UNIVERSIDADE ESTADUAL PAULISTA
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Campus de Araçatuba

GABRIEL PEREIRA NUNES

Influência da incorporação de trimetafosfato de sódio, com substituição parcial ou não por cálcio, em formulações clareadoras, e avaliação sistemática da adição de agentes a base de cálcio em géis clareadores

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Tese apresentada à Faculdade de Odontologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Araçatuba, para obtenção de título de Doutor em Ciências - Área de Concentração: Biomateriais.

Orientador: Prof. Tit. Alberto Carlos Botazzo Delbem

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Orientador: Prof. Alberto Carlos Botazzo Delbem

1. Clareamento dental 2. Fosfatos 3. Efeitos colaterais e reações adversas relacionados a medicamentos 3. Esmalte dentário 4. Teste de materiais I. T.

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Dedicatória

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"O tempo muito me ensinou: ensinou a amar a vida, não desistir de lutar, renascer na derrota, renunciar às palavras e pensamentos negativos, acreditar nos valores humanos, e a ser otimista. Aprendi que mais vale tentar do que recuar... Antes acreditar do que duvidar, que o que vale na vida, não é o ponto de partida e sim a nossa caminhada".

Cora Coralina

Resumo Geral

Nunes, GP. **Influência da incorporação de trimetafosfato de sódio, com substituição parcial ou não por cálcio, em formulações clareadoras, e avaliação sistemática da adição de agentes a base de cálcio em géis clareadores,** 2025, 144f. Tese (Doutorado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba 2025.

RESUMO

O objetivo geral deste estudo foi avaliar, *in vitro*, novas formulações de géis clareadores à base de peróxido de hidrogênio (H_2O_2) contendo trimetafosfato de sódio (TMP), com substituição parcial ou não por cálcio (CaNaTMP), sobre as alterações induzidas pelo procedimento clareador no esmalte dentário. Em adição, foi realizada uma revisão sistemática e metanálise sobre o efeito da incorporação de agentes à base de cálcio em géis clareadores. ***Artigo I:*** Avaliar o efeito da adição de nanopartículas de TMP (TMPnano) e fluoreto de sódio (F) em géis clareadores com 17,5% H_2O_2. Discos de esmalte/dentina bovina (n = 180) foram randomizados nos seguintes grupos experimentais: 17,5% H_2O_2; 17,5% H_2O_2 + 0,1% F; 17,5% H_2O_2 + 1% TMPnano; 17,5% H_2O_2 + 0,1% F + 1% TMPnano; e 35% H_2O_2. Avaliaram-se parâmetros como alteração de cor, propriedades mecânicas e morfológicas do esmalte, além da difusão transamelodentinária de H_2O_2. Os resultados indicaram que o grupo contendo F/TMPnano apresentou similar efeito estético, e redução significativa na desmineralização (dureza superficial e subsuperficial), rugosidade do esmalte e difusão de H_2O_2, em comparação aos géis de 17,5% ou 35% H_2O_2. As análises morfológicas evidenciaram alterações menores no esmalte tratado com F/TMPnano. ***Artigo II:*** Avaliar a influência de diferentes concentrações (0,25%, 0,5% e 1%) de trimetafosfato de sódio substituído por cálcio (CaNaTMP) em géis clareadores com 17,5% e 35% H_2O_2. Discos de esmalte/dentina bovina (n = 288) foram distribuídos de acordo com os grupos experimentais: (1) 35% H_2O_2; (2) 35% H_2O_2 + 0,25% CaNaTMP; (3) 35% H_2O_2 + 0,5% CaNaTMP; (4) 35% H_2O_2 + 1% CaNaTMP; (5) 17,5% H_2O_2; (6) 17,5% H_2O_2 + 0,25% CaNaTMP; (7) 17,5% H_2O_2 + 0,5% CaNaTMP; e (8) 17,5% H_2O_2 + 1% CaNaTMP. Além das metodologias descritas no Artigo 1, foi incluída a análise do conteúdo mineral do esmalte por microtomografia computadorizada. Observou-se que a adição de CaNaTMP, especialmente na concentração de 1%, reduziu significativamente a perda mineral, a rugosidade e a difusão transamelodentinária de H_2O_2, além de promover a precipitação de apatita amorfa na superfície do esmalte, mantendo eficácia clareadora similar aos géis

convencionais. ***Artigo III:*** A revisão sistemática e metanálise avaliou a influência de agentes bioativos à base de cálcio incorporados em géis clareadores sobre a preservação estrutural do esmalte, a eficácia do clareamento e a sensibilidade dentária associada. Após análise de 4.289 artigos, 56 estudos (6 ensaios clínicos e 50 *in vitro*) foram incluídos. Os agentes analisados, como íons de cálcio, gluconato de cálcio, fosfato de cálcio amorfo, fosfopeptídeo de caseína-fosfato de cálcio amorfo e polifosfato de cálcio, reduziram significativamente os efeitos adversos do clareamento, como desmineralização, alterações morfológicas e sensibilidade dentária, sem interferir na eficácia estética. Esses resultados sugerem que tais agentes aumentam a segurança e a biocompatibilidade da terapia clareadora. ***Conclusão Geral:*** A incorporação de TMPnano e fluoreto, CaNaTMP e outros agentes à base de cálcio em géis clareadores com H_2O_2 demonstrou ser uma estratégia eficaz para minimizar os danos estruturais ao esmalte e os efeitos adversos induzidos pelo procedimento clareador. Esses agentes não comprometeram a eficácia estética, representando uma abordagem promissora para otimizar a segurança e os resultados do clareamento dental

Palavras-chave: Clareamento Dentário. Efeitos colaterais e reações adversas relacionados a medicamentos. Esmalte Dentário. Fosfato. Teste de Materiais.

General Abstract

Nunes, GP. **Influence of sodium trimetaphosphate incorporation, with or without partial substitution by calcium, in whitening formulations, and a systematic evaluation of calcium-based agents in bleaching gels,** 2025, 144f. Tese (Doutorado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba 2025.

ABSTRACT

The general aim of this study was to evaluate, *in vitro*, new formulations of hydrogen peroxide (H_2O_2)-based whitening gels containing sodium trimetaphosphate (TMP), with partial or no substitution by calcium (CaNaTMP), regarding their effects on enamel alterations induced by the bleaching procedure. Additionally, a systematic review and meta-analysis were conducted to assess the effect of incorporating calcium-based agents into whitening gels. ***Article I:*** This study evaluated the effect of adding TMP nanoparticles (TMPnano) and sodium fluoride (F) to whitening gels containing 17.5% H_2O_2. Bovine enamel/dentin discs (n = 180) were randomized into the following experimental groups: 17.5% H_2O_2; 17.5% H_2O_2 + 0.1% F; 17.5% H_2O_2 + 1% TMPnano; 17.5% H_2O_2 + 0.1% F + 1% TMPnano; and 35% H_2O_2. Parameters such as color change, mechanical and morphological properties of enamel, and trans-enamel and dentin diffusion of H_2O_2 were assessed. Results showed that the group containing F/TMPnano achieved comparable whitening efficacy and significantly reduced demineralization (surface and subsurface hardness), enamel roughness, and H_2O_2 diffusion compared to the 17.5% and 35% H_2O_2 gels. Morphological analyses revealed fewer alterations in enamel treated with F/TMPnano. ***Article II:*** This study investigated the influence of different concentrations (0.25%, 0.5%, and 1%) of calcium-substituted sodium trimetaphosphate (CaNaTMP) in whitening gels with 17.5% and 35% H_2O_2. Bovine enamel/dentin discs (n = 288) were divided into the following experimental groups: (1) 35% H_2O_2; (2) 35% H_2O_2 + 0.25% CaNaTMP; (3) 35% H_2O_2 + 0.5% CaNaTMP; (4) 35% H_2O_2 + 1% CaNaTMP; (5) 17.5% H_2O_2; (6) 17.5% H_2O_2 + 0.25% CaNaTMP; (7) 17.5% H_2O_2 + 0.5% CaNaTMP; and (8) 17.5% H_2O_2 + 1% CaNaTMP. In addition to the methodologies described in Article 1, mineral content analysis of enamel via micro-computed tomography was performed. The study revealed that adding CaNaTMP, especially at a 1% concentration, significantly reduced mineral loss, enamel roughness, and H_2O_2 diffusion, while promoting amorphous apatite precipitation on the enamel surface. These effects were achieved without compromising the whitening efficacy.

Article III: The systematic review and meta-analysis evaluated the influence of calcium-based bioactive agents incorporated into whitening gels on enamel structural preservation, whitening efficacy, and bleaching-associated sensitivity. From 4,289 articles, 56 studies (6 clinical trials and 50 in vitro studies) met the inclusion criteria. The agents evaluated, including calcium ions, calcium gluconate, amorphous calcium phosphate (ACP), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and calcium polyphosphate, significantly reduced adverse bleaching effects such as demineralization, morphological changes, and tooth sensitivity, without interfering with whitening outcomes. These findings suggest that such agents improve the safety and biocompatibility of bleaching therapy. ***General Conclusion:*** The incorporation of TMPnano and fluoride, CaNaTMP, and other calcium-based agents into H_2O_2 whitening gels proved to be an effective strategy to minimize structural damage to enamel and adverse effects induced by the bleaching procedure. These agents did not compromise the aesthetic efficacy, representing a promising approach to optimize the safety and outcomes of dental bleaching.

Keywords: Dental Bleaching, Drug-Related Side Effects and Adverse Reactions, Dental Enamel, Phosphates, Materials Testing.

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Lista de Apêndices

Lista de Abreviaturas e Siglas

ACP Fosfato de Cálcio Amorfo

ANOVA Análise de Variância

ºC Graus Celsius

CPP Caseína Fosfopepetídeo

Ca Cálcio

CaF^{+} Íon Fluoreto de Cálcio

CaF_2 Fluoreto de Cálcio

CaGlu Gluconato de Cálcio

Ca^{+2} Íon cálcio

CaNaTMP Trimetafosfato de Sódio e Cálcio

CPP-ACP Caseína Fosfopeptídeo Fosfato de Cálcio Amorfo

F Fluoreto

F^{-} Íon fluoreto

g Grama

h Hora

HF^{0} Fosfato de hidrogênio neutro

KHN Dureza knoop

lbs Libras

L Litro

M Molar

Min Minutos

mg Miligramas

mg/g Miligramas por grama

mL Mililitro

mm Milímetro

Mol/L Mol por litro

mol L^{-1} Mol por litro

mV Milivolts

n Número de voluntários

Na^{+} Íon sódio

NaF Fluoreto de sódio

NaOH Hidróxido de sódio

P Fósforo

pH Potencial hidrogeniônico

s Segundos

SHi Dureza de superfície inicial

SHf Dureza de superfície final

%SH Porcentagem de perda de dureza de superfície

TMP Trimetafosfato de sódio

TMP nano Trimetafosfato de sódio nanoparticulado

µg Micrograma

µL/mg Microlitro por miligrana

µm Micrometro

ΔKHN Perda integrada de dureza de subsuperfície

β Beta

α Alfa

Sumário

Introdução Geral

1. Introdução Geral

Nos últimos anos, a odontologia estética tem ganhado destaque, especialmente em relação aos tratamentos de clareamento dental (Wittich et al., 2024; Joiner & Luo, 2017). Esse interesse crescente tem impulsionado o desenvolvimento de técnicas de clareamento dentário cada vez mais eficazes e trazendo satisfação aos pacientes (Ludovichetti et al., 2024). O peróxido de hidrogênio (H_2O_2) continua sendo o agente mais amplamente utilizado para esse propósito devido às suas propriedades oxidantes, que permitem a quebra de moléculas cromóforas no esmalte, resultando em um efeito clareador visível (Aragão et al., 2024; Kihn, 2007). A técnica de clareamento realizada em consultório, que envolve a aplicação de géis de H_2O_2 em altas concentrações, é comumente empregada por profissionais da área para alcançar resultados rápidos e eficientes (Favoreto et al., 2024; Maram et al., 2020). Contudo, apesar de sua popularidade e eficácia na melhora estética, preocupações têm sido levantadas sobre os possíveis efeitos adversos desse tratamento nas estruturas dentárias (Aragão et al., 2024).

O principal mecanismo de ação do H_2O_2 no clareamento está em sua capacidade de gerar espécies reativas de oxigênio (EROs) que quebram as moléculas pigmentadas no esmalte (Kwon & Wertz, 2015; Carey, 2014). No entanto, esse processo de oxidação não se limita aos cromóforos, podendo impactar negativamente as propriedades físicas do esmalte (Aragão et al., 2024; Magalhães et al., 2012). Estudos têm consistentemente relatado que géis à base de H_2O_2 em altas concentrações provocam alterações como redução da microdureza, perda mineral, aumento da rugosidade da superfície e mudanças na morfologia do esmalte (Ortiz et al., 2023; Gruba et al., 2023; Elfallah et al., 2015). Essas alterações estruturais não se restringem às camadas superficiais, podendo atingir regiões mais profundas do tecido dental (Elfallah et al., 2015), levantando preocupações sobre a integridade a longo prazo do esmalte e o aumento da sensibilidade dental (Kury et al., 2022; Kielbassa et al., 2015), especialmente quando o gel se difunde pelo esmalte, alcançando a dentina e a polpa (Donato et al., 2024).

Embora concentrações elevadas de H_2O_2 (variando entre 35% e 40%) proporcionem resultados rápidos e visíveis, a exposição dos tecidos dentários a esses agentes agressivos traz riscos consideráveis (Aragão et al., 2024; Donato et al., 2024). Quanto maior o tempo de exposição e a concentração de H_2O_2, maior é a produção de EROs, o que aumenta a probabilidade de efeitos adversos, como desmineralização do esmalte e sensibilidade dental (Maran et al., 2020; Goldberg et al., 2010). Consequentemente, há um interesse crescente

em desenvolver alternativas mais seguras e menos invasivas para os procedimentos de clareamento. Pesquisas recentes têm focado na redução da concentração de H_2O_2, mantendo sua eficácia clareadora (Maran et al., 2020; Fernández et al., 2017). Além disso, a inclusão de compostos bioativos, como trimetafosfato de sódio (TMP) e fluoreto (F), tem sido investigada para minimizar os efeitos negativos no esmalte (Felipe Akabane et al., 2021; Junior et al., 2021; Dos Santos et al., 2020). O TMP incorporado em géis de alta concentração de H_2O_2 demonstrou ter alguns efeitos protetores, atenuando a desmineralização do esmalte e reduzindo alterações superficiais; no entanto, sua eficácia é limitada quando usado isoladamente (Felipe Akabane et al., 2021).

Estudos recentes exploraram a possibilidade de aumentar as propriedades protetoras do TMP incorporando-o em escala nanométrica (Gruba et al., 2023). As nanopartículas de TMP têm mostrado maior capacidade de proteção ao esmalte (Emerenciano et al., 2018; Danelon et al., 2015), potencialmente prevenindo a desmineralização e alterações estruturais típicas dos procedimentos de clareamento (Gruba et al., 2023). Além disso, o TMP tem sido combinado ao fluoreto de sódio para melhorar sua eficácia por sinergismo, já que o fluoreto pode fornecer proteção adicional ao promover a remineralização do esmalte. Apesar do progresso alcançado no desenvolvimento de agentes clareadores concentrados mais biocompatíveis, a necessidade de uma solução que combine eficácia clareadora com efeitos mínimos ainda persiste. Assim, as avaliações de agentes com reduzida concentração de H_2O_2 são sugeridas.

Outra abordagem promissora que tem emergido nos últimos anos é a substituição iônica de compostos (Imran et al., 2024; Ullah et al., 2023; Meanwell, 2017), de modo que pode propiciar a incorporação de íons cálcio à molécula de TMP. O uso de trimetafosfato de cálcio e sódio (CaNaTMP), que combina as propriedades protetoras do TMP com os benefícios remineralizantes do cálcio, pode oferecer uma solução mais abrangente para os efeitos adversos dos agentes clareadores à base de H_2O_2. O cálcio é um componente essencial para a manutenção da integridade estrutural do esmalte (Abou Neel et al., 2016; García-Godoy et al., 2008), e sua adição ao TMP pode potencializar sua capacidade de prevenir a perda mineral e danos estruturais durante os tratamentos clareadores.

Em resumo, o desafio atual nas pesquisas sobre clareamento dentário é desenvolver géis que proporcionem resultados estéticos eficazes, minimizando os efeitos adversos nas estruturas dentárias. Ao aumentar a biocompatibilidade dos agentes clareadores e proteger o esmalte contra os danos causados pelo estresse oxidativo, essa abordagem pode oferecer uma alternativa mais segura e eficaz para os procedimentos de clareamento dentário na

prática clínica. Diante do exposto, o objetivo deste estudo foi explorar a eficácia do TMP em escala nanométrica em géis clareadores contendo concentrações reduzidas de H_2O_2 (17,5%), avaliando a eficácia clareadora, microdureza, rugosidade, morfologia e composição química do esmalte. Adicionalmente, foi investigado a influência da incorporação de diferentes concentrações de CaNaTMP em formulações com 17,5% e 35% de H_2O_2 sobre as mesmas propriedades do esmalte e avaliação do conteúdo mineral por microtomografia computadorizada. Além disso, também foi conduzido uma revisão sistemática e metanálise analisando o desempenho de géis clareadores enriquecidos com agentes a base de cálcio e seus efeitos protetores sobre as alterações dentárias decorrentes do tratamento clareador.

Objetivo Geral

Este estudo teve como objetivo avaliar, em modelo *in vitro*, novas formulações de géis clareadores contendo peróxido de hidrogênio (H_2O_2) associado ao trimetafosfato de sódio (TMP), com ou sem substituição parcial por cálcio (CaNaTMP), e o impacto dessas formulações nas alterações induzidas pelo processo clareador no esmalte dentário. Além disso, foi conduzida uma revisão sistemática e metanálise sobre o efeito da adição de agentes à base de cálcio em géis clareadores.

Artigo I

2.0 Effect of a novel Low-Concentration Hydrogen Peroxide Bleaching Gel Containing Nano-Sized Sodium Trimetaphosphate and fluoride

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2.1 Abstract

Objectives: To evaluate *in vitro* the effects of nano-sized sodium trimetaphosphate (TMPnano) and sodium fluoride (F) added to a 17.5% hydrogen peroxide (H_2O_2) bleaching gel on the color change, enamel mechanical and morphological properties, and H_2O_2 transamelodentinal diffusion.

Materials and Methods: Bovine enamel/dentin discs (n = 180) were divided according to the bleaching gel: 17.5% H_2O_2 (17.5% HP); 17.5% H_2O_2 + 0.1% F (HP/F); 17.5% H_2O_2 + 1% TMPnano (HP/TMPnano); 17.5% H_2O_2 + 0.1% F + 1% TMPnano (HP/F/TMPnano) and 35% H_2O_2 (35% HP). The gels were applied for 40 min on three sessions, each session spaced 7 days apart. The total color change (ΔE^*_{ab}) according to the Commission Internationale de l'Eclairage (CIE) L*a*b* color change measured by CIEDE2000 (ΔE_{00}), whitening index (ΔWI_D), surface hardness (SH), surface roughness (Ra), cross-sectional hardness (ΔKHN), and transamelodentinal diffusion were assessed. Enamel surfaces were examined using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDS) analysis. The data were analyzed using ANOVA, followed by the Student-Newman-Keuls test ($p < 0.05$).

Results: ΔE^*_{ab}, ΔE_{00}, and ΔWI_D values were comparable among the gels that produced a bleaching effect post-treatment ($p < 0.001$). The HP/F/TMPnano group exhibited lower mineral loss (SH and ΔKHN), Ra, and H_2O_2 diffusion compared to the 17.5% HP and 35% HP groups, which had the highest values ($p < 0.001$). SEM/EDS analysis revealed surface changes in all bleached groups, though these changes were less pronounced with F/TMPnano.

Conclusions: The 17.5% HP gel containing F/TMPnano maintains the bleaching effect while reducing enamel demineralization, roughness, H_2O_2 diffusion, and enamel morphological changes.

*Clinical Relevance***:** Low-Concentration H_2O_2 bleaching gel containing F/TMPnano can be used as a novel approach to enhance safety and maintain the performance of aesthetic effects.

Keywords: Tooth Bleaching Agents; Hydrogen Peroxide; Phosphates; Nanoparticles; Sodium Fluoride.

2.2 Introduction

Currently, the appreciation of aesthetics is more present in modern society [1], tooth whitening has gained prominence in this scenario [2], which has led to an increase in the search for the procedure. Hydrogen Peroxide (H_2O_2) is widely used in different concentrations as a whitening agent [3-5]. Its efficacy is mainly based on the oxidation of organic structures [6], where the breakdown of pigmented macromolecules occurs through the release of oxygen, transforming them into smaller hydroxyls that are more easily eliminated [7].

In-office bleaching technique is a widely employed in dental esthetic treatment due to its high success rate [8]. However, challenges persist regarding this treatment modality, such as providing patients with a comfortable, rapid technique with the least possible risk of developing tooth sensitivity during or after the bleaching procedure [9-11]. Presently, the indiscriminate use of bleaching agents is a growing concern, as it can result in damage to dental tissues. Alterations such as mineral loss, depressions, surface porosity, susceptibility to wear, and decreased microhardness have been consistently reported in several studies [12-14].

It is known that the higher the concentration of H_2O_2 and the longer the exposure time of dental tissue to the bleaching product [14], the greater the release of reactive oxygen species, and their effects on mineralized tissues and pulp have been increasingly questioned [15,16]. Recent studies have shown that gels with reduced H_2O_2 concentration (6-20%) exhibit satisfactory bleaching performance and reduce H_2O_2 diffusion, thus minimizing adverse effects on dental structure [14,17-23]. However, even with this reduction, the deleterious effect has not been completely eliminated.

Thus, distinct protocols from conventional therapies are sought, as well as the development of more biocompatible bleaching gels. Recently, studies have investigated the association of sodium fluoride (F) and sodium trimetaphosphate (TMP) in the composition of concentrated bleaching gel (35% H_2O_2) [12, 24,25], demonstrating reduced demineralization, morphological and roughness alterations of enamel, and trans-amelodentinal diffusion of H_2O_2 [12,25]. However, even so, it was substantially cytotoxic [25]. Furthermore, when TMP was tested on a nanometric scale (TMPnano), it potentiated its protective effects on the bleaching gel; however, it was not able to prevent these alterations entirely [12]. Considering the adverse effects of highly concentrated agents [13,25-27], it would be interesting to evaluate the impact of TMP nanoparticles on enamel alterations resulting from a gel with reduced H_2O_2 concentration.

Therefore, it becomes relevant to explore the minimum exposure required to mitigate side effects while preserving effective performance. Based on this assumption, this study evaluated *in vitro* the addition of nanoparticles of TMPnano at a concentration of 1%, associated with 0.1% sodium fluoride and 17.5% hydrogen peroxide, on the aesthetic efficacy, microhardness, roughness, morphology, and chemical composition of dental enamel, as well as the trans-amelodentinal diffusion of H_2O_2. The null hypothesis of this study is that there is no difference in the bleaching effect and alterations in dental enamel exposed to therapy with TMPnano and sodium fluoride when compared to the conventional agent.

2.3 Material and Methods

2.3.1 Experimental Design

Enamel/dentin discs were obtained from bovine incisor teeth. The sample size per group was determined based on a previous study [25], with 12 enamel/dentin discs allocated per group. Primary outcomes included surface and cross-sectional hardness, with mean differences between groups of 10 and 2800, respectively, and standard deviations of 4 and 1500, respectively. An α error of 5% and a β error of 10% were adopted. Consequently, 60 discs were designated for color change analysis, 60 for hardness, roughness, and SEM/EDS, and an additional 60 discs for transmelodentinal diffusion analysis. The allocation of discs among the 5 experimental groups (n = 12) was randomly performed using Microsoft Excel. Subsequently, these discs were further randomly assigned to five different experimental groups based on the treatment regimens: 1) Bleaching gel containing 17.5% hydrogen peroxide (17.5% HP); 2) Bleaching gel containing 17.5% H_2O_2 and 0.1% NaF (HP/F); 3) Bleaching g gel containing 17.5% H_2O_2 and 1% TMPnano (HP/TMPnano); 4) Bleaching gel containing 17.5% H_2O_2, 0.1% NaF, and 1% TMPnano (HP/F/TMPnano); and 5) Bleaching gel containing 35% H_2O_2 (35% HP). The treatment involved the application of the gels once, during 3 sessions lasting 40 min each, with each session spaced 7 days apart. The discs were stored in artificial saliva between treatment sessions, with the artificial saliva being renewed daily. Following the treatment protocol, total color changes, hardness and roughness surface, cross-sectional hardness, H_2O_2 transamelodentinal diffusion, and enamel morphology and chemical composition were determined (Fig 1).

2.3.2 Enamel/Dentin Disc Preparation

Enamel/dentin discs were prepared from bovine incisors obtained from animals aged 24 to 36 months. Following extraction, the teeth were cleaned, stored in a thymol solution to prevent bacterial growth [12,25]. The roots were removed at the cementoenamel junction, and the crowns were mounted on a bench drill to produce 5.7 mm enamel/dentin discs from the buccal surface using a diamond glass-cutting tip under continuous irrigation. The discs were then sanded to a thickness of 3.5 mm (1.3 mm enamel, 2.2 mm dentin ± 0.2 mm) using 400 and 600 grit aluminum oxide sandpapers, and cleaned ultrasonically in deionized water at 40 Hz and 135 W for 20 min [12].

2.3.3 Composition of Bleaching Gels and Experimental Groups

The bleaching gels were prepared without pigmentation (transparent) and were in dispensed in application syringes. The gel formulation included of a thickening agent (12% Carbopol) and a bleaching agent (Hydrogen Peroxide at 17.5% or 35%). Glycerin and water (q.s.p.) were present in the formulation, along with the necessary NaOH to achieve a gel pH of approximately 7.0. Depending on the experimental group, 0.1% NaF and/or 1% TMPnano were added to the formulation, whose concentrations were based on previous studies [12,25]. Thus, five experimental groups were defined: 1) 17.5% HP; 2) HP/F; 3) HP/TMPnano; 4) HP/F/TMPnano; and 5) 35% HP.

2.3.4 Experiment for Color Change Analysis

2.3.4.1 Selection and Initial Distribution of Enamel/Dentin Discs for Color Change Analysis

After the discs were obtained, an initial color measurement was conducted in accordance with the standards prescribed by the Commission Internationale de l'Eclairage – CIE (International Commission on Illumination), which allows the specification of color perceptions in three-dimensional models. For this purpose, a UV-Visible Reflectance Spectrophotometer (Model UV-2450, Shimadzu, Kyoto, Japan) was used, which employs the CIE L* a* b* color system [25,28,29]. For the readings, the samples were lightly dried with absorbent paper (Kimberly Clark., 30240325, SP, Brazil), ensuring that the enamel did not become dehydrated. After obtaining the L* a* b* values, the average was calculated for the entire sample, and initially, the 200 dental discs that displayed L* a* b* values closest to the average were selected, with a tolerance of 5%.

2.3.4.2 Pigmentation of Enamel/Dentin Discs

The pigmentation procedure involved enamel/dentin discs (n=12 per group), making a total of 60 samples, which were stored in eppendorf-type microtubes filled with 1 mL of black tea infusion at room temperature. This infusion was prepared using 1.6 g of black tea (Chá Matte Leão, Curitiba, PR, Brazil) for every 100 mL of distilled/deionized water [25,29]. The pigmentation process was monitored for six days, during which the infusion was replaced daily. Both the tubes with the infusion and the specimens were maintained at room temperature throughout the entire procedure. Following the pigmentation treatment, a second selection of the samples was carried out in the same manner as previously described.

