC 1.10.3.2)

Laccase is an enzyme of the group of "blue oxidases", named due to the copper present in their active sites, which gives a blue color to these proteins. This enzyme catalyzes the oxidation of several compounds, mainly phenolic compounds, including monophenols, o- and p-diphenols, methoxyphenol, aminophenols, in addition to aryl amines and even some inorganic ions (potassium ferrocyanide) (Chauhan, Goradia, Saxena, 2017; Whitaker, Voragen Wong, 2003). They use molecular oxygen as electron acceptors, forming water as a reaction byproduct.

This enzyme features potential applications in the food, paper and cellulose, fabrics, synthetic chemistry, and cosmetics industry, in addition to use in the bioremediation and bio-degradation of pollutant phenolic compounds (Shraddha *et al.*, 2011). Considering that, as mentioned before, dark stains on teeth can have a polyphenolic character, and keeping this oxidation capacity of phenolic compounds through laccase in mind, Colgate-Palmolive listed in 1997 a patent request (WO9706775) of formulations containing this enzyme as a tooth-whitening agent (Aaslyng *et al.*, 1997). This patent also vindicates the use of other oxidoreductases in dental bleaching formulation, such as glucose oxidase, hexose oxidase, L-amino acid oxidase, xylitol oxidase, galactose oxidase, pyranose oxidase, alcohol oxidase, peroxidase and haloperoxidase (Aaslyng *et al.*, 1997). Years later, the Kobayashi-Pharmaceutical and the Takasago Perfumery also requested a patent (JP4637494) involving the application of laccase in dental bleaching. However, in this case, only a cleaning solution was tested, in artificial teeth.

Peroxidase (EC1.11.1)

Peroxidases are a group of oxidoreductases that use the peroxide as an electron acceptor instead of oxygen (Whitaker, Voragen Wong, 2003). The main specific enzymes of this group are NADH peroxidase (EC 1.11.1.1), glutathione peroxidase (EC 1.11.1.9) and lactoperoxidase (EC 1.11.1.7), in addition to non-specific enzymes collectively referred to as peroxidase (Hamid, Rehman, 2009).

Peroxidases are capable of oxidizing a broad range of substrates, using hydrogen peroxide or other peroxides as the electron acceptor. Thus, these enzymes feature a large number of industrial and analytical applications, such as in preparations of enzyme-linked immunosorbent assay (ELISA), bioremediation, synthetic dye degradation and bleaching in the paper and cellulose industry (Shraddha *et al*, 2011).

A recent study described that the horseradish peroxidase activation of high concentration bleaching gel accelerates the degradation of hydrogen peroxide, which could decrease the adverse effects on odontoblast-like cells (Ortecho-Zuta *et al.*, 2018).

Similar to laccases, the degradation properties of several color compounds allow peroxidase enzymes to have potential application as dental bleaching agents, with the advantage of being able to be used concomitantly with peroxides (reducing agent). Starting from such a premise, the company Kin Laboratories patented (EP1224925) the use of peroxidase in toothpaste for dental bleaching (Pons *et al.*, 2001). The efficiency of toothpaste containing lactoperoxidase (5%) combines with carbamide peroxide (3%) was evaluated in 20 volunteers for 21 days (Pons *et al.*, 2001; Forner *et al.*, 2012). The treatment consisted of teeth brushing using the toothpaste for 2 minutes, three times per day. Teeth color before and after treatment was recorded using spectrophotometry and analyzing

color variations in teeth using the CIE L*a*b* standard. The authors have concluded that the use of the product containing low amounts of carbamide peroxide, when activated by peroxidase, was effective in whitening teeth even with a short time of exposure. Thus, the use of peroxidase proves to be able to increase the efficiency of dental bleaching by carbamide peroxide (Forner *et al*., 2012).

*Llen*a et al., (2016) observed increased luminosity and reduced b* component compared to the control group in double-blind clinical trials with the same toothpaste involving 48 participants with teeth colored A3 or higher than the *Vita classical guide*. The color variation among groups was not significant (p<0.064) but, according to the authors, the toothpaste containing enzyme could effectively modify the teeth color, progressively starting from the third to the ninth week of treatment, stabilizing after this period.

