UNIVERSIDADE FEDERAL DE PELOTAS

Faculdade de Odontologia

Programa de Pós-Graduação em Odontologia

Dissertação

Desenvolvimento e avaliação da efetividade de agentes potencialmente clareadores livres de peróxidos

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Pelotas, 2017

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Odontologia, área de concentração Dentística.

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Pelotas, 2017

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

R484d Ribeiro, Juliana Silva

Desenvolvimento e avaliação da efetividade de agentes potencialmente clareadores livres de peróxidos / Juliana Silva Ribeiro ; Rafael Guerra Lund, orientador ; Adriana Fernandes da Silva, coorientadora. — Pelotas, 2017.

69 f.

Dissertação (Mestrado) — Programa de Pós-Graduação em Dentística, Faculdade de Odontologia, Universidade Federal de Pelotas, 2017.

1. Clareamento dental. 2. Cor. 3. Dureza. 4. Rugosidade. I. Lund, Rafael Guerra, orient. II. Silva, Adriana Fernandes da, coorient. III. Título.

Black : D2

Elaborada por Fabiano Domingues Malheiro CRB: 10/1955

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Dissertação aprovada, como requisito parcial, para obtenção do grau de Mestre em Odontologia, Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia de Pelotas, Universidade Federal de Pelotas.

Data da defesa: 24/02/2017

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Agradecimentos

À Deus, pois até aqui ele tem cuidado de nós.

À Universidade Federal de Pelotas, na pessoa do Magnífico Reitor Pedro Rodrigues Curi Hallal.

À Faculdade de Odontologia, nas pessoas do Excelentíssimo Diretora Profª Drª Adriana Etges e Excelentíssimo Vice-Diretor Profº Drº Luis Eduardo Rilling da Nova Cruz.

Ao Programa de Pós-Graduação em Odontologia, na pessoa do Coordenador do PPGO, Profº Drº Rafael Ratto de Moraes.

Aos meus pais, e ao meu querido irmão por todo apoio e suporte, por serem meu porto seguro nas horas mais difíceis, todo o amor e cuidado a distância nunca nos separou mesmo distante sempre estiveram perto. Amo vocês.

Aos tios, primo e amigos e amigas que mesmo estando longe sempre acreditaram em mim e sempre me apoiaram.

Ao meu orientador Professor Doutor Rafael Guerra Lund, agradeço por toda a parceria, a confiança, e por todas as oportunidades que me proporcionou até aqui. E, a minha coorientadora Adriana Silva, pelas considerações e apoio à realização do trabalho.

As amigas sempre presentes no CDC Leina e Andressa, muito obrigada pela amizade de vocês, foram sem dúvida um grande apoio, nos momentos mais difíceis, uma companhia sempre, uma parceria sempre, um suporte quando mais precisei, sempre estavam lá.

Ao Carlos, que sempre esteve pronto a ajudar, em todos os ensaios no CDC. A Katiele sempre animada, Raissa, Rafaela e Cristina muito obrigada pela amizade de vocês.

A Andressa Barbosa, por todo o auxilio que me deu durante os ensaios, sempre muito esforçada e compromissada com as atividades do laboratório, fazendo de tudo para ajudar.

Aos demais amigos do Laboratório de Microbiologia e também do CDC, aos técnicos, pela ajuda quando precisei e pelo tempo que passamos juntos nos laboratórios.

A todos aqueles que colaboraram com e elaboração desse trabalho, direta ou indiretamente.

À banca avaliadora, por prontamente aceitarem meu convite e ajudarem a melhorar meu trabalho.

Obrigada.

Notas Preliminares

A presente dissertação foi redigida segundo o Manual de Normas para Dissertações, Teses e Trabalhos Científicos da Universidade Federal de Pelotas de 2013, adotando o Nível de Descrição 3 – estrutura em “Capítulos não convencionais”, descrita no Apêndice D do referido manual. <http://sisbi.ufpel.edu.br/?p=documentos&i=7> Acesso em: 24 de janeiro de 2016.

O projeto de pesquisa desta dissertação foi apresentado e aprovado em sua forma final após qualificação realizada em 03 de setembro de 2015 e aprovado pela Banca Examinadora composta pelos Professores Doutores Rafael Guerra Lund, Giana Lima da Silveira e Rudimar Antonio Baldissera.

Resumo

RIBEIRO, Juliana Silva. **Desenvolvimento e avaliação da efetividade de agentes potencialmente clareadores livres de peróxidos** 2017. 69 f. Dissertação (Mestrado em Odontologia) – Programa de Pós Graduação em Odontologia. Universidade Federal de Pelotas, Pelotas, 2017.

A demanda de procedimentos estéticos tem crescido nos consultórios odontológicos. Entre os procedimentos mais procurados está o clareamento dentário. Dentre os compostos utilizados nesse procedimento, estão o peróxido de hidrogênio e o peróxido de carbamida. No entanto é relatado na literatura que esses sistemas apresentam efeitos colaterais como sensibilidade pós-operatória e toxidade as células pulpares. Esse trabalho está dividido em dois capítulos, no primeiro foi analisada a eficácia clínica desses materiais por meio de uma revisão sistemática e de um monitoramento tecnológico da área a fim de analisar o contexto atual para obter oportunidades de prospecção tecnológica do setor. O segundo foi formular e avaliar agentes clareadores não peróxidos experimentais à base de endopaptidases cisteínicas. Materiais e métodos: A revisão sistemática foi realizada de acordo com as normas PRISMA (MOHER et al., 2009). A busca de artigos foi realizada por duas pessoas independentemente, em sete bases de dados: MedLine (PubMed), Lilacs, Ibecs, Web of Science, Scopus, Scielo e The Cochrane Library e para o levantamento tecnológico em bases de dados de patentes os seguintes bancos de dados de patentes de adição serão utilizados: Google Patentes, questel orbit, USPTO (Patentes dos Estados Unidos e da marca registrada Office), IEP (Instituto Europeu de Patentes), JPO (Japan Patent Office), INPI (Instituto Nacional da Propriedade Intelectual do Brasil), Derwent Innovations Index e PATENTSCOPE. Para o estudo laboratorial foram preparados e caracterizados géis experimentais à base de papaína, ficina e bromelina. Foram obtidos 50 discos de dentes bovinos (6 × 6 mm^2, 4 mm de espessura), polidos e submetidos a uma pigmentação em solução

de café. As amostras foram distribuídas aleatoriamente em quatro grupos (n = 10), de acordo com o agente de clareamento utilizado: 20% de peróxido de carbamida (controle positivo); gel de papaína a 1%, gel de ficina a 1% e gel de bromelina a 1%, e nenhum agente (controle negativo). Os materiais foram aplicados uma vez por semana, em três seções de 15 minutos, durante 4 semanas, seguindo segundo as instruções do fabricante do controle positivo. As avaliações de cor foram obtidas através dos parâmetros individuais do CIEDE2000 (L *, a *, b *, C * e h-) em dois períodos diferentes. A dureza de Knoop foi analisada sob carga de 50 g durante 15 s, e a rugosidade por um perfilômetro cada espécime foi medida 5 vezes em diferentes locais foi realizada antes e após os tratamentos. Os dados foram analisados estatisticamente com um nível de significância α = 0,05. Resultados: Os artigos e nas patentes analisadas, apontam para uma variedade significativa de agentes potencialmente clareadores isentos de peróxidos. Os géis a base de ficina, bromelina e peroxido de carbamida apresentaram resultados estatisticamente semelhantes. Além disso, os géis baseados em enzimas apresentaram menos danos a superfície do esmalte. Conclusão: Com base nos artigos e nas patentes encontradas, pode-se concluir que há alternativas ao uso do peróxido, no entanto, mais estudos, são necessarios afim de caracterizar os efeitos desses agentes no dente, bem como a sua eficácia clínica.

Palavras-chave: clareamento dental; cor; dureza; rugosidade;

Abstract

RIBEIRO, Juliana Silva. **Development and evaluation of the effectiveness of potentially bleaching agents free of peroxides** 2017. 69f. Dissertation Project (Master Degree em Dentística) - Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas, Pelotas, 2015.

The demand for aesthetic procedures has grown in dental offices. Among the most sought after procedures are tooth whitening. Among the compounds used in this procedure are hydrogen peroxide and carbamide peroxide. However, it is reported in the literature that these systems have side effects such as postoperative sensitivity and toxicity to the pulp cells. This work is divided into two chapters; the first one analyzed the clinical efficacy of these materials through a systematic review and a technological monitoring of the area in order to analyze the current context to obtain technological prospecting opportunities in the sector. The second was to formulate and evaluate experimental non-peroxidizing bleaching agents based on cysteine endopaptidases. Materials and methods: The systematic review was performed according to PRISMA standards (MOHER et al., 2009). The search for articles was carried out by two people independently in seven databases: MedLine (PubMed), Lilacs, Ibecs, Web of Science, Scopus, Scielo and The Cochrane Library and for the technological survey in patent databases the following Patent databases of addition will be used: Google Patents, questel orbit, USPTO (United States Patent and Trademark Office), IEP (European Patent Office), JPO (Japan Patent Office), INPI Intellectual Property of Brazil), Derwent Innovations Index and PATENTSCOPE. For the laboratory study, experimental gels based on papain, ficin and bromelain were prepared and characterized. It was obtained 50 discs of bovine teeth (6 × 6 mm2, 4 mm of thickness), polished and submitted to a pigmentation in coffee solution. The samples were randomly distributed into four groups (n = 10), according to the whitening agent used: 20% carbamide peroxide (positive control); 1% papain gel, 1% ficin gel and 1% bromelain gel, and no agent (negative control). The materials were applied once a week, in three 15-minute sections, for 4 weeks, following the instructions of the manufacturer of the positive control. The color

evaluations were obtained through the individual parameters of CIEDE2000 (L *, a *, b *, C * and h-) in two different periods. The hardness of Knoop was analyzed under a load of 50 g for 15 s, and the roughness by a profilometer each specimen was measured 5 times at different locations was performed before and after treatments. Data were analyzed statistically with a significance level α = 0.05. Results: The articles and patents analyzed point to a significant variety of potentially bleaching agents that are free of peroxides. The gels based on ficin, bromelain and carbamide peroxide presented statistically similar results. In addition, enzyme-based gels presented less damage to the surface of the enamel. Conclusion: Based on the articles and patents found, it can be concluded that there are alternatives to the use of peroxide, however, more studies are necessary to characterize the effects of these agents on the tooth, as well as their clinical efficacy.