2.3.4.3 Treatment with Bleaching Gels

Following the selection of pigmented enamel/dentin discs (n=60) as specified anteriorly, the bleaching gels were applied to the enamel surface (0.04 mL) for 40 min using a dispensing syringe and microbrush. The bleaching sessions were conducted every seven days over a period of 21 days, totaling three treatments. To remove the gels after each session, gauze and a 30-second wash with deionized water were used to eliminate any remaining residues from the discs. Between treatments, the discs were stored in individual containers filled with 2 mL of artificial saliva [12,27] consisting of 1.5 mmol/L $Ca(NO_3)_2.H_2O$, 0.9 mmol/L $NaH_2PO_4.H_2O$, and 150 mmol/L KCl in a 0.1 mol/L sodium cacodylate buffer, pH 7.0 [25,27]. This artificial saliva was replaced daily.

2.3.4.4 Color Measurements

The enamel/dentin discs were fixed in black silicone holders with dimensions of 5.7 mm in diameter and 3.5 mm in thickness, ensuring consistent light beam incidence in the UV-2450 Visible Ultraviolet Reflection Spectrophotometer (Shimadzu, Kyoto, Japan). This setup, with a wavelength range from 400 nm to 700 nm, used standard D65 lighting and an illumination/observation angle of 45/0°. Color measurements were taken on the vestibular surface of the enamel after disc preparation and pigmentation, following the 1st, 2nd, and 3rd bleaching sessions, and on the 7th and 14th days post-bleaching. The absolute differences (Δ) in color coordinates (L*, a*, b*) were calculated between the various time points (post-staining, post-bleaching sessions, and 7 and 14 days post-bleaching) and the initial values: ΔL* (positive indicates increased brightness, negative indicates darkness), Δa* (positive indicates redness, negative indicates greenness), and Δb* (positive indicates yellowness, negative indicates blueness). Total color change was calculated using the formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 +$

$(\Delta b^*)^2]^{1/2}$ [25,28,29]. The Whitening Index for Dentistry (ΔWI_D) was then determined using the equation: $\Delta WI_D = 0.511L^* - 2.324a^* - 1.100b^*$ [30]. The CIEDE2000 system calculated ΔE_{00} values with the formula [31]: $E_{00} = [(\Delta L/KL \times SL) + (\Delta C/KC \times SC)^2 + (\Delta H/KH \times SH)^2 + RT \times (\Delta C/KC \times SC) \times (\Delta H/KH \times SH)^{0.5}]$, where ΔL^*, ΔC^*, and ΔH^* represent differences in brightness, chroma, and hue between two samples. RT (rotation function) explains the interaction between chroma and hue differences in the blue region. SL, SC, and SH are weighting functions for lightness, chroma, and hue components. KL, KC, and KH are parametric constants set to 1. Color differences were evaluated using the 50:50% perceptibility ($PT_{00} = 0.81$) and acceptability ($AT_{00} = 1.81$) thresholds [31,32].

2.3.5 Surface and Cross-Sectional Hardness and Roughness Analyses

The enamel surface of the enamel/dentin discs (n=60) was leveled and polished to remove approximately 120 µm of the surface enamel, in line with previous research [25]. Post-polishing, the samples underwent initial surface hardness (SH) assessments (Micromet 5114, Buehler, Lake Bluff, IL, USA) utilizing a Knoop diamond under a static vertical load of 25 g for 10 s [12,25]. Additionally, initial surface roughness (Ra) was measured using a profilometer (model SJ-401, Mitutoyo, Kawasaki, Japan) with a radius of 2 mm, operating at a constant speed of 0.1 mm/s, a load of 5 N, and a cut-off value of 0.25 mm [12]. The enamel/dentin discs with initial SH values between 347.0 and 372.6 KHN were randomly allocated (Excel, Microsoft Corporation, USA) into five predefined experimental groups (n = 12). After completing the three bleaching sessions as previously described, surface hardness was re-evaluated (final SH), and the final Ra was recorded.

Following the determination of SH and Ra, the enamel/dentin discs were bisected, and one half was embedded in acrylic resin and polished [25]. Knoop hardness was measured in cross-sections using a load of 5 g for 10 s at depths of 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 180 µm from the surface. The integrated area under the hardness curve (KHN × µm) was calculated using the trapezoidal rule with GraphPad Prism software (Prism 7 for Windows, version 7.00, San Diego, CA, USA) [25,27]. The values were then subtracted from the integrated area for sound enamel to determine the integrated loss of subsurface hardness (ΔKHN; KHN × µm) [12,25,27].

2.3.6 Determination of Trans-Amelodentinal Diffusion of H_2O_2

Enamel/dentin discs (n = 12/group) were fixed in the upper compartment of artificial pulp chambers using silicone rings and sealed with pink wax number 7 (Wilson®, Polidental,

Cotia, SP, Brazil). The lower compartments, which exposed the dentin surface, were submerged in 1 mL of sodium acetate buffer solution (2.0 mol/L, pH 4.5, adjusted with acetic acid, Sigma Chemical Co., St. Louis, MO, USA) and placed in 24-well cell culture plates (Costar Corp., Cambridge, MA, USA) [25]. The bleaching gels were applied once as previously described, while maintaining a humid environment at 37°C.

To quantify the H_2O_2 present in the buffer solution, a colorimetric method by Mottola et al. [33] was employed. After 40 min of bleaching, 1 mL of leucocrystal violet (0.5 mg/mL in HCl, Sigma Chemical Co., St. Louis, MO, USA), 50 µL of horseradish peroxidase enzyme (1 mg/mL in water, Sigma Chemical Co., St. Louis, MO, USA), and 3 mL of deionized water were added [25]. A standard curve for H_2O_2 concentrations (ranging from 0.5 to 5.0 µg/mL) was prepared under the same conditions as the samples, and measurements were taken using a spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) at a wavelength of 596 nm.

2.3.7 Scanning Electron Microscopy Surface Examination

Two samples from each experimental group underwent examination using scanning electron microscopy (SEM - Carl Zeiss, EVO ILS15, Carl Zeiss NTS LTD, Germany). To prepare the samples, all discs underwent a dehydration process using an increasing ethanol concentration series (ranging from 50% to 100%). Subsequently, they were coated with a layer of gold using a Quorum Q150T E sputter coater. The SEM analysis was then performed at an accelerating voltage of 20 kV, with a magnification level set at ×10000.

For further analysis, the chemical composition of the enamel surface was determined through energy dispersive X-ray spectroscopy (EDS) using an Oxford Instruments INCAx-act system with an energy resolution of 133 eV from England. The spatial resolution during EDS analysis was approximately 2 µm, and each sample was analyzed for a count time of 7000 s. This analysis focused on quantifying the atomic percentages of calcium (Ca), phosphorus (P), oxygen (O), and fluoride (F) present on the enamel surface [12].

2.3.8 Statistical Analysis

Statistical analysis was conducted using Sigmaplot® version 12.0 for Windows, with a significance level set at 5%. The color change data were analyzed considering different bleaching gels and time as factors of variation, with variables including ΔE^*_{ab}, ΔWI_D, ΔE_{00}, SHF, %SH, and Ra. For the analysis of ΔKHN and H_2O_2 diffusion, the values were treated as variables, while the bleaching gels were considered as the factor of variation. The data were

assessed for homogeneity and subjected to ANOVA, followed by the Student-Newman-Keuls multiple comparison test.

2.4 Results

The bleaching agents evaluated produced a significant change in ΔE^*_{ab} after the bleaching sessions ($p < 0.001$) in previously pigmented enamel. All treatments performed similarly, regardless of the evaluation period ($p > 0.05$). The color change was progressive and continuous from the first bleaching session (Fig. 2A). Regarding ΔWI_D, all groups achieved gradual and continuous color changes after the bleaching sessions, making the samples whiter ($p < 0.001$) (Fig. 2B). No differences in ΔWI_D were observed among the groups in any bleaching application or throughout the bleaching sessions ($p > 0.05$), thus all gels maintained a stable chromatic effect 14 days after the treatment. For color changes assessed by ΔE_{00}, the bleaching protocols showed clinically noticeable color changes ($\Delta E_{00} > 0.8$), where all gels produced a similar bleaching effect, regardless of the timing of the analysis ($p > 0.05$) (Fig. 2C).

The bleaching treatment resulted in a reduction in hardness values across all experimental groups evaluated ($p < 0.05$; Table 1). The 35% HP group exhibited the most significant loss in hardness ($p < 0.001$), suggesting a dose-response pattern to the concentration of hydrogen peroxide. The addition of F and/or TMPnano reduced surface hardness loss (SHf: $p < 0.001$; %SH: $p < 0.001$). The HP/F/TMPnano bleaching gel showed the lowest levels of surface and in-depth hardness loss (SHf: $p < 0.001$; %SH: $p < 0.001$; ΔKHN: $p < 0.001$). The bleaching gels (35% HP and 17.5% HP) increased the surface roughness of enamel following the bleaching process ($p < 0.05$). Experimental gels containing F and/or TMPnano did not alter surface roughness, displaying roughness values statistically similar to those measured before the bleaching process ($p > 0.05$; Table 1). The 35% HP bleaching gel had the highest level of trans-amelodentinal H_2O_2 penetration ($p < 0.001$), while HP/F/TMPnano had the lowest ($p < 0.001$; Table 1).

The images obtained by scanning electron microscopy show that the 17.5% HP and 35% HP bleaching gels caused greater exposure of the enamel prisms and interprismatic enamel, as the "keyhole" or honeycomb-like structures that characterize these features became more evident when compared to healthy enamel (Fig. 3). The bleaching gels containing TMPnano and F/TMPnano show amorphous material precipitated, mainly in the interprismatic spaces, compared to their counterparts, making the "honeycomb" appearance of the enamel surface less evident. The elements detected on the enamel surface were Ca, P, and O, regardless of the bleaching gel composition, with a Ca/P ratio between 1.56 and 1.61.

2.5 Discussion

This study investigated the impact of adding 1% sodium trimetaphosphate nanoparticles (TMPnano), with or without 0.1% sodium fluoride (F), to bleaching gels containing 17.5% hydrogen peroxide on the mechanical properties of bleached enamel. The first null hypothesis of this study was accepted, as the addition of these agents to the bleaching gels did not compromise the bleaching efficacy. However, the second null hypothesis was rejected, as the addition of F and/or TMPnano to the bleaching gels reduced trans-amelodentinal diffusion, as well as changes in roughness, hardness, and morphology of the enamel after bleaching.

The concentration of hydrogen peroxide and the incorporation of F and/or TMPnano in the bleaching agents did not result in significant differences in bleaching effectiveness across therapies, regardless of the evaluation time, confirming previous results with high-concentration H_2O_2 gels and TMP at the micrometric scale [24,25]. Notably, color changes were more pronounced in the first bleaching session, reinforcing previous findings that hydrogen peroxide has a greater redox effect during initial exposure, with less pronounced chromatic changes in subsequent sessions [25,27,34]. Overall, all bleaching protocols were equivalent in terms of color change across all chromatic evaluation tools (ΔE^*_{ab}; ΔWI_D e ΔE_{00}). This color change was considered clinically perceptible to the naked eye, as the ΔE values obtained were greater than 3.3 [35,36]. It is assumed that the free radicals produced by 35% H_2O_2 are already sufficient to oxidize the organic component of dentin and promote significant bleaching in the first session, which explains the less pronounced changes in subsequent sessions.

To assess color stability over time, we evaluated dental bleaching results 7 and 14 days after the final session. The color rebound observed in all experimental groups was neither significant nor clinically perceptible ($\Delta E \leq 3.3$). These findings are relevant as they confirm the hypothesis that the addition of the evaluated agents does not influence the amount of oxidizing molecules present in the dentin structure. Furthermore, they suggest the potential to avoid exposing the tooth to a high-concentration H_2O_2 gel (35%) [14,20], as the reduced concentration H_2O_2 gel (17.5%), even with the incorporation of F and/or TMPnano, promotes a similar effect and post-bleaching chromatic stabilization. It is assumed that in vital teeth, the behavior would be similar, as a recent randomized clinical trial observed that the addition of 1% sodium hexametaphosphate, another inorganic polyphosphate, to the bleaching gel maintained the same bleaching efficacy and reduced postoperative sensitivity [37]. These results offer a promising perspective for the clinical use of experimental gels and suggest the need for future investigations to evaluate, under real oral conditions, whether TMPnano also does not affect the

clinical performance of the bleaching agent and can reduce the deleterious effects of bleaching therapy.

Regarding hardness and roughness analysis, the 35% HP group exhibited the lowest hardness values (KHN) and the highest positive variation in roughness values (Ra) compared to the other bleached groups. These changes are likely related to peroxide-based gels, as mineral loss may have been caused by the release of H^+ ions from the anionic dissociation of H_2O_2, resulting in enamel dissolution and consequently in structural alterations [38]. These findings are corroborated by the dose-dependent response observed in the present study, as halving the concentration of the H_2O_2 gel (17.5% HP group) resulted in less mineral loss, roughness alteration, and trans-amelodentinal diffusion of H_2O_2 compared to the 35% HP group. Studies suggest that 35% hydrogen peroxide diffuses more rapidly through tissues than a reduced concentration and can alter the structural and biochemical properties of dental tissues [6,39].

It is important to highlight that all bleaching gels were adjusted to a neutral pH (7) to avoid possible demineralization and increased roughness due to the acidity of the bleaching gel [12,25], as a drop in pH is one of the main causes of mineral loss in enamel. Additionally, pH neutrality contributes to maintaining the degree of whitening [40]. Stoichiometric experiments indicate that the formation of the perhydroxyl ion is influenced by pH; thus, the higher the pH, the more ions are formed, leading to a greater production of free radicals [41]. Gels containing only hydrogen peroxide (HP), without the addition of TMP and/or F, showed significantly lower surface hardness values. The combinations of F/TMPnano with HP proved beneficial in preserving enamel hardness values, proving to be more effective in preventing demineralization.

The presence of 0.1% sodium fluoride in the HP/F gel contributed to the microstructural repair of defects in the bleached enamel, as fluoride ions have the ability to enhance crystal growth and slow down the dissolution of enamel minerals [42]. These ions aid in repairing microstructural defects in the enamel through the adsorption and precipitation of calcium and phosphate present in saliva. Previous studies have reported that the addition of calcium and fluoride to the bleaching gel increases its saturation and allows the incorporation of these ions into the enamel structure, consequently increasing its resistance to demineralization [26,42]. However, the use of F or TMPnano alone in the gel composition was not sufficient to minimize the loss of hardness in depth, mainly due to its limited action on the superficial region of the enamel. This can also be explained by the similar diffusion of H_2O_2 among the HP/F, HP/TMPnano, and 17.5% HP groups. Nonetheless, the presence of F/TMPnano provided a synergistic effect of the agents, resulting in greater prevention of demineralization on the

surface and in the depth of the enamel, as well as reducing the diffusion of H_2O_2 and alteration of surface roughness.

These findings are further clarified by enamel topography, where the inclusion of TMPnano or F/TMPnano in the bleaching gels facilitated the deposition of amorphous material, mainly in the interprismatic spaces. This process minimized morphological alterations in the enamel (Fig. 3). The literature also reports minimal variation in the Ca/P ratio, which could indicate either mineral loss or increased enamel roughness, as demonstrated by the reduction in hardness following treatment with bleaching gels. However, the remaining enamel structure after these treatments is predominantly composed of calcium-deficient apatite or carbonated apatite, with a Ca/P ratio ranging from 1.56 to 1.61 [43,44]. Conversely, the addition of F or TMPnano to bleaching agents promotes the adsorption of these components to the enamel, reducing solubility and increasing resistance in the presence of hydrogen peroxide. These changes are corroborated by SEM analyses, which indicate the precipitation of amorphous material, mainly in the interprismatic spaces, in samples treated with whitening gels containing TMPnano and F/TMPnano. In addition, it is important to mention that, according to recent studies, EDS reveals minimal changes in enamel after treatment with bleaching agents [45-47].

It is known that when TMP is adsorbed onto dental substrates, this interaction can alter selective permeability and the diffusion of ions into its interior [48-50]. Thus, TMP possibly retains positively charged ions like CaF^+ and Ca^{2+}, substituting Na^+ in the cyclic structure and promoting a reduction in acid diffusion [48,49]. Additionally, during demineralization and remineralization processes, TMP facilitates the formation and flow of neutral species ($CaHPO_4^0$ e HF^0) in the enamel, which have diffusion coefficients a thousand times greater than their charged counterparts [49]. This action is enhanced when phosphate is present in an appropriate molar ratio relative to fluoride (F/TMP), as both components will act synergistically [12,25,49-51]. Furthermore, the reduction of TMP particle size to the nanometric level optimized its effect in the formulation, as it provides a high surface area-to-volume ratio and a high percentage of surface atoms compared to larger particles, making them more reactive [49,51]. This mechanism also seems to explain that the smaller alterations observed in the enamel were not only due to the reduced concentration of H_2O_2 but also to the effect of TMP nanoparticles, especially when compared to data obtained in previous studies with TMP on a micrometric scale [24,25,52]. It is important to note that this study is the first to evaluate the addition of F/TMP to gels with a low concentration of H_2O_2 (17.5%) for in-office whitening applications. The results are particularly noteworthy. When comparing the effectiveness of these agents in 17.5% H_2O_2 gels with previous studies that investigated their use in 35% H_2O_2 gels, the lower

concentration gel proved more effective in preventing enamel alterations. This effectiveness is highlighted in comparison to gels without these additives [12,24,25]. Specifically, the 17.5% concentration gel resulted in less loss of enamel hardness, reduced trans-enamel-dentin diffusion of H_2O_2, and fewer changes in enamel roughness and topography. The lower hydrogen peroxide concentration not only caused fewer alterations to the enamel but also enhanced the performance of F/TMPnano. Furthermore, the protective effects of F or TMPnano on enamel roughness, microhardness, and topography were more pronounced in the low-concentration gel, a benefit not observed in the high-concentration gel.

Thus, it has been shown that gels with high concentrations of H_2O_2 cause greater alterations in dental tissues [14,17,19,20], without providing additional aesthetic benefits [14,19], corroborating the results found in this study. Therefore, using a highly concentrated agent does not seem advantageous, considering the reported adverse effects and similar whitening efficacy. Clinical studies have also demonstrated that bleaching gels with 15-20% H_2O_2 can cause significant color change, comparable to that achieved with 35% H_2O_2 gel [53-55]. It is noteworthy that in these studies, the incidence of dental sensitivity ranged from 24% to 78%, considered mild in severity [53-55]. These findings were confirmed in a published systematic review with meta-analysis [19]. The present study corroborates the observed clinical esthetic outcomes and also provides significant additional value. Although clinical studies suggest that whitening gels with reduced concentrations of H_2O_2 can provide satisfactory aesthetic results with a lower risk of tooth sensitivity, the literature still lacks *in vitro* studies comparing whitening efficacy and the effects of gels with different concentrations of H_2O_2 (17.5% and 35%) on dental enamel. Thus, it is possible to perform an efficient and less aggressive in-office dental bleaching protocol. The association of F/TMPnano was chosen to maximize the protection of dental tissues during bleaching, with the clinical hypothesis that this combination can minimize sensitivity and other adverse effects, without compromising the effectiveness of the bleaching process. However, *in vivo* studies on vital human teeth are necessary to evaluate the efficacy of dental bleaching and the pulpal responses following the application of the bleaching protocols evaluated in this study.

In addition, the present study had some limitations. Firstly, it utilized an experimental *in vitro* model using artificially pigmented bovine teeth. While widely employed in dental bleaching studies and yielding clinically relevant outcomes [29], the in vitro model allows for the standardization of conditions and samples that may not be feasible in clinical settings. However, it remains unclear whether our results can be extrapolated to other pigmentation agents or if the behavior of formulations would differ in clinical tests, including the reduction

of dental sensitivity. Furthermore, investigation into potential adverse effects of the experimental gel on adhesion, dentin mechanical properties, and biological effects is warranted. Given these limitations, the findings thus far for the gel containing TMPnano and F in combination with a reduced concentration of H_2O_2 are promising. It is pertinent to assess the performance of the experimental gel in future clinical research, which could lead to the development of in-office bleaching therapies that are aesthetically viable and biologically compatible with the dentin-pulp complex, effectively reducing the side effects of dental bleaching and enhancing procedure safety.

2.6. Conclusion

Considering the obtained data and the limitations of the experimental model, it can be concluded that the addition of F/TMPnano to the 17.5% H_2O_2 bleaching gel significantly reduces surface and cross-sectional hardness loss, surface roughness, trans-amelodentinal diffusion of H_2O_2, and morphological changes in the enamel, as well as does not interfere with the bleaching efficacy.

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Figure 1. Representative diagram of the study's experimental design. A. Enamel/Dentin Discs Preparation. B. Experimental Gels and Treatment. C. Color Alteration Analysis. D. Surface Hardness. E. Surface Roughness. F. Cross-sectional Hardness (KHN x µm). G. Trans-amelodentinal Diffusion of H_2O_2. H. SEM/EDS Analysis.

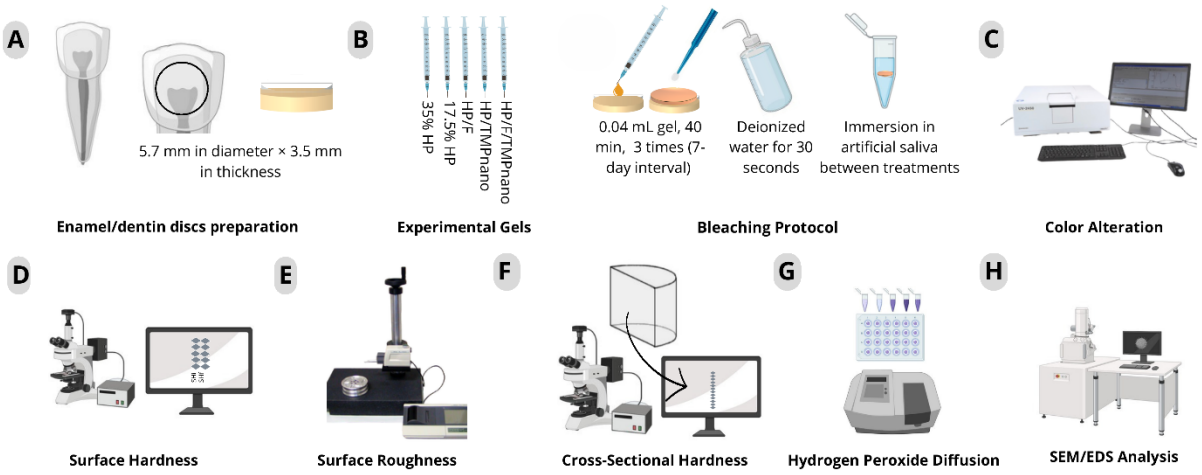

Figure 2. Mean values of the alteration of: (A) total color alteration (ΔE^*_{ab}), (B) whitening index in dentistry (ΔWI_D), and (C) color alteration by CIEDE2000 (ΔE_{00}) according to bleaching gels and time of analysis (n = 12). Different subscript lowercase letters indicate statistical differences between whitening gels at each time of analysis and between times of analysis for each bleaching gel (Student-Newman-Keuls; $p < 0.05$).

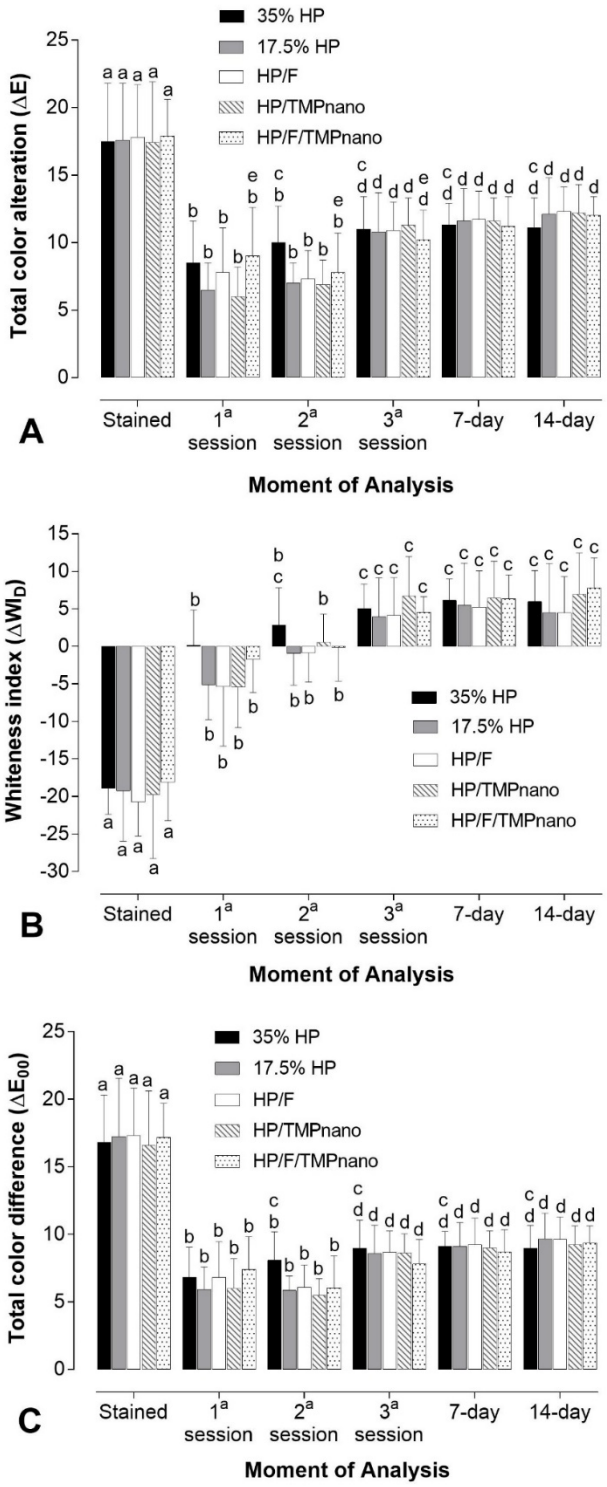

Figure 3. Representative micrographs obtained by scanning electron microscopy of the enamel surface after bleaching gels (10,000× magnification). The black arrows indicate interprismatic enamel. Open arrows point to the enamel prisms.