In an *in vitro* study, the aesthetic effectivity and cytotoxicity of a toothpaste containing 10% hydrogen peroxide and activated by radish peroxidase were evaluated. The authors observed the treatments with 10% peroxide plus enzyme presented statistically similar results (p<0.05) to that of the positive control (gel with 35% hydrogen peroxide). In addition, the authors have observed that the treatment containing radish peroxidase did not present cytotoxic effects, being statistically similar to the negative control (Duque *et al*., 2018).

Tannases (EC 3.1.1.20)

Tannase (EC 3.1.1.20), also known as tannin acyl hydrolase (TAH), is an enzyme that hydrolyzes ester or depsidic bindings in hydrolyzable tannins, such as tannic acid, methyl gallate, ethyl gallate, propyl gallate and isoamyl gallate.

As explained before, many teeth stains are based on tannins. Thus, tannase has been considered an enzyme for dental bleaching. The use of tannase can be useful for removing tannin compounds linked to the teeth proteins; this strategy has already been suggested in patents of oral hygiene products from Colgate-Palmolive (WO2004019899 and WO03094879). These documents cite the potential use of purified tannase of *Aspergillus niger* and *A. allianceus* to ease the removal of stains in the extrinsic portion of the tooth (Szeles *et al*., 2003; Wiliams, Prencipe, Masters, 2004). However, the use of these enzymes is not declared in the requests of the respective patents, as only the use of formulations containing papain and glucoamylase are vindicated (Szeles *et al*., 2003; Wiliams, Prencipe, Masters, 2004).

DISCUSSION

Dental bleaching has become a major ally for dentistry, being able to reestablish teeth aesthetics by achieving good results while conservating dental structure (Joiner, 2006); however, they also feature limitations and risks. Given this context, the personal hygiene and cosmetics industry increasingly seeks to launch new whitening products that maintain healthy dental structure the most, preserving dental tissues.

Dental bleaching is currently much used in dentistry, is considered an effective treatment and is associated to an improvement in the quality of life (De Geus *et al*., 2016). Effective whitening treatments manage to break molecules of chromogens in dental depth, especially in the dentin and not being restricted to the surface. Some of the aforementioned agents could be incorporated into conventional gels to promote catalysis of bleaching reactions. Additionally, proteolytic or chromogen-specific enzymes could be associated with different vessels to potentiate the diffusion of such agents through the dental structure. Some of the compounds described may act in extrinsic pigmentation or in chromogen biofilms associated to the acquired pellicle of the enamel, which could reduce staining, especially in risk patients who are frequently exposed to pigments. In this category, these agents could be associated with oral-care products such as toothpaste and rinses, which provide frequent and daily exposure to the patient. Biotechnology agents discussed in this article are presented as promising alternatives. However, clinical trials addressing the effectivity and safety of these assets must be devised to confirm the findings described in this review.

In addition to the enzymes mentioned in this review, the study of other proteolytic enzymes and

oxidoreductases in dental bleaching also presents considerable potential since the number of enzymes studied up to the moment is limited. Thus, we highlight the potential of new proteolytic enzymes, e.g. those obtained from microorganisms present in the dental microbiota, in dental bleaching. Furthermore, the use of oxidoreductases traditionally used in the treatments of biomass (lignin peroxidase and manganese peroxidase) are also interesting options to be tested. It is well known that such enzymes present greater redox potential than laccases and could degrade a broader range of polyphenols (Llena *et al.*, 2016). Such enzymes are known for degrading pigments, a fact that has been even used as a way to quantify the activity of these enzymes (Hamid, Rehman, 2009). The use of new enzymes requires several studies, from *in vitro* trials to extensive clinical studies, to monitor possible secondary or adverse effects.

CONCLUSIONS

Although currently used conventional tooth whitening techniques such as hydrogen peroxide and carbamide peroxide together with their mechanism of action are well reported in the literature, some adverse reactions are reported, such as enamel demineralization, which can be caused both by concentration of the bleaching agent used, application time to obtain the desired color, increase in surface roughness, decrease in microhardness, bond strength of enamel and dentin, and a significant decrease in the concentration of calcium, phosphate and carbon in dental enamel. The use of biotechnology, together with new formulations, aims to promote teeth whitening, or the control of stains, and can provide fewer adverse reactions, which can render great interest in the industrial sector.

ACKNOWLEDGMENTS

This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Financial Code 0001.