Keywords: tooth bleaching agents; color; hardness; rugosity; materials testing

Sumário

1 Introdução

Os procedimentos clareadores mais comumente utilizados são o clareamento caseiro e o de consultório. O clareamento caseiro é considerado um tratamento mais conservador para dentes vitais quando comparado com outras modalidades de tratamento. É relatado na literatura o alto nível de satisfação e eficácia dos sistemas de clareamento caseiros (MEIRELES et al., 2008; RITTER et al., 2002). Estes adotados como terapia de primeira escolha para clareamento dental vital. A popularidade de clareamento caseiro aumentou devido à possibilidade da autoaplicação pelo paciente, bem como a influência da mídia (PERDIGAO; BARATIERI; ARCARI, 2004).

Embora não seja um procedimento novo na odontologia, os protocolos de clareamento não estão totalmente esclarecidos quanto à maneira de utilização e seus efeitos nas estruturas dentais, ou seja, ainda há muitas informações controversas na literatura (MATIS; COCHRAN; ECKERT, 2009). Estudos demonstram que a utilização dos géis clareadores acarreta a perda da dureza superficial do esmalte, perdas de cálcio e fósforo, aumento da rugosidade superficial, alterações de morfologia, bem como aumento de permeabilidade (ALQAHTANI, 2014; BISTEY et al., 2007; DE ARRUDA et al., 2012; EFEOGLU; WOOD; EFEOGLU, 2005; EFEOGLU; WOOD; EFEOGLU, 2007; RODRIGUES et al., 2005; SCHIAVONI et al., 2006; TEZEL et al., 2007).

Outro fato relatado na literatura e bastante controverso a respeito dos agentes de clareamento são os efeitos deletérios sofridos pela polpa devido ao procedimento, sensibilidade dentária e irritação gengival, que são geralmente ligeiros a moderados e transitórios. Alguns estudos relatam não ocorrer danos pulpares significativos quando o agente clareador for bem utilizado e indicado (DO AMARAL et al., 2012; FELIZ-MATOS; HERNANDEZ; ABREU, 2014; HE et al., 2012; LI; GREENWALL, 2013). Outros sugerem que os agentes clareadores causam sérios danos pulpares (COLDEBELLA et al., 2009; DE ALMEIDA et al., 2015; SOARES et al., 2014; SOARES et al., 2015; SOARES et al., 2013).

Outro problema atribuído ao clareamento é a sensibilidade; no entanto, sua causa ainda não foi totalmente elucidada, apesar de ser fortemente associada ao uso do peróxido (BERGA-CABALLERO; FORNER-NAVARRO; AMENGUAL-

LORENZO, 2006; BERNARDON et al., 2015; DAHL; PALLESEN, 2003; MARKOWITZ, 2010; REZENDE et al., 2016).

Os agentes disponíveis no mercado para o clareamento de dentes vitais são o peróxido de hidrogênio e de carbamida, eles se apresentam de acordo com a concentração do peróxido, modo de aplicação e tempo de exposição (MEIRELES et al., 2012). O peróxido de carbamida é o mais utilizado no mercado, quando em contato com os tecidos da cavidade oral se dissocia em peróxido de hidrogênio e ureia. O peróxido de hidrogênio decompõe-se em radicais livres de oxigênio e agua, e a ureia dissocia-se em amônia e dióxido de carbono. Essas radicais hidroxilas são capazes de converter moléculas maiores em menores, provável mecanismo do clareamento (HEGEDUS et al., 1999; MEIRELES et al., 2012).

Os agentes que compõem os sistemas de clareamento são divididos em agentes ativos e inativos. Os compostos ativos são o peróxido de hidrogênio ou peróxido de carbamida. E os principais agentes inativos são agentes espessantes, tensoativos, dispersantes de pigmento, conservantes, aromatizantes e em alguns casos dessensibilizantes (ALQAHTANI, 2014).

O peróxido de hidrogênio é uma molécula termicamente instável. Altas concentrações deste agente químico apresentam alto poder oxidativo (BITTER, 1998). As reações oxidativas e os danos causados por radicais livres são os principais mecanismos responsáveis pela toxidade de compostos contendo peróxido (HEGEDUS et al., 1999).

Este agente apresenta baixo peso molecular e transita livremente pelos espaços interprismáticos do esmalte e também na dentina, provocando a oxidação dos pigmentos presentes nestas estruturas (BITTER, 1998; GOPINATH et al., 2013; HEGEDUS et al., 1999; MONDELLI et al., 2009) e esta reação química envolvendo os pigmentos é que proporciona o efeito clareador (PLOTINO et al., 2008).

Embora seja relatado que o oxigênio livre é capaz de degradar moléculas orgânicas complexas, que são as responsáveis pela discromia dos dentes, o exato mecanismo pelos quais os dentes são branqueados ainda não foi completamente compreendido. Tem sido relatado que o baixo peso molecular de H_2O_2 torna essas moléculas capazes de se difundir através de esmalte e dentina, podendo alcançar o espaço pulpar (SHACKELFORD; KAUFMANN; PAULES, 2000; TREDWIN et al., 2006). Sendo assim, o H_2O_2 e seus produtos de degradação desempenham um papel imprescindível no clareamento dentário; no entanto, podem causar sérios

danos ao tecido pulpar, especialmente aos odontoblastos que estão subjacentes a dentina (DIAS RIBEIRO et al., 2009; TREDWIN et al., 2006). Segundo COSTA et al., 2010(COSTA et al., 2010) o clareamento realizado com peroxido de hidrogênio a 38% por 45 minutos, causa danos irreversíveis em incisivos.

Vários estudos *in vitro* envolvendo culturas de células avaliarem a citotocidade de agentes de clareamento, tem sido relatado que aproximadamente 55% dos pacientes submetidos ao clareamento se queixam sobre a sensibilidade pós-operatória, moderada a severa, principalmente em seus dentes anteriores (TAY et al., 2009). Devido a isso, é de grande importância o desenvolvimento de produtos odontológicos que não provoquem danos pulpares aos dentes, incluindo danos irreversíveis como a morte de células pulpares.

A Fitoterapia é um tratamento caracterizado pela utilização de plantas medicinais em uma variedade de produtos farmacêuticos (SOUSA et al., 2010), sendo um dos principais recursos na produção de fármacos (VIEIRA et al., 2010). Os fatores que contribuem para a propagação desta terapia são: a origem natural das drogas, a crença na sua utilização segura e a alegada falta de efeitos colaterais (BAPTISTAI; DE SOUSA BRITTOI, 2008; JUNIOR; PINTO; MACIEL, 2005; SANTOS et al., 2011; VIEIRA et al., 2010).

Recentemente, o uso de medicamentos à base de plantas em todo o mundo cresceu profusamente (BAPTISTAI; DE SOUSA BRITTOI, 2008). Prova disso é que no Brasil já existe uma Política Nacional de Plantas Medicinais e Fitoterápicos, respaldada pelo o decreto federal 5.813 de 22 de junho de 2006 (SANTOS et al., 2011).

O surgimento desta politica fez com que a utilização da fitoterapia se tornasse uma alternativa mais possível através da ampliação da utilização de opções terapêuticas (SANTOS et al., 2011; VIEIRA et al., 2010). Com isso, os compostos fitoquímicos naturais tornaram-se uma alternativa em potencial, segura, econômica e eficaz (KESLER SHVERO et al., 2013).

A papaína e a bromelína fazem parte do grupo das cisteína-proteases. As cisteínas-proteases, são amplamente distribuídas entre os organismos vivos. A maioria das proteases cisteínicas vegetais pertencem às duas grandes famílias da papaína e legumaína (SCHALLER, 2004). Dentro da família das papaínas, se encontram proteinases tais como quimopapaína, papaína, caricaina, bromelina,

actinidina, ficina, e aleuraina, e as catepsinas lisossomais B, H, L, S, C e K (TURK; TURK; TURK, 1997).

A papaína é uma peptidase importante extraída do mamão (Carica papaya). Tem uma alta capacidade proteolítica, hidrolizando grandes proteínas e, pequenos peptídeos e aminoácidos e apresenta atividade antioxidante. Além disso, não apresenta potencial tóxico ou mutagênico (MARTINS et al., 2009; TURK; TURK; TURK, 1997).

A bromelina é uma enzima proteolítica derivada do ananás (Ananas Comosus), e é um membro da família Bromeliaceae. Alguns estudos demonstraram que a bromelina tem um efeito antimicrobiano, bem como atividade anti-helmíntica. Ela é considerada um suplemento alimentar e está disponível para o público em geral em lojas de alimentos saudáveis e farmácias. Diversos estudos "in vitro" e "in vivo" demonstraram que a bromelina tem baixo potencial tóxico e mutagênico (ASSIS, 2014; CÂNDIDO DA SILVA, 2005; PAVAN; JAIN; KUMAR, 2012).

Ficina, é um nome genérico dado à fração endoproteolítica do látex do figo. Ela também que pertence a família das proteases cisteína da papaína (família C1, clã CA). A ficina de látex do figo é conhecida como a enzima com atividade de coagulação do leite (utilizada para a fabricação de queijo). Além disso, apresenta uma elevada actividade proteolítica. Além de atividades antimicrobianas e antifúngicas.

A papaína, a bromelina e a ficina são amplamente utilizadas por suas propriedades bem reconhecidas, tais como seus efeitos anti-inflamatórios, antitrombóticos e fibrinolíticos, atividade anticancerígena e efeitos imunomoduladores, na indústria alimentícia, remédios e Indústrias farmacêuticas e em procedimentos técnicos para testes laboratoriais (DUTTA; BHATTACHARYYA, 2013; MAURER, 2001; RATHNAVELU et al., 2016). Sendo assim, a origem natural destas enzimas confere a elas uma segurança quanto ao comportamento biológico não citotóxico.

Não há estudos que avaliem a eficiência de sistemas claeadores livres de peróxido contendo Ficina. Só há um estudo que avalia as propriedades da papaína e da bromelina como géis clareadores sobre o esmalte dentário pigmentado (MUNCHOW et al., 2016). Além disso, outros dois estudos avaliam a bromelina e a papaína como agentes clareadores em dentifrícios (CHAKRAVARTHY; ACHARYA, 2012; KALYANA et al., 2011).

Partindo desses pressupostos, os objetivos deste trabalho são formular agentes clareadores não-peróxido, bem como avaliar a sua ação clareadora e efeito no esmalte dentário. A hipótese a ser testada é que os agentes clareadores experimentais terão efeito branqueador similar aos peróxidos já disponíveis no mercado.

2 Capitulo 1 – Efficacy of non-peroxide bleaching agents: Systematic review, meta-analysis and technology prospecting

Short title. Non-peroxide bleaching agents

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Keywords: non-peroxide, tooth-bleaching agent, tooth bleaching, review.

Artigo formatado segundo as normas do periódico Journal of Dentistry

https://www.elsevier.com/journals/journal-of-dentistry/0300-5712/guide-for-authors

Abstract

Objectives: To analyze the efficacy of non-peroxide bleaching agents and the current technological development in this research field.

Data: Five laboratory studies and twenty-five patents were included. Data regarding non-peroxide bleaching agent used, application protocol and the main findings of studies were analyzed.