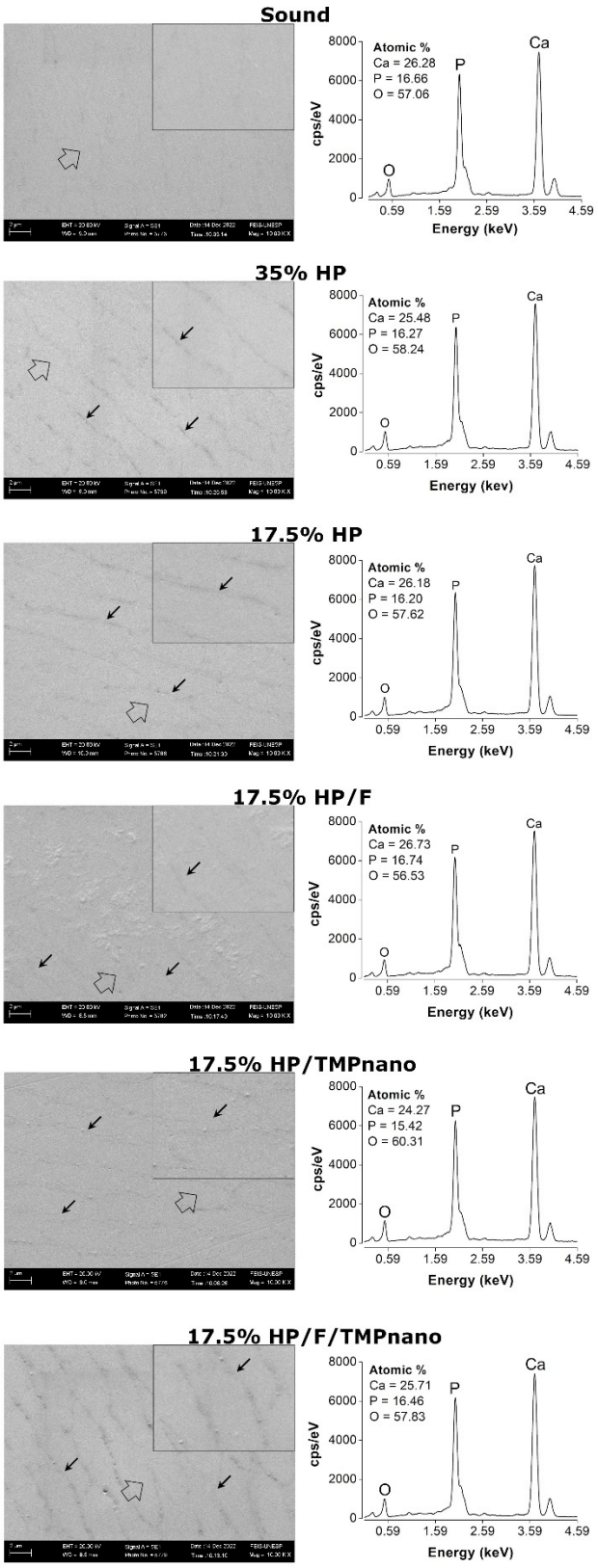

Table 1. Mean values (SD) of roughness (Ra), surface hardness (SH), surface hardness and percentage of surface hardness change (%SH), trans-amelodentinal diffusion of H_2O_2, and hardness in longitudinal section according to groups (n=12)

Groups	Variables						
	Ra		SH		%SH	H_2O_2 (µg/mL)	ΔKHN (KHN x µm)
	Initial*	Final	Initial*	Final			
35% HP	0.018[a] (0.003)	0.027[b] (0.003)	362.2[a] (5.6)	289.2[b] (5.7)	-20.1[a] (1.4)	7,16[a] (0.66)	18.395[a] (777)
17.5% HP	0.017[a] (0.004)	0.026[b] (0005)	362.1[a] (5.9)	306.7[c] (6.2)	-15.3[b] (1.1)	5,65[b] (0.57)	19.436[b] (340)
HP/F	0.016[a] (0.004)	0.019[a] (0.005)	362.0[a] (5.7)	314.3[d] (2.3)	-13.2[c] (1.1)	5.51[b] (0.68)	20.027[b] (662)
HP/TMPnano	0.016[a] (0.004)	0.018[a] (0.005)	362.2[a] (5.1)	312.6[d] (4.9)	-13.7[c] (1.0)	5.47[b] (0.46)	19.802[b] (603)
HP/F/TMPnano	0.017[a] (0.005)	0.018[a] (0.005)	362.1[a] (5.7)	343.3[e] (6.0)	-5.2[d] (1.3)	4.59[c] (0.57)	21.864[c] (562)

Lowercase letters indicate a significant difference between the means of each variable (repeated measures ANOVA, Student-Newman-Keuls, $p<0.05$). *Baseline: values of surface hardness and roughness before bleaching treatments.

Artigo II

3.0 Synthesis and application of calcium cyclotriphosphate in bleaching formulations: effects on dental enamel properties

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3.1 Abstract

Objectives: This study aimed to synthesize and evaluate the *in vitro* effects of varying concentrations (0.25%, 0.5%, and 1%) of calcium-substituted sodium trimetaphosphate (CaNaTMP) incorporated into 17.5% and 35% hydrogen peroxide (HP) whitening formulations on enamel color change, microhardness, morphology, surface roughness, mineral content, and transamelodentinal diffusion of HP.

Materials and Methods: Enamel/dentin discs (N = 288) were allocated into eight groups according to the bleaching gel: (1) 35% HP; (2) 35% HP + 0.25% CaNaTMP; (3) 35% HP + 0.5% CaNaTMP; (4) 35% HP + 1% CaNaTMP; (5) 17.5% HP; (6) 17.5% HP + 0.25% CaNaTMP; (7) 17.5% HP + 0.5% CaNaTMP; and (8) 17.5% HP + 1% CaNaTMP. Gels were applied for 40 min across three weekly sessions. Evaluated parameters included total color change according to CIELab (ΔE), CIEDE2000 equation (ΔE_{00}), whiteness index (ΔWI_D), surface hardness (SH), surface roughness (Ra), enamel mineral content ($g_{HAp} \times cm^{-3} \times \mu m$), and transamelodentinal of HP. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) analyses were used to assess enamel surface characteristics. Data were analyzed using ANOVA and the Student-Newman-Keuls test ($p < 0.05$).

Results: All bleaching gels caused significant color changes after treatment ($p < 0.001$), with similar ΔE, ΔE_{00}, and ΔWI_D values among them. Mineral loss (SH, $g_{HAp} \times cm^{-3} \times \mu m$), Ra, and HP diffusion were highest with the 35% HP gel ($p < 0.001$) and lowest in groups containing CaNaTMP, particularly at 1% ($p < 0.001$). SEM/EDX analysis revealed no visible surface alterations in the 17.5% HP group, with prominent amorphous apatite precipitation in the 1% CaTMP-containing group. Incorporating CaNaTMP, particularly at 0.5% and 1%, into the 35% HP gel reduced surface changes.

Conclusions: This study demonstrated that the addition of CaNaTMP to bleaching gels with 17.5% and 35% HP reduces mineral loss, changes in roughness, and surface morphology of enamel, as well as decreasing trans-amelodentinal diffusion of H_2O_2, without compromising the bleaching efficacy. The incorporation of 1% CaNaTMP was particularly more effective in protecting the dental enamel.

Clinical Relevance: The incorporation of CaNaTMP to 17.5% and 35% HP bleaching gels enhances safety and biocompatibility by reducing enamel damage. The lower HP concentration (17.5%) combined with 1% CaNaTMP provides a safer whitening option, maintaining effectiveness while minimizing adverse enamel changes, aimed at improving patient comfort during treatment.

Keywords: Dental Enamel, Tooth Whitening, Phosphate, Calcium, Hydrogen Peroxide.

3.2 Introduction

Dental bleaching is widely recognized as an effective clinical procedure for enhancing the aesthetics of discolored teeth [1]. For this purpose, hydrogen peroxide (HP) is the most used bleaching agent worldwide, available in various concentrations [2]. In the in-office technique, performed by professionals during clinical sessions, high concentrations of HP, usually ranging from 35% to 40%, are applied for a short duration [3,4]. This compound acts as a powerful oxidizing agent, generating free radicals capable of breaking the bonds of chromophore molecules within dental tissues, thereby producing the whitening effect [5]. Despite its aesthetic efficacy and the satisfaction, it provides to patients, the oxidizing action of HP is not confined to whitening and may lead to adverse effects on dental tissues [5,6].

Recent studies indicate that high-concentration hydrogen peroxide-based gels generate greater amounts of reactive oxygen species, which can lead to significant alterations in dental structure [5-7]. These alterations include reduced microhardness, mineral loss due to enamel dissolution, increased surface roughness, and changes in the morphology and mechanical properties of dental substrates [5,8,9]. Such evidence has raised growing concerns regarding the adverse effects on dental hard tissues and pulp integrity [5,6]. Furthermore, the high transamelodentinal diffusion capacity of HP, combined with the presence of residual toxic substances in the dentin, may reach the pulp chamber [10-12]. Consequently, there are reports of high rates of tooth hypersensitivity induced by this procedure, particularly in in-office treatments [13,14].

New approaches are being investigated to prevent dental alterations, ensuring patient comfort and safety. Recent studies suggest reducing HP concentrations in bleaching gels [8,15]; however, this strategy alone does not fully prevent the structural changes observed in dental tissues. In this context, the incorporation of bioactive agents into bleaching gels has gained prominence [16-18]. The combination of sodium trimetaphosphate (TMP) and sodium fluoride (F) in bleaching gels has been explored, demonstrating protective effects on dental tissues [7,8,12]. However, TMP alone exhibits limited effects, and while its association with F enhances performance, it is still insufficient to fully prevent the alterations caused by whitening agents on enamel. Furthermore, TMP demonstrates a potent deep remineralizing effect on hard tissues [19,20], whereas F's action is largely restricted to superficial layers [19,21]. Thus, to address these limitations, the development of compounds that integrate these protective properties, making them more reactive and biomimetic to the complex oral cavity environment, could offer more comprehensive protection against the adverse effects of tooth whitening. This represents a promising strategy to enhance the safety and efficacy of this therapy.

The remineralization and structural integrity of dental enamel are directly associated with the presence of calcium and phosphate ions [22,23], making the provision of an additional source of these ions a promising strategy to avoid demineralization [22]. Furthermore, calcium ions have demonstrated protective benefits against the adverse effects associated with whitening therapy. Based on these findings, it is hypothesized that partially replacing sodium ions with calcium in the TMP molecule could result in a polyphosphate with optimized properties and enhanced performance compared to conventional TMP. Thus, it would be relevant to explore the effect of calcium sodium trimetaphosphate (CaNaTMP) in bleaching gels containing traditional and reduced concentrations of hydrogen peroxide (HP), assessing its ability to prevent alterations in dental enamel. This is particularly relevant given the prevalence of both high concentrations of HP in clinical practice and the ongoing investigations into gels containing lower HP concentrations. This study aimed to synthesize and characterize CaNaTMP and evaluate its in vitro performance at concentrations of 0.25%, 0.5%, and 1% in bleaching formulations containing 17.5% and 35% HP. The following null hypotheses were tested: (a) the incorporation of CaNaTMP into bleaching gels would not alter their whitening efficacy; and (b) the addition of CaNaTMP to bleaching gels would not influence the properties of dental enamel.

3.3 Materials and Methods

3.3.1 Synthesis and Characterization of CaNaTMP

The calcium-substituted trimetaphosphate compound (CaNaTMP) was synthesized by diluting 30 g of sodium trimetaphosphate ($Na_3P_3O_9$, CAS 7785-84-4, Sigma-Aldrich Co., St. Louis, MO, USA) in 200 mL of deionized water (pH 5.7). The resulting solution was processed in a glass column (2 cm × 30 cm) containing strong cationic ion exchange resin in the H^+ form (PUROLITE C100H, Purolite®,Philadelphia, PA, USA), with a flow rate of 1.0 mL/min, to partially substitute sodium ions with calcium ions. After passing through the column (pH 1.11), the filtrate was combined with 4.39 g of calcium hydroxide ($Ca(OH)_2$, Exôdo, Sumaré, SP, Brazil), previously dissolved in 800 mL of deionized water, and the pH was adjusted to the original value of the NaTMP solution. The mixture was incubated at 37°C for 24 h to allow for the precipitation of CaNaTMP [24]. The precipitate was filtered using quantitative filter paper and a Buchner funnel under vacuum (-600 mmHg), followed by drying in an oven at 70°C for 24 h. Finally, the material was homogenized in a ball mill (Planetary Micro Mill PULVERISETTE 7, Fritsch GmbH, Idar-Oberstein, Germany) for 5 min.

The CaNaTMP was characterized for crystallinity by X-ray diffraction (XRD) using a Shimadzu XRD 6000 diffractometer with CuKα radiation (30 kV, 30 mA), in continuous

scanning from 5° to 85° (2θ) at 0.2° min^{-1}, with diffractograms compared to JCPDS-PDF standards. The morphology of the particles was analyzed by scanning electron microscopy (SEM, Carl Zeiss EVO ILS15, 20 kV, magnification of 3,000×) after gold coating. The chemical composition was determined by energy-dispersive X-ray spectroscopy (EDS, Oxford Instruments INCAx-act, 20 keV, spatial resolution of 2 μm), evaluating phosphorus (P), oxygen (O), sodium (Na), and calcium (Ca).

3.3.2 Formulation of Bleaching gels and Determination of Experimental Groups

The bleaching gels were formulated without pigmentation, resulting in transparent products, and stored in applicator syringes. The gel base consisted of a thickening agent (Carbopol at 12%) and a bleaching agent (hydrogen peroxide at 17.5% and 35%). Glycerin and water were added in sufficient quantity (q.s.p.) to complete the formulation. Depending on the experimental group, CaNaTMP was incorporated into the gels at concentrations of 0.25%, 0.5%, and 1%, and NaOH was used to adjust the gel pH, which ranged from 7.01 to 7.04 across all groups.

For the definition of the experimental groups, enamel/dentin discs with a diameter of 5.7 mm were obtained from bovine incisor teeth. These discs were randomly assigned to eight groups based on the bleaching gels used: 1) hydrogen peroxide at 35%; 2) HP 35% + 0.25% CaNaTMP; 3) HP 35% + 0.5% CaNaTMP; 4) HP 35% + 1% CaNaTMP; 5) HP 17.5%; 6) HP 17.5% + 0.25% CaNaTMP; 7) HP 17.5% + 0.5% CaNaTMP; 8) HP 17.5% + 1% CaNaTMP. The sample size (n = 12 discs per group) was calculated based on a pilot study, considering the primary outcomes as the mean values of color change, surface microhardness (SH), and the integrated loss of subsurface microhardness (ΔKHN). The minimum detectable difference between the means was 10, 12, and 1900, respectively, with expected standard deviations of 4, 7, and 1200, considering a type I error of 5% and a type II error of 10% [12].

3.3.3 Evaluation of Color Change

3.3.3.1 Preparation and Staining of Enamel/Dentin Discs

Enamel/dentin discs with a total thickness of 3.7 mm (comprising 1.3 mm of enamel and 2.4 mm of dentin, ± 0.2 mm) were prepared from bovine incisor teeth. The discs were obtained using an 8-mm diameter grinding wheel under constant water cooling at 4 °C. After preparation, the discs were carefully rinsed with deionized water and stored in a physiological saline solution containing 0.1% thymol, maintained at 4 °C [12].

The initial color values were measured based on the CIE L* a* b* system using a calibrated spectrophotometer (UV-2450 model, Shimadzu, Kyoto, Japan), with a wavelength range of 400–700

nm, under standard D65 illumination and a 45/0° illumination/observation angle. Subsequently, the discs were transferred to microtubes (Kasvi K6-0150, 1.5 mL capacity, São José dos Pinhais, PR, Brazil) containing 1 mL of black tea infusion at room temperature to induce staining. The black tea solution was prepared at a concentration of 1.6 g of tea (Chá Matte Leão, Curitiba, PR, Brazil) per 100 mL of deionized water, following the protocol described by Felipe Akabane et al. (2021) [12]. The staining procedure was conducted over six days, with daily replacement of the solution. After this period, the color values were remeasured using the same CIE L* a* b* system, and the samples were randomized into eight experimental groups.

3.3.3.2 Application of Bleaching Gels

After the staining period, bleaching gels were applied directly to the enamel surface of the discs in a uniform layer approximately 0.04 mm thick, using a dispensing syringe and a microbrush (KG Sorensen, Cotia, SP, Brazil). The whitening protocol consisted of three sessions conducted at seven-day intervals.

Each session lasted 40 min, after which the gels were carefully removed with gauze. The discs were then rinsed for 30 s with deionized water to remove any gel residue. Between sessions, the discs were individually stored in plastic containers containing 2 mL of artificial saliva, as described by Gruba et al. (2023) [7]. The artificial saliva solution was replaced daily to maintain its chemical and biological integrity throughout the experiment.

3.3.3.3 Evaluation of Total Color Change and Whiteness Index in Dentistry

After that, post-whitening measurements were conducted similarly to the first two color readings. Chromatic changes (ΔE and ΔE_{00}) and the dental whiteness index (ΔWI_D) were recorded at various experimental time points: T_0 (initial reading), T_1 (after staining), T_2 (after the first whitening session), T_3 (after the second whitening session), and T_4 (after the third whitening session). Additionally, to evaluate chromatic stability, the specimens were reanalyzed 14 days after treatment completion (T_5).

Absolute differences (Δ) in the color coordinates (L*, a*, b*) were calculated between the initial values (T_0) and subsequent readings. Total color variation (ΔE), The dental whiteness index (ΔWI_D), and total color change by CIEDE2000 system (ΔE_{00}) were determined using the formulas:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \text{(I)}$$

$$\Delta WID = 0.511L^* - 2.324a^* - 1.100b^* \quad \text{(II)}$$

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L^*}{k_L \cdot S_L}\right)^2 + \left(\frac{\Delta C^*}{k_C \cdot S_C}\right)^2 + \left(\frac{\Delta H^*}{k_H \cdot S_H}\right)^2 + R_T \cdot \left(\frac{\Delta C^*}{k_C \cdot S_C}\right) \cdot \left(\frac{\Delta H^*}{k_H \cdot S_H}\right)} \quad \text{(III)}$$

The perception threshold (PT) and acceptance threshold (AT) limits (50:50%) for color difference (ΔE_{00}) were set at 0.81 (PT) and 1.8 (AT) units, respectively. Similarly, the thresholds for whiteness index difference (ΔWI_D) were 0.7 (PT) and 2.6 (AT) units, respectively [25].

3.3.4 Surface Microhardness and Roughness Analyses of Enamel

3.3.4.1 Sample Preparation and Processing

The enamel surfaces of the discs were prepared through sequential polishing using water-cooled silicon carbide paper discs with grit sizes of 600, 800, and 1200 (Extec, Enfield, CT, USA). This process was performed using an automatic polishing machine (Vector-Phoenix Beta, Buehler, Lake Bluff, IL, USA) set to operate at 4 lbs of pressure, 200 rpm rotation, and continuous water cooling [7]. Following the initial polishing, a final polishing step was conducted using a felt disc moistened with aqueous diamond suspension (0.25 µm, Extec, Enfield, CT, USA) to ensure a smooth and uniform surface. Between polishing stages, the discs were cleaned with deionized water in an ultrasonic bath (Unique USC 1400, Indaiatuba, SP, Brazil) operating at 40 Hz and 135 W for 20 min at room temperature [19].

Throughout the preparation process, the discs were stored in a humid environment, immersed in a 0.1% thymol solution at 4 °C [12] to prevent dehydration and contamination. Only discs with flat surfaces, free from scratches, cracks, or signs of hypoplasia were included in the analyses as per the inclusion criteria.

3.3.4.2 Surface Microhardness and Roughness Evaluations of Enamel

Following enamel surface planing and polishing, the initial surface microhardness (SHI) was assessed, with values ranging from 351.4 to 374.2 KHN. A microhardness tester with a Knoop indenter (Micromet 5114, Buehler, Lake Bluff, USA) was used for this analysis. Enamel discs (n = 12/group) were evaluated at different time points: baseline, 24 h after each bleaching session (1st, 2nd, and 3rd), and 14 days after the final bleaching session. Microhardness measurements were performed with five indentations on the central region of each disc, applying a 25-gf load for 10 s, with 100 µm spacing between indentations in the horizontal direction [7]. To determine the final surface microhardness (SHF), the mean of the five measurements for each

disc at each time point was calculated. The percentage of surface microhardness loss (%SH) was determined using the formula:

$$\%SH = \frac{\text{SHF} - \text{SHI}}{\text{SHI}} \times 100$$

Similarly, surface roughness (Ra) was also evaluated at the same time points using a surface roughness tester (Model SJ-401, Mitutoyo, Kawasaki, Japan). Measurements were performed with three readings per specimen at distinct locations (parallel, oblique, and perpendicular). The equipment parameters included a cutoff length of 0.25 mm, a reading length of 0.25 mm, a scanning speed of 0.1 mm/s, and a load of 5 N. The average of the three readings was considered the representative surface roughness value for each evaluation period [7].

3.3.5 Analysis of Enamel Mineral Content by X-ray Microcomputed Tomography (Micro-CT)

The mineral content of enamel was evaluated using a high-resolution microcomputed tomography system (SkyScan 1272, Bruker MicroCT, Kontich, Belgium) with reconstruction algorithms from the NRecon software to determine 3D mineral concentration ($g_{HAp} \times cm^{-3}$). The MicroCT operational parameters were as follows: 70 kV, 142 µA, 0.5 mm aluminum filter, spatial resolution of 1.5 µm, rotation step of 0.400°, and random movement set to 10 [26]. Projections were reconstructed using NRecon software (version 1.6.10.2, Bruker) with level 3 smoothing, level 9 ring artifact correction, and 60% beam hardening correction. After reconstruction, two-dimensional virtual slices in the coronal plane were obtained using Data Viewer software (version 1.5.1.2, Bruker). The stacked two-dimensional slices were imported into ImageJ software (version 1.47, National Institute of Mental Health, Bethesda, Maryland, USA) to generate mineral concentration profiles (MC; $g_{HAp} \times cm^{-3}$) as a function of depth (µm). The analysis was based on a calibration curve constructed using mineral reference standards (0, 0.25, and 0.75 $g_{HAp} \times cm^{-3}$) [19,26].

3.3.6 Evaluation of Transamelodentinal Diffusion of HP

Transamelodentinal diffusion of HP was assessed using 96 enamel/dentin discs (12 per group), individually adapted to artificial pulp chambers (APCs) and assembled into a 24-well cell culture plate (Costar Corp., Cambridge, MA, USA). The lower compartments of the APCs, in contact with the dentin surface, were filled with 1 mL of sodium acetate buffer solution (2.0 mol/L, pH 4.5, adjusted with acetic acid; Sigma Chemical Co., St. Louis, MO, USA). Under humid conditions at 37°C, bleaching gels were applied as previously outlined.

After 40 min of gel exposure, the following were added to the buffer solution: 1 mL of leucocrystal violet (0.5 mg/mL in HCl; Sigma Chemical Co.), 50 μL of horseradish peroxidase (1 mg/mL in water; Sigma Chemical Co.), and 3 mL of deionized water. The HP concentration in the buffer solution was determined using a colorimetric method [27]. For quantification, a standard curve of HP concentrations (0.5–5.0 μg/mL) was constructed under identical experimental conditions. Measurements were performed using a spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) at a wavelength of 596 nm.

3.3.7 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

Two discs from each group, as well as sound enamel, were prepared for SEM and EDS analyses. Surface morphology was observed using scanning electron microscopy (SEM, Carl Zeiss, EVO ILS15, Carl Zeiss NTS LTD, Germany), while the chemical composition of the enamel was assessed using energy-dispersive X-ray spectroscopy (EDS, Oxford Instruments, INCAx-act, 133 eV, UK). All discs were gradually dehydrated in an ascending ethanol series (50%, 60%, 70%, 80%, and 100%) and then gold-coated (Quorum - Q150T E). The analyses were performed on a scanning electron microscope (SEM) at 20 kV, and images were captured at a magnification of ×10,000. The atomic weight of the elements calcium (Ca), phosphorus (P), and oxygen (O) on the enamel surface was determined by energy-dispersive X-ray microanalysis, with a spatial resolution of approximately 2 μm and a counting time of 7000 s.

3.3.8. Statistical Analysis

All the statistical analyses were performed in the Sigmaplot® for Windows program, version 12.0, at a significance level of 5%. The different bleaching gels and time points were considered as factors of variation for the variables ΔE, ΔWI_D, ΔE_{00}, SHF, %SH, and Ra. For the variables HP (μg/mL) and $g_{HAp} \times cm^{-3}$, only the different bleaching gels were considered as factors of variation. The data were subjected to analysis of variance (ANOVA), followed by the Student-Newman-Keuls multiple comparison test.

3.4 Results

3.4.1 CaNaTMP Characterization

Scanning electron microscopy (SEM) images revealed larger crystals with irregular morphology for NaTMP (Figure 1A), whereas CaNaTMP exhibited smaller and more uniform grains (Figure 1B). Energy-dispersive X-ray spectroscopy (EDS) analysis identified peaks corresponding to the chemical elements Na (1.04 keV), O (0.57 keV), and P (2.03 keV), present in both NaTMP and CaNaTMP samples (Figures 1C and 1D). Additionally, peaks at 0.26 and

3.69 keV, characteristic of the element Ca, were exclusively detected in CaNaTMP (Figure 1D). Quantitative analysis showed a lower mass percentage of P and Na in the CaNaTMP samples compared to NaTMP, whereas the percentage of O was higher in CaNaTMP.

3.4.2 Whitening Perfomance

The pigmentation of the enamel samples resulted in a significant reduction in luminosity ($p < 0.001$), an increase in red ($p < 0.001$) and yellow hues ($p < 0.001$) (Fig. 2), and a substantial overall color variation across the analyzed coordinates ($p < 0.001$; Fig. 2). No statistically significant differences were observed between the groups treated with conventional gels and those containing CaNaTMP ($p > 0.05$). After bleaching, the enamel exhibited higher luminosity ($p < 0.001$), reduced red hue ($p < 0.05$), and a more bluish tone ($p < 0.001$), contributing to an increase in total color variation (Fig. 3). The evaluated bleaching agents induced significant ΔE changes after the whitening sessions ($p < 0.001$), with progressive and continuous color changes starting from the first session (Fig. 3). Regarding ΔE, 35% HP showed superior performance compared to 17.5% HP during the first and second bleaching sessions (ΔE; $p < 0.05$). However, both treatments achieved the same aesthetic outcome by the end of the third session and during the color stabilization period (14 days post-bleaching) (ΔE; $p > 0.05$; Fig. 3). Both ΔE_{00} and ΔWI_D analyses demonstrated comparable whitening effects between the 17.5% and 35% HP gels across all evaluation periods ($p > 0.05$), except for ΔE_{00} during the first session, where differences were noted. The addition of CaNaTMP (0.25%, 0.5%, and 1%) to gels containing 17.5% and/or 35% HP did not alter the whitening performance of these gels compared to their respective counterparts for any of the evaluated color parameters (ΔE, ΔWI_D, and ΔE_{00}; $p > 0.05$), regardless of the evaluation period.

3.4.3 Whitening effects on Surface Roughness

The whitening treatment resulted in an increase in surface roughness across all experimental groups (Fig. 4). The greatest changes were observed in the group treated with 35% HP, where roughness values progressively increased with each session ($p < 0.001$). Incorporating CaNaTMP into the bleaching gels (17.5% and 35% HP) effectively reduced these changes compared to their respective controls ($p < 0.001$; Fig. 4). While no significant differences were observed among the 0.25%, 0.5%, and 1% CaNaTMP concentrations in the 17.5% HP gels ($p > 0.05$), the 35% HP gel containing 1% CaNaTMP provided the strongest protection against roughness increase.

3.4.4 Impact on Enamel Surface Hardness

All bleaching gels resulted in significant reductions in surface hardness post-treatment ($p < 0.001$; Table 1). The percentage loss in hardness (%SH) was directly correlated with both the hydrogen peroxide concentration (17.5% and 35%) and the number of bleaching sessions ($p < 0.001$; Table 1). Gels enriched with CaNaTMP consistently reduced the extent of hardness loss ($p < 0.001$), with 0.5% and 1% CaNaTMP showing comparable effects regardless of the peroxide concentration ($p > 0.05$). Among all formulations, gels with 1% CaNaTMP maintained the highest absolute hardness (SH) values and demonstrated the lowest %SH losses ($p < 0.001$).