The authors thank Espaço da Escrita – Pró-Reitoria de Pesquisa - UNICAMP - for the language services provided.

CONFLICT OF INTEREST

The authors of this article 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.

REFERENCES

Aaslyng D, Tsuchiya R, Smith SF, Gaffar A. European Patent No EP844864. Tooth bleaching. 1997.

Al-Salehi SK, Wood DJ, Hatton PV. The effect of 24h non-stop hydrogen peroxide concentration on bovine enamel and dentine mineral content and microhardness. J Dent. 2007;35(11):845-850.

Attin T, Schmidlin PR, Wegehaupt F, Wiegand A. Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review. Dent Mater. 2009;25(2):143-57.

Alqahtani MQ. Tooth-bleaching procedures and their controversial effects: A literature review. Saudi Dent J. 2014;26(2):33–46.

Banerjee A, Friedman J. European Patent 1224925. Dental bleaching gel composition, activator system and method for activating a dental bleaching gel. 2002.

Bernardon JK, Sartori N, Ballarin A, Perdigão J, Lopes GC, Baratieri LN. Clinical performance of vital bleaching techniques. Oper Dent. 2010;35(1):3-10.

Berger SB, Cavalli V, Ambrosano GM, Giannini M. Changes in surface morphology and mineralization level of human enamel following in-office bleaching with 35% hydrogen peroxide and light irradiation. Gen Dent. 2010;58(2):E74-E79.

Bizhang M, Chun YH, Damerau K, Singh P, Raab WH, Zimmer S. Comparative clinical study of the effectiveness of three different bleaching methods. Oper Dent. 2009;34(6):635-41.

Camargo SEA, Valera MC, Camargo CHR, Mancini MNG, Menezes MM. Penetration of 38% hydrogen peroxide into the pulp chamber in bovine and human teeth submitted to office bleach technique. J Endod. 2007;33(9):1074-1077.

Carey CM. Tooth whitening: what we know. J Evid Based Dent Pract. 2014;14:70-76.

Cavalli V, Rosa DAD, Silva DPD, Kury M, Liporoni PCS, Soares LES, et al. Effects of experimental bleaching agents on the mineral content of sound and demineralized enamels. J Appl Oral Sci. 2018;4(26):E20170589.

Chauhan PS, Goradia B, Saxena A. Bacterial laccase: recent update on production, properties and industrial applications. 3 Biotech. 2017;7(5):323.

Chavez-González M, Rodriguez- Duran LV, Balagurusamy N, Prado- Barragán A, Rodriguez R, Contreras JC. Biotechnological advances and challenges of tannase: An overview. Food Bioprocess Tech. 2012;5(2):445–459.

Côrtes G, Pini NP, Lima DA, Liporoni PC, Munin E, Ambrosano GM, et al. Influence of coffee and red wine on tooth color during and after bleaching. Acta Odontol Scand. 2013;71(6):1475-1480.

D'Amario M, D'Attilio M, Baldi M, De Angelis F, Marzo G, Vadini M, et al. Histomorphologic alterations of human enamel after repeated applications of a bleaching agent. Int J Immunopathol Pharmacol. 2012;24(4):1021-1027.

De Geus JL, Wambier LM, Kossatz S, Loguercio AD, Reis A. At-home vs In-office Bleaching: A Systematic Review and Meta-analysis. Oper Dent. 2016;41(4):341-56.

Duque CCO, Soares DG, Leite MLAS, Anovazzi G, Zuta UO, Bordini EAF, et al. Aesthetic and biological analysis of na experimental tooth-bleaching gel. Dent Maters. 2018;34(SI).

Eimar H, Siciliano R, Abdallah MN, Nader SA, Amin WM, Martinez PP, et al. Hydrogen peroxide whitens teeth by oxidizing the organic structure. J Dent. 2012;40(2):e25–33.

Fasanaro TS. Bleaching teeth: history, chemicals, and methods used for common tooth discolorations. J Esthet Dent.1992;4(3):71-8.

Féliz-Matos L, Hernández LM, Abreu N. Dental Bleaching Techniques; Hydrogen-carbamide Peroxides and Light Sources for Activation, an Update. Mini Review Article. Open Dent J. 2014;8(1):264-8.

Food and Agriculture Organization of the United Nation. FAO. (n.d.) Tannic acid. Retrieved online January 8, 2019 from: http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/Additive-454.pdf.