Sources: Two reviewers performed a literature search up to December 2016 in fifteen databases: PubMed (Medline), Lilacs, Ibecs, Web of Science, Scopus, BBO, Scielo, The Cochrane Library, Espacenet, Google patents, INPI, JPO, PatentScope, Questel Orbit, USPTO.

Study selection: It was included laboratory studies that evaluating non-peroxide bleaching agent used with or without peroxides and patents related to non-peroxide bleaching agents. Clinical trials, reviews, editorial letters, case reports, case series and studies published in a language other than English were not included.

Results: Five *in vitro* studies evaluated different non-peroxide bleaching agents, such as papain, bromelain, chlorine dioxide, sodium chloride plus vinegar and sodium bicarbonate used as peroxide-free agents and sweet potato extract, lactoperoxidase and peroxidase were associated to peroxide-used were included. Twenty-five patents were included; among the most cited are the papain and the chlorine with their variations. The meta-analysis of in vitro studies comparing non-peroxide agent with control showed no statistically significant differences among groups ($p>0.05$). However, the subgroup analysis showed that non-peroxide agents incorporated to peroxide were statistically significant different than control ($p<0.05$), with an improvement in bleaching effect with the incorporation of these non-peroxide agents. On the other hand, the peroxide materials showed a higher bleaching effect that were statistically significant different than peroxide free agents ($p<0.05$).

Conclusions: The available studies and patents suggest there is a significant variety of potentially peroxide free bleaching agents that need to be further studied, and their effects on whitening teeth as well as their clinical effectiveness have not been fully elucidated.

Clinical significance: The literature available suggests non-peroxide bleaching agents incorporated to peroxide may improve the bleaching effect. However, further studies are needed to evidence if these effects may be translated to clinical practice.

The current available literature does not support the use of non-peroxide bleaching agents alone to dental bleaching.

Keywords: non-peroxide, tooth-bleaching agent, tooth bleaching, systematic review.

Introduction

Despite the bleaching systems are well accepted for showing good efficiency aesthetics [1, 2]. Bleaching procedures most commonly used are the home whitening and the office. The home whitening is considered a more conservative treatment for vital teeth when compared with other treatment modalities. It is reported in the literature the high level of satisfaction and effectiveness of home whitening systems [1, 2]. However, the in office bleaching has advantages in terms of clinician control, quick whitening results, reduced treatment time, and avoidance of material ingestion and discomfort from wearing trays [3].

However, the harmful effects suffered by the pulp due to the procedure, tooth sensitivity and gum irritation, which are usually mild to moderate and transient, is quite controversial. Some studies report no significant pulp damage occur when the whitening agent is properly used and indicated [4-6]. Others suggest that bleaching agents cause serious damage pulp [7-12] and sensitivity. Though, its cause has not been fully elucidated, the sensitivity is strongly associated with the use of peroxide a study was reported that approximately 55% of patients undergoing whitening complain about postoperative sensitivity, moderate to severe, especially in their front teeth [3, 13-16].

When in contact with the tissues of the oral cavity the carbamide peroxide dissociates into hydrogen peroxide and urea [17, 18]. Since the hydrogen peroxide decomposes into free radicals of oxygen and water, and urea dissociates to ammonia and carbon dioxide [18, 19]. This agent has a low molecular weight and moves freely through interprismatic enamel spaces as well as dentin, causing oxidation of the pigments present in these structures and this reaction chemistry involving the pigments is that it provides the whitening effect [19].

While it is reported that the free oxygen is able to degrade complex organic molecules, which are responsible for dyschromia teeth, the exact mechanism by which the teeth are bleached has not been fully understood [19]. It has been reported that low molecular weight H_2O_2 makes these molecules capable of diffusing through enamel and dentin, can reach the pulp chamber [20, 21]. Thus, the H_2O_2 and its degradation products play an essential role in tooth whitening; however, can cause serious damage to the pulp tissue, especially the odontoblasts underlying dentin [21, 22].

Therefore, any attempt to achieve high efficiency of dental bleaching with less or no deleterious effect on the enamel, pulp and oral mucosa, would be a welcome change in dentistry. Therefore, the aim of this study was to analyze the efficacy of non-peroxide bleaching agents in dental bleaching and to map the current technological development of non-peroxide bleaching agents. Our hypothesis evaluated was that non-peroxide bleaching agents would present a similar performance than peroxide bleaching agents.

Materials and methods

The protocol of this review was registered in the PROSPERO international database for systematic reviews (CRD42014010018) and this review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA Statement) [23]. In addition The PICO was carried out to formulate the question from evidence-based practice: Population: Pigmented teeth; Intervention: Dental bleaching; Comparison: Unbleached teeth; and Outcome: Bleaching. The research question was: What are the non-peroxide bleaching agents currently available? Are they efficient in dental bleaching?

Search strategies

The literature search was carried out by two independent reviewers of studies published during or before December 2016. Eight databases were screened, including PubMed (Medline), Lilacs, Ibecs, Web of Science, Scopus, BBO, Scielo and The Cochrane Library, using the search strategy developed for PubMed (Medline) (Table 1) and adapted for other databases. In addition, seven patents databases: Espacenet, Google patents, INPI, JPO, PatentScope, Questel Orbit, USPTO. Besides, the online system Questel Orbit (Paris, France) was used to search of relevant patents. The references cited in the included papers were also checked to identify other potentially relevant articles. After the identification of articles in the databases, the articles were imported into Endnote X7 software (Thompson Reuters, Philadelphia, PA, USA) to remove duplicates.

Study selection

Two authors independently assessed the titles and abstracts of all of the documents. For the patents, it was assessed the titles, abstracts and claims. As inclusion criteria: clinical, in vitro, in vivo, in situ studies using non-peroxide dental bleaching agents;

studies evaluating bleached teeth agents associated or not to non-peroxide components; and patents related to non-peroxide bleaching agents. As exclusion criteria: Non-controlled clinical trials, reviews, editorial letters, case reports, case series and studies published in a language other than English, Portuguese or Spanish.

Full copies of all the potentially relevant studies were identified. Those appearing to meet the inclusion criteria or for which there were insufficient data in the title and abstract to make a clear decision were selected for full analysis. The full-text papers were assessed independently and in duplicate by two authors. Any disagreement regarding the eligibility of the included studies was resolved through discussion and consensus or by a third reviewer. Only papers that fulfilled all of the eligibility criteria were included.

Data extraction

The data were extracted using a standardized form. If there was some information missing, the authors of the included papers were contacted via e-mail to retrieve any missing data. The following data were tabulated: author, publication year, country, number and type of teeth evaluated, storage, color system and follow-up (Table 2). The characteristics of the included studies, such as selection criteria, non-peroxide agents, control group, application protocols and main findings were also analyzed (Table 3). The technological survey including demographic data, inventors and non-peroxide bleaching agent claimed were analyzed in Table 4.

Assessment of risk of bias

Two reviewers assessed the methodological quality of the studies and these were evaluated and classified according to Cochrane guidelines [24] for the following items: sample size calculation, presence of control group (negative and positive) and coefficient of variation.

Statistical analysis

The analyses were performed using Review Manager Software version 5.2 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). The global analysis was carried out using a random-effects model, and pooled-effect estimates were obtained by comparing the mean difference of values regarding CIE Lab, ΔE and ΔL (in one article) of each non-peroxide agent with the conventional

material (control) or non-peroxide agent associated to peroxide. A p value < 0.05 was considered to be statistically significant. Subgroup analysis for non-peroxide agents incorporated to peroxide or peroxide free agents compared with the conventional material were also performed. Multiple groups from the same study were analyzed according to Cochrane guidelines for combining groups [25]. Statistical heterogeneity of the treatment effects among studies were assessed using the Cochran's Q test and the inconsistency I^2 test, in which values greater than 50% were considered to be indicative of substantial heterogeneity [24].

Results

Search strategy

Six thousand four hundred and thirty four potentially relevant records were identified from all of the databases. No additional studies were identified as relevant after a search of the reference lists. Fig. 1 is a flowchart that summarizes the article selection process according to the PRISMA Statement [23]. After the title and abstract examination, 3129 studies were excluded because they did not meet the eligibility criteria. Of the 34 documents retained for detailed review, 5 studies and 25 patents, fulfilled all of the selection criteria and were included in the qualitative analysis and 4 studies were included in the meta-analysis.

Descriptive analysis

The studies were published between 2008 and 2016. All studies were laboratory. A total of 170 teeth were evaluated in this review (82 human and 88 bovine). The staining protocols were based on stored in: artificial saliva, chlorehexidine, tea, tetracycline, and coffee.

In the analyzed papers, 8 potentially bleaching agents were described, such as papain, bromelain, chlorine dioxide, sodium chloride plus vinegar and sodium bicarbonate used as peroxide-free agents. Sweet potato extract, lactoperoxidase and peroxidase were associated to peroxide-used.

In the analyzed patents, an infinite number of potentially bleaching agents were found and a combination among then. It was because the documents do not establish which the chemical components are responsible by dental bleaching in the part of claims. They only cite that the components claimed could be used as dental whitening or some combination among the possible listed agents.

The papain was cited in 14 documents and the chlorine and its derivatives appeared in 14 documents. In 5 documents were claimed enzymes as teeth bleaching. In one document, were listed malic acid (apple, strawberry), citrus acid (Orange, lemon, carrot), and bromelain (pineapple). In one document, was cited a chormophore agent but then do not specified which is. In the other patents the following agents are listed: tetrasodium pyrophosphate, sodium acid pyrophosphate, sodium hex metaphosphate, sodium tripolyphosphate, sodium potassium tripolyphosphate, tetrapotassium pyrophosphate, sodium bicarbonate, nitric acid calcium, methionine, cystein and taurine as for under including amino acids; ursodeoxycholic acid, tauroursodeoxycholic acid, vitamin A, C and E.

Risk of bias of included studies

Concerning the quality assessment (Fig. 2), these studies presented a low risk of bias for most of the biases that were analyzed. All papers showed control groups and all showed good coefficient of variation. Only one study [26] did not report randomization of the specimens.

Meta-analysis

A meta-analysis Fig. 3 was performed for 4 studies. The global comparison of non-peroxide agent with control showed no statistically significant differences among groups ($p>0.05$). However, the subgroup analysis showed non-peroxide agents incorporated to peroxide were statistically significant different than control ($p<0.05$), with an improvement in bleaching effect with the incorporation of these non-peroxide agents. On the other hand, the peroxide materials showed higher bleaching effect that was statistically significant different than peroxide free agents ($p<0.05$). Besides, substantial heterogeneity was observed in all analysis.

Discussion

The hypothesis that was evaluated was partially rejected once our meta-analysis demonstrated that when used the non-peroxide bleaching agents without peroxides, they were not effective as the peroxide. However, when used as a coadjuvant, they increased the bleaching effect of the peroxide. All revised studies were laboratory, and in general, they presented a low risk of bias. Meanwhile, the included studies presented substantial heterogeneity, which was probably due to the different non-

peroxide agents, different type and group of teeth evaluated, follow-up periods, evaluation criteria and outcomes that were assessed.