3.4.5 HP Transamelodentinal Diffusion and Enamel Mineral Content

Specimens treated with 35% HP exhibited the highest levels of transamelodentinal diffusion of HP ($p < 0.05$; Table 2). However, the addition of CaNaTMP to the gels reduced diffusion in a concentration-dependent manner ($p < 0.05$). Notably, gels containing 1% CaNaTMP achieved the lowest diffusion levels, with no significant differences between the 17.5% and 35% HP gels ($p > 0.05$). Mineral concentration analysis by micro-CT revealed the greatest mineral loss in enamel treated with 35% HP gels ($p < 0.001$; Table 2). The addition of CaNaTMP also reduced mineral loss in a concentration-dependent way ($p < 0.05$), with comparable outcomes for gels with the same CaNaTMP concentration, regardless of the hydrogen peroxide content ($p > 0.05$; Table 2).

3.4.6 Enamel Morphology by SEM-EDS

SEM analysis of the sound enamel and the groups treated with 17.5% HP did not reveal visible changes on the enamel surface. In the group with the 35% HP gel, enamel prisms and interprismatic enamel were observed, displaying characteristics consistent with "lock-holes" or honeycomb-like structures. The addition of CaNaTMP to the 35% HP gel reduced the exposure of enamel prisms due to the formation of an amorphous apatite layer. The combination of 17.5% HP with CaNaTMP resulted in a more pronounced precipitation of an amorphous apatite layer, especially at the 1% concentration (Fig. 5). The Ca/P ratio ranged from 1.53 to 1.58 in the experimental gels, similar to that observed in the non-bleached enamel.

3.5 Discussion

The quest for safer and more biocompatible whitening therapies for dental tissues has driven the development of innovative bleaching formulations compared to conventional options. In this context, the incorporation of bioactive compounds into bleaching gels emerges as a promising strategy to counteract dental alterations induced by the whitening treatment

[8,16,17,28]. The results of this study demonstrated that incorporating CaNaTMP into bleaching gels at various concentrations effectively protected dental substrates by minimizing the alterations caused by the bleaching treatment without compromising its aesthetic efficacy. Thus, the first null hypothesis, related to whitening performance, was accepted. Moreover, CaNaTMP significantly reduced mineral loss, trans-amelodentinal diffusion of HP, and changes in enamel morphology and roughness. These findings led to the rejection of the second null hypothesis, highlighting the potential of CaNaTMP as a protective additive in bleaching gels.

In this study, the addition of CaNaTMP to hydrogen peroxide-based beaching gels did not result in a significantly distinct whitening effect compared to conventional bleaching gels, regardless of the CaNaTMP concentration or the bleaching agent evaluated. These findings align with previous studies investigating the incorporation of various inorganic phosphate salts [9,12,16]. It is hypothesized that these phosphates, when included in gel formulations, exert a neutral effect on whitening performance without compromising product efficacy. The three-session bleaching protocol demonstrated the most substantial color change during the first session, with diminishing effects in the subsequent sessions. This outcome likely reflects heightened redox activity during the initial interaction with dental pigments [29]. Furthermore, these findings suggest a more substantial whitening effect in the first session with the high-concentration gel (35% HP). However, the outcomes of subsequent applications and at the end of treatment were comparable to those of the lower-concentration gel (17.5% HP). This observation indicates that additional exposures allow the 17.5% HP gel to act more comprehensively, potentially reaching pigments that the high-concentration gel removes in a single application. Conversely, 35% HP may generate sufficient free radicals to oxidize the organic components of dentin and achieve significant whitening during the first application. This would explain the less pronounced color changes in subsequent applications, equalizing their performance with that of lower-concentration gels [8].

It is worth noting that, at the end of treatment and 14 days post-bleaching, results were documented using three different color assessment tools (ΔE, ΔE_{00}, and ΔWI_D). These findings confirmed the bleaching efficacy of the gels analyzed, irrespective of the addition of CaNaTMP and/or the HP concentration. Different objective metrics were employed to measure color changes. ΔE was chosen for its widespread use in the literature, enabling broad comparability with prior studies. Additionally, ΔE_{00} was applied for its enhanced precision in interpreting perceptible changes, particularly in relation to human visual perception, as it accounts for interactions between chroma and hue. Finally, the Whiteness Index for Dentistry (ΔWI_D) was

included as a crucial tool for objectively evaluating the degree of dental whitening, being widely recommended for studies focusing on bleaching treatment efficacy.

It has been reported that bleaching agents can contribute to significant enamel hardness loss and increased surface roughness, and these changes are independent of peroxide concentration [8] or exposure time [30]. They are also linked to the oxidative process, the effects of free radicals, and the gel composition regarding the presence or absence of ions such as Ca^{2+} and F^{-} [31]. Previous studies have shown that highly concentrated bleaching gels with acidic pH cause alterations in enamel chemical composition, calcium loss, demineralization, and hardness reduction [31]. However, other studies have demonstrated that gels with neutral pH can also promote morphological changes on the dental enamel surface [7,32]. These changes are characterized by increased surface porosity, degradation of the organic matrix, and loss of Ca^{2+} and PO_4^{3-}, leading to reduced surface hardness [32]. In our study, all experimental gels were prepared with a neutral pH. Nevertheless, a significant decrease in hardness and surface roughness alteration was observed regardless of the H_2O_2 concentration evaluated. Only the gels containing CaNaTMP maintained hardness values within the tolerable limit established by ISO 28399.

According to findings in the literature, in addition to the non-specific oxidative effect, the low concentration of Ca^{2+} and PO_4^{3-} in the formulation of the bleaching gel makes it undersaturated in relation to hydroxyapatite, promoting enamel demineralization regardless of pH [12,33]. On the other hand, unstable and non-specific oxygen free radicals released by H_2O_2 gels act not only on the chromophore groups of molecules but also on the organic structure of enamel, causing oxidation of proteins present in the enamel (amelogenins and enamelins) [34]. Thus, it can be considered that the mineral loss and increase in surface roughness of enamel, observed in our study primarily for gels without CaNaTMP, were caused by the release of H^{+} ions during the decomposition of HP (ionic dissociation), leading to enamel dissolution and structural modifications, as observed in other studies [33-35].

Although the literature on tooth whitening is extensive, studies on the in-office technique have not assessed the effects of sessions on enamel properties throughout the treatment, generally limiting measurements to baseline (before the procedure) and post-treatment stages. Similarly, there are few studies investigating the effects of the at-home technique during the therapy [36,37]. Our study aimed to analyze the effect of the number of bleaching sessions on the microhardness and roughness of dental enamel, aiming to understand the influence of repeated exposure to the treatment and the behavior of enamel throughout the protocol. As with the evaluation of chromatic changes, the greatest loss of hardness (for gels with 17.5% and 35% H_2O_2) was

observed during the first session, with subsequent worsening, but less pronounced. However, this was not observed for surface roughness, which intensified between the second and third applications of the bleaching gel. These results suggest that changes in enamel properties are cumulative, worsening with the increased number of whitening sessions. These alterations are attributed to modifications in the inorganic and organic composition of dental tissues caused by peroxide-based bleaching agents [34].

Moreover, our study evaluated mineral loss using microcomputed tomography, a technique recognized as the gold standard for measuring mineral content. The results corroborated the data on microhardness, roughness, and HP diffusion, highlighting the importance of precise quantitative analysis with minimal interference from external factors and a more detailed direct visualization of the evaluated substrate. Moreover, this technique has been infrequently used in the literature of experimental studies on whitening gels, making it an innovative and robust approach that provides an accurate and more detailed analysis of the effects of these products on enamel mineral loss. Additionally, during the process, free radicals generated react non-selectively with organic structures, increasing enamel porosity [38,39]. Furthermore, SEM analysis confirmed the exposure of enamel prisms, especially in the group treated with 35% HP [8,40]. Previous studies indicate that techniques such as surface polishing, fluoride application, and the use of remineralizing solutions are effective in reducing the roughness of bleached enamel [38,41]. However, in the present study, a difference in surface roughness values before and after bleaching was observed in all groups, with lower intensity in the applications with gels containing 1% CaNaTMP. This suggests that the polyphosphate may attenuate the changes in the surface morphology of enamel, even without the use of fluoride products or additional therapeutic protocols. In addition, this is an interesting and significant finding and highlights the main distinguishing feature of the synthesized compound. TMP, by itself, only shows an effect when associated with fluoride, with an action limited to the enamel surface. However, the addition of calcium to the compound improved its performance, showing promising results even in the absence of fluoride.

The positive results found with CaNaTMP gels are presumably related to the TMP's ability to adsorb to dental enamel and increase anionic sites, thereby electrostatically attracting Ca^{2+} to the phosphate to create crystallization nuclei and apatite precipitation [42,43]. This interaction may alter the selective permeability and ion diffusion into the enamel [20]. Each calcium atom binds to two TMP molecules in the sodium substitution process, forming a network structure [44]. This modification in the molecular bonds gave the new compound the ability to reduce acid penetration into the enamel, as demonstrated by the lower trans-amelodentinal diffusion of HP,

which attenuates mineral loss during the bleaching sessions. These results explain the promising effects observed in gels containing CaNaTMP, compared to control gels. Furthermore, the gel with 35% HP proved to be more aggressive to enamel than the gel with the lower concentration of HP, confirming that the adverse effects of the product are directly related to the concentration of peroxide [8]. Adding 1% CaNaTMP in both concentrations of HP minimized enamel changes, making the effects of the two gels similar. Notably, the formulation of 35% HP + 1% CaNaTMP was less harmful to enamel than the conventional gel with low H_2O_2 concentration. Moreover, the HP diffusion data corroborates the results of mineral content and enamel roughness. The gel with 35% HP caused greater changes, likely due to the higher penetration of peroxides and free radicals into the dental substrate. In contrast, the presence of CaNaTMP in the bleaching gels interestingly minimized this penetration, as it preserved the mineral content and roughness of the enamel without negatively influencing the whitening effect.

Moreover, the results obtained show that, after three whitening sessions, the gel with the lower HP concentration (17.5%) exhibited a whitening effect similar to that of the gel with the higher concentration (35% HP). However, when considering other evaluated parameters, the gel containing 17.5% HP demonstrated less significant changes in hardness, roughness, peroxide diffusion, enamel mineral content, and enamel morphological alterations compared to the 35% HP gel. These differences were mainly evident when CaNaTMP was incorporated into the formulation. The addition of CaNaTMP resulted in a significant reduction in the observed changes, especially at the 1% concentration. These results suggest that, clinically, the use of a gel with a lower HP concentration (17.5%) combined with the incorporation of 1% CaNaTMP could offer a safer and more biocompatible alternative for the whitening procedure, maintaining an effective whitening effect while minimizing adverse impacts on the properties of dental enamel. This combination not only provides maintain the structural integrity of the enamel but also supports the viability of using formulations with lower HP concentrations without compromising whitening efficacy and could represent a safer and less aggressive treatment option.

This study has typical limitations of *vitro* assays, as this experimental model cannot fully simulate the complexity of the oral cavity, including factors such as the use of human teeth, saliva profile and acquired pellicle, salivary flow, different pigmentation agents, ion exchange between the environment and the dental surface, as well as patient behavioral aspects. However, the *in vitro* approach has been widely adopted in tooth whitening research and serves as an initial stage in the development of biomaterials, allowing for the standardization of conditions and samples, something that is not feasible under clinical conditions. While it is not possible to directly extrapolate to clinical practice, additional methodologies are still required. The evaluation of

mineral content by micro-CT, considered the gold standard, can be complemented by future studies investigating the behavior of native enamel without the need for specimen planning. Additionally, assessing trans-amelodentinal cytotoxicity in pulp cells is an important area to explore. The results obtained for the gels with CaNaTMP are promising, but future clinical evaluations are essential to determine their in vivo behavior and their role in protecting against dental sensitivity, one of the main concerns associated with dental bleaching.

3.6 Conclusion

This study demonstrated that the addition of CaNaTMP to bleaching gels with 17.5% and 35% HP reduces mineral loss, changes in roughness, and surface morphology of enamel, as well as decreasing trans-amelodentinal diffusion of H_2O_2, without compromising the bleaching efficacy. The incorporation of 1% CaNaTMP was particularly more effective in protecting the dental enamel. These results suggest that CaNaTMP can enhance the safety and biocompatibility of bleaching gels, providing a promising strategy for the procedure, and being less aggressive to dental enamel.

3.7 Acknowledgments

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Figure 1. Scanning electron microscopy photomicrograph of the NaTMP (A) and CaNaTMP (B) samples, and their respective EDS histograms (C and D).

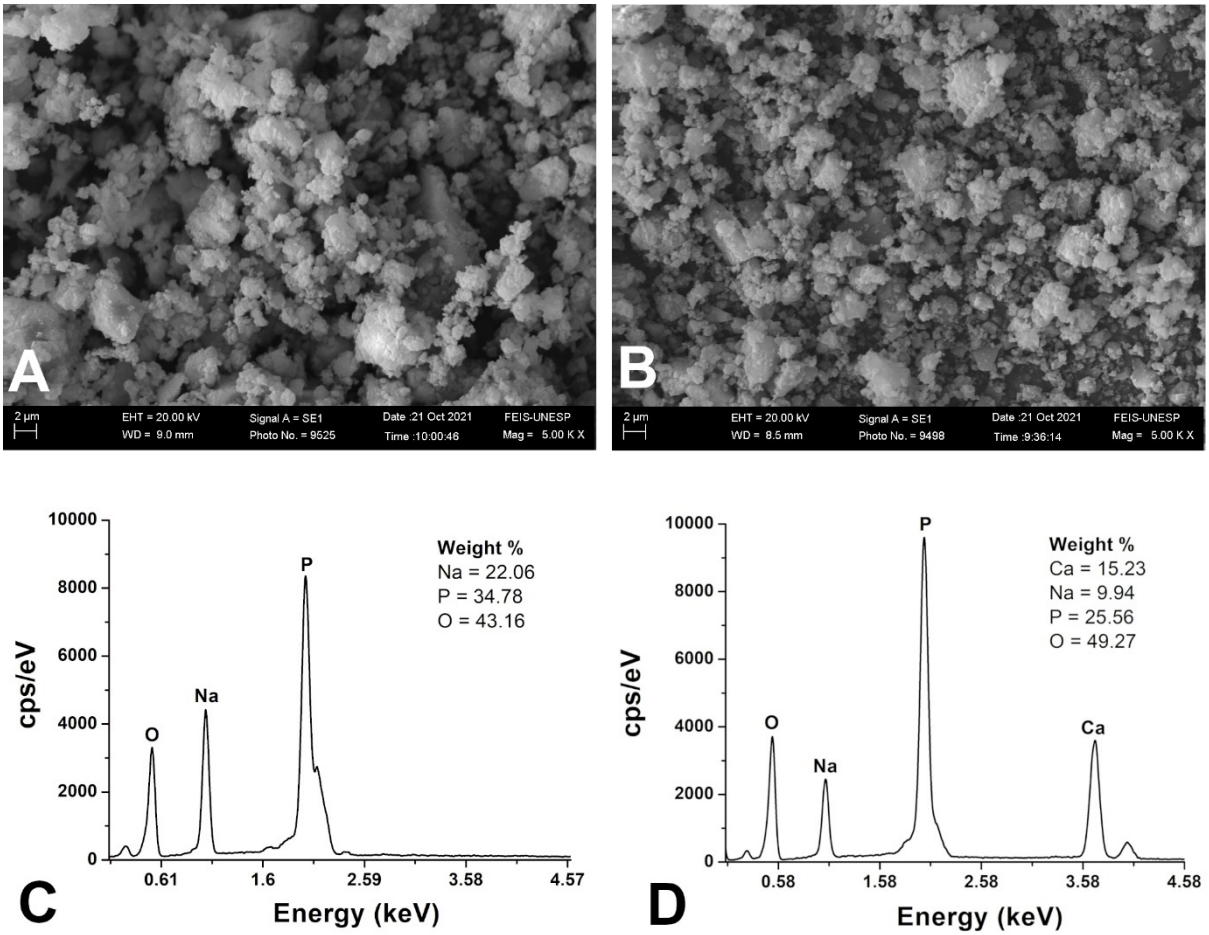


Figure 2. Mean values (±SD) for changes in: (A) lightness (ΔL*), (B) green/red axis (Δa*), and (C) blue/yellow axis (Δb*), based on the Commission Internationale de l'Éclairage (CIE) system, according to bleaching gels and evaluation time (n = 12). Distinct lowercase letters indicate statistical differences between the bleaching gels at each analysis time and between the analysis times for each bleaching gel (Student-Newman-Keuls; p <0.05).

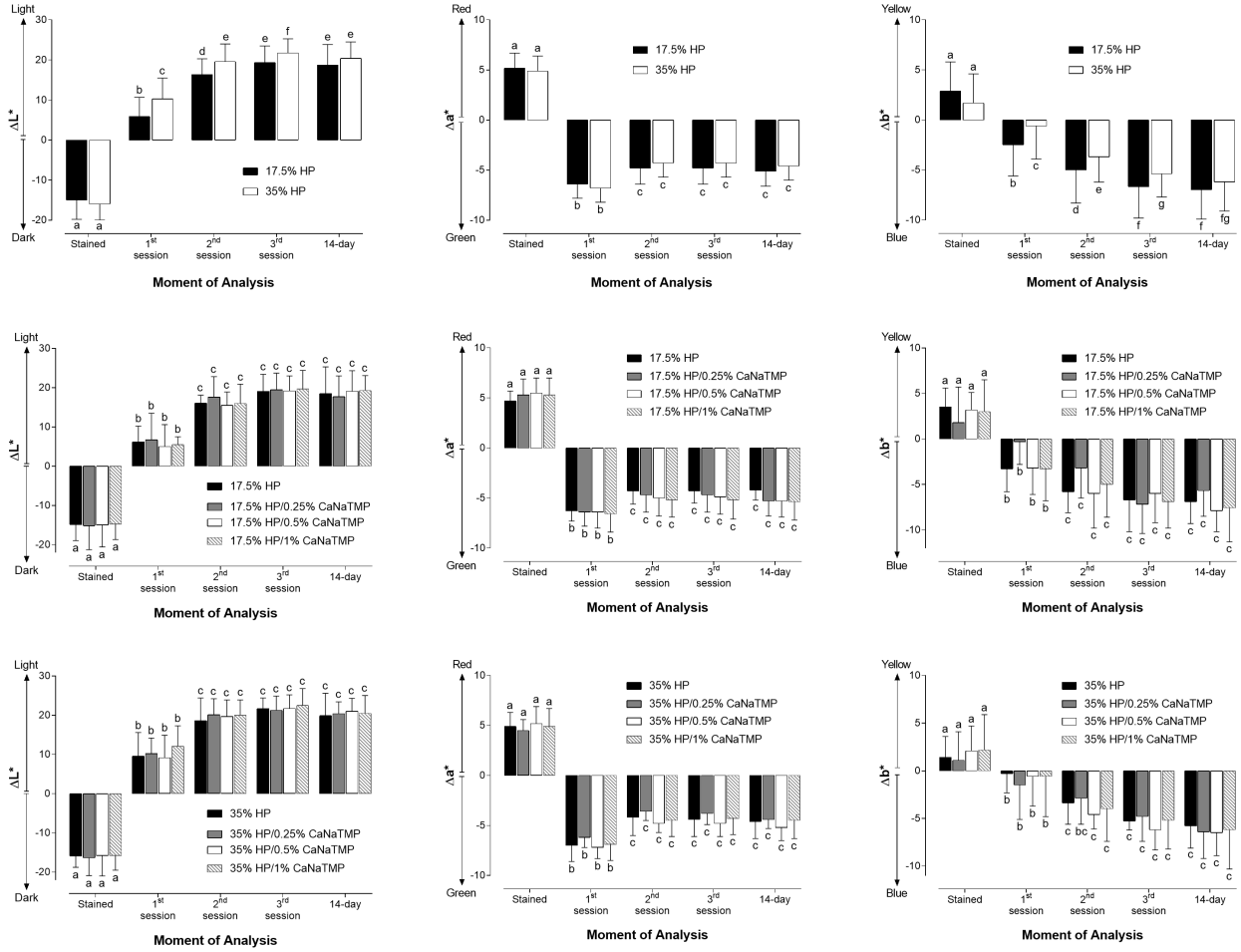


Figure 3. Mean values (±SD) of total color change (ΔE) by the International Commission on Illumination; dental whiteness index (ΔWI_D), and color change by CIEDE00 (ΔE_{00}), according to bleaching gels and analysis time (n=12). Distinct lowercase letters indicate statistical differences between the bleaching gels at each analysis time and between the analysis times for each bleaching gel (Student-Newman-Keuls; $p < 0.05$).

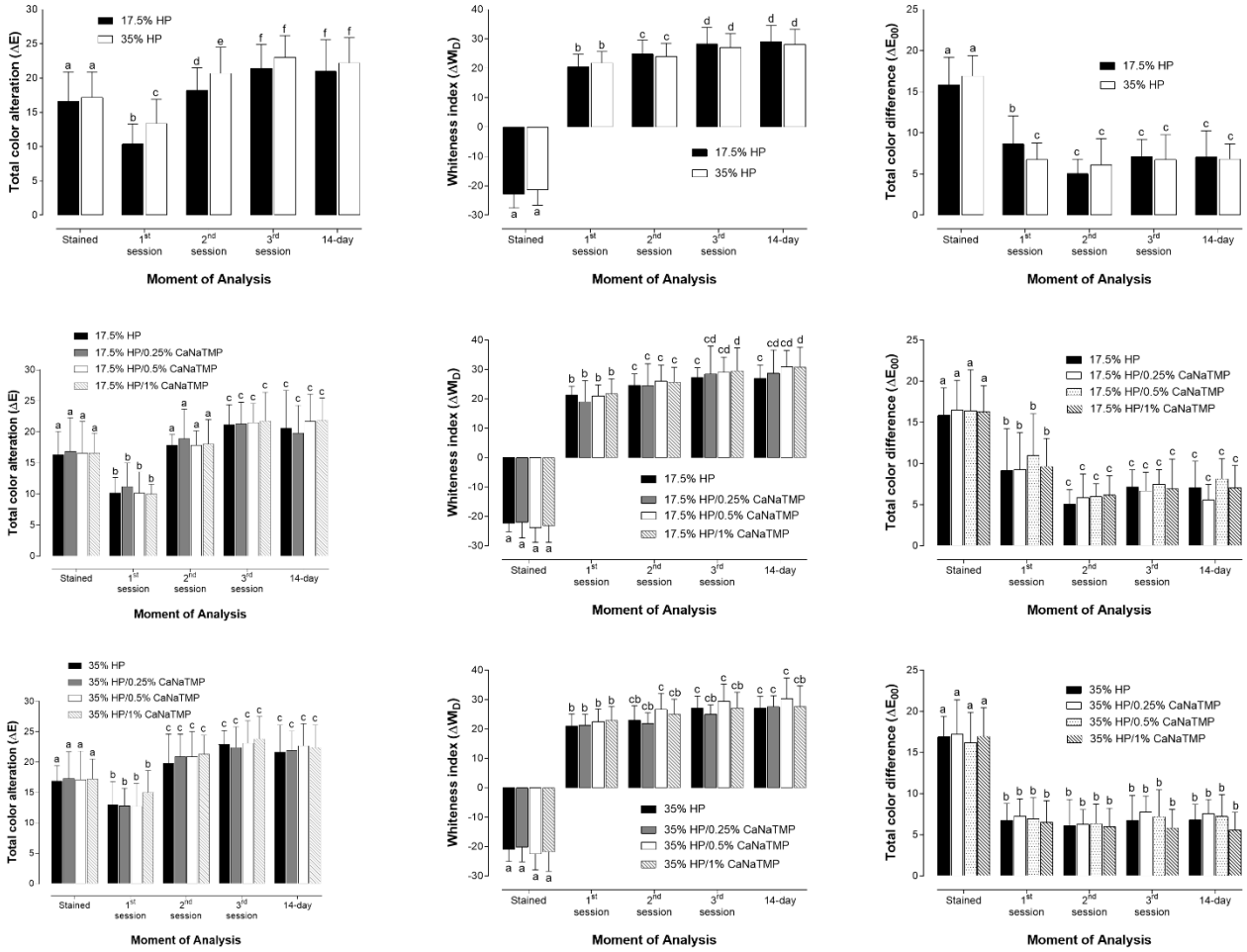


Figure 4. Mean values (±SD) of surface roughness (Ra) according to the bleaching gels and analysis time (n=12). Distinct lowercase letters indicate statistical differences between the bleaching gels at each analysis time and between the analysis times for each bleaching gel (Student-Newman-Keuls; p <0.05).

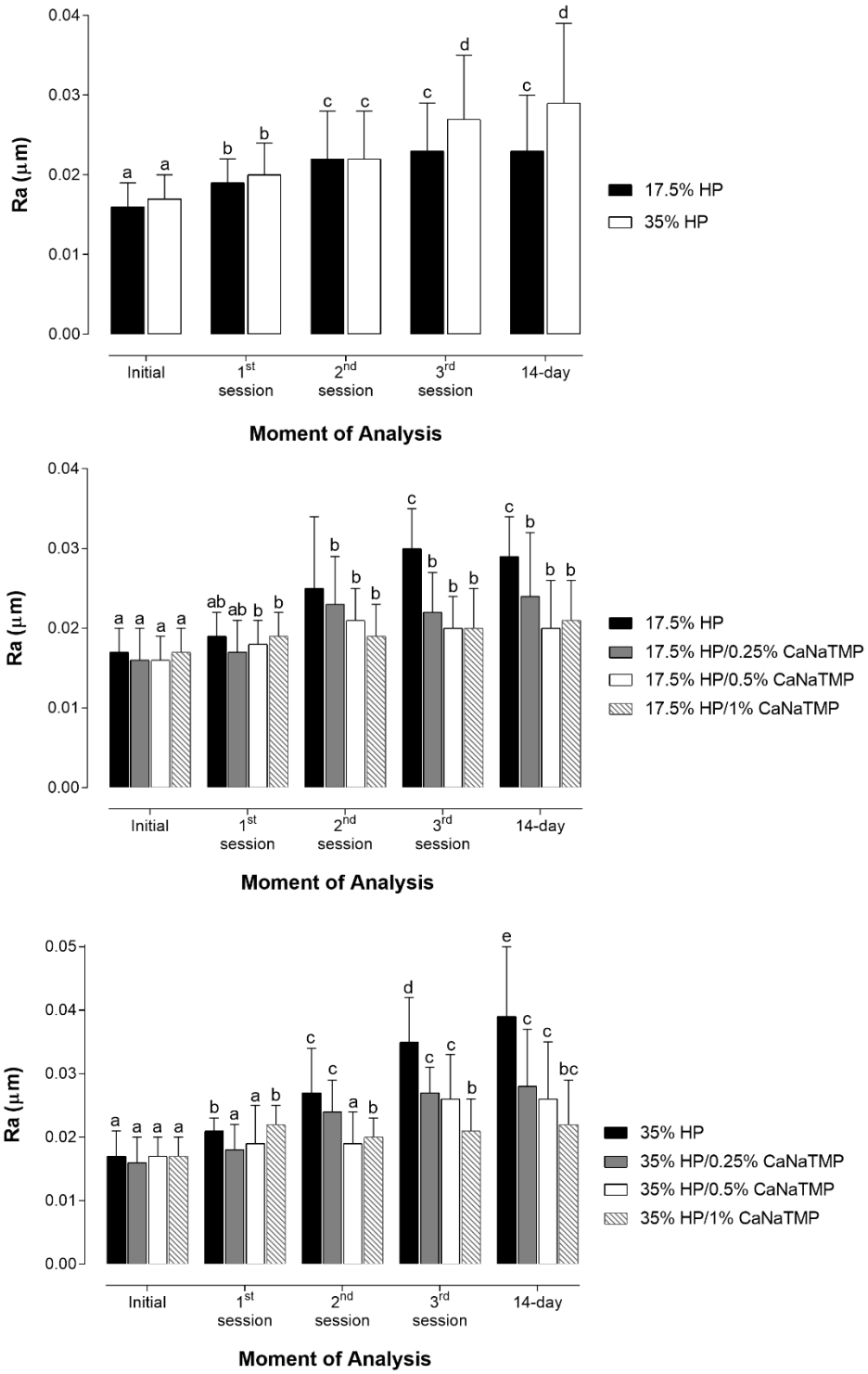


Figure 5. Representative images obtained by scanning electron microscopy of the enamel surface and their respective EDX spectra according to the experimental groups (10,000× magnification). The black arrows indicate interprismatic enamel. Open arrows point to the enamel prisms.