Forner L, Amengual J, Liena C, Riutord P. Therapeutic Effectiveness of a New Enzymatic Bleaching Dentifrice. Eur J Esthet Dent. 2012;7(1):62–70.

Filho Menezes PF, Barros CHO, De Noronha JAA, Melo Junior PC, Cardoso RM. Critical evaluation of smile. Int J Dent. 2006;1(1):14-19.

Fox CA. Baby care skin protectant. Cosmet Toiletries. 2005;8:34-38.

Goldberg M, Grootveld M, Lynch E. Undesirable and adverse effects of tooth-whitening products: A review. Clil Oral Investig. 2010;14(1):1-10.

Gouveia TH, Públio Jdo C, Ambrosano GM, Paulillo LA, Aguiar FH, Lima DA. Effect of at-home bleaching with different thickeners and aging on physical properties of a nanocomposite. Eur J Dent. 2006;10(1):82-91.

Gouveia TH, Públio JC, Ambrosano GM, Paulillo LA, Aguiar FH, Lima DA. Evaluation of physical properties of a nanocomposite after aging, bleaching and staining. J App Biomater Funct Mater. 2016;14(3):E256-65.

Govindarajan RK, Revathi S, Rameshkumar N, Krishnan M, Kayalvizhi N. Microbial tannase: Current perspectives and biotechnological advances. Biocatal Agric Biotechnol. 2016;6:168–175.

Grazioli G, Valente LL, Isolan CP, Pinheiro HA, Duarte CG, Münchow EA. Bleaching and enamel surface interactions resulting from the use of highly-concentrated bleaching gels. Archi Oral Biol. 2018;87:157-162.

Hamid M, Rehman KU. Potential applications of peroxidases. Food Chem. 2009;115(4):1177-1186.

Harrisson JWE, Salisbury GB, Abbott DD, Packman EW. Effect of enzyme-toothpastes upon oral hygiene. J Periodont. 1963;34(4):334-337.

Haywood VB, Heymann HO. Nightguard vital bleaching. Quintessence Int. 1989;20(3):173-6.

He LB, Shao MY, Tan K, Xu X, Li JY. The effects of light on bleaching and tooth sensitivity during in-office vital bleaching: a systematic review and meta-analysis. J Dent. 2012;40(8):644-653.

Hertel S, Potschke S, Basche S, Delius J, Hoth- Hannig W, Hannig M, et al. Effect of Tannic Acid on the Protective Properties of the in situ Formed Pellicle. Caries Res. 2017;51(1):34–45.

Hosoya N, Honda K, Iino F, Arai T. Changes in enamel surface roughness and adhesion of Streptococcus mutans to enamel after vital bleaching. J Dent. 2003;31(8):543-548.

Joiner A. Tooth colour: a review of the literature. J Dent. 2004;32(1):3-12.

Joiner A. The bleaching of teeth: A review of the literature. J Dent. 2006;34(7):412-419.

Joiner A. Review of the effects of peroxide on enamel and dentine properties. J Dent. 2007;35(12):889-96.

Joiner A. Whitening toothpastes: a review of the literature. J Dent. 2010;38(2):17-24.

Kalyana P, Shashidhar A, Meghashyam B, Sweta S. Stain removal efficacy of a novel dentifrice containing papain and Bromelain extracts – an in vitro study. Int J Dent Hyg. 2011;9(3):229–233.

Kang EJ, Campbell RE, Bastian E, Drake MA. Invited review: Annatto usage and bleaching in dairy foods. J Dairy Sci. 2010;93(9):3891–3901.

Kihn PW. Vital tooth whitening. Dent Clin North Am. 2007;51(2):319-331.

Kugel G, Petkevis J, Gurgan S, Doherty E. Separate whitening effects on enamel and dentin after fourteen days. J Endod. 2007;33(1):34-7.

Kwon SR, Wertz PW. Review of the mechanism of tooth whitening. J Esthet Restor Dent. 2015;27(5):240-257.

Li Y. Safety controversies in tooth bleaching. Dent Clin North Am. 2011;55(2):255-63.

Lima MJP, Araújo JPC. Study in vitro of the action bleaching of to 35% hydrogen peroxide. J Dental Sci. 2006;21(54):376-86.