When the non-peroxide bleaching agents were used without peroxides, they were not as effective as the peroxide based gels. The papain and bromelain were tested in the same study and they were comparing to carbamide peroxide [27]. The carbamide peroxide showed greater stain removal effect values when compared to the experimental gels. However, it is important to highlight that bromelain and papain was used in a concentration 20 times lower than the peroxide. Bromelain and papain are cysteines proteases; they probably can breakdown macromolecules/stains into smaller parts, thus increasing light reflection from the tooth surface, thereby resulting in a whitening effect [27]. These mechanisms are different from hydrogen/carbamide peroxide-based products act by creating reactive oxygen species, which alter the structural and biochemical properties of dental hard and soft pulp tissues [28].

Other peroxide-free agent found was the chlorine dioxide, which was compare with the hydrogen peroxide in a similar concentration [29]. Chlorine dioxide whitens teeth at a faster rate than hydrogen peroxide. They suggest that this bleaching effect was relayed to the dehydration of teeth, because of temperature generated by light activation, could be a reason for the immediate [29]. Despite this, prolonged exposures did not improve color change. Only, this research evaluated the ClO_2 whitening effects. Thus, this limits conclusions about the efficacy of the ClO_2 and supports the need for more studies for proper comparisons.

Sodium chloride and sodium bicarbonate are commonly used as bleaching agents as they have been propagated in various electronic media as the bleaching agent without appropriate scientific studies conducted [30]. The sodium chloride dissolved in vinegar were significantly effective in removing the intrinsic tooth stain whereas sodium bicarbonate dissolved in vinegar demonstrated no significant change. The mechanism of action sodium chloride plus vinegar containing 4% acetic acid is still unknown. This effect may be associated with the low pH presented by this solution [30]. In other study, it was relate that the vinegar can cause more decrease in hardness, changes in color and morphological characteristics enamel surface than hydrogen peroxide[11] however it was not included in this review because they was in chinese language.

In papers that associated the use of peroxide with other chemical substances, it was observed that they increased the bleaching effect of the peroxide. One study

evaluated the effect of lactoperoxidase on tetracycline decoloration [26]. In this study, the lactoperoxidase increased the rate of tetracycline decoloration. After 8 h or more of contact of the lactoperoxidase system, the rate of tetracycline decoloration was greater than carbamide peroxide [26]. Carbamide peroxide could be discomposed in secondary products (H_2O and O_2) during the bleaching, whereas the lipoperoxidase drives the carbamide peroxide to react specifically with the tetracycline and to produce the decoloration of the teeth, which is its main reaction[26]. The enzyme increased the availability of the oxygenation potential to drive the substrate to the unique reaction catalyzed by the enzyme, and to produce a higher degree of efficiency in the decolorating process [26]. The hydrogen peroxide/lactoperoxidase system also could act on the enamel surface, as being the hydrogen peroxide, but also arrive to the teeth inner through the microtubules [26].

In the study that evaluated addition of sweet potato extract to hydrogen peroxide, it was observed that the extract produced greater color variation when compared to groups in which no sweet potato extract was added [31]. This can be attributed to the enzymatic action of catalase present in sweet potato which, when added to the hydrogen peroxide, reduces its activation energy and increases the rate of release of the free radicals [32]. In addition, it can also be attributed to the presence of enzymatic and non-enzymatic antioxidants in the extract; they exert an action of elimination of the free radicals, thus limiting its deleterious effects on the enamel [31, 32].

Besides the search in the articles bases, an additional search was made in the patent banks because this search gives us a broader view of the state of development of these dental bleaching agents. If the review had been carried out only on the basis of articles we would have a limited view of the sector. In this search, we found 25 documents, various bleaching agents have been claimed, which can be used individually or in combination. Among the listed agents are the papain, the chlorine and its derivatives some enzyms, malic acid, citrus acid, bromelain, a chormophore agent but then do not specified which is. In addition, it was also reported other potential non-peroxide agents in patents, such as tetrasodium pyrophosphate, sodium acid pyrophosphate, sodium hex metaphosphate, sodium tripolyphosphate, sodium potassium tripolyphosphate, tetrapotassium pyrophosphate, sodium bicarbonate, nitric acid calcium, methionine, cystein and taurine as for under including amino acids; ursodeoxycholic acid, tauroursodeoxycholic acid, vitamin A, C

and E. However, patents did not inform the action mechanism of these substances nor did they assess their action and their combinations on the surface of the tooth or pulp tissue. Further studies are needed to evaluate if the claimed non-peroxide agents are effective in dental bleaching.

Because of this high heterogeneity and the small amount of studies, that evaluating the efficacy of the non-peroxide bleaching agents it is necessary wariness when making other comparisons. In addition, no randomized controlled trials evaluating these systems were found. Basis on this documents further studies comparing different peroxide free whiteness agents are need to be performed to determine the best treatment option and, elucidate the effects of these agents on the surface of the dental enamel as well as the effects on the pulp.

Conclusions

The available literature suggests that non-peroxide bleaching agents incorporated to peroxide can improve dental bleaching. However, the studies showed high heterogenenity, it being that more studies are needed to determine which the best non-peroxide agent and if its effect can be translated to clinical practice. Besides, the overview of technological development showed many other potential non-peroxide bleaching agents that need to be further investigated by researchers. Up to now, it seems that the available literature does not support the use of non-peroxide bleaching agents alone to the dental bleaching.