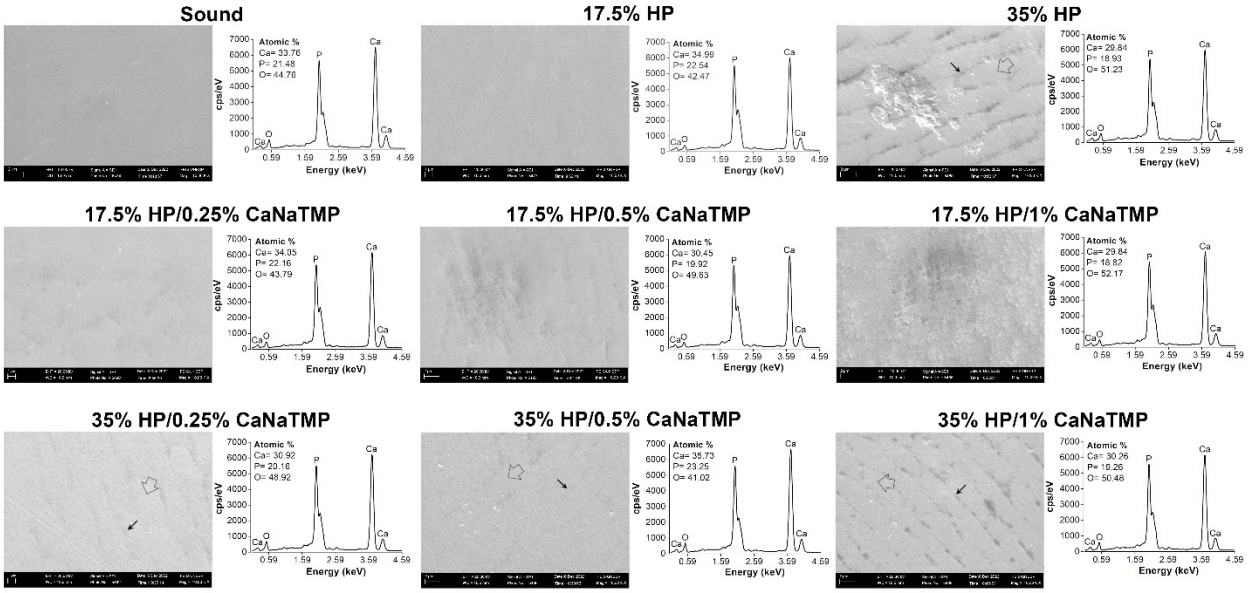


Table 1. Mean values (±SD) of surface hardness (SH) and percentage of surface hardness change (%SH) according to hydrogen peroxide and CaNaTMP concentration and experimental phases (n=12).

% CaNaTMP	Experimental Phases and Hydrogen Peroxide Concentration									
	Baseline*		1ª session		2ª session		3ª session		Post 14-days	
	17.5% HP	35% HP	17.5% HP	35% HP	17.5%PHP	35% HP	17.5% HP	35% HP	17.5% HP	35% HP
Knoop Surface Hardness (SH)										
0%	362.0^{aA} (5.0)	362.2^{aA} (5.8)	330.7^{bA} (9.4)	317.9^{cA} (8.4)	323.3^{cA} (9.5)	303.1^{dA} (7.5)	311.2^{eA} (9.4)	292.7^{fA} (6.4)	312.6^{eA} (8.5)	292.5^{fA} (7.6)
0.25%	361.9^{aA} (5.2)	362.0^{aA} (5.1)	350.9^{bB} (8.3)	338.8^{cB} (7.8)	342.1^{cB} (7.9)	333.9^{dB} (7.4)	338.0^{eB} (7.2)	330.9^{dB} (7.8)	338.9^{eB} (7.3)	332.1^{dB} (8.1)
0.5%	362.0^{aA} (5.9)	362.2^{aA} (5.7)	354.3^{bB} (8.5)	351.5^{bC} (9.8)	346.0^{cBC} (8.7)	348.2^{cC} (8.2)	343.9^{dBC} (9.0)	342.8^{dC} (8.2)	342.4^{dB} (9.1)	341.7^{dC} (8.4)
1%	362.1^{aA} (5.6)	362.1^{aA} (5.1)	357.4^{bB} (6.1)	355.1^{bC} (9.7)	351.9^{cC} (8.5)	352.2^{cC} (9.8)	349.6^{cC} (8.4)	348.3^{cC} (9.1)	349.4^{cC} (7.9)	348.1^{dD} (8.7)
Percentage of Surface Hardness Change (% SH)										
0%	–	–	−8.7^{aA} (2.0)	−12.2^{bA} (1.5)	−10.7^{bA} (1.9)	−16.3^{cA} (1.3)	−14.0^{cA} (2.0)	−19.2^{dA} (1.3)	−13.7^{cA} (1.7)	−19.3^{dA} (1.4)
0.25%	–	–	−3.0^{aB} (1.7)	−6.4^{bB} (1.8)	−5.5^{bB} (1.7)	−7.7^{cB} (1.9)	−6.6^{cB} (1.6)	−8.6^{dB} (2.1)	−6.4^{cB} (1.5)	−8.2^{dB} (2.2)
0.5%	–	–	−2.1^{aBC} (1.2)	−3.0^{aC} (1.7)	−4.4^{bB} (1.8)	−3.9^{bC} (1.4)	−5.0^{bcC} (1.9)	−5.4^{cC} (1.4)	−5.4^{cB} (1.7)	−5.7^{cC} (1.5)
1%	–	–	−1.3^{aC} (0.9)	−1.9^{aD} (1.8)	−2.8^{bC} (1.5)	−2.7^{bC} (1.7)	−3.4^{bD} (1.5)	−3.8^{cD} (1.5)	−3.5^{bcD} (1.5)	−3.9^{cD} (1.7)

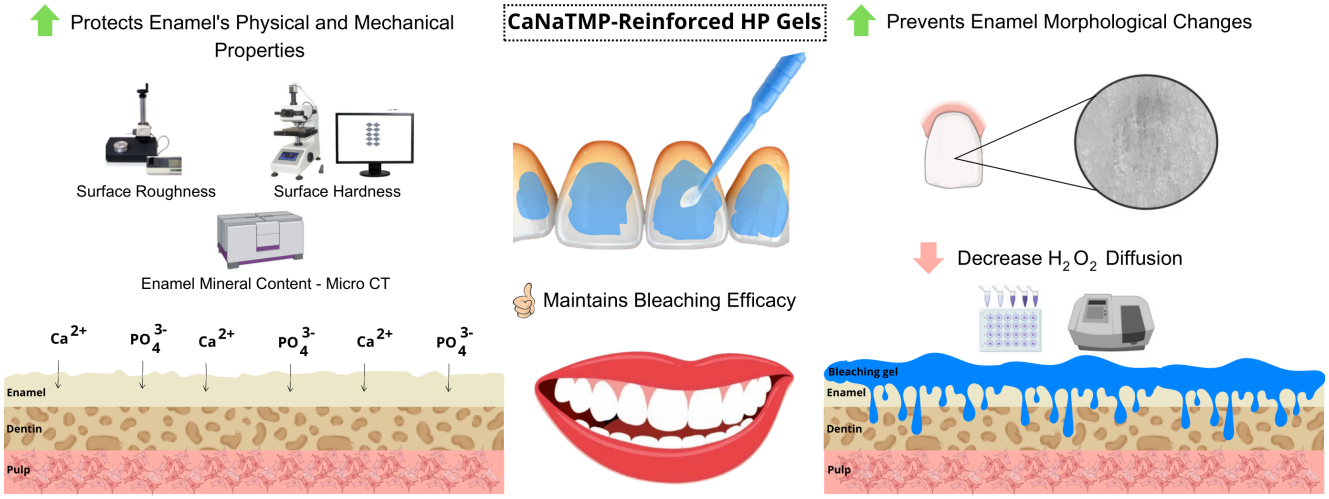
Lowercase letters indicate significant differences between the conditions/%HP for each %CaNaTMP. Identical uppercase letters indicate no significant differences between %CaNaTMP at each %HP and condition (two-way repeated measures ANOVA, Student-Newman-Keuls, $p<0.05$). *Baseline: surface hardness values of sound enamel blocks prior to treatments.

Table 2. Mean values (±SD) of HP transamelodentinal diffusion (µg/mL) and percentage of enamel mineral loss assessed by Micro-CT ($g_{HAp} \times cm^{-3} \times \mu m$) according to experimental groups (n=12).

% de CaNaTMP	HP Concentration	
	17.5%	**35%**
HP Diffusion (µg/mL)		
0%	5.60 (0.43)[a]	6.95 (0.47)[d]
0.25%	4.93 (0.42)[b]	5.65 (0.57)[a]
0.5%	4.75 (0.37)[b,c]	5.20 (0.42)[e]
1%	4.39 (0.42)[c]	4.48 (0.47)[c]
Percentage of Enamel Mineral Loss ($g_{HAp} \times cm^{-3} \times \mu m$)		
0%	−14.38 (3.11)[a]	−16.96 (3.77)[b]
0.25%	−6.67 (0.78)[c]	−6.64 (0.74)[c]
0.5%	−3.36 (0.98)[d,f]	−3.86 (1.22)[d]
1%	−0.68 (0.22)[e]	−1.17 (0.99)[e,f]

Lowercase letters indicate a significant difference between the mean values considering the percentage of HP and CaNaTMP in the whitening gels ($p<0.05$; Two-way ANOVA, Student-Newman-Keuls).

Graphical Abstract



Artigo III

4.0 Protective role of calcium-based agents in dental bleaching gels: insights from a systematic review and meta-analysis of clinical and laboratory evidence

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***Artigo nas normas e submetido ao periódico Clinical Oral Investigations**

4.1 Abstract

Objectives: This systematic review and meta-analysis (SRM) evaluated the effect of incorporating calcium-based bioactive agents in bleaching gels on dental structure preservation and whitening efficacy.

Data sources: The protocol for this review was registered in PROSPERO (CRD42024520242). The SRM adhered to PRISMA 2020 guidelines and employed the PICO framework. Studies were identified through comprehensive searches in databases: PubMed, Scopus, Cochrane, Embase, and Web of Science, with data collected up to November 2024.

Study Selection: Two independent reviewers screened and selected clinical trials and in vitro studies that assessed the effects of calcium-based bioactive agents incorporated into bleaching gels on dental substrates, following predefined inclusion criteria. Data were extracted using standardized forms, and the quality of the studies was evaluated with the Cochrane Collaboration tool and the Joanna Briggs Institute Critical Appraisal Tools. Meta-analysis was performed using RevMan software, with the mean difference as the effect measure.

Results: Out of 4,289 articles identified, 56 met the inclusion criteria, consisting of 50 in vitro studies and 6 clinical trials. The agents evaluated included calcium ions, calcium gluconate, amorphous calcium phosphate, CPP-ACP, and calcium polyphosphate. Overall, all agents reduced the adverse effects associated with bleaching therapy, such as minimizing changes in the mechanical and morphological properties of enamel, reducing transamelodentinal diffusion, and clinically decreasing tooth sensitivity. None of the calcium-based agents interfered with the bleaching effect. In short, the studies demonstrated a low risk of bias.

Conclusions: Calcium-based agents incorporated into bleaching gels reduced bleaching-induced changes in dental tissues and demonstrated clinical aesthetic results comparable to conventional gels. These agents offer additional benefits by enhancing the biocompatibility and safety of whitening therapy. However, further clinical trials are warranted to optimize formulations and application protocols.

Clinical significance: This SRM provides evidence that the addition of calcium-based agents in bleaching gels offers significant clinical advantages over conventional treatments, guiding clinicians in material selection for whitening procedures.

Keywords: Tooth Bleaching, Hydrogen Peroxide, Carbamide Peroxide, Calcium, Dentin Desensitizing Agents.

4.2 Introduction

The smile is significantly associated with self-esteem, aesthetics, and social interactions [1, 2]. In this context, dental bleaching represents one of the most frequently sought-after options in dental practices, emerging as a viable solution for patients dissatisfied with the pigmentation of their teeth [3, 4]. The most commonly used products for tooth whitening are peroxide-based bleaching agents, primarily hydrogen peroxide (HP) and carbamide peroxide (CP) [5-7]. These agents can be applied using both in-office and at-home techniques [6, 8, 9]. Although it provides satisfactory aesthetic results, tooth whitening may induce adverse effects on dental structures [10-14].

The literature has shown that bleaching treatments can reduce mechanical strength, decrease mineral content, and alter the chemical composition, roughness, and morphology of enamel [15-19]. As dental bleaching operates via oxidation, peroxide degradation occurs with the release of H^+ ions through chemical dissociation [7, 16, 19], subsequently leading to the formation of reactive oxygen species (ROS). These byproducts penetrate the hard dental tissues and can reach the pulp, often resulting in dental sensitivity [10, 20, 21]. To minimize these effects, the incorporation of bioactive ingredients into bleaching gels has been investigated as a promising alternative [11, 22-25].

Among these bioactive agents, calcium-based compounds have emerged as a promising strategy to enhance the biocompatibility of bleaching gels [22, 26-29]. These agents, when interacting with dental tissues, can form a physical barrier against demineralization by precipitating on the enamel surface [18, 30]. Furthermore, saturating the whitening agent with calcium and phosphate allows for ion replenishment through ionic exchange, which helps mitigate demineralization effects and preserves enamel morphology [16, 31, 32]. Additionally, these compounds inhibit the degradation of free radicals and H^+ dissociation, significantly reducing the diffusion of ROS into the pulp, thereby decreasing dental sensitivity [28, 29, 33].

Recently, a systematic review and meta-analysis demonstrated that the application of calcium-containing bioactive desensitizers, whether administered prior to or following whitening treatments, did not compromise aesthetic efficacy while significantly reducing dental sensitivity [34]. Although the incorporation of these agents into bleaching gels has been extensively studied, no systematic review or meta-analysis has specifically assessed their efficacy in mitigating the adverse effects of bleaching procedures on dental structure. Therefore, this systematic review and meta-analysis aimed

to evaluate whether bleaching gels formulated with calcium-based agents preserve aesthetic efficacy and prevent or minimize the structural alterations associated with whitening treatments.

4.3 Materials and Methdos

4.3.1 Protocol and Registration

This systematic review was conducted following the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 checklist [35]. The research protocol for this review was also registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (CRD42024520242) and conducted also based on recent publications [36, 37].

4.3.2 PICO question and Search Strategy

A specific research question was formulated based on the Population, Intervention, Comparison, Outcome (PICO) criteria: “Does the presence of calcium-based agents in the composition of the bleaching gel minimize the changes induced by the bleaching process?”. The search strategy was structured using the PICO framework, incorporating controlled vocabulary terms (MeSH) and free keywords frequently appearing in article titles and abstracts. The elements of the PICO acronym are as follows:

- Population (P): Individuals or specimens undergoing dental bleaching procedures, with no restrictions regarding gender, occupation, or type of tooth sample.
- Intervention (I): Bleaching gels containing calcium-based bioactive agents.
- Comparison (C): Conventional bleaching gels.
- Outcome (O): Outcomes related to dental changes (e.g. color changes, hardness, roughness, chemical composition, morphology, color change and dental sensitivity).

The literature search was carried out across several databases, including PubMed, Scopus, Web of Science, Embase, and the Cochrane Library. The search strategy was initially formulated for PubMed and subsequently adjusted for compatibility with the other databases (see Supplementary Material – Appendix A). Additionally, a manual search was performed to capture any studies that may have been missed. Unpublished or ongoing studies were sought by querying the ClinicalTrials.gov registry, and gray literature was examined via the System for Information on Gray Literature in Europe (SIGLE) database. There were no limitations regarding publication date or language. Two

independent reviewers (GPN and ROA) conducted the electronic searches for studies available up to November 05, 2024. The retrieved references were organized using EndNote X8 software (Clarivate Analytics, Philadelphia, PA).

4.3.3 Eligibility criteria

The eligibility criteria for this review were as follows: (a) inclusion of adult participants, with no restrictions on gender or occupation, and acceptance of both in situ and in vitro research; (b) evaluation of the effects of calcium-based bioactive agents incorporated into bleaching gel formulations compared to a conventional treatment group; (c) assessment of dental substrate alterations as the primary outcome, along with secondary outcomes; (d) focus on individuals without prior exposure to bleaching treatments containing calcium, with specimen collection.

The exclusion criteria were as follows: (a) absence of a control group; (b) lack of evaluation of the outcomes of interest; (c) assessment of calcium-based agents applied before or after the bleaching agent; (d) combination of calcium-based agents with other active substances or treatments; (e) lack of incorporation of calcium-based agents into the gel formulation; (f) focus on desensitizing agents other than calcium-based ones; (g) absence of a placebo or non-desensitizing agent group for comparison; (h) review articles.

4.3.4 Selection of studies and data collection

Two independent reviewers (ROA and GRP) conducted a systematic two-step screening of references to identify studies meeting the eligibility criteria. In Step 1, initial selection was based on title and abstract evaluation according to predefined criteria. Duplicate records from multiple databases were counted only once. Titles and abstracts retrieved from the search were assessed to identify studies for preliminary inclusion. In Step 2, a full-text assessment was performed on the remaining records, with inclusion limited to studies that fully met all eligibility requirements. Extracted data were then organized into tables and reviewed for accuracy. In instances of disagreement, discussions were held until consensus was achieved, with a third reviewer (GPN) consulted to resolve any unresolved issues [38].

Details of the study were gathered using customized extraction forms that documented the following parameters: authors and year of publication, country of origin, study design, study group details, sample characteristics, bleaching protocol, assessment methods, results (outcomes), conclusions, and intervention effects. Data were separately recorded for in vitro and in situ studies (Table 1) and clinical/RCT studies (Table 2).

When needed, authors were contacted via email for additional information. Inter-examiner agreement during the selection process was measured using the kappa statistic. Discrepancies were addressed through discussion and mutual agreement among all authors.

4.3.5 Quality assessment and risk of bias of individual studies

The risk of bias for each study was evaluated using the appropriate tool corresponding to its specific design. Two independent reviewers (ROA and PTA) conducted quality assessments of the selected trials, and any discrepancies between reviewers during data selection and quality assessment were resolved through discussion, with a third reviewer (GPN) consulted when necessary to reach a consensus.

For randomized controlled trials (RCTs), the Cochrane Collaboration's tool was employed to assess bias, considering factors such as random sequence generation, allocation concealment, blinding procedures, incomplete outcome data, and selective reporting. The risk of bias for each quality assessment criterion was evaluated in line with the guidelines provided in the Cochrane Handbook for Systematic Reviews of Interventions, version 6.5.0 (http://handbook.cochrane.org) [38]. At the individual domain level, each item was categorized as "yes" for a low risk of bias, "no" for a high risk of bias, or "unclear" when there was insufficient information or ambiguity regarding potential bias. For the overall study assessment, a study was categorized as having a low risk of bias when all key domains for each outcome were judged to have a low risk of bias. If one or more key domains were assessed as having an unclear risk of bias, the study was classified as having an unclear risk of bias. Conversely, if at least one key domain was identified as having a high risk of bias, the study was considered to have a high risk of bias overall.

Pre-clinical studies were evaluated based on their level of evidence, following the Checklist for Quasi-Experimental Studies (non-randomized experimental studies) from the Joanna Briggs Institute Critical Appraisal Tools, as adapted by Reis-Prado et al., to enhance the methodological assessment of in pre-clinical research [38]. The criteria assessed included: a clearly defined objective, justification of sample size, sample randomization, comparability between control and experimental groups, and the adequacy of statistical analysis. Each item of JBI scale was scored as follows: 0, not reported or reported inadequately, which indicated a high risk of bias; 1, reported and adequate, which indicated a low risk of bias. The risk of bias was classified according to

the total number of “Y's” received by each study, as follows: 0 to 3 = high; 4 to 5 = medium; 6 to 10 = low risk of bias.

4.3.6 Data synthesis and Meta-analysis

A meta-analysis was performed using Review Manager software (RevMan, The Nordic Cochrane Centre, Copenhagen, Denmark) to assess the effects of incorporating 0.25%–3% calcium into 35% HP gel on extracted bovine and human teeth. Variance and heterogeneity among the included studies were evaluated using the I^2 test [38]. Continuous variables, including surface roughness, hardness, transamelodentinal diffusion, and color change, were compared using mean differences (MD), analyzed with a random-effects model [39, 40], and reported with 95% confidence intervals (CI).

4.4 Results

4.4.1 Study selection

The study selection results are summarized in Fig. 1. An initial search across databases recovered 4,289 studies: 1,265 from PubMed/MEDLINE, 1,363 from Scopus, 786 from Embase, 803 from Web of Science, 68 from the Cochrane Library, and 4 from manual searches. After removing duplicates, 2,748 studies remained. In Step 1, screening of titles and abstracts resulted in 79 studies progressing to full-text assessment based on the eligibility criteria (Step 2), where 23 studies were excluded, with reasons provided in Fig. 1. Consequently, 56 studies met the eligibility criteria and were included in the systematic review, comprising six randomized clinical trials [12, 17, 21, 25, 28, 29, 33, 41, 42] and 50 in vitro studies [13, 16, 18, 22, 26, 27, 30-32, 43-79]. The kappa coefficient for inter-rater agreement among investigators on the included articles indicated an excellent level of concordance, with a value of k = 0.875 across the databases.

4.4.2. Characteristics of the included studies

Table 1 provides detailed characteristics of the randomized controlled clinical trials, while Table 2 covers pre-clinical laboratory studies. Of the 56 included studies, the majority (n=49) were conducted in Brazil, with two each in Turkey and Iran, and one each in the Republic of Korea, Peru, and the United States.

Randomized Clinical Trials: The six studies included in this review comprised a total of 261 participants, with a mean age of approximately 18 years. Three studies investigated the in-office bleaching technique [28, 29, 33], and three examined the at-home method [21, 41, 42]. HP was the primary bleaching agent, except in Giniger et al.,

which used CP [42]. Four studies incorporated calcium gluconate as a bioactive additive in the bleaching gel [21, 28, 29, 41], while the other two examined casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [33] and amorphous calcium phosphate (ACP) [42]. All studies that performed in-office bleaching protocols conducted the treatments over two sessions [28, 29, 33]. The control group underwent three applications of 15 min per session, while the intervention group received 40 min per session [28, 29]. In Borges et al., both groups received three applications of 15 min per session [33]. At-home bleaching protocols were applied over 7 days [41] and 14 days [21, 42]. The daily exposure time was either 30 min [21, 41] or 4 h [42]. In general, all studies assessed tooth sensitivity and aesthetic performance as primary outcomes. Only one study [33] included an evaluation of enamel morphology using high-precision impressions.

*Pre-Clinical studies***:** Among the 50 studies, 58% (n=29) examined the effects of bleaching agents on human tooth specimens, while the remaining 42% (n=21) conducted tests using bovine tooth samples. Sample sizes within experimental groups ranged from 5 to 30, resulting in a total of 1,822 specimens evaluated, comprising 1,196 bovine teeth and 626 human teeth. Among the studies included, 62% (n=31) evaluated in-office whitening protocols, 36% (n=18) focused on at-home whitening techniques, and 2% (n=1) assessed both approaches. Regarding the bleaching agents used, 76% (n=38) employed HP, 18% (n=9) used CP, and 6% (n=3) investigated both agents. In the studies evaluating in-office techniques, HP was the predominant bleaching agent, with a concentration of 35% used in most cases. Notable exceptions included five studies that examined other concentrations: 20% [59], 38% [13], and 40% [56, 57]. For at-home techniques, the most common HP concentrations were 10% and 7.5%. Among studies using CP for at-home bleaching, 75% (n=9) applied a concentration of 10%, followed by 17% (n=2) with 16%, and one study (8%) evaluated both concentrations [30]. Calcium-based bioactive agents in bleaching gels were predominantly represented by calcium gluconate (n= 24; 48%). Other studied compounds included calcium ions (n=11; 22%), amorphous calcium phosphate (n=9; 18%), casein phosphopeptide-amorphous calcium phosphate (n=6; 12%), calcium polyphosphates (n=4; 8%), and calcium silicate (n=1; 2%). In studies evaluating in-office bleaching protocols, 41% (n = 13) employed three sessions, 34% (n = 11) used one session, 22% (n = 7) used two sessions, and 3% (n = 1) used five sessions. Regarding gel application time per session, 47% (n = 15) used a 40-min exposure, 28% (n = 9) 45 min (3 applications of 15 min), 13% (n = 4) 30 min, another 6 % (n = 2) used 1 hour, and 6 % (n = 2) 50 min. Regarding at-home bleaching protocols,

treatment duration varied across studies, with 11% (n=2) applying treatment for one week, 61% (n=11) for two weeks, 22% (n=4) for three weeks, and 6% (n=1) for five weeks. Among studies using at-home protocols, exposure time to bleaching gels differed based on gel composition. For HP-based gels, application times included 30 min (11% of studies; n=2), 1 h (32%; n=6), and 2 h (11%; n=2). For CP gels, application times were longer, ranging from 4 h (26%; n=5) to 6 h (21%; n=4) and 8 h (21%; n=4). Regarding the analyses conducted, 64% (n=32) of the eligible studies measured enamel surface hardness, while 40% (n=20) evaluated chromatic alterations related to aesthetic performance, and another 40% (n=20) investigated morphological changes in enamel. Additionally, 34% (n=17) examined the chemical composition of enamel post-bleaching, 22% (n=11) assessed enamel surface roughness, and 14% (n=7) focused on trans-amelodentinal diffusion and cross-sectional microhardness.

4.4.3 Calcium-Bioactive agents and outcomes-related

4.4.3.1 Calcium gluconate (CaGlu)

Twenty-eight studies evaluated the incorporation of CaGlu into hydrogen peroxide (HP)-based bleaching gels [16, 17, 21, 25, 28, 29, 41, 43, 45-49, 51-55, 58-60, 62-64, 66, 67, 79, 80]. Of these, most studies (n = 23) incorporated CaGlu into highly concentrated bleaching agents containing 35% HP for in-office techniques. The remaining 5 studies examined distinct concentrations: 4 studies used HP concentrations ranging from 6% to 10% for at-home applications [21, 41, 54, 55], and one study evaluated both in-office (35%) and at-home (7.5%) HP concentrations [64]. Most studies evaluated CaGlu at a 2% concentration, with five studies examining concentrations of 0.5% [52, 54, 55] and 3% [43, 45].

Across the studies reviewed, 61% (n=17) reported a protective effect of calcium gluconate (CaGlu) against adverse effects related to whitening treatments, while 35% (n=10) observed no effect [16, 17, 46, 48, 49, 52, 58, 60, 79, 80], and 4% (n=1) noted a negative effect [47]. Among the 13 studies assessing enamel microhardness, 54% (n = 7) reported greater enamel hardness following whitening treatment with CaGlu-containing gels, 38% (n = 5) found no additional effect of CaGlu, and 8% (n = 1) observed a reduction in enamel hardness compared to conventional gels. Regarding enamel surface roughness, half of the studies (n=4; 50%) showed reduced roughness alterations with CaGlu, while the other half reported similar changes as conventional gels. For enamel

morphology, 66.7% (n=4) concluded that CaGlu offers protective effects against whitening-induced morphological changes, while 33.3% (n=2) found no effect.