Lima DA, De Alexandre RS, Martins AC, Aguiar FH, Ambrosano GM, Lovadino JR. Effect of curing lights and bleaching agents on physical properties of a hybrid composite resin. J Esthet Restor Dent. 2008;20(4):266-73.

Lima AF, Fonseca FM, Freitas MS, Palialol AR, Aguiar FH, Marchi GM. Effect of bleaching treatment and reduced application time of an antioxidant on bond strength to bleached enamel and subjacent dentin. J Adhes Dent. 2011;13(6):537-42.

Lima FG, Rotta TA, Penso S, Meireles SS, Demarco FF. In vitro evaluation of the whitening effect of mouth rinses containing hydrogen peroxide. Braz Oral Res. 2012;26(3):269-274.

Llena C, Esteve I, Forner L. Effect of hydrogen and carbamide peroxide in bleaching, enamel morphology, and mineral composition: in vitro study. J Contemp Dent Pract. 2017;18(7):576-582.

Llena C, Oteo C, Oteo J, Amengua J, Forner L. Clinical efficacy of a bleaching enzyme-based toothpaste: A double-blind controlled clinical trial. J Dent. 2016;44:8–12. https://doi.org/10.1016/j.jdent.2015.08.004.

Lo Giudice R, Pantaleo G, Lizio A, Romeo U, Castiello G, Spagnuolo G, et al. Clinical e spectrophotometric evaluation of LED and laser activated teeth bleaching. Open Dent J. 2016;10:242–250.

Markovic L, Jordan RA, Lakota N, Gaengler P. Micromorphology of enamel surface after vital tooth bleaching. J Endod. 2007;33(5):607-610.

Marson FC, Sensi LG, Vieira LCC, Araújo E. Clinical evaluation of in-office dental bleaching treatments with and without the use of light-activation sources. Oper Dent. 2008;33(1):15-22.

Miranda TAM, Moura SK, Amorim VHO, Terada RSS, Pascotto RC. Influence of exposure time to saliva and antioxidant treatment on bond strength to enamel after tooth bleaching: an *in situ* study. J App Oral Sci. 2013;21(6):567-74.

Navarra CO, Reda B, Diolosà M, Casula I, Di Lenarda R, Breschi L, et al. The effects of two 10% carbamide peroxide nightguard bleaching agents, with and without desensitizer, on enamel and sensitivity: an in vivo study. Int J Dent Hyg. 2014;12(2):115-20.

Ontiveros JC, Paravina RD. Color change of vital teeth exposed to bleaching performed with and without light. J Dent. 2009;37(11):840-847.

Orladelli RC, Specian V, Felber AC, Pamphile JA. Enzimas de interesse industrial: Produção por fungos e aplicações. Rev Saúde Biol. 2012;7(3):97-109.

Ortecho-Zuta U, De Oliveira Duque CC, Leite ML, Bordini E, Basso FG, Hebling J, et al. Effects of enzymatic activation of bleaching gels on hydrogen peroxide degradation rates, bleaching effectiveness,and cytotoxicity. Oper Dent. 2019;44(4):414-423. doi: 10.2341/17-276-L (2018).

Patil PA, Ankola AV, Hebbal MI, Patil AC. Comparison of effectiveness of abrasive and enzymatic action of whitening toothpastes in removal of extrinsic stains – a clinical trial. Int J Dent Hyg. 2015;13(1):25–29.

Pons BA, Tur MJA, Riutord SP, Tauler RP, Gimeno FI, Balash R.I, et al. European Patent 1230908. Teeth whitening product. 2001.

Pretty IA, Edgar WM, Higham SM. The erosive potential of commercially available mouthrinses on enamel as measured by quantitative light-induced fluorescence (QLF). J Dent. 2003;31(5):313-319.

Priest G, Lindke L. Tooth color selection and characterization accomplished with optical mapping. Pract Periodontics Aesthet dent.1999;12(5):497-503.

Rotstein I, Wesselink PR, Bab I. Catalase protection against hydrogen peroxide induced injury in rat oral mucosa. Oral Surg Oral Med Pathol. 1993;75(6):744–750.

Shraddha, Shekher R, Sehgal S, Kamthania M, Kumar A. Laccase : microbial sources, production, purification, and potential biotechnological applications. Enzyme Res. 2011; 21, Article ID 217861.