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Figures

Figure 1

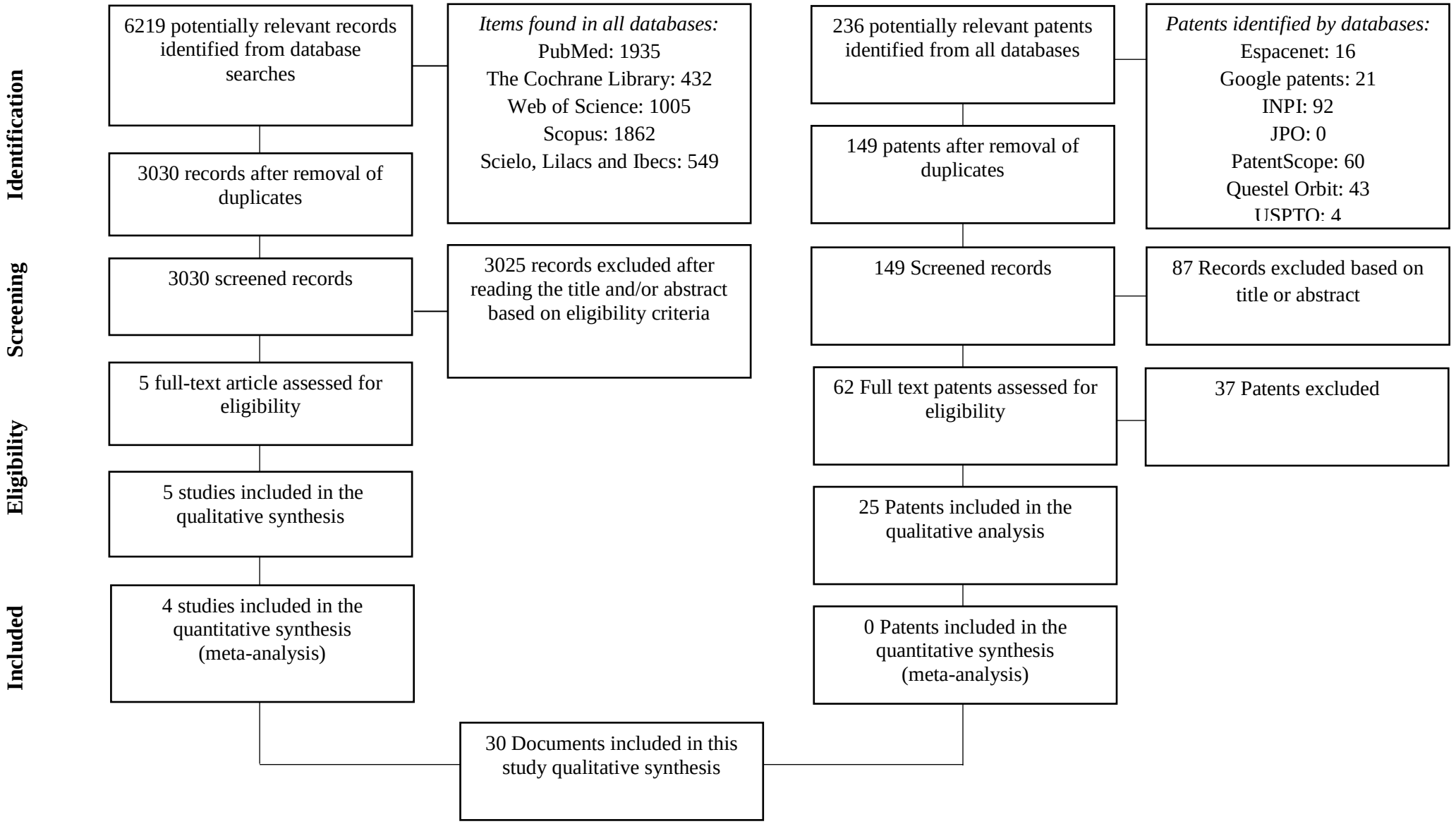


Figure 2

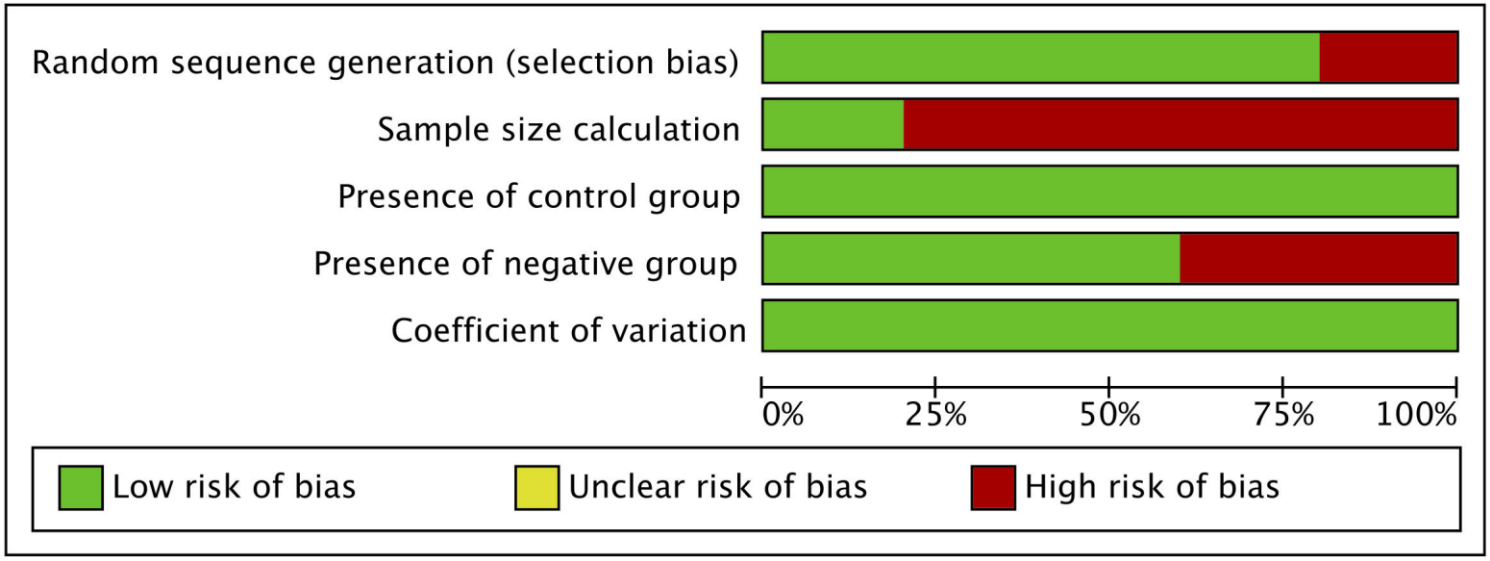
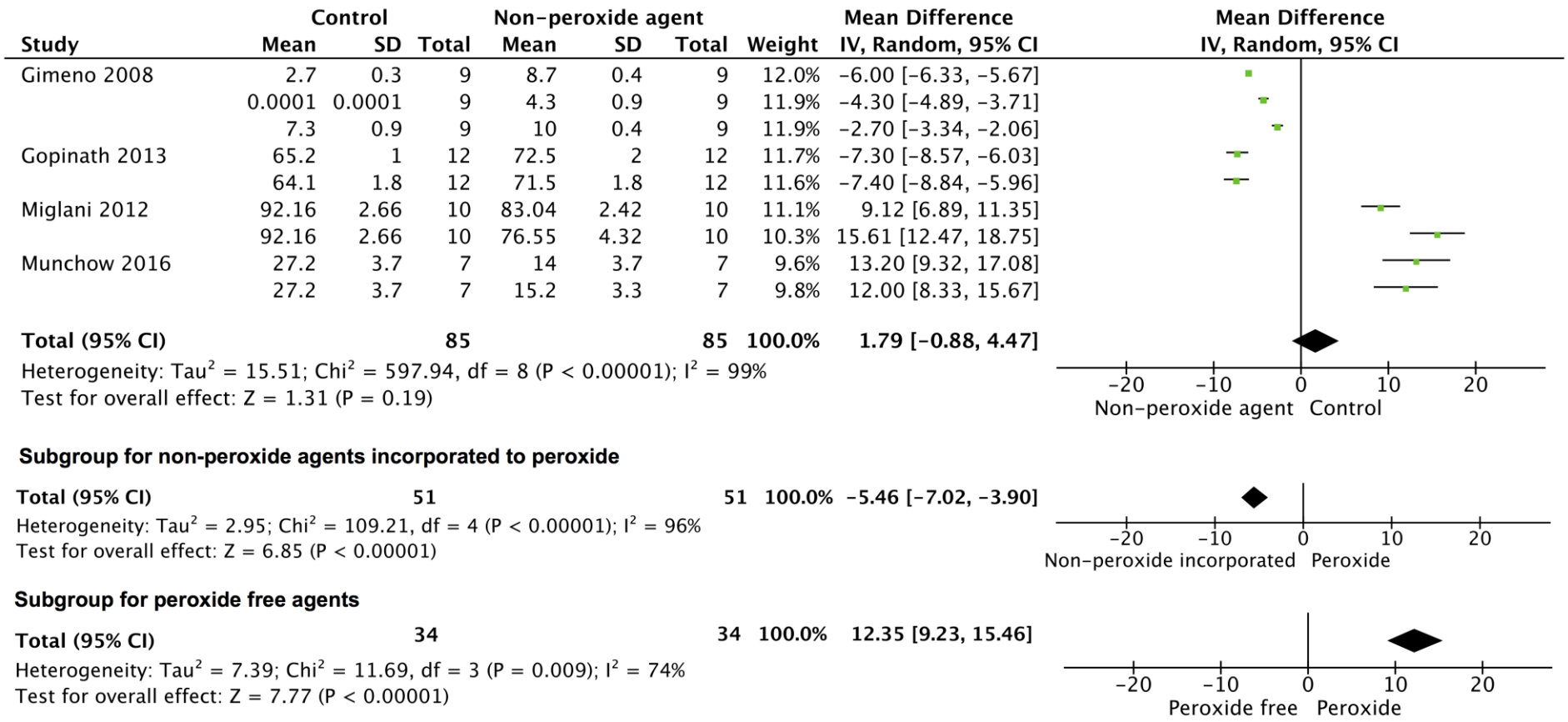

Random sequence generation (selection bias)
Sample size calculation
Presence of control group
Presence of negative group
Coefficient of variation
0%
25%
50%
75%
100%
Low risk of bias
Unclear risk of bias
High risk of bias

Figure 3


Control
Non-peroxide agent
Mean Difference
Mean Difference
Study
Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Gimeno 2008 2.7 0.3 9 8.7 0.4 9 12.0% -6.00 [-6.33, -5.67]
0.0001 0.0001 9 4.3 0.9 9 11.9% -4.30 [-4.89, -3.71]
7.3 0.9 9 10 0.4 9 11.9% -2.70 [-3.34, -2.06]
Gopinath 2013 65.2 1 12 72.5 2 12 11.7% -7.30 [-8.57, -6.03]
64.1 1.8 12 71.5 1.8 12 11.6% -7.40 [-8.84, -5.96]
Miglani 2012 92.16 2.66 10 83.04 2.42 10 11.1% 9.12 [6.89, 11.35]
92.16 2.66 10 76.55 4.32 10 10.3% 15.61 [12.47, 18.75]
Munchow 2016 27.2 3.7 7 14 3.7 7 9.6% 13.20 [9.32, 17.08]
27.2 3.7 7 15.2 3.3 7 9.8% 12.00 [8.33, 15.67]
Total (95% CI) 85 85 100.0% 1.79 [-0.88, 4.47]
Heterogeneity: Tau² = 15.51; Chi² = 597.94, df = 8 (P < 0.00001); I² = 99%
Test for overall effect: Z = 1.31 (P = 0.19)
-20 -10 0 10 20
Non-peroxide agent Control
Subgroup for non-peroxide agents incorporated to peroxide
Total (95% CI) 51 51 100.0% -5.46 [-7.02, -3.90]
Heterogeneity: Tau² = 2.95; Chi² = 109.21, df = 4 (P < 0.00001); I² = 96%
Test for overall effect: Z = 6.85 (P < 0.00001)
-20 -10 0 10 20
Non-peroxide incorporated Peroxide
Subgroup for peroxide free agents
Total (95% CI) 34 34 100.0% 12.35 [9.23, 15.46]
Heterogeneity: Tau² = 7.39; Chi² = 11.69, df = 3 (P = 0.009); I² = 74%
Test for overall effect: Z = 7.77 (P < 0.00001)
-20 -10 0 10 20
Peroxide free Peroxide

Tables

Table 1 - Search strategy used in PubMed (*MedLine*).

	Search Terms
#4	Search #1 AND #2 AND #3
#3	Search dental OR tooth OR teeth OR dentistry OR odontology OR dentist
#2	Search Natural extracts OR natural extract OR Fruit OR Fruits OR Plant Capsule OR Capsule, Plant OR Capsules, Plant OR Plant Capsules OR Plant Aril OR Aril, Plant OR Arils, Plant OR Plant Arils OR Berries OR Berry OR Legume Pod OR Legume Pods OR Pod, Legume OR Pods, Legume OR extract OR essential oil OR Volatile Oils OR Oils, Essential OR Essential Oils OR Oils, Volatile [Mesh] OR Citrus sinenses OR sinenses, Citrus OR sinensis, Citrus OR Orange Tree OR Orange Trees OR Tree, Orange OR Trees, Orange OR Oranges OR Citrus bergamia OR Citrus bergamias OR bergamia, Citrus OR bergamias, Citrus OR Citrus hystrix OR Citrus hystrices OR hystrices, Citrus OR hystrix, Citrus OR Kaffir Lime OR Kaffir Limes OR Lime, Kaffir OR Limes, Kaffir OR Fruit, Citrus OR Citrus Fruits OR Fruits, Citrus OR Citrus Fruit OR Citrus máxima OR Citrus máximas OR maxima, Citrus OR maximas, Citrus OR Pomelo Tree OR Pomelo Trees OR Tree, Pomelo OR Trees, Pomelo OR Pummelo Tree OR Pummelo Trees OR Tree, Pummelo OR Trees, Pummelo OR Citrus grandis OR Citrus grandi OR grandi, Citrus OR grandis, Citrus OR Citrus medica OR Citrus medicas OR medica, Citrus OR medicas, Citrus OR Citron Tree OR Citron Trees OR Tree, Citron OR Trees, Citron OR Citrus reticulata OR Citrus reticulatas OR reticulata, Citrus OR reticulatas, Citrus OR Tangerine Tree OR Tangerine Trees OR Tree, Tangerine OR Trees, Tangerine OR Orange Tree, Mandarin OR Mandarin Orange Tree OR Mandarin Orange Trees OR Orange Trees, Mandarin OR Tree, Mandarin Orange OR Trees, Mandarin Orange OR Citrus aurantium OR Citrus aurantiums OR aurantium, Citrus OR

aurantiums, Citrus OR Orange Tree, Bitter OR Bitter Orange Tree OR Bitter Orange Trees OR Orange Trees, Bitter OR Tree, Bitter Orange OR Trees, Bitter Orange OR Orange Tree, Seville OR Orange Trees, Seville OR Seville Orange Tree OR Seville Orange Trees OR Tree, Seville Orange OR Trees, Seville Orange OR Orange Tree, Sour OR Orange Trees, Sour OR Sour Orange Tree OR Sour Orange Trees OR Tree, Sour Orange OR Trees, Sour Orange OR Citrus limon OR Citrus limons OR limon, Citrus OR limons, Citrus OR Lemon Tree OR Lemon Trees OR Tree, Lemon OR Trees, Lemon OR actinidin OR actinidin [Mesh] OR Papain OR Tromasin OR Cysteine Endopeptidases OR OR Bromelains [Mesh] OR Bromelains OR Bromelin OR Bromelins OR Bromelain OR Bromelain-POS OR Bromelain POS OR BromelainPOS OR Ursapharm Brand of Bromelains OR Bromelains Ursapharm Brand OR Dayto Anase OR Debrase OR Teva Pharmaceutical's Brand of Bromelain OR Extranase OR Rottapharm Brand of Bromelains OR Bromelains Rottapharm Brand OR Rhône-Poulenc Rorer Brand 3 of Bromelains OR Rhône Poulenc Rorer Brand 3 of OR Bromelains OR Mucozym OR Mucos Brand of Bromelains OR Bromelains Mucos Brand OR Proteozym OR Wiedemann Brand of Bromelains OR Bromelains Wiedemann Brand OR Traumanase OR Rhône-Poulenc Rorer Brand 2 of Bromelains OR Rhône Poulenc Rorer Brand 2 of Bromelains OR Nattermann Brand of Bromelains OR Bromelains Nattermann Brand OR Ananase OR Rhône-Poulenc Rorer Brand 1 of Bromelains OR Rhône Poulenc Rorer Brand 1 of Bromelains OR Dontisanin OR Aventis Brand of Bromelains OR Bromelains Aventis Brand OR Ficain [Mesh] OR Ficain OR Ficin OR Cysteine Endopeptidases OR Bromelains OR Calpain OR Caspases OR Caspases, Effector OR Caspases, Initiator OR Chymopapain OR Separase OR Fragaria [Mesh] OR Fragaria OR Fragarias OR Strawberry OR Strawberries OR Malus[Mesh] OR Malus OR Malus domestica OR Malus domesticas OR domestica, Malus OR domesticas, Malus OR Apple OR Apples OR Musa [Mesh] OR Musa OR Musas OR Banana Plant OR Banana Plants OR Plant, Banana OR