On hydrogen peroxide transamelodentinal diffusion, 43% of studies (n=3) observed reduced HP diffusion with CaGlu, another 43% (n=3) found no significant difference from control gels, and 14% (n=1) reported an increase in diffusion. In terms of enamel chemical composition, all studies [17, 58, 60, 80] indicated that CaGlu did not affect post-treatment calcium and phosphate concentrations in enamel. Regarding whitening efficacy, all in vitro studies (n = 13) indicated that CaGlu did not compromise the whitening effect; furthermore, one study reported a superior whitening effect in the CaGlu group compared to the control [60]. In the RCTs included [21, 28, 29, 41], none demonstrated an effect of CaGlu on bleaching efficacy. Regarding tooth sensitivity, three studies [28, 29, 41] reported a reduction in sensitivity associated with CaGlu use, while one study noted an increase in sensitivity; in this case, however, different HP concentrations (4% vs. 10% HP with CaGlu) were compared [21].

4.4.3.2 Calcium ion

Eleven studies assessed the incorporation of calcium ions in bleaching gels. [18, 31, 32, 43, 45, 68, 71, 74, 76, 78], including four studies assessing calcium addition to 35% HP gels [31, 43, 45, 77], two studies with 4–7.5% HP gels [68, 78], and five studies with 10% CP gels [18, 32, 71, 74, 76]. Calcium ion concentrations varied from 0.05% to 0.5%. Among the studies reviewed, 82% (n=9) reported a protective effect of calcium ions against adverse effects associated with bleaching therapies, while 18% (n=2) observed no effect.

Among the six studies evaluating enamel microhardness, five reported increased enamel hardness in specimens treated with calcium-containing gels [71, 74, 76-78], while one study [18] found no additional effect of calcium. For enamel morphology, all four studies [31, 32, 74, 78] indicated that calcium minimized morphological changes induced by bleaching. Regarding enamel chemical composition, two studies found that adding calcium to gels increased calcium, phosphate, and carbonate concentrations in enamel [31, 76], while another study noted no impact on post-treatment calcium and phosphate levels [18]. For hydrogen peroxide transamelodentinal diffusion, the presence of calcium ions in HP gels did not reduce HP diffusion [43, 45] and had no effect on bleaching efficacy compared to the control group.

4.4.3.3 Amorphous calcium phosphate (ACP)

A total of ten studies evaluated the effect of ACP into bleaching gels [18, 42, 56, 57, 61, 65, 68, 72, 73, 75]. These studies included two that assessed the addition of ACP to 40% hydrogen peroxide (HP) gels [56, 57], three with 7.5% HP gels [61, 68, 75], three with 16% carbamide peroxide (CP) gels [42, 65, 72], and two with 10% CP gels [18, 73]. Among the studies reviewed, 60% (n=6) reported a protective effect of ACP in minimizing adverse effects associated with bleaching therapies, while 40% (n=4) observed no significant effect.

Among the six studies evaluating enamel hardness, half (50%, n=3) demonstrated reduced alterations in hardness with the incorporation of ACP [56, 72, 75], while the other half reported similar changes to those observed with conventional gels [18, 61, 73]. Regarding enamel surface roughness, two studies found that ACP reduced enamel roughness changes [56, 75], while one study found no significant difference [61]. In terms of enamel morphology, three studies indicated that ACP protected against morphological changes induced by bleaching [56, 72], while one study did not show a protective effect [61].

With respect to enamel chemical composition, the incorporation of ACP into bleaching gels did not influence calcium and phosphate levels in enamel [18]. As for aesthetic efficacy, the presence of ACP in HP gels did not impact the bleaching effect in vitro [61]. Additionally, in clinical evaluation, CP bleaching gel containing ACP were clinically effective, exhibiting reduced overall tooth sensitivity during treatment compared to the control group [42].

4.4.3.4 Casein Phosphopeptide -Amorphous Calcium Phosphate (CPP-ACP)

The effects of CPP-ACP were investigated in seven studies [13, 22, 30, 33, 50, 69, 70]. Among these, four studies focused on high-concentration hydrogen peroxide (HP) gels (35–38%) [13, 22, 33, 50], two assessed 7.5% HP gels [69, 70], and three evaluated 10% carbamide peroxide (CP) gels [30, 69, 70]. CPP-ACP was incorporated at varying concentrations: 1% and 3% [22], 0.2 mL gel [33], 1.5 mL [13], and in ratios of 2:1, 1:2, and/or 1:1 [30, 50, 69, 70]. All studies reported that CPP-ACP offered a protective benefit, reducing the potential damage associated with bleaching agents.

All four studies evaluating enamel hardness indicated that CPP-ACP in bleaching gels helped prevent or minimize hardness changes [13, 22, 30, 69]. Regarding enamel surface roughness, no significant difference was observed compared to the control group [69]. However, enamel morphology changes were reduced in two out of three in vitro

studies [22, 69]. Findings on chemical composition were inconsistent: one study reported increased calcium and phosphate levels in enamel [22], while another found no significant difference [13]. One study reported that incorporating CPP-ACP into the gel reduced hydrogen peroxide transamelodentinal diffusion [50]. In terms of aesthetic efficacy, all studies confirmed that CPP-ACP did not affect the whitening effect of the gels [13, 22, 30, 50, 70]. Moreover, in a randomized clinical trial, bleaching efficacy was also confirmed, and the CPP-ACP-containing gel potentially reduced the intensity of tooth sensitivity and attenuated early post-bleaching morphological changes in enamel [33].

4.4.3.5 Calcium polyphosphates and Calcium Silicate

Four recent studies [12, 16, 26, 44] investigated the effects of calcium polyphosphates in bleaching gels. Three of these studies assessed its incorporation into gels with a high concentration of hydrogen peroxide (35%) [12, 16, 26], while one study focused on gels with lower concentrations: 6% hydrogen peroxide and 10% carbamide peroxide [44]. The tested concentrations of calcium polyphosphates ranged from 0.25% [16], to 0.5% [12, 26], and up to 1.5% [44]. Collectively, these studies concluded that adding calcium polyphosphate to whitening gels provides a protective effect on enamel, reducing the adverse effects associated with whitening treatments.

The four studies assessing enamel hardness demonstrated that incorporating calcium polyphosphates into bleaching gels contributed to reducing microhardness loss [12, 16, 26, 44]. In terms of enamel morphology, two of the three studies indicated that these agents minimized topographic changes [16, 44], while one study found greater surface alterations in the intervention group [12]. For enamel surface roughness, calcium glycerophosphate was associated with fewer roughness alterations [16], whereas another study found no significant difference with calcium polyphosphate compared to the control gel [12]. Analysis of chemical composition revealed that calcium and phosphate levels were maintained similarly to the control gel [12]. Regarding bleaching performance, all studies confirmed that these calcium bioactive agents did not compromise the whitening effect of the gels [12, 16, 26, 44].

In a single study, the effects of incorporating different concentrations of hydrated calcium silicate (0.25%, 0.5%, 1%, and 2%) into 35% HP whitening gels were evaluated [27]. The findings showed that all concentrations of calcium silicate maintained the bleaching efficacy of the gels. Moreover, adding calcium silicate contributed to reducing

loss of microhardness in the enamel caused by HP and preserved enamel surface morphology.

4.4.4. Assessment of the risk of bias

The risk of bias analyses, presented in Figure 2 (RCT studies) and Table 3 (in vitro studies), assess the methodological quality of the included clinical studies. For RCT studies, three studies were classified as having a low risk of bias [28, 29, 42], while three others showed a high risk of bias [21, 33, 41]. A critical issue identified was “selection bias”, with two studies failing to provide clear information on the allocation concealment process [33, 41]. Additionally, the “blinding of outcome assessors” was not clearly described in three studies [28, 33, 41]. In the "other biases" domain, two studies were classified as having a high risk of bias due to the use of hydrogen peroxide gels with different concentrations for the intervention and control groups [21, 41]. Despite these limitations, the remaining assessed domains generally indicated a low risk of bias (Fig. 2).

In the in vitro studies, a generally low risk of bias was identified, with final scores ranging from 7 to 10 domains classified as adequately reported. However, only 28% (n = 14) of the included studies provided justification for sample size calculation [12, 17, 25-27, 43, 45-47, 49, 50, 52, 64, 76]. Furthermore, three studies did not describe whether random allocation of samples to experimental groups was performed [49, 67, 72]. Blinding of outcome assessors, a critical aspect to reduce measurement bias, was explicitly reported in only two studies [50, 62]. Despite these limitations, the other evaluated items were adequately reported, reflecting a generally low risk of bias and good methodological rigor. Among the most consistent aspects were a clear definition of objectives, detailed descriptions of experimental protocols, consistent outcome measurements across groups, reliability of analyses, and appropriate statistical methods (Table 3).

4.4.5. Meta-analysis

Data from 16 studies were included in the meta-analyses [16, 17, 25-27, 46-49, 51, 53, 60, 62, 63, 66, 67]. A comparison was made between 35% HP and 0.25%-0.5% calcium polyphosphates, as well as 2-3% calcium gluconate, using bovine and human teeth for the evaluated outcomes (Figures 3 and 4).

The incorporation of 2% calcium gluconate did not significantly influence the roughness of bovine teeth compared to bleaching with 35% HP alone (MD = -0.01, 95%

CI = -0.02–0.01, I^2 = 80%; Figure 3A). However, 2% calcium gluconate significantly increased the hardness of bleached bovine dentin (MD = 8.66, 95% CI = 2.78–14.55, I^2 = 93%; p = 0.004; Figure 3B). Similarly, lower concentrations of calcium polyphosphates (0.25%-0.5%) significantly improved the hardness of bovine teeth samples compared to bleaching alone (MD = 12.26, 95% CI = 10.96–14.28, I^2 = 0%, p < 0.0001; Figure 3C).

The incorporation of 2% calcium gluconate into 35% HP did not significantly affect the transamelodentinal diffusion of HP (MD = 0.30, 95% CI = -0.45–1.06, I^2 = 94%; p= 0.43; Figure 4A) or the color change in bovine teeth after 7 days of bleaching (MD = 0.84, 95% CI = -0.78–2.46, I^2 = 86%; p= 0.31; Figure 4B). Likewise, 3% calcium gluconate/HP gel did not significantly affect the color of human teeth bleached with 35% HP (MD = 0.11, 95% CI = -2.69–2.92, I^2 = 81%; p= 0.94; Figure 4C).

4.5 Discussion

Dental bleaching, although one of the most common procedures in clinical practice, presents challenges due to its potential to cause alterations in dental substrates and the occurrence of post-operative sensitivity [81]. To address these issues, the incorporation of calcium-based bioactive agents into bleaching gels has been investigated as a strategy to mitigate bleaching-related effects. This systematic review and meta-analysis confirms that these agents effectively reduce bleaching-induced changes. While the calcium sources were evaluated among different compounds, they all demonstrated protective properties and promising outcomes during whitening therapy.

The analysis of the evaluated outcomes underscores the potential of calcium-based agents in enhancing the performance of dental bleaching gels, particularly through their remineralizing and protective effects on dental tissues. Calcium, in its various forms−such as CaGlu, ACP, CPP-ACP, and calcium polyphosphates−provides essential ions for the prevention and/or repair of enamel's mineral structure [82-85]. These agents promote remineralization in demineralized areas and strengthen the hydroxyapatite matrix [86]. Such mechanisms explain the ability of these agents to preserve the mechanical properties of enamel and minimize the topographical changes induced during whitening therapy, as demonstrated in this study. Moreover, studies suggest that these agents also play a crucial role in forming a protective layer over the enamel, reducing its permeability and transamelodentinal diffusion [50, 76], thereby contributing to decreased sensitivity associated with bleaching procedures [33]. This effect is strongly linked with the interaction between Ca^{+2} with PO_4^{-3} ions, which increases mineral saturation and helps

prevent structural damage caused by the oxidative process of peroxides [17, 86]. In addition, most of these agents enable the controlled release of calcium ions, a critical factor in maintaining enamel crystal stability while neutralizing changes caused by ionic dissociation and the formation of ROS [17, 31]. These pathways likely explain the benefits associated with incorporating such compounds, enhancing the biocompatibility and safety of dental bleaching while minimizing adverse impacts on dental tissues.

In the present study, the majority of the included studies did not observe significant changes in enamel surface roughness after the use of bleaching agents, regardless of the addition of calcium-based compounds. This result is likely related to the minimal variation in roughness induced by dental bleaching, with changes that, according to the literature, are not clinically significant [87, 88]. The roughness induced by bleaching is generally of insufficient magnitude to warrant additional benefits from the inclusion of these agents, which may explain the lack of significant differences in comparative studies. In addition, it is well-established that alterations in surface roughness can be naturally restored by human saliva or through extended storage in artificial saliva, as demonstrated in most studies.

Another important point to highlight is that most of the analyzed results involved bleaching gels with neutral to alkaline pH, ensuring similarity across comparative groups. Generally, these gels presented a pH higher than the critical enamel pH (5.5), reflecting a focus on minimizing adverse effects on dental tissues, such as demineralization associated with product acidity. Moreover, it is crucial to consider that the pH of bleaching gels may experience instability during application, particularly with in-office gels [89, 90], where pH tends to decrease with prolonged contact with enamel. This scenario can lead to demineralized enamel surfaces, facilitating the diffusion of hydrogen peroxide into the pulp cavity and increasing risks to dental tissues. In addition, regarding the source of the sample, calcium-containing gels demonstrated superior results compared to conventional gels, regardless of whether they were evaluated on human or bovine teeth. These findings support previous evidence that human and bovine teeth share a similar evolutionary origin [91]. Additionally, due to the close resemblance in enamel microstructure between the two species, bovine teeth have proven to be an excellent alternative to human teeth in dental research, offering advantages in terms of availability and ethical considerations.

Factors such as the type of peroxide-based bleaching agent (hydrogen or carbamide), its concentration, the technique used (at-home or in-office), and the

administration protocols (e.g., application exposure time, treatment duration) are crucial in determining the outcomes of dental bleaching. Controlling these variables is essential for an accurate assessment of therapeutic effects and for a better understanding of the results. However, calcium-based agents have demonstrated the ability to minimize adverse effects associated with bleaching and promote similar effects regardless of these variables. This suggests that, regardless of the type of peroxide used, its concentration, the application technique, or the administration protocols, the addition of these agents can play a consistent role in preserving dental properties, offering protection against additional enamel damage and promoting a positive response in bleaching treatments.

Studies evaluating different concentrations of calcium-based agents have not demonstrated a dose-dependent relationship in improving observed effects [12, 22, 44, 69-71], except for one study analyzing calcium silicate [27]. The concentrations assessed primarily ranged from 0.05% to 3%, with the most favorable outcomes observed for calcium gluconate (CaGlu) at 2-3%, calcium, ACP, and calcium polyphosphate at 0.2-0.5%, and CCP-ACP in a 1:1 ratio. In experimental models using hydrogen peroxide (HP) at concentrations between 7.5% and 40%, calcium-based agents effectively reduced adverse bleaching effects, despite the potential for high HP concentrations and prolonged exposure to compromise dental substrates. In the context of at-home therapy with carbamide peroxide, all eligible studies using the agent at 16% reported improved outcomes with the inclusion of calcium-based agents. For concentrations of 10%, only 2 out of 10 studies observed no effect [71, 73], likely influenced by the low calcium concentrations used (0.05% and 0.1%). In long-duration protocols (at-home therapy) or a higher number of sessions (in-office therapy), gels containing calcium-based agents also outperformed conventional products. However, although it is plausible to assume that the duration or frequency of exposure to bleaching agents could influence the efficacy of calcium additives, this relationship could not be clearly established in the analyzed studies. Nevertheless, these findings highlight the protective role of calcium-based agents against bleaching-induced changes, even under varied experimental conditions.

Tooth sensitivity associated with whitening is likely a consequence of peroxide-induced damage to the connective tissues within the dental structure. Due to its low molecular weight, strong oxidative potential, and rapid diffusion, CP/HP penetrate the tooth's hard tissues, reaching the pulp chamber and triggering an acute inflammatory response that may result in pain. Although the relationship between the amount of peroxide reaching the pulp and clinical symptoms remains unclear, the evaluation of

obliterating materials is based on the hypothesis that these compounds could limit hydrogen peroxide penetration, delay the onset of inflammation, and allow pulp tissues more time to manage oxidative stress. However, the findings of the present study did not show a reduction in the occurrence of spontaneous sensitivity, indicating that the tested bioactive agents (CaGlu, ACP, and CPP-ACP) were not effective in preventing this discomfort. These results align with previous laboratory studies demonstrating that none of the desensitizers evaluated so far have completely blocked peroxide diffusion into the pulp [17, 43, 45, 50, 52]. Although high concentrations of hydrogen peroxide can increase the permeability of enamel and dentin, thereby raising the risks of pulp inflammation and patient discomfort, the direct correlation between these factors and the development of dental sensitivity must be analyzed with caution and rigor. It is important to emphasize that these findings cannot be directly extrapolated to clinical practice, as experimental conditions often differ significantly from real-world scenarios. In addition, while calcium-based agents may reduce the intensity of sensitivity, they are still not capable of full prevention. This underscores the need for more effective strategies to address whitening-induced sensitivity.

Moreover, in all the studies evaluated, both in vitro and clinical, the presence of calcium-based agents did not alter the aesthetic outcome of the bleaching treatment. This finding is important, as it is essential that any additives to bleaching gels remain functionally inert, ensuring that they do not compromise the bleaching efficacy. Thus, calcium-based agents play a crucial role in protecting and optimizing whitening formulas. They maintain the whitening effect, promote remineralization, stabilize mineral changes in enamel, and reduce the intensity of sensitivity - critical factors for the safety and efficacy of dental bleaching procedures.

The meta-analyses conducted in this review were designed to minimize heterogeneity and methodological differences among the included studies. To achieve this, rigorous criteria were applied, taking into account factors such as sample type, application technique, type of peroxide used (hydrogen or carbamide), and comparisons exclusively between compatible concentrations of whitening agents and calcium-based agents. As a result of this stringent approach, only about 30% of the initial studies met the inclusion criteria for the meta-analyses. Nevertheless, the findings for the agents evaluated in quantitative analyses, such as calcium gluconate and calcium polyphosphate, were consistent with the general observations from the qualitative analysis. This

consistency reinforces the validity of the data and highlights the relevance of these agents in mitigating the adverse effects of dental bleaching.

The risk of bias analysis revealed varying methodological rigor among the included studies, emphasizing areas requiring improvement to enhance the quality of evidence. In RCTs, the classification of half the studies as having a high risk of bias highlights persistent challenges, particularly in allocation concealment and blinding of outcome assessors, which may compromise internal validity through selection and measurement biases. A critical flaw in two studies [21, 41] was the use of differing hydrogen peroxide concentrations between intervention and control groups, undermining the comparability of treatments and obscuring the isolated effects of calcium-based agents. Although hydrogen peroxide concentration did not affect aesthetic outcomes, its variability could influence dental tissue integrity, making it impossible to accurately determine the evaluation of calcium additives. In contrast, in vitro studies generally demonstrated low risk of bias with robust methodological reporting. However, gaps such as inadequate sample size justification, unclear randomization, and lack of blinding highlight opportunities for refinement. Addressing these limitations is crucial to strengthening the reliability and applicability of findings in both experimental and clinical contexts.

Although most of the studies included in this review presented a low risk of bias, significant methodological variations identified among them pose a key constraint to performing more robust comparisons. Differences in the administration protocols of whitening agents, the techniques adopted, the types and concentrations of peroxides and bioactive or remineralizing agents used, as well as the application times and frequencies, complicate the generalization of results. These discrepancies reflect the diversity of approaches often tailored to the specific needs of each investigation, providing a comprehensive perspective on the efficacy and effects of whitening treatments. However, such methodological variations can also directly influence whitening effectiveness, the occurrence of adverse effects, and the benefits associated with the addition of protective agents, such as calcium-based compounds. Given the importance of these differences, the need for greater standardization in future studies becomes evident. Standardization in protocols and experimental conditions would enable more consistent comparisons and the development of optimized clinical guidelines. This approach could not only enhance the understanding of the effects of whitening and calcium-based bioactive agents but also

contribute to the development of safer, more effective, and widely reproducible clinical protocols.

Furthermore, while this systematic review highlights promising results regarding the use of calcium-based bioactive agents in dental bleaching gels, it is limited by the predominance of *in vitro* studies. Although these models allow for controlled and replicable conditions, they fail to fully replicate the dynamic environment of the oral cavity, including interactions with saliva, oral microbiota, and patient behavioral factors. The scarcity of clinical studies further hinders the extrapolation of findings to clinical practice, particularly concerning long-term safety and efficacy, emphasizing the need for further investigation in this area. To the best of our knowledge, no prior meta-analyses have simultaneously addressed the efficacy of these agents, and the adverse effects associated with dental bleaching. Therefore, this study provides relevant contributions to contemporary scientific literature. Overall, the findings suggest significant potential for the development of safer whitening formulations that reduce damage to enamel and dentin while minimizing post-treatment sensitivity intensity. Future studies should prioritize robust clinical trials to evaluate both the efficacy and biocompatibility of these formulations. Additionally, new application strategies and protocols should be explored to balance aesthetic outcomes with safety, fostering more personalized approaches for patients.

4.6 Conclusion

Within the limitations of this study, the evidence from this systematic review and meta-analysis suggests that bleaching gels containing calcium-based bioactive agents can reduce tooth changes induced by bleaching and yield clinical aesthetic results comparable to conventional gels. These agents show promising potential in enhancing the safety and biocompatibility of whitening treatments.

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Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart.

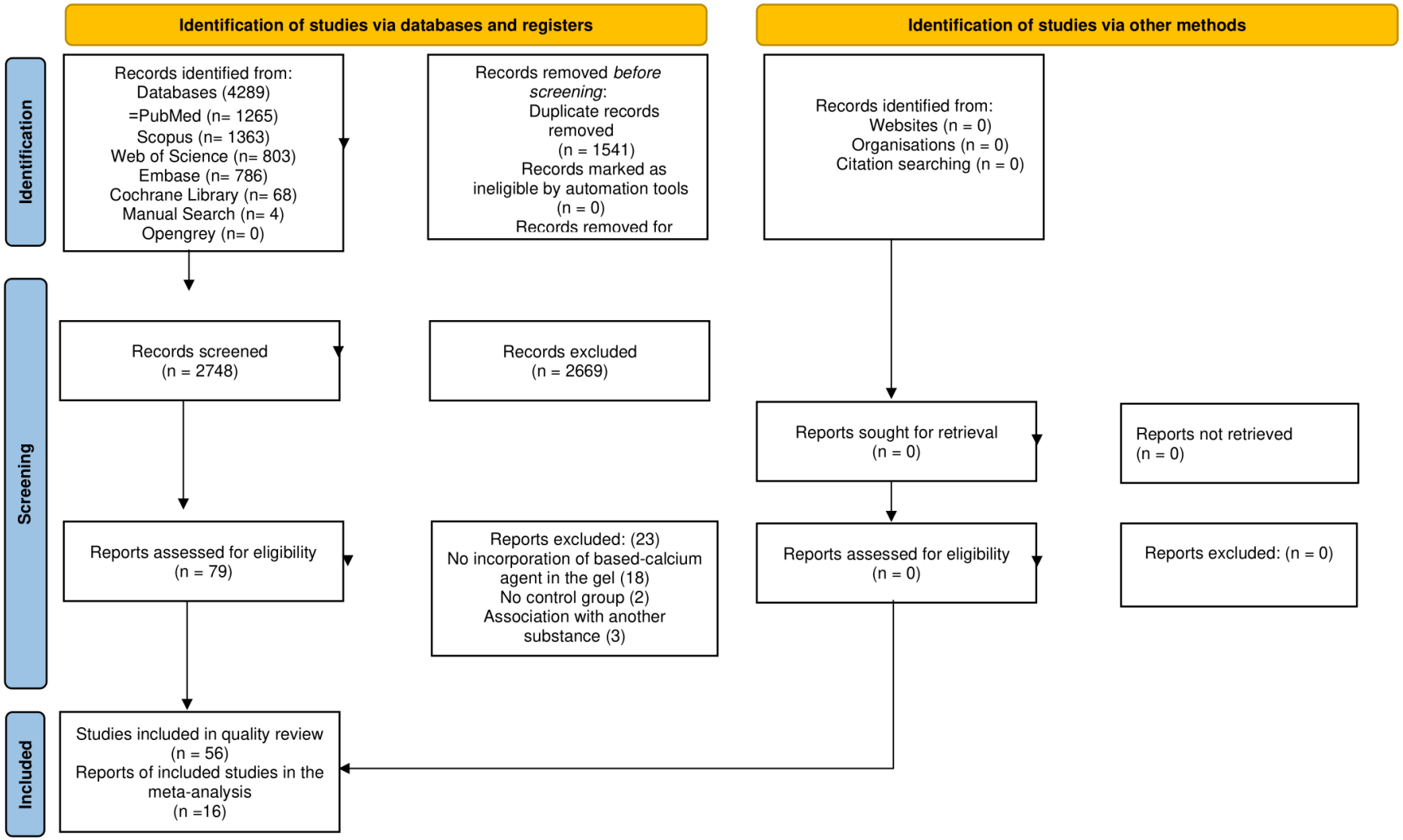


Figure 2. Overview of Risk of Bias Evaluation Utilizing the Cochrane tool.

Figure 3. Meta-analysis comparison between conventional bleaching (35% hydrogen peroxide) gel and bleaching gel containing calcium-based agents. A: Metanalysis of enamel roughness using 2% calcium gluconate. B: Metanalysis of enamel hardness using 2% calcium gluconate. C: Metanalysis of enamel hardness using 0.25-0.5% calcium polyphosphates

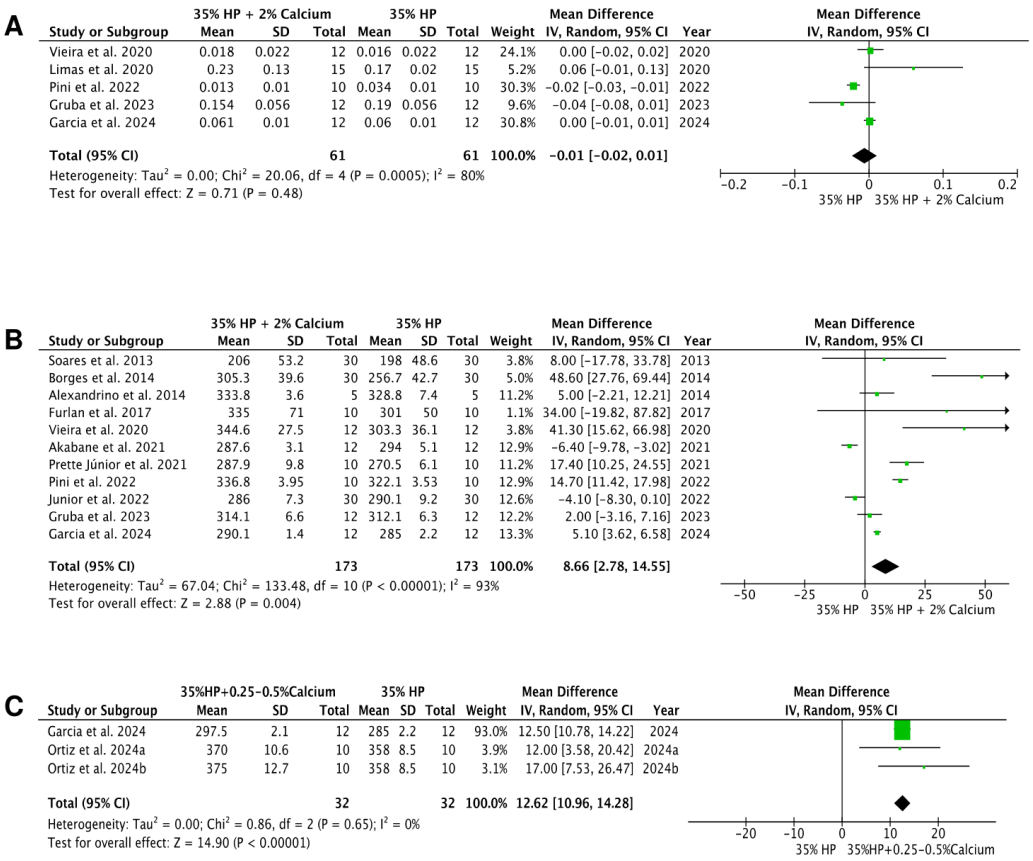


Figure 4. Meta-analysis comparison between conventional bleaching (35% hydrogen peroxide) gel and bleaching gel containing calcium-based agents. A: Metanalysis of transamelodentinal diffusion of hydrogen peroxide using 2% calcium gluconate. B: Metanalysis of color change in bovine teeth after applying 2% calcium gluconate. C: Metanalysis of color change in human teeth after applying 3% calcium gluconate.