Silva RB, Bezerra JJ, Figueiroa AFA, Selva TMG, Eurípedesoliveira J, Silva GHN, et al. Brazilian Patent 102014010685-5 A2. Maximizador do clareamento dental. 2014.

Soares DG, Ribeiro AP, Sacono NT, Loguércio AD, Hebling J, Costa CAS. Effect of Fluoride-Treated Enamel on Indirect

Cytotoxicity of a 16% Carbamide Peroxide Bleaching Gel to Pulp Cells. Braz Dent J. 2013;24(5):517-521.

Sulieman M, Addy M, Macdonald E, Ress JS. A safety study in vitro for the effects of an in-office bleaching system on the integrity of enamel and dentine. J Dent. 2004;32(4):295-9.

Sulieman M, MacDonald E, Rees JS, Newcombe RG, Addy M. Tooth bleaching by different concentrations of carbamide peroxide and hydrogen peroxide whitening strips: an in vitro study. J Esthet Restor Dent. 2006;18(2):93-100.

Szeles LH, Liu X, Williams M, Carale MTR, Prencipe M, Masters JG. European Patent 1503727. Antibacterial dentifrice exhibiting enhanced antiplaque and breath freshening properties. 2003.

Telang A, Narayana IH, Madhu KS, Kalasaiah D, Ramesh P, Nagaraja S. Effect of staining and bleaching on color stability and surface roughness of three resin composites: An *in vitro* study. Contemp Clin Dent. 2018;9(3):452-456.

Ten Bosch JJ, Coops JC. Tooth color and reflectance as related to light scattering and enamel hardness. J Dent Res. 1995;74(1):374-380.

Torres CRG, Barcellos DC, Batista GR, Borges AB, Cassiano KV, Pucci CR. Assessment of the effectiveness of light-emitting diode and diode laser hybrid light sources to intensify dental bleaching treatment. Acta Odontol Scand. 2011;69(3):176-181.

Ubaldini AL, Baesso ML, Medina Neto A, Sato F, Bento AC, Pascotto RC. Hydrogen peroxide diffusion dynamics in dental tissues. J Dent Res. 2003;92(7):661–665.

Van der Geld P, Oosterveld P, Van Heck G, Kuijpers-Jagtman AM. Smile attractiveness: self-perception and influence on personality. Angle Orthod. 2007;77(5):759-765.

Viegas CM, Paiva SM, Carvalho AC, Scarpelli AC, Ferreira FM, Pordeus IA. Influence of traumatic dental injury on quality of life of Brazilian preschool children and their families. Dent Traumatol. 2014;30(5):338-47.

Vieira-Junior WF, Lima DA, Tabchoury CP, Ambrosano GM, Aguiar FH, Lovadino JR. Effect of toothpaste application prior to dental bleaching on whitening effectiveness and enamel properties. Oper Dent. 2006;41(1):E29-E38.

Vieira-Junior WF, Vieira I, Ambrosano GM, Aguiar FH, Lima DA. Correlation between alteration of enamel roughness and tooth color. J Clin Exp Dent. 2018;10(8):e815-e820.

Vieira-Junior WF, Ferraz LN, Giorgi M, Ambrosano G, Aguiar F, Lima D. Effect of Mouth Rinse Treatments on Bleached Enamel Properties, Surface Morphology, and Tooth Color. Oper Dent. 2019;44(2):178-187.

Watts A, Addy M. Tooth discolouration and staining: a review of the literature. Br Dent J. 2001;190(6):309–331.

Whitaker JR, Voragen AGJ, Wong DWS. Handbook of food enzymology. Marcel Dekker, New York. 2003.

Wiliams M, Prencipe M, Masters JG. US Patent 2004042977. Dual component dental composition containing enzyme. 2004.

Yey ST, Su Y, Lu YC. Surface changes and acid dissolution of enamel after carbamide peroxide bleach treatment. Oper Dent. 2005;30(4):507-515.

Zeczkowski M, Tenuta LMA, Ambrosano GMB, Aguiar FHB, Lima DANL. Effect of different storage conditions on the physical properties of bleached enamel: An in vitro vs. in situ study. J Dent. 2015;43(9):1154-1161.

Received for publication on 19th July 2019
Accepted for publication on 22nd May 2020