Plants, Banana OR Banana OR Bananas OR Prunus OR Prunus OR Almond Tree OR Almond Trees OR Tree, Almond OR Trees, Almond OR Apricot OR Apricots OR Cherry OR Cherries OR Cherry Tree OR Cherry Trees OR Tree, Cherry OR Trees, Cherry OR Peach OR Peaches OR Plum OR Plums OR Prunus armeniaca OR Apricot Tree OR Apricot Trees OR Tree, Apricot OR Trees, Apricot OR Prunus persica OR Peach Tree OR Peach Trees OR Tree, Peach OR Trees, Peach OR Prunus serotina OR Black Cherry OR Cherry, Black OR Almond OR Almonds OR Chokecherry Tree OR Chokecherry Trees OR Tree, Chokecherry OR Trees, Chokecherry OR Psidium [Mesh] OR Psidium OR Psidiums OR Goiaba OR Goiabas OR Guava OR Guavas OR Psidium guajava OR Psidium guajavas OR guajava, Psidium OR guajavas, Psidium

#1

Search Tooth Bleaching OR Bleaching, Tooth OR Teeth Whitening OR Whitening, Teeth OR Tooth Whitening OR Whitening, Tooth OR Teeth Bleaching OR Bleaching, Teeth OR tooth color change OR teeth color change OR Dental color change OR Dental bleaching OR Tooth Discoloration OR Discoloration, Tooth OR Discolorations, Tooth OR Tooth Discolorations OR Tooth Bleaching Agents OR Agents, Tooth Bleaching OR Bleaching Agents, Tooth OR Teeth Whitening Agents OR Agents, Teeth Whitening OR Whitening Agents, Teeth OR Tooth Whitening Agents OR Agents, Tooth Whitening OR Whitening Agents, Tooth OR Teeth Bleaching Agents OR Agents, Teeth Bleaching OR Bleaching Agents, Teeth OR peroxides OR Hydrogen Peroxide OR Peroxide, Hydrogen OR Hydrogen Peroxide (H2O2) OR Hydroperoxide OR Superoxol OR Oxydol OR Perhydrol OR Strip OR Strips OR Tape OR Whitening strips OR whitening strip OR whitening strips OR bleaching strip OR bleaching strips OR bleaching tape OR bleaching tapes OR whitening tape OR whitening tapes

Table 2 - Description of demographic data, study design and main objectives of included studies.

Study (authors)	Year	Country	Teeth	Number of Teeth (per group)	Storage	Evaluation Criteria	Follow-up
Ablal	2013	United Kington	Bovine central incisor	20	Artificial saliva, chlorehexidine and tea	CIE Lab	2 and 30 min
Gimeno	2008	Spain	Human teeth	9	Tetracycline	CIE Lab*	8, 24, 48 and 72 hours
Gopinath	2013	India	Maxillary central incisor	12	Tea	CIE Lab	2 weeks
Miglani	2012	India	Premolars	10	-	CIE Lab	0, 7, 14 and 21 day
Munchow	2016	United States	Bovine central incisor	7	Coffee	CIE Lab	1, 2, 3 and 4 weeks

*Only L (Luminosity)

Table 3 - Description of bleaching agents, protocols and main findings of included studies.

Study (authors)	Non-peroxide bleaching agent	Control	Application protocol	Main findings
Ablal	Clorine dioxide (ClO_2)	Hydrogen peroxide (H_2O_2) and Deionised water (H_2O).	Non-peroxide: Application during 2min and 30 min Control: 2min, after added 30 min	ClO_2 whitens teeth at a faster rate than hydrogen peroxide. However, in extended exposures ClO_2 and H_2O_2 were similar.
Gimeno	Carbamide peroxide + peroxidase	Hydrogen peroxide (H_2O_2) Carbamide peroxide $CH_6N_2O_3$	Non-peroxide: 20min, after added 8,24,48 and 72 hours Control: 20min, after added 8,24,48 and 72 hours	The enzymes could be used as whitening catalysts to increase the rate H_2O_2 of tetracycline decoloration.
Gopinath	Hydrogen peroxide	Hydrogen	Non-peroxide:	The addition of sweet potato extract

	(H_2O_2) + sweet potato extract	peroxide (H_2O_2)	3x 10 min Control: 3x 10 min	to H_2O_2 improve the whitening effect and reduced the damage caused by H_2O_2 when used alone.
Miglani	Sodium chloride + vinegar containing 4% acetic acid Sodium bicarbonate + vinegar containing 4% acetic acid	Hydrogen peroxide (H_2O_2) and Distilled water (H_2O)	Non-peroxide: 5 min per day Control: 5 min per day	The solution sodium chloride + vinegar, was effective in removing the intrinsic tooth stain and similar to control. Nonetheless, the solution of sodium bicarbonate demonstrated no significant change.
Munchow	Papain 1% Bromelain 1%	Hydrogen peroxide (H_2O_2) and Distilled water (H_2O)	Non-peroxide: 3x 15 min per week Control: No stain removal agent day	Both enzymes produced color change, however H_2O_2 showed statistically greater results.

Table 4 - Description of technological survey, demographic data, inventors, and non-peroxide bleaching agent claimed of included documents.

Patent	Country	Title	Year	Inventors	Non-peroxide bleaching agent claimed
IN201621008773	India	Teeth whitener	2016	Munver Uttam Munver Ravi	Malic acid (apple, strawberry) and citrus acid (Orange, lemon, carrot), bromelain (pineapple)
KR20150111646	South Korea	Patch for attaching to teeth or tissues surrounding teeth	2014	Jae Ahn Jong Kim Ji Kim In Lee	Tetrasodium pyrophosphate, sodium acid pyrophosphate, sodium hexametaphosphate, sodium tripolyphosphate, sodium potassium tripolyphosphate, tetrapotassium pyrophosphate, sodium chlorate, the sodium hypochlorite, the papain, the sodium bicarbonate, the nitric acid calcium and vitamin E.
KR20150057749	South Korea	Composition for abirritating tooth-whitening product and kit containing the composition	2013	Jong Kim Jae Ahn Ji Kim In Lee	Methionine, cystein and taurine as for under including amino acids; ursodeoxycholic acid, tauroursodeoxycholic acid, vitamin A, C

					and E
CA2930230Al	Canada	Oral composition for tooth whitening product, and kit comprising same	2013	Jong-Hoon Kim Jae-Hyun Ahn Ji-Hye Kim In-Ho Lee	Chloride
CN103800210	China	Dental and oral formulation containing protease and preparation method thereof	2012	Liqun Yue Anzhen Huang Yiwen Wu	Chloramine-T and protease
US20080181855	USA	Tooth whitener and maintenance with bleach bumpers	2006	Jennifer Jablow	Papain
US20110085991	USA	Compositions for enhancing effects of other oral care compositions	2005	Martin Giniger	Papain
US20070098650	USA	Dental formulation	2005	Robert Miller John Karolchyk Bernard Covalesky	Papain

KR20060097172	South Korea	Improved tooth whitening systems for oral care conveniences	2005	Sei Yun Sang Kwak Han Kim Hoo Kim Sang Kim Sug Chang	Sodium percarbonate, sodium perborate, tetrasodium pyrophosphate peroxidate, tetrasodium pyrophosphate, sodium acid pyrophosphate, sodium hexametaphosphate, sodium tripolyphosphate, sodium potassium tripolyphosphate, tetrapotassium pyrophosphate, acidic sodium meta-polyphosphate, acidic sodium polyphosphate, papain and vitamin E and the sodium bicarbonate, the sodium chlorate, the sodium hypochlorite.
US20060198797	USA	Multi-component oral care compositions	2005	Martin Giniger	Chlorous acid
US20070110682	USA	Non-peroxide preparation for whitening natural and manufactured teeth	2005	Chantal Bergeron	Actinidin
WO200689139	USA	Oral care cleaning compositions and methods	2005	Martin Giniger	Peracetic acid and a salt of chlorous acid

KR20060097177	South Korea	Soft peel-off systems of tooth whitening strips	2005	Hae Yang Han Kim Sang Kwak Sei Yun Sug Chang Andrew Choi Ji Kim	Fatigue phosphoric acid natrium, acid fatigue phosphoric acid natrium, meta phosphoric acid natrium, pulley phosphoric acid natrium, the fatigue phosphoric acid natrium potassium, the fatigue monopotassium phosphate and the acid sodium meta - phosphate, acid pulley phosphate, papain and vitamin E and the sodium bicarbonate, the sodium chlorate, the sodium hypochlorite.
KR20060081533	South Korea	The tooth whitening strip increasing safety	2005	Sug Chang Andrew Choi Ji-Young Kim Hae Yang Sang Kwak Han Kim Sei Yun	Acid fatigue phosphoric acid natrium, meta phosphoric acid natrium, pulley phosphoric acid natrium, a fatigue phosphoric acid natrium potassium, a fatigue monopotassium phosphate and a ultra-phosphoric acid salt and acidic sodium polyphosphate, the papain, vitamin E, the sodium bicarbonate and

					the chlorine
KR20050117679	South Korea	Improved adhesion systems for oral care substances to tooth	2004	Hae Yang Sei Yun Sang Kwak Ji Kim Choi Andrew	Sodium percarbonate, sodium perborate, tetrasodium pyrophosphate peroxidate, tetrasodium pyrophosphate, sodium acid pyrophosphate, sodium hexametaphosphate, sodium tripolyphosphate, sodium potassium tripolyphosphate, tetrapotassium pyrophosphate, acidic sodium meta-polyphosphate, acidic sodium polyphosphate, papain and vitamin E and the sodium bicarbonate, the sodium chlorate, the sodium hypochlorite.
KR20050046049	South Korea	Improved tooth whitening patches adhesive to tooth	2003	Sug Chang Jong Kim Sei Yum	Sodium pyrophosphate, papain, vitamin E, soda, sodium chlorite, sodium hypochlorite