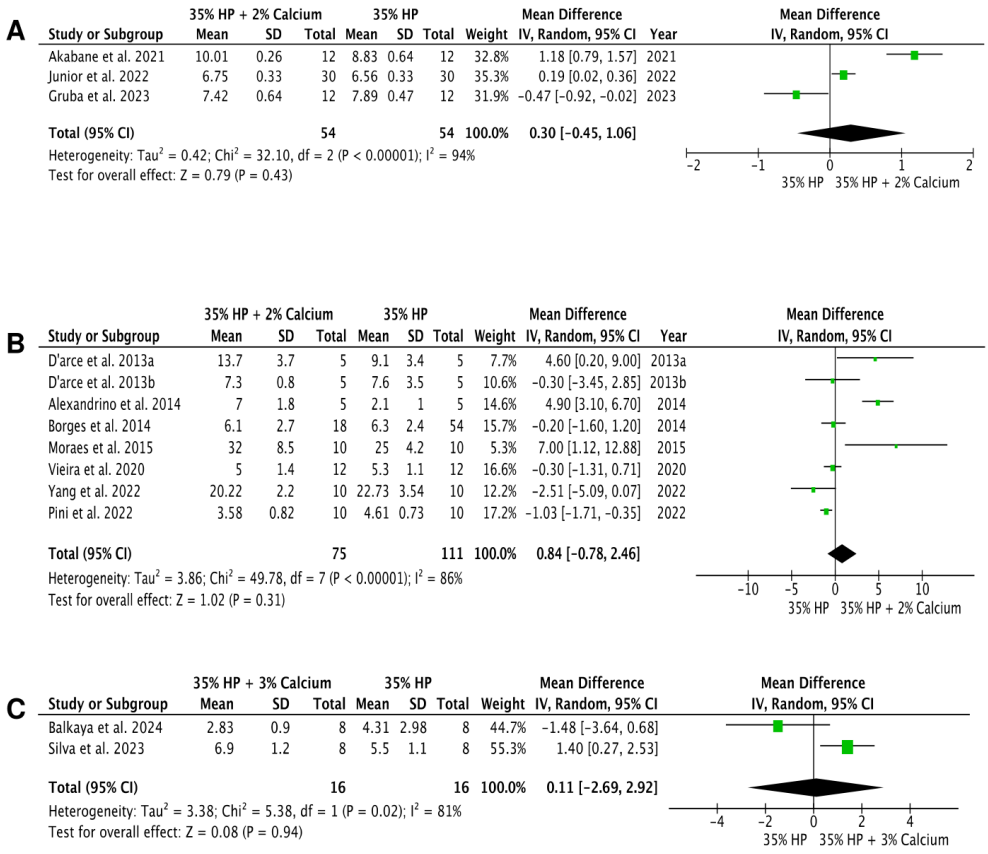


Table 1. General characteristics from RCT eligible studies.

Author/ year (Country)	G1 - Bleaching gel based-calcium G2 – Bleaching gel conventional (N sample)	Bleaching Protocol	Assessment Method	Results: Outcomes [Mean ± SD] or percentage or [Mean (minimum and maximum value)]	Results - Morphology Enamel (Qualitative analyses)	Conclusion	Intervention Effect
Chemin *et al.,* 2018 (Brazil)	G1: HP 10 % + 2% calcium gluconate G2: HP 4% n/per group: 39	Each patient received the bleaching tray and his or her respective bleaching product. Subjects were instructed all participants to wear the tray with the bleaching agent for 30 minutes twice a day for 14 days.	Color Evaluation (Vita Classical; Vita Bleachedguide 3D-MASTER and spectrophotometer) Tooth sensitivity (numeric rating scale and visual analog scale (VAS))	Tooth Sensitivity (number of patients) G1: 25 (64%) / G2: 15 (38%) Tooth Sensitivity Intensity Numeric Rating Scale (0-4) / Visual Analogue Scale (0-10) G1: 0.8 ± 0.9 / 1.2 ± 2.1 G2: 0.5 ± 0.8 / 0.7 ± 1.5 Color Evaluation 1 week/ 2weeks/ 1 month *ΔSGU* (Vita Classical) G1:3.7 ± 1.2/ 4.2 ±0.9 / 4.1±0.9 G2: 3.0 ± 1.3 / 4.0 ± 1.3/ 4.1 ± 1.3 *SGU* (Vita Bleachedguide 3-D) G1: 5.1 ± 2.3 / 6.6 ± 2.6 / 6.1 ± 2.5 G2: 4.1 ± 1.6 / 3.7 ± 1.8 / 5.3 ± 2.3 *(ΔE*)* G1: 7.4 ± 3.6 / 9.0 ± 4.2 / 8.4 ± 3.5 G2: 6.5 ± 3.2 / 6.8 ± 3.0 / 7.9 ± 4.5	NS	Both HP concentrations (4% and 10%) were equally effective after two weeks of at-home bleaching. The gel containing only 4% HP showed a lower risk and intensity of dental sensitivity.	Null
Gonçalves *et al.,* 2017 (Brazil)	G1.1: HP 35% + 2% Calcium gluconate G1.2: HP 20% +2% Calcium gluconate G2: HP 35%	G1.1: Applied the gel for 40 min- one session. G1.2: Applied gel for 50 min in a single session. G2: Applied gel to the for 15 minutes. Use a brush or microapplicator to move the gel 3-4 times. If needed,	Tooth sensitivity (Friedman Test) Color analysis (Vita Classical Shade)	Sensitivity (VAS Sensitivity Score) 1ª session / 2ª session G1.1: ~ 7 ± 1.4 / 5.8 ± 2.8 G1.2: ~ 5 ± 2.2 / 1.8 ± 1.5 G2: ~ 8 ± 1.5 / 7.5 ± 2.1 Color Change G1.1: ~ 6.1 ± 3.4 G1.2: ~ 6 ± 3.3	NS	The addition of calcium in the bleaching gels appears to help reduce sensitivity without affecting the bleaching efficacy.	Positive

	n/per group: 17; G1.2: 19	reapply up to two more times.		G2: ~ 5.9 ± 3.7			
Pinto *et al.,* (2017) (Brazil)	G1.1: HP 7.5% + 2% calcium gluconate G1.2: HP 6% + 2% calcium gluconate G2: HP 10% n/per group: 10	For the groups containing calcium, the gel was applied for 1 hour a day for 7 days. For G2, the gel was applied for 30 minutes, twice a day, for 7 days.	Colorimetric change (Vita 3D-Master color scale) Satisfaction, sensitivity and discomfort (questionnaire)	Color Change (median) 7d / 15d / 30d G1.1: ~ 4.5 / 3 / 2.5 G1.2: ~ 4 / 2 / 2 G2: ~ 2.2 / 1.8 / 1.8 7 days/15 days Tooth Sensitivity (number of patients) G1.1: 5 (50%) / 1 (10%) G1.2: 8 (80%) / 3 (30%) G2: 8 (80%) / 0 (0%)	NS	The calcium-containing whitening gels had a similar effect to the whitening gel containing only hydrogen peroxide.	Positive
Borges *et al,* 2012 (Brazil)	G1: HP 35% + 0.2mL CPP-ACP G2: HP 35% n/per group: 3	The bleaching gels were applied in three applications of 15 min, in two bleaching sessions carried out separately on the first and seventh day.	Tooth sensitivity (scale from 0 to 10)- SEM: Polyvinyl siloxane-based material- impression technique	Tooth Sensitivity Baseline: 1.5 ± 0.2 G1:1.9 ± 0.3 G2:7.5 ± 0.9 Color Assessment: bleaching techniques produced an overall change in color values of at least 2 U	G1: The group maintained an enamel surface without any morphological alterations. G2: The group showed small irregularities and depressions.	The bleaching gel containing CCP-ACP may potentially lower tooth sensitivity and prevent early post-bleaching morphological changes in enamel.	Positive
Kossatz *et al,* 2012 (Brazil)	G1: HP 35% + 2% calcium gluconate G2: HP 35% n/per group: 20	G1: The bleaching gels were applied for 15 min three times in each appointment. the appropriate teeth using a different brush for each. G2: The bleaching gels were applied for 40 min.	Color Change (Shade evaluation); Tooth sensitivity (scale from 0 to 4) - spontaneous perception	Color Change One week / two week G1: 4.7 ± 1.5 / 1.4 ± 0.7 G2: 4.3 ± 1.57 / 1.8 ± 0.6 Participants' experience of tooth sensitivity / mean participants' tooth sensitivity intensity scores G1: 8 (40%) / 0.55 G2: 16 (80%) /1.85	NS _____	The CC hydrogen peroxide gel caused less TS during in-office dental bleaching without any deleterious effects on bleaching effectiveness.	Positive
Giniger *et al,* 2005 (United States of America)	G1: CP 16 % + 0.5% ACP G2: CP 16 % n/per group: 27	The bleaching gel was applied for 4 h (overnight) daily for 14 days	Vita classical shade guide; visual analogue scale (VAS)	Color change (Vita index) 5 days / 90 days / 180 days G1: 8.0 ± 0.7 / 6.4 ± 0.5 / 6.2 ±0.5 G2: 7.8 ± 0.6 /6.0 ± 0.4 / 5.4 ± 0.5 0 days / 180 days Thermal Sensitivity G1: 0.2 ± 0.1 / 0.2 ± 0.1 G2: 0.3 0.1 / 0.2 ± 0.1	NS	The bleaching gel containing ACP was clinically effective, exhibiting reduced overall tooth sensitivity during the treatment.	Positive

				Tactile Sensitivity G1: 0.3 0.1 / 0.3 ± 0.1 G2: 0.4 ± 0.1 / 0.4 ± 0.1 Self-reported Sensitivity G1: 0.2 0.1 / 0.2 ± 0.1 / G2: 0.3 0.2 / 0.3 ± 0.2			

Abbreviations ACP: Amorphous calcium phosphate; CPP-ACP Casein Phosphopeptide -Amorphous Calcium Phosphate; CP: Carbamide peroxide; HP: Hydrogen peroxide; NS: Not studied.

Table 2. General characteristics from eligible *in vitro* studies.

Author/ year (Country)	Design Study (Sample Type)	G1 - Bleaching gel based-calcium G2 – Bleaching gel conventional (N sample)	Bleaching Protocol	Assessment Performed	Results: Outcomes [Mean ± SD] or percentage or [Mean (minimum and maximum value)]	Results - Morphology Enamel (Qualitative analyses)	Conclusion	Intervention Effect
Centenaro et al., 2024 (Brazil)	*In vitro* (Human enamel)	G1.1: HP 35% + calcium G1.2: HP 35% + 3% CaGlu G2: HP 35% n/per group: 8	Bleaching gel in an attachable syringe was applied using a brush tip, starting from the cervical region and spreading uniformly. Each session lasted 40 min, with a one-week interval between the two sessions.	Color analysis, HP penetration into the pulp chamber	G1.1 / G1.2 / G2 HP Diffusion (µg/mL) *Tip without Brush* 0.48 ± 0.16 / 0.16 ± 0.07 /0.25 ± 0.08 *Tip with brush* 0.23 ± 0.08 / 0.07 ± 0.06 / 0.14 ± 0.06 Color Change (ΔE*) *Tip without Brush* 2.7 ± 1.3 / 4.2 ± 1.6 / 4.0 ± 1.0 *Tip with brush* 2.8 ± 1. 0 / 4.5 1.5 / 3.8 ± 1.1	NS	The addition of calcium in the bleaching agent does not affect aesthetic effectiveness and reduces the concentration of diffuse hydrogen peroxide in the pulp chamber	Positive
Garcia et al., 2024 (Brazil)	*In vitro* (Bovine enamel)	G1.1: HP 35% + 0.25% CaGP G1.2: HP 35% + 2% CaGlu G2: HP 35% n/per group: 12	Gels were applied for 40 min, in 3 whitening sessions (7-day intervals) conducted weekly. Enamel specimens were stored in containers with artificial saliva (2 ml) at 37°C between sessions.	Enamel surface hardness analysis; Enamel surface Roughness Analysis; Integrated Hardness Area Analysis; SEM.	G1.1 / G1.2 / G2 Roughness: 0.05 ± 0.01 / 0.061 ± 0.01 /0.06 ± 0.01 Surface Hardness: 297.5 ± 2.1 / 290.1 ± 1.4 / 285.0 ± 2.2 Cross sectional Hardness (KHN x µm) 18,787.8 ± 1202.0 / 18,789.3 ± 1357.2 / 18,754.2 ± 1269.9	Post-treatment, all groups showed varying degrees of enamel surface changes such as depressions and fissures. The HP and CaGlu groups exhibited exposed enamel prisms resembling fish-scale or honeycomb structures.	It can be concluded that CaGP with the 35% HP gel significantly changed tooth enamel demineralization in terms of surface, roughness, and enamel morphology.	Positive
Balkaya et al., 2024 (Turkey)	*In vitro* (Human enamel)	G1.1: HP 35% + 1% CPP-ACP G1.2: HP 35% + 3% CPP-ACP	The bleaching gels were applied using an applicator following a 20-minute application	Evaluation of color measurements; Determination of surface hardness	G1.1 / G1.2 / G2 Color change ΔE: 4.5 ± 2.66 / 2.83 ± 0.9 / 4.31 ± 2.98 Vicker's microhardness (VHN) (Before/After bleaching) G1.1: 362.5 ± 122.7 / 364 ± 56.1	SEM micrographs showed a significant accumulation of CPP-ACP on the	The addition of 1% CPP-ACP to the bleaching agent had positive effects	Positive

		G2: HP 35% n/per group: 8	period, the existing bleaching agents were removed with a highly absorbent suction cup, and a second application was performed for another 20 minutes (20 x 2 min [40 min]).	changes (Vicker's mocrohardness (VHN); changes in enamel mineral content (EDX) and evaluation of enamel surface structure (SEM).	G1.2: 312.8 ± 30.3 / 385 ± 73.9 G2: 354.7 ± 54.7 / 191.3 ± 68.7 Elemental content values (%) *Carbon:* 4.1 ±2.4 / 5.8 ±2.7 /10.3± 7.8 *Nitrogen:* 3.1 ± 2 / 3.6 ± 0.4 /3.6 ± 0.8 *Oxygen:* 37.7 ± 7 /36.2 ± 2 /34.9 ± 1.7 *Phosphorus:* 16.8 ±2/18.4 ±0.7/15.7 ±2 *Calcium:* 38.4 ±0.8 /36 ± 1.5 /35.6 ± 5	surface after the application of bleaching agents containing 1% and 3% CPP-ACP. This accumulation was more intense in the 3% CPP-ACP group (G1.2).	on the mineral content and surface hardness of the enamel, and did not negatively affect the whitening effectiveness.	
Ortiz et al., 2024 (Brazil)	*In vitro* (Bovine enamel and dentin discs)	G1.1: HP 35% + 0.5% calcium-polyphosphate + water based G1.2: HP 35% + 0.5% calcium-polyphosphate + tris-based G2: HP 35% n/per group: 10	Specimens were submitted to three weekly bleaching sessions, each consisting of three 15-minute applications. Specimens were stored in artificial saliva at 37°C during and up to 14 days post-treatment.	Color (ΔE, ΔE00, ΔWID), Surface microhardness (SMH) and Cross-Sectional Microhardness (CSMH) assessments.	G1.1 / G1.2 / G2 Surface hardness 370 ± 10.6 /~375 ± 12.7/~ 358 ± 8.5 Cross-sectional hardness Distance (µm) *10:* 261.5 ± 44 /268 ± 41/229.2 ± 48 *20:* 327.2 ± 29 /338.7 ± 44/299.1 ± 42 *40:* 352.0 ± 26 / 35.9 ± 36 /343.4 ± 25 *60:* 359.0 ± 23/359.2 ± 37/352.9 ± 26 *80:* 352.4 ±18 /362.2 ±36 /345.5± 29 *100:* 359.7 ±18/367.3 ±40 /346.5 ± 30 *120:* 369.5 ±19/ 367.0 ±39 /354.9 ±25 *140:* 370.3 ± 15/368.8 ± 48/342.7 ±31 *160:* 364.6 ± 18/ 370.2 ±36/350.0 ±39 *180:* 369.9 ±23 /365.9 ±36 /348.3± 38 Color: No statistically significant differences were observed between the gels.	NS	ALP activity was highest at pH 9, reduced in G2, and stable in Tris or water. Also, similar color changes (ΔE; ΔE00; ΔWID) and Surface Microhardness (SMH) were observed in all groups.	Positive
Dos Anjos et al., 2024 (Brazil)	*In vitro* (Bovine enamel and dentin blocks)	CP 10% G1.1: 10% CP + 0.5% Calcium polyphosphate sub-microparticles (CaPPs) G1.2: CP 10%+ 1.5% Calcium polyphosphate sub-microparticles G2: CP 10% HP 6%	Two-week home bleaching with bleaching gel applied to the tooth surface for 4 hours (CP groups) or 2 hours (HP groups) as per manufacturer recommendations. Between applications, all specimens were	Color analysis; pH; Surface micrroharness analysis (KHN); Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX).	G1.1 / G1.2 / G2 Color analysis *ΔE* 10% CP: 19.0 ± 4.0 / 19.7 ± 3.7 / 17.9 ± 4.1 6% HP: 20.2 ± 2.9 / 18.9 ± 2.3 / 18.1 ± 4.2 *ΔE_{00}* 10% CP: 12.8 ± 2.4 / 13.3 ± 2.3 / 12.1 ± 2.5 6% HP: 13.8 ± 1.7 / 12.8 ± 1.3 / 12.0 ± 2.7	SEM images showed a uniform surface in groups CP with 0.5% CaPPs, CP with 1.5% CaPPs, commercial HP, and HP with 1.5% CaPPs. While particle deposition was not confirmed, tissue preservation was more evident in these groups. In contrast, the	The incorporation of CaPPs in low concentration whitening gels reduces its negative effects on microhardness without interfering with their bleaching efficacy.	Positive

		G1.1: HP 6% + 0.5% Calcium polyphosphate sub-microparticles G1.2: HP 6% + 1.5% Calcium polyphosphate sub-microparticles G2: HP 6% n/per group: 24	stored in artificial saliva at 37°C.		*ΔWI$_D$* 10% CP: 27.2 ± 6.0 / 28.4 ± 5.6 / 23.6 ± 5.9 6% HP: 30.0 ± 4.7 / 27.1 ± 4.2 / 24.7 ± 8.3 Surface Microhardness (KHN) 10% CP: ~ 345 ± 17/ ~ 340 ± 28 / ~ 285 ± 21 6% HP: ~ 270 ± 28 / ~ 342 ± 31 / ~ 305 ± 35	commercial CP and HP with 0.5% CaPPs groups exhibited surface irregularities and increased porosity.		
Gruba et al., 2023 (Brazil)	*In vitro* (Bovine enamel and dentin discs)	G1: HP 35% + 2% CaGlu G2: HP 35%) n/per group: 12	Gels were applied to the enamel surface (0.04 mL) for 40 min, 3 bleaching sessions with 7-day intervals. The gels were removed with gauze, followed by washing with deionized water for 30 s. Between bleaching sessions, the discs were kept in 2 mL of artificial saliva and renewed every day.	Color alteration, surface and cross-sectional hardness , surface roughness, and HP diffusion. Enamel surfaces were evaluated by Scanning Electron Microscopy (SEM) and X-ray Dispersive Energy.	G1 / G2 Roughness: 0.154 ± 0.056 / 0.190 ± 0.058 Surface Hardness: 314.1 ± 6.6 / 312.1 ± 6.3 Integrated loss of subsurface hardness (ΔKHN) (KHN x µm) 18,241.2 ± 503.1 / 18,486.9 ± 491.9 Transamelodentinal diffusion of H_2O_2 (µg/mL): 7.42 ± 0.64 / 7.89 ± 0.47	The SEM images confirmed the results for the HP group (G2), showing greater demineralization and an increase in the number of striations, scratch marks, and pitting on the enamel surface, when compared to G1.	Gels containing CaGlu interfere with the bleaching effect and reduce enamel demineralization , roughness, H_2O_2 diffusion, and morphological changes, when compared to control group.	Positive
Ortiz et al., 2023 (Brazil)	*In vitro (*Human enamel/dentin spectimens)	G1.1: HP 35% + Calcium polyphosphate sub-microparticles 0.5% G1.2: HP 35% + Calcium polyphosphate sub-microparticles 1.5% G2.1: HP 35% (Whiteness-HP-Maxx) G2.2: HP 35% experimental n/per group: 24	Bleached groups underwent three weekly sessions, each with three 15-minute applications. Specimens cleaned and non-bleached areas protected. 0.03 g bleaching agent applied, kept moist at room temperature. Gel removed with gauze, rinsed with distilled water. After final application,	Spectro-photometer; SEM; EDX; the color (ΔE/ΔE$_{00}$) and bleaching index (ΔWI$_D$); surface roughness; microhardness (KHN) and Raman spectroscopy.	Colo/r variation *[Mean (*minimum and maximum value)] *ΔE (3rd session)* G1.1: 7.04 (6.06; 8.80) G1.2: 6.92 (4.78; 10.48) G2.1: 7.77 (5.68; 9.30) G2.2: 6.24 (3.89; 8.02) *ΔE$_{00}$ (3rd session) / ΔE$_{WID}$* G1.1: 3.73 (3.17; 4.54) / 9.24 (5.00; 13.55) G1.2: 3.68 (2.36; 5.43) / 9.74 (5.13; 13.84) G2.1: 4.02 (3.01; 5.59) / 9.13 (5.21; 14.56) G2.2: 3.50 (2.04; 4.34) / 8.60 (2.53; 12.47)	The SEM images show the experimental bleaching gel caused the most significant enamel alterations.	In human enamel, CaPP did not alter the bleaching effectiveness or roughness, and additionally, CaPP containing gels increased the microhardness and preserved the mineral content when compared to the	Positive

			specimens rinsed, dried, stored in 1.5 mL artificial saliva at 37°C, with daily renewal for 14 days post-treatment.		*Roughness values Ra* *(3rd session)* G1.1: 0.026 (0.018; 0.035) G1.2: 0.024 (0.016; 0.036) G2.1: 0.023 (0.015; 0.040 G2.2: 0.023 (0.013; 0.053) *Microhardness (KHN)* *3rd session* G1.1: 373.48 (346,09; 402,35) G1.2: 365.33 (292,91; 393,61) G2.1: 358.33 (318,69; 379,44) G2.2: 330.02 (252,89; 373,44) *Ca (at%), P (at%), and Ca:P ratio* G1.1: 65.32 / 34.68 / 1.88 G1.2: 65.60 / 34.40 / 1.91 G2.1: 65.39 / 34.28 / 1.91 G2.2: 65.02 / 34.98 / 1.86		experimental without CaPP.	
Silva et al., 2023 (Brazil)	*In vitro* (Human teeth)	G1.1: HP 35% + Calcium G1.2: HP 35% + 3% CaGlu G2: HP 35% n/per group: 8	Gel applied for 30 min, one session removed, area washed.	HP diffusion into the pulp chamber; color change evaluation	G1.1 / G1.2 / G2 ΔE_{ab} 4.4 ± 1.3 / 6.9 ± 1.2 / 5.5 ± 1.1 ΔE_{00} 2.8 ± 0.8 / 4.7 ± 1.1 / 3.1 ± 0.9 H_2O_2 Diffusion ~ 0.45 ± 0.04 / ~ 0.2 ± 0.05 / ~ 0.5 ± 0.1	NS	A single application was able to produce a bleaching efficacy. Caglu into gel reduces the HP diffusion into the pulp chamber.	Positive
Júnior et al., 2022 (Brazil)	*In vitro* (bovine teeth)	G1: HP 35% + 2% CaGlu G2: HP 35% n/per group: 30	Bleaching gels (0.04 mL) applied to enamel surface for 40 min. Three sessions over 14 days at 7-day intervals. Discs stored in 2 mL artificial saliva, renewed daily.	Color alteration analysis; surface and cross-sectional hardness analysis; determination of trans-amelodentinal diffusion of H_2O_2.	G1 / G2 Surface Hardness: 286.0 ±7.3 /290.1 ±9.2 Percentage of loss surface hardness (%SH): -21.4 ± 1.5 / -20.1 ± 1.6 Cross sectional Hardness (KHN x µm) 17,062.8 ± 784.9 / 18,019.1 ± 1,174.8 H_2O_2 diffusion (µg/mL) 6.75 ± 0.33 / 6.56 ± 0.33	NS	It is possible to conclude that the addition of gluconate calcium in the in-office bleaching agent did not interfere with the bleaching and presented an effect similar in	Null

							enamel microhardness.	
Yang et al., 2022 (Republic of Korea)	*In vitro* (bovine teeth)	G1.1: HP 35% + 0.25% hydrated calcium silicate G1.2: HP 35% + 0.5% hydrated calcium silicate G1.3: HP 35% + 1% hydrated calcium silicate G1.4: HP 35% + 2% hydrated calcium silicate G2: HP 35% n/per group: 10	Tooth specimen covered with experimental solution for 15 min repeated three times Surface rinsed with distilled water and dried for 10 s with air spray. Treatment repeated three times with freshly prepared solution.	XRD; SEM; EDS; pH change analysis; Calcium ion release; Color measurement; Microhardness analysis.	Color analysis ΔE^* G1.1: 21.84 ± 2.12 G1.2: 22.76 ± 2.70 G1.3: 21.63 ± 2.33 G1.4: 20.22 ± 2.20 G2: 22.73 ± 3.54 Percentage of microhardness loss (%) G1.1: ~ 15 G1.2: ~ 2.5 G1.3: ~ + 8 G1.4: ~ +19 G2: ~ +18	Surfaces treated with G2 exhibited weakly polished, rough patterns, whereas those treated with G1 groups displayed polished patterns without erosion. G1.4 treatment left aggregated particles identified as Ca and Si on the enamel.	This study suggests that all groups containing HP calcium silicate (hCS) is effective in bleaching efficacy. In addition, hCS could also minimize the microhardness loss of tooth structure caused by HP and maintain enamel surface morphology.	Positive
Pini *et al.,* (2022) Brazil	*In vitro* (Bovine teeth)	G1: HP 35% + 2% CaGlu G2: HP 35% n/per group: 10	The gel was applied 3x 15 min, each time, totaling 45 min. The specimens were kept in remineralizing solution, simulating artifcial saliva at 37 °C, for 24 h before and 24 h after the bleaching session.	Color measurement (refectance spectrophotometer); Surface roughness (contact proflometer); Surface microhardness (Knoop microhardness tester);	Microhardness (KHN) G1: 336.8 ± 3.95 G2: 322.1 ±3.53 Color analysis ΔE^* G1: 3.58 ± 0.82 G2: 4.61 ± 0.73 Surface Roughness G1: 0.013 ± 0.01 G2: 0.034 ± 0.01	Slight surface changes were observed in all groups, with the most pronounced changes in Group 2. Group 1 showed intermediate surface changes, while Group 2 exhibited a demineralization pattern, including pores and depressions.	Although commercial gels containing calcium decreased the negative impact on the evaluated properties of teeth	Positive