KR20040076179	South Korea	Stick for whitening tooth surfaces which is made using emulsion system	2003	Tae Hwang Yeong Jung Ho Nam	Chlorine, phosphate, vitamin – E, titanium D oxide, sodium hypochlorite.
KR20050041807	South Korea	The oral product of the solidity which is stabilized a whitening ingredient	2003	Jae Lee	Chloric bleaching agent, dextranase and papain, sodium hypochlorite
US20030003059	USA	Dentifrice compositions	2001	Dana Frederic	Sodium hypochloride, potassium hypochloride
US20040191729	USA	Light emitting oral appliance and method of use	2001	Gregory Altshuler Valery Tuchin	Chormophore
WO200343518	USA	Methods and products for oral care	2001	David Bar-Or	Glycine, alanine, valine, leucine, isoleucine, serine, threonine, aspartic acid, isoaspartic acid, asparagine, glutamic acid, isoglutamic acid, glutamine, lysine, hydroxylysine, histidine, arginine, ornithine, phenylalanine, tyrosine, tryptophan, cysteine, methionine, a-hydroxymethylserine

KR20020096263	South Korea	Patches for tooth whitening	2001	Jang Seok Jang Ji Kim Jong Kim Se Yoon	Dextranase, glucose oxidase and papain.
WO200164175	Belgium	Tooth whitening products and procedures	2000	Lucas Huybrechts	Tannase, dioxirane, laccase, hydroxybenzotriazole, dioxirane or oxone, papain, a laccase, an oxidase, lyzozyme, cysteine, cystine, EDTA, mercaptoethanol, dithiothreitol, metabisulfite salts, N-bromosuccinimide
KR19970073569	South Korea	Dental whitening composition	1996	Ji-Young Kim Sung-Keun Oh Ho-Jung Ahn	Papain
GB9410224	United Kingdom	Tooth-whitening compositions containing both chlorite and chlorate salts	1994	Rayson Guy Butcher Greg	Chlorate and chlorite salts, glucose oxidase, papain, alkaline proteinases and/or neutral proteinases

3 Capitulo 2 – Novel in-office peroxide-free tooth whitening gels

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Artigo formatado segundo as normas do periódico Clinical Oral Investigations
http://www.springer.com/medicine/dentistry/journal/784?detailsPage=pltci_1060698

Abstract

Objectives: The aims of this study were to evaluate the stain removal effect of novel bleaching gel-based formulations containing papain, ficin or bromelain and to investigate their effect on enamel surface properties.

Materials and methods: Experimental papain, ficin and bromelain based stain removal gels were prepared and characterized. One hundred forty bovine dental slabs (6 mm diameter x 4 mm height) were obtained, polished and submitted to artificial aging in coffee solution. After artificial aging, the samples were randomly allocated into four groups (n = 10), according to the stain removal agent applied: 20 wt.% carbamide peroxide (positive control); papain-based; ficin-based; bromelain-based and no agent (negative control). The materials were applied once a week, three times of 15 minutes per day, during 4 weeks, following the directions of use from positive control. Color measurement were obtained by CIEDE2000 individual parameters (L*, a*, b*, C* and h-). Knoop microhardness and roughness was also measured. All measurements were performed before and after the stain removal treatments. Data were statistically analyzed at α=0.05 significance level.

Results: All stain removal agents produced greater color change (ΔE*) than the negative control ($p<0.001$). Bromelain and ficin based gels resulted in greater color change being statistically similar to positive control. The carbamide peroxide showed the highest decrease of hardness and the highest increase of roughness.

Clinical significance: The proposed gels containing proteolytic bromelain, papain or ficin may hold significant clinical potential as active agents for the preparation of stain removal agents free of hydrogen/carbamide peroxide.

Keywords: Non-peroxide. Bleaching. Extrinsic stain removal. Carbamide peroxide. Proteolytic enzymes.

Introduction

Among the most sought after dental procedures are dental bleaching[1, 2]. The use and effectiveness of these systems is very well consolidated in the literature and in dental practice. The most commonly used agents for vital tooth bleaching are the carbamide and the hydrogen peroxide[3].

Even its consolidated efectiveness, several studies have demonstrated the deleterious action of peroxide-based bleaching gels on tooth enamel[4-11]. It has been reported the deleterious effects on pulp, tooth sensitivity and gingival irritation, which are generally mild to moderate and transient. The presence of peroxides activates the defense system of the pulp cells, releasing several endogenous antioxidant agents, such as peroxidases and catalases, which promote an enzymatic degradation of H_2O_2 to avoid excessive tissue damage[12]. Therefore, the development of dental products that do not cause tooth damage to the teeth, including irreversible damage such as the pulp cells death, is of great value.

Recently, some enzymes have been described with bleaching potential.[13-15] The natural origin of these enzymes gives them a certainty about non-cytotoxic biological behavior. Papain, bromelain and ficin are part of the cysteine protease group which are widely distributed among living organisms. Most plant cysteine proteases belong to the two large families of papain and legumaine [16]. Proteins such as chymopapain, papain, caricaine, bromelain, actinidine, ficin, and aleurain, and lysosomal cathepsins B, H, L, S, C and K are found within the papain family [17].

The objective of this work was to evaluate *in vitro* the bleaching potential of non-peroxide new whitening gels, containing bromelain, papain and ficin. The null hypothesis tested is that the different whitening gels do not affect the color change, roughness and microhardness of the enamel-dentin discs.

2.3 Materials and methods

Characterization of bleaching systems

Preparation of the peroxide-free gels

The experimental gels were prepared using the reagents shown in Table 1. The formulation was prepared as viscous gel at room temperature. Carbopol®, which was used as a thickener, was incorporated into propylene glycol, and mixed until a homogeneous gel was obtained. Water-soluble components such as potassium oxalate, sodium fluoride and preservatives were previously solubilized in ultrapure water and added to the gel until its homogenization. Then, the enzymes were incorporated until the formation of a homogeneous and transparent gel resulting in 1% of bromelain-, ficin- or papain-based gel, respectively.

In order to utilize the enzymes in their maximal activity, pH of the gels was adjusted according to the optimum pH for proteolytic activity (Table 2). pH was measured using a previously calibrated digital pH meter (Quimis®, Diadema - SP - Brazil).

Evaluation of the effectiveness of bleaching systems

Preparation of specimens and staining procedure

Bovine teeth were randomly selected and stored in 0.1% thymol solution for 1 week. Standard enamel-dentin discs 6 mm in diameter and 4 mm in thickness were cut from the buccal enamel surface using a water-cooled trephine drill. The specimens were wet-polished with 600- and 1200-grit SiC papers. Before their staining process, all specimens were etched with 37% phosphoric acid (Total-etch; Ivoclar-Vivadent, Amherst, NY, USA) for 60 s^3. Then, the prepared specimens were stored in coffee solution by boiling 12g of coffee (melitta, Avaré, SP, Brazil) in 200ml of destilled water for 5 min followed by filtering in a coffee maker (Melitta) for 1 week. Storage media was replaced with fresh solution daily. Upon completion of the staining process, the specimens were washed with distilled water and placed in wells of 24-well plates. Each well was filled with distilled water and were kept at 37° C for 24 h.

Stain removal protocol

After completing baseline measurements, all specimens were randomly allocated to four groups (n = 10), according to the stain removal gel applied: ContrastPM+ (Botica Pelotense, Pelotas, Brazil) which is comprised of 20 wt.% carbamide peroxide (positive control); bromelain-based; papain-based; ficin-based; and no stain removal agent (negative control). Taking into consideration that the experimental materials had no standard application protocol, all were applied based on a previously study. Munchow et al 2016[13]. In brief, the gels were applied over the buccal surface of each specimen and left to rest for 15 min. The gel was then removed and the surface was cleaned using gauze embedded in water. This protocol was repeated two more times. In addition, while the gel was in contact with the specimen, the latter was kept under a wet environment by placing it over a cotton pellet embedded in distilled water and placing it into the wells of 24-well plates. After the stain removal protocol, the specimens were stored into the original wells and kept in distilled water for 1 week. All stain removal protocols were repeated three more times, corresponding to a total of four clinical applications of 1-week intervals.

Measuring the color change

To evaluate the color change efficiency of the specimens, the CIEL*a*b* color coordinates of specimens were measured with a spectrophotometer (SP60, X-Rite, Grand Rapids, MI, USA). The spectrophotometer was plugged into a voltage stabilizer to avoid changes in light source intensity. The equipment was calibrated on the standard tiles provided by the manufacturer. The specimens were evaluated over white (L* = 93.1, a* = 1.3, b* = 5.3) and black (L* = 27.9, a* = 0.0, b* = 0.0). The evaluation was performed before (baseline color) and after the bleaching protocols. The color differences (ΔE) were obtained with the CIEDE 2000 by the following formula:

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

2.3.2.3 Evaluation of hardness loss

Prior to exposure to bleaching agents, the enamel surface microhardness (MH1) was obtained using a microhardness tester (MicrohardnessTester, FM 700, Future-Tech Corp, Japan) with a Knoop indentor using a 25 g load applied for 10s. Three indentations, at 25, 50 and 100 µm from the margins were performed parallel to the surface interface of the dental enamel. After the bleaching process, the microhardness measurement was repeated under the same conditions (MH2). The percentage of surface hardness loss (% SS) was calculated using the following formula:

%SS = (MH1 - MH2) × 100 / MH1.

Evaluation of surface roughness

The enamel surface roughness evaluation was performed using a profilometer (Surftest SJ-301, Mitotoyo, Japan) using a screening length of 1.25 mm and a cut-off point of 0.25 mm to maximize the filtration surface, ripple and a measurement speed of 0.5 mm/s. Each specimen was measured 5 times at different locations and in different directions near the center of the sample, and the mean roughness was obtained. One calibration block was used every six test pieces to verify the

performance of the profilometer. Measurements were performed before (initial) and after (final) the bleaching protocols.

Statistical analysis

The results of color stability, microhardness and surface rougness were analysed using IBM SPSS Statistics 20 statistical package (SPSS Inc., USA) by one-way analysis of variance (ANOVA) and Student Newman Keuls's post hoc test. A significance level of α = 5 % was set for all analyses.

Results

The color change (ΔE*) results are shown in Fig. 1. After the intervention, all gels resulted in greater color change when compared to the negative control. Bromelain and ficin based gels resulted in greater color change, statistically similar to positive control.

Table 3 shows mean values and standard deviation of the hardness and roughness variation after bleaching procedures. The carbamide peroxide showed the highest decrease of hardness, followed by bromelain. After the bleaching procedure the highest increase of roughness was showed by the carbamide peroxide.

Discussion

This work evaluated the action of natural enzymes (ficin, bromelain and papain) as non-peroxides dental whitening agents, and its effect on the dental enamel surface. The null hypothesis tested was rejected, the ficin and bromelain based gels were more effective in removing stains than the papain-based gel. Moreover, the enzyme based gels presented less damage in the enamel surface than carbamide peroxide gel.

The ficin, bromelain and papain-based gels were effective in removing stains of the tooth samples. All experimental gels produced a color change (Fig. 1), however, only the color change caused by bromelain and ficin was greater than the human perceptibility threshold. The bromelain and ficin showed similary color variation when compare to carbamide peroxide. To the best of our knowledge, there is only another study who evaluated papain and bromelain as dental whitening agents, however, they showed a lower stain removal effect when compared to the carbamide peroxide[13]. This result could be explained because in such study, enzymes used

were obtained by soy proteases, different of the present study where the enzymes were extracted from the plant itself, ensuring high purity. Also, in this study the pH of the whitening gels was adjusted in order to ensure the optimum media for maximal enzyme activity[18].

Some studies have reported that the stain removal effect of whitening agents could be caused by the rupture and removal of the portion of the protein from the spots attached to the surface of the enamel[14]. Any macromolecule or stain impregnated onto the tooth surface reduces light reflection, reducing the light reflection and white appearance of the tooth[19]. As the enzymes used in this study are cysteine proteases, they can breakdown such macromolecules, reducing the strains into smaller parts and increasing the lightness of tooth, causing a bleaching effect.

Another similar study,[20] tested the sweet potato (Ipomoea batatas L.) extract as tooth whitening agent. They used the sweet potato extract as an additive to hydrogen peroxide, showing that the addiction of the extract maintained the whitening potential of the system and reduced the deleterious alteration of enamel morphology caused by the use of hydrogen peroxide alone. Despite of being different components, the sweet potato extract contains used natural enzymes (polyphenol peroxidase, catalase, and superoxide dismutase), which can explain this protective effect. In this study, all experimental groups showed less enamel damage when compare to the carbamide peroxide (Table 1). The carbamide peroxide caused the biggest loss of hardness and the highest roughness increment. During the bleaching process, the peroxide decomposes easily when it encounters substances with which it can react and results in the release of free radicals. By passing the tooth saturation point, in the course of decomposition, hydrogen peroxide can interact with the organic components of the tooth, such as proteins and lipids, removing them. In addition, it dissolves the inorganic components of the enamel by penetrating the intra- and interprismatic regions [21], making the surface of the enamel rougher.

The papain and ficin gel based behaved as a protection factor increasing the hardness. This outcome is accepted once one of the most important advantages of using natural products to whiten teeth rather than using peroxide-based agents is the less aggressive process produced by the former. In addition, there is no release of free radicals as occurs with the peroxides based gel, then the oxidative reactions and the free radicals are the main mechanisms responsible for the toxicity and structural

and biochemical damages on the dental hard and soft pulp tissues of peroxide-containing compounds[22].

Papain, bromelain and ficin are widely used for their well-recognized properties, such as their anti-inflammatory, antithrombotic and fibrinolytic effects, anticancer activity and immunomodulatory effects in the food industry, pharmaceuticals and pharmaceutical industries, and in technical procedures for laboratory testing [23-25]. Therefore, the natural origin of these enzymes gives them a certainty about non-cytotoxic biological behavior. Therefore, these results might reduce the tooth sensitivity, since this post-operative pain is related to the cytotoxic of carbamide/hydrogen peroxide.

Conclusions

The non-peroxide bromelain and ficin gels were effective in dental bleaching, being similar to the commercially available gel. Moreover, they showed good comportment on enamel surface, causing less damage when compare to the carbamide peroxide. Therefore, these enzymes showed up promising results, representing significant clinical potential as active of free of peroxide whitening agents.

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Figure

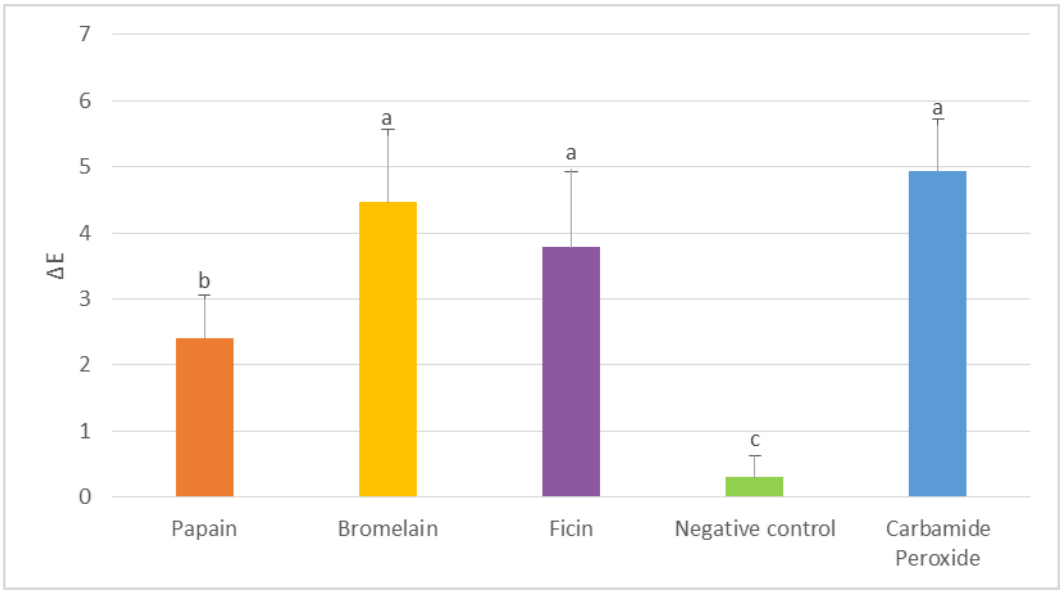

Figure 1 Comparison of whitening gels. Mean values and standard deviations of ΔE values of enamel-dentin discs.*

Tables

Table 1. Components of gel formulation used as vehicle

Component	Percentage by weight relative to total
Potassium oxalate	0.3%
Sodium Fluoride	0.2%
Propyleneglycol	35%
Carbopol®	1%
Sodium benzoate	0.2%
Ultrapure water	Qsp

Table 2. pH values of bleaching gels adjusted for optimal proteolytic activity of enzymes

	pH
Bromelain	7,2 -7,5
Ficin	7-8
Papain	6,5

Table 3. Variation in hardness and roughness (%) after bleaching procedures (Mean ± SD)

	Hardness (%)	Roughness (%)
Carbamide peroxide	-18.72± 4.21^{d}	131.69 ± 11.3 ^{c}
Bromelain	-2.92± 1.5^{c}	96.86 ± 9.8 ^{bc}
Ficin	5.92± 2.5^{a}	71.39 ± 14.9 ^{bc}

Papain	18.58± 3.4[b]	17.21 ± 4.8 [ab]
Negative control	2.51± 2.2[a]	2.55 ± 1.6 [a]

Within a test parameter values denoted with similar lower letters are not significantly different (α=0.05)

4 Considerações finais

Os géis a base de bromelina, papaína e ficina se mostraram promissores para que possam ser usados como agentes de clareamento dental, sendo que a bromelina e a ficina apresentaram resultados semelhantes ao peroxido de carbamida usado como controle comercial. Além disso, apresentaram bom comportamento na superfície do esmalte, causando menos danos quando comparados ao peróxido de carbamida. Portanto, estas enzimas apresentaram um grande potencial, sendo assim, torna-se interessante a realização de mais estudos laboratoriais para uma melhor caracterização dos efeitos sobre o esmalte e a polpa dentária.

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Apêndices

Apêndice A – Nota da Dissertação

Desenvolvimento e avaliação da efetividade de agentes potencialmente clareadores livres de peróxidos
Development and evaluation of the effectiveness of potentially bleaching agents free of peroxides

A presente dissertação de mestrado desenvolveu agentes clareadores experimentais, livres de peróxidos. Apesar da técnica de clareamento dental e seus efeitos no esmalte dentário estar bem consolidada, há um grande incoviniente da técnica, a sensibilidade pós operatória. Por isso, se faz necessário o desenvolvimento de agentes clareadores livres de peróxidos, biocompatíveis, afim de proporcionar ao paciente uma experiencia mais satisfatória, levando a uma maior aceitação do procedimento e consequentemente ao sucesso da técnica. A ação do gel sobre o esmalte foi testada através da análise da mudança de cor, porcentagem da perda de dureza, rugosidade.

Campo da pesquisa: Dentística, Materiais Odontológicos,

Candidato: Juliana Silva Ribeiro, Cirurgiã-Dentista pela Universidade Federal de Pelotas (2017)

Data da defesa e horário: 24/02/2017 – 9h

Local: Sala 603 da Faculdade de Odontologia da Universidade Federal de Pelotas. 6º andar da Faculdade de Odontologia de Pelotas. Rua Gonçalves Chaves, 457.

Membros da banca: Prof. Dr. Rafael Guerra Lund, Profª. Drª. Cristina Pereira Isolan, Profª. Drª. Gabriela Romanini Basso, Profª. Drª. Sonia Luque Peralta (Suplente).

Orientador: Prof. Dr. Rafael Guerra Lund

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Apêndice B – Súmula do currículo do candidato

Súmula do currículo

Juliana Silva Ribeiro nasceu em 04 de fevereiro de 1992, em Cachoeiro de Itapemirim, Espirito Santo. Completou o ensino fundamental e médio em Escola na mesma cidade. No ano de 2010 ingressou na Faculdade de Odontologia da Universidade Federal de Pelotas (UFPel), tendo sido graduada cirugiã-dentista em 2015. No mesmo ano, ingressou no Mestrado do Programa de Pós-graduação em Odontologia da Universidade Federal de Pelotas (UFPel), área de concentração Dentística, sob orientação do Profº. Drº. Rafael Guerra Lund.

Publicações

CORREA, C.F.; SANTANA, L.R.; SILVA, R.M.; NOREMBERG, B.S.; LUND, R.G.; RIBEIRO, J.S.; MOTTA, F.V.; BOMIO, M.R.D.; NASCIMENTO, R.M.; CARREÑO, N.L.V. Antimicrobial activity from polymeric composites-based polydimethylsiloxane/TiO2/GO: evaluation of filler synthesis and surface morphology **Polymer Bulletin** 1-12. 2016. ISSN 0170-0839.

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