				Scanning electron microscopy (SEM)				
Júnior et al., 2021 (Brazil)	*In vitro* (Enamel bovine teeth)	G1: HP 35% + 2% CaGlu G2: HP 35% n/per group: 10	Bleaching gels were applied for 40 min, in 3 bleaching sessions, interval 7 days. Blocks were stored in artificial saliva (5 mL) at 37 °C. During the bleaching the samples were or were not exposed to fluoride dentifrice: ND - *No treatment with fluoride dentifrice; TD - Treatment with fluoride dentifrice*	Enamel hardness analysis (SHf, %SH, KHN x µm)	G1 / G2 Final Surface Hardness (SHf) *ND:* 256.4 ± 19.2 / 270.5 ± 6.1 *TD:* 287.9 ± 9.8 / 285.0 ± 5.7 Percentage of loss surface hardness (%SH) *ND:* -25.5 ± 4.9 /-20.4 ± 2.0 *TD:* -15.2 ± 2.9 / -16.0 ± 3.2 Cross sectional Hardness (KHN x µm) *ND:* 52,750.4 ± 870.6 / 53,384.4 ± 2,142.3 *TD:* 56,369.7 ± 895.2 / 57,081.8 ± 2,272.2	NS	The addition of CaGlu to a 35% HP (G1) bleaching gel no reduced the mineral loss compared to 35% HP (G2)	Null
Felipe Akabane et al., 2021 (Brazil)	*In vitro* (Enamel/dentin bovine teeth)	G1: HP 35% + 2% CaGlu G2: HP 35% n/per group: 12	After pigmentation, whitening gels (0.04 mL) applied to enamel for 40 min. Over 21 days, 3 whitening sessions at 7-day intervals. Disks stored between sessions in individual plastic containers with 2 mL artificial saliva, renewed daily.	Color alteration analysis; Surface and cross-sectional hardness analysis; Determination of H_2O_2 trans-amelodentinal penetration;	G1 / G2 Surface Hardness (SH) 287.6 ± 3.1 / 294.0 ± 5.1 Percentage of loss surface hardness (%SH): -21.6 ± 1.1 / -19.7 ± 1.0 Cross sectional Hardness (KHN x µm) 51,455.5 ± 1,979.2 / 55,035.8 ± 2,678.7 H_2O_2 diffusion (µg/mL) 10.01 ± 0.26 / 8.83 ± 0.64	NS	It is possible to conclude that the addition of CaGlu (G1) to the in-office bleaching agent did not interfere with the bleaching process but resulted in higher losses in enamel microhardness and trans-amelodentinal penetration of	Negative

							H_2O_2 compared to G2.	
Limas et al., 2020 (Peru)	*In vitro* (Bovine enamel)	G1: HP 35%+ 2% CaGlu G2: 35% HP n/per group: 15	The teeth were bleaching in one session (3x15 min). Gel applied 0.5-1 mm thick. The specimens were stored in artificial saliva	Microroughness Assessment	Microroughness Assessment G1: 0.19 ± 0.05 / G2: 0.18 ± 0.02 Microroughness *(Tooth surface) - (MV, V, DV)* *MV / V / DV* G1: 0.18 ±0.04 / 0.23±0.13 / 0.17±0.01 G2: 0.17 ±0.05 / 0.17±0.02 / 0.18±0.01	NS	There were no significant differences in surface microroughness on comparing the Whiteness HP Maxx group with the control group and the Whiteness HP Blue group.	Null
Barbosa et al., 2020 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 35% + CPP-ACP at proportion 1:1 G2: HP 35 % n/per group: 10	The teeth were bleaching in one session (30 min). Gel applied 0.04 mL. The specimens were stored in artificial saliva.	Color alteration analysis and H_2O_2 transamelodentinal penetration	Immediate / 24h Color Change G1: 3.44 ± 0.69 / 3.80 (3.28–4.66) G2: 3.19 ± 0.41 / 3.77 (2.26–4.79 Transamelodentinal diffusion of H_2O_2 (µg/mL) G1: 1.82 (1.26–2.31) G2: 3.11 (2.56–3.85)	NS	The mixture of MI Paste Plus and bleaching gel reduces H_2O_2 penetration and maintains bleaching effectiveness.	Positive
Vieira et al., 2020 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 35% + 2% CaGlu G2: HP 35% n/per group: 12	G1: Two sessions with an interval of 7 days. In each session, the gel was applied once for 40 min G2: Two sessions with an interval of 7 days. In each session, the gel was applied three times, each for a duration of 15 min (45 min total),	Color Measurements Microhardness (KHN) Surface Roughness (Ra)	Color Change G1: 5.0 ± 1.4 G2: 5.3 ± 1.1 Microhardness (KHN) G1: 344.6 ± 27.5 G2: 303.3 ± 36.1 Roughness Variation G1: 0.018 ± 0.022 G2: 0.016 ± 0.008	NS	The presence of Ca in bleaching gels did not interfere with bleaching efficacy. However, only the enamel exposed to the bleaching gel containing Ca obtained microhardness	Positive

							values similar to unbleached enamel.	
Moura et al., 2019 (Brazil)	*In vitro* (Bovine enamel)	G1.1: CP 10% + Calcium G1.2: CP 10% + ACP G2: CP 10% n/per group: 12	The bleaching agent was applied on the enamel surface (1 mm thickness) for 6 h/day at 37°C ± 1°C, after individually stored in artificial saliva. The bleaching treatments were performed for 21 consecutive days.	Microhardness (KHN); Ca/P ratio. Micro energy-dispersive X-ray fluorescence spectrometry (μ-EDXRF).	Microhardness (KHN) Baseline / Final G1.1: 221.7 ± 11.4/ 116.6 ± 6.9 G1.2: 217.6 ± 7.3 / 109.2 ± 12.5 G2: 223 ± 8.2 / 113.5 ± 7.7 Ca/P ratio G1.1:1.91 ± 0.06 G1.2: 1.97 ± 0.09 G2: 1.96 ± 0.04	NS	Calcium into 35% HP gel reduced the peroxide diffusion through the tooth structure and the negative effects over the microhardness without interfering with bleaching efficacy.	Positive
Torres *et al.,* 2019 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 35% + 0.5% CaGlu G2: HP 35% n/per group: 20	2-mm layer of the respective bleaching gel was applied on the enamel surface for 30 min (3x10 min).	Microhardness Measurement (KHN). Colorimetric reflectance spectrophotometer.	G1 / G2 Hardness: 190.4 ± 38.2 /185.8 ± 47.2 HP Diffusion: 0.49 ± 0.67 / 0.49 ± 0.66 Color Change Δ*E:* 4.3 ± 0.71 / 4.2 ± 0.7	NS	The addition of calcium in the gel did not affect bleaching efficacy, peroxide diffusion and the bleached enamel hardness loss.	Null
Kutuk et al., 2018 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 38%: 1.5 mL of bleaching agent was mixed + 0.5 mL CPP-ACP G2: HP 38% n/per group: 13	Three 15-min applications were performed for each specimen, consecutively. Single session and after application of bleaching and	Vickers hardness (VHN) Scanning Electron Microscopy (SEM). Energy dispersive	Vickers hardness (VHN) 1 /14 days G1: 453.4 ±117.8 / 492.4 ± 72.1 G2: 325.6 ± 95.0 9 / 420.4 ± 110.4 Ca, P and F B1/B14 days G1 Ca: 67.3 ± 2.9 / 69.4 ± 19	No changes were observed on the enamel surfaces in the test groups at any evaluation time.	The use of desensitizing agents containing CPP-ACP, after in-office bleaching or mixed in bleaching agent	Positive

			desensitization procedures, the samples were stored in freshly prepared artificial saliva for 14 days 13. An artificial saliva solution was changed daily.	spectrometry (EDS) microhardness loss (PML) Calcium (Ca), phosphorous (P) and fluoride (F) were measured.	P: 30.7 ± 15 / 30.7 ± 13 F: 2 ± 2.8 /1.2 ± 3.5 Ca/P: 2.2 ± 0.1 /2.4 ± 0.2 G2 Ca: 66.7 ± 2.9 /67.5 ± 2.3 P: 29.5 ± 2 / 29.4 ± 3.6 F: 1.5 ± 4 /1.5 ± 4 Ca/P: 2.2 ± 0.1 /2.4 ± 0.2 Percentage of microhardness loss (PML) 1 day and 14 days G1/G2 G1: -10.2 / -19.6 G2: 22.1 / -0.6 Color differences among the 1 and 14days (*ΔE**) G1: 14.7 ± 7.4 G2: 12 ± 12.9		did not inhibit the bleaching effect. However, they all recovered microhardness of enamel 14 days after in-office bleaching.	
Cavalli *et al.,* 2018 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 35% + 0.2% Calcium G2: HP 35% n/per group: 20	Sound and demineralized enamel were treated with bleaching agents, being applied for 15 min, repeated 3 times, two bleaching sessions (Interval 7 days). All the samples were stored in in remineralizing solution between procedures.	Fourier Transform Raman spectroscopy (FT-Raman); X-ray fluorescence spectroscopy (μ-EDXRF); Cross-sectional microhardness (CSMH); Polarized light microscopy (PLM).	G1/G2 Sound enamel Cross-sectional microhardness: Enamel mineral loss area (ΔZ) 509.4 ± 45.7 / 565.3 ± 87.2 Phosphate (PO_4^{3-}) peak v2 (431–449 cm^{-1}) / peak v4 (582–611 cm^{-1}): G1: 6.89 ± 0.97 / 6.72 ± 0.87 G2: 3.57 ± 0.33 / 2.89 ± 0.55 Carbonate (CO_3^{2-}) peak v3 (1070 cm^{-1}) 6.55 ± 0.89 / 3.13 ± 0.24 Ca/P ratio: 2.32 ± 0.07 / G2: 1.67 ± 0.05 Demineralized enamel Cross-sectional microhardness: Enamel mineral loss area (ΔZ) 868.8 ± 69.6 / 962.6 ± 121.8 Relative areas of phosphate (PO_4^{3-}) peak v2 (431–449 cm^{-1}) / peak v4 (582–611 cm^{-1}): G1: 6.57 ± 0.12 / 5.89 ± 0.58 G2: 4.79 ± 0.41 / 4.55 ± 0.31	Polarized light microscopy (PLM): Sound enamel: Calcium group showed subsurface demineralization, but the mineral loss pattern denoted an intermediary phase compared to control group. Demineralized enamel: Control group showed a slightly more prominent demineralization than the calcium group, and calcium group presented	Even though experimental bleaching agents with Ca reduced mineral loss for both sound and demineralized enamel surfaces, these agents were unable to reverse the enamel subsurface demineralization.	Positive

					Relative areas of carbonate (CO_3^{2-}) peak v3 (1070 cm^{-1}) 5.38 ± 0.88 / 4.11 ± 0.31 Ca/P ratio: 1.68 ± 0.09 /1.99 ± 0.04	that the top enamel surface was not as demineralized as the enamel subsurface.		
Furlan *et al.,* 2017 (Brazil)	*In vitro* (Bovine enamel	G1: HP 35%+ 2% CaGlu G2: HP 35% n/per group: 10	Three sessions: G1: 40 min (each session); G2: 15 min × 3 = 45 min; Teeth stored in artificial saliva during the protocol.	Knoop microhardness (KHN)	Microhardness (KHN) G1: 335 ± 71 G2: 301 ± 50	NS	Microhardness values decreased over time with the addition of calcium in gel.	Positive
Moreira *et al.,* 2017 (Brazil)	*In vitro* (Bovine enamel	G1: HP 35% with 2% CaGlu G2: 3 HP 35% n/per group: 5	G1: 3x15 min, G2: 45 min. 5 sessions were held for each group, and the specimens were stored in ultra-pure water between sessions.	The technique of XRF (x-ray fluorescence.)	Intensity of calcium in the groups (intensity relative media) G1 / G2 Session 1: 1.01 /1.00 Session 2: 1.02 / 1.00 Session 3: 0.99 / 0.99 Session 4: 0.99 / 1.00 Session 5: 1.02 / 0.99	NS	The whitening gels showed no potential of modifying the content of calcium and phosphate of human dental enamel.	Null
Santos *et al,* 2016 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 7.5 % + CaGlu 0.5% G2: HP 7.5 % n/per group: 20	A 2 mm layer of gel was applied daily for 1 h, after washed and placed in artificial saliva. Subsequently, specimens underwent cyclic erosive challenges, being immersed in unstirred soft drink four times daily for 2 min each.	Profilometric contact (wear)	After 7 days/ After 14 days Surface Loss (um) G1:3.3 ± 0.3 / 6.7 ± 1.0 G2:4.1 ± 0.5 / 10.3 ± 1.5	NS	The supplementation of bleaching gels with calcium has been shown to reduce the susceptibility of bleached enamel to erosion, especially when considering the cumulative detrimental effect of HP	Positive

Borges *et al.,* 2016 (Brazil)	*In vitro* (Bovine enamel and dentin specimens)	G1: HP 7.5 % + CaGlu 0.5% G2: HP 7.5% n/per group: 10	A 2 mm thick layer of bleaching gel was applied daily to the specimens for 1 h. Afterward, the gel was removed and the surface rinsed with ultrapure water for 20 s.	Contact profilometry	G1 / G2 Surface Roughness of enamel (Ra): Immediately after bleaching High abrasive: 0.088 ± 0.038 / 0.090 ± 0.035 Low abrasive: 0.043 ± 0.03 / 0.05 ± 0.025 1 h after bleaching High abrasive: 0.07 ± 0.027 / 0.075 ± 0.035 Low abrasive: 0.041 ± 0.017 / 0.048 ± 0.01	NS	Supplementation of bleaching gel with calcium and delaying brushing for one hour after bleaching reduced surface loss	Positive
Khoroushi *et al.,* 2016 (Iran)	*In vitro* (Human molars enamel)	G1: HP 40% + ACP G2: HP 40% n/per group: 12	The bleaching agents applied for 60 min. All the samples were stored in distilled water at 37°C between procedures. The procedure was repeated twice a week for 2 weeks.	Microhardness tester. Atomic force microscopy (AFM)	Microhardness (KHN): G1: 384.8 ± 22.6 G2: 299.0 ± 7.2	Atomic force microscopy (AFM): G1: The surface of specimens looked more prominent and denser. G2: AFM evaluation exhibited irregular surface topography	The incorporation of the ACP in the bleaching gel as remineralizing agent was effective in decreasing enamel microhardness change after in-office bleaching.	Positive
Moraes *et al,* 2015 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 35 % + CaGlu 2%: G2: HP 35%: n/per group: 10	The bleaching protocol consisted of three sessions, each lasting 40 minutes, with one-week intervals between sessions. The specimens were stored in artificial saliva and replenished daily.	Color analysis; Energy-Dispersive X-Ray Spectroscopy (EDX); Scanning Electron Microscopy (SEM)	Percentage changes in Ca/P G1: 100% / 98% G2: 100% / 95% Total color change (ΔE) G1: ~ 32 ± 8.5 G2: ~ 25 ± 4.2	Both groups exhibited similar alterations on their enamel surfaces, including increased surface porosity, an etching-like appearance, and enamel pitting erosion with secondary pitting occurring inside cavities. These changes alternated	Adding calcium to a 35% HP-based bleaching agent did not contribute to the prevention or reduction of morphological changes nor enhance calcium concentration on bleached enamel surface.	Null

						with areas devoid of any cavities.		
Khoroushi *et al,* 2015 (Iran)	*In vitro* (Human teeth)	G: HP 40% + ACP G2: HP 40% n/per group: 12	The bleaching agents applied for 60 min. The procedure was repeated twice a week for 2 weeks. Samples were stored in distilled water at 37°C	Surface Roughness (Atomic force microscopic)	Surface roughness (Ra): Post-treatment G1: 19.9 ± 10.1 G2: 31.4 ± 14.0	NS	The bleaching procedures have a detrimental effect on enamel surface roughness which can be minimized by the subsequent use of, ACP, powders as remineralizing agent.	Positive
Basting *et al.,* 2015 (Brazil)	*In vitro* (Human molars enamel)	G1: HP 35%+ CaGlu 2% G2: HP 35% n/per group: 10	G1: The gel (approximately 1 mm thick) was applied to the buccal, lingual, and proximal surfaces of the teeth for 40 min. Subsequently, the teeth were individually placed in containers with artificial saliva. This protocol was conducted once a week for three weeks.	Calcium and phosphorus concentrations (µg/mL) for enamel specimens by colorimetric test.	G1/G2 Calcium Concentrations 1st application: 7.8 ± 1.9 / 7.5 ± 2 2nd application: 8.2 ± 4.9 / 7.8 ± 7.2 3rd application: 6.9 ± 1.2 / 7.2 ± 1.9 7d post treatment: 2.7 ± 1.7 / 2.7 ± 1 14d post treatment: 5.6 ± 1.9 / 5.0 ± 2.2 Phosphorus Concentrations Baseline: 1.5 ± 0.4 / 1.6 ± 1.0 1s application: 1.8 ± 0.3 / 2.0 ± 0.4 2nd application: 2.3 ± 0.7 / 2.0 ± 0.4 3rd application: 0.5 ± 0.7 / 1.7 ± 0.3 7 d post treatment: 1.5 ± 0.4 / 1.5 ± 0.4 14d post treatment: 1.8 ± 0.2 / 1.8 ± 0.3	NS	The levels of calcium and phosphorus concentration on the enamel surfaces of extracted teeth treated with 35% HP containing 2% CaGlu showed no significant difference compared to teeth exposed to	Null

			G2: The bleaching protocol was consistent with that used in Group 1, but the gel was applied for 15 minutes				35% HP without CaGlu.	
Mena-Serrano *et al.,* 2015 (Brazil)	*In vitro* (Human teeth)	G1.1: HP 35 % + CaGlu 2% G1.2: HP 20 % + CaGlu 2% G2.1: HP 35 % G2.2: HP 20 % n/per group: 10	G1.1: A single 40-min application. G1.2: A single 40-minute application. G2.1: 3 x 15 min each G2.2: 3 x 15 min each	HP penetration into the pulp	HP penetration into the pulp HP Concentration (µg/mL) G1.1: 0.20 ± 0.18 /G1.2: 0.11 ±0.08 G2.1: 1.15 ± 0.33 /G2.2: 0.94 ± 0.48 HP Weight (µg/mL) G1.1: 0.64 ± 0.55 / G1. 2: 0.66 ±0.98 G2.1: 3.46 ± 1.01 / G2.2: 3.25 ± 1.17	NS	The calcium-containing bleaching gel resulted in lower penetration of HP into the pulp compared to the bleaching gel containing only 35% HP.	Positive
Rauen *et al,* 2015 (Brazil)	*In vitro* (Human enamel)	G1: HP 35 % + CaGlu 2% G2: HP 35 % n/per group: 18	G1: The bleaching gel was applied in one session, with each application lasting 45 min. G2: The bleaching gel was applied in three sessions, with each session lasting 3x 15 min.	Digital roughness meter; Vickers microhardness test; permeability test	Baseline / post-treatment Surface Roughness (Ra): G1: 0.3 ± 0.0 / 0.4 ± 0.0 G2: 0.2 ± 0.1 / 0.4 ± 0.0 Microhardness (KHN): G1: 342.1 ± 34.1 / 258.1 ± 34.6 G2: 413.1 ± 74.6 / 369.7 ± 47.1 Permeability degree (median) G1:1.5 / G2: 3	NS	All bleaching agents decreased microhardness, and increased permeability and surface roughness of human enamel; thus, the addition of calcium was not beneficial.	Null
Pizani *et al,* 2015 (Brazil)	*In vitro* (Human enamel)	6% HP G1: HP 6 % + calcium G2: HP 6 % 7.5% HP G1: HP 7.5 % + calcium G2: HP 7.5 % n/per group: 20	Specimens received bleaching agent application once daily for 1 h by 5 days. A calibrated syringe delivered 0.02 mL of each bleaching agent to the specimens, which were then rinsed with distilled	Micohardness tester. Scanning electron microscopy (SEM) analysis	Microhardness (KHN) Baseline / post-treatment HP 6% G1: 307.9 ± 25.7 / 95.9 ± 3.6 G2: 281.4 ± 21.5 / 87.5 ± 3.0 HP 7.5% G1: 272.3 ± 18.2 / 110.9 ± 7.4 G2: 309.8 ± 13.7 / 85.0 ± 2.7	SEM: The images reveal structural changes on the enamel surface following the application of bleaching agents. Erosions and discontinuities were observed on the enamel surface in all groups.	All bleaching gels resulted in a decrease in hardness of human dental enamel. However, dental bleaching agents containing calcium led to a lesser loss of microhardness.	Positive

			deionized water to remove the agents.					
Sasaki *et al,* 2015 (Brazil)	*In vitro* (Human enamel)	G1: HP 7.5 % + ACP G2: HP 7.5 % n/per group: 10	A calibrated syringe delivered 0.02 ml of bleaching gel onto the dental specimens. Time-points: 7, 14, 21, 24, and 35 days. The specimens were subsequently stored in 1.5 mL of artificial saliva.	Microhardness tester; Reflectance spectrophotometer; mechanical profilometer; Scanning electron microscopy (SEM)	G1 / G2 Microhardness (KHN) 7d: 258.8 ± 26.8 / 292.7 ± 19.8 14d: 267.1 ± 36.5 / 276.1 ± 54.4 21d: 233.6 ± 26.6 / 288.4 ± 65.7 28d: 276.1 ± 37.0 / 306.5 ± 15.8 35 d: 311.2 ± 22.3 / 321.0 ± 12. Color change: After 7d: 7.5 ± 1.8 / 7.9 ± 2.9 After 14d: 8.4 ± 2.3 / 11.0 ± 2.8 After 21d: 8.03 ± 1.4 / 10.7 ± 2.1 After 28d: 9.3 ± 1.7 / 9.5 ± 1.6 After 35d: 9.2 ± 1.6 / 10.8 ± 2.6 Surface Roughness (Ra): After 7 days: 0.4 ± 0.0 / 0.3 ± 0.0 After 14 days: 0.4 ± 0.0 / 0.3± 0.0 After 21 days: 0.4 ± 0.0 / 0.3 ± 0.0 After 28 days: 0.4 ± 0.0 / 0.3 ± 0.0 After 35 days: 0.3 ± 0. / 0.3± 0.0	Scanning Electron Microscopy (SEM): Micromorphological changes such as irregularity and porosity presence and deposition of crystalline structures on the enamel surface.	The inclusion of ACP in the whitening gel diminishes the enamel's KHN and surface roughness post tooth whitening, showing similar effects to the group that solely received hydrogen peroxide.	Null
Alexandrino *et al,* 2014 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 35% + CaGlu 2% G2: HP 35 % n/per group: 5	G1: Gel applied for 40 min, for 3 sessions spaced 7 days apart. Specimens were stored in artificial saliva at 37°C. The saliva was replenished daily. G2: Gel was applied 3x15 min each session, following the same protocol as in group 1.	Future Tech Microdurometer; Chroma Meter CR-400 Colorimetry. Scanning electron microscopy (SEM)	Baseline /post-treatment Microhardness (KHN): G1: 344.5 ± 10.1 / 333.8 ± 3.6 G2:355.0 ± 13.6 / 328.8 ± 7.4 Color change (ΔE*) G1: 11.3 ± 2.0 G2: 9.3 ± 2.9	The SEM morphological analysis reveals the preservation of surface morphology in both groups.	This study found that adding 2% calcium to 35% HP was able to prevent changes in enamel hardness and morphology without reducing bleaching efficacy.	Positive

Borges *et al,* 2014 (Brazil)	*In vitro / in situ* (Bovine enamel)	G1: HP 35 % + CaGlu 2% G2: HP 35 % n/per group: 30	The resulting mixed gel was applied to the enamel surface for 50 minutes at room temperature. The bleaching procedure was conducted ex-vivo in a single session.	Microhardness tester	Microhardness (KHN) *Sound enamel* Post- Bleaching: G1: 305.3 ± 39.6 / G2: 256.7 ± 42.7 After 1 day: G1: 297.5 ± 25.0 / G2: 296.7 ± 49.7 After 7 days: G1: 320.9 ± 54.8 / G2:319.2 ± 46.3 *Demineralized enamel* Post- Bleaching: G1: 197.3 ± 49.5 / G2: 162.7 ± 18.0 After 1 day: G1: 207.9 ± 53.0 / G2: 186.3 ± 43.2 After 7 days: G1: 210.3 ± 45.8 / G2: 228.0 ± 30.6	NS	As a conclusion, the addition of calcium in to bleaching gel should be encouraged as they might play an important role in maintaining the enamel microhardness or inducing remineralization of artificially demineralized bovine enamel right after bleaching procedures with 35% HP gel.	Positive
Borges *et al.,* 2013 (Brazil)	*In vitro* (Bovine enamel)	HP 35% G1: HP 35% + 2% CaGlu G2: HP 35%: HP 7.5 % G1: HP 7.5% + 2% CaGlu G2: HP 7.5 % n/per group: G1: 18; G2: 54	In the at-home bleaching technique (7.5% HP), a 2 mm gel layer was applied to the enamel for 1 hour daily over 14 days. In the in-office technique (35% HP), the same amount of gel was applied for 50 min at 37°C, followed by immersion in artificial saliva for 7 days and a second gel application session to simulate a two-session treatment.	Color change	Color change (ΔE) HP 35% G1: ~ 6.1 ± 2.7 G2: ~ 6.3 ± 2.4 HP 7.5 % G1: ~ 6.3 ± 2.5 G2: ~ 6.6 ± 2.4	NS	The incorporation of calcium into the bleaching gel containing 35% HP or 7.5% does not affect its whitening efficacy.	Positive

Machado *et al,* 2013 (Brazil)	*In vitro* (Bovine enamel)	G1: CP 16% + ACP G2: CP at 16% n/per group: 12	0.5 mm thick layer gels were applied constituting one cycle. Fourteen cycles were completed over a 15-day interval after the whitening treatment before bonding the brackets to the teeth.	Shear bond strength test	Shear bond strength (MPa): G1: 3.7 G2: 5.4	NS	There was a considerable reduction in bond strength in the groups. Both whitened groups failed to achieve a clinically efficient bond strength	Positive
D'arce *et al,* 2013 (Brazil)	*In vitro* (Bovine enamel and dentin)	G1: HP 35 % + CaGlu 2%: G2: HP 35 % n/per group: 5	A 2.0 mm and 3.5 mm thick layer of gel was applied for 40 min during each session, with three sessions conducted and a seven-day interval between each session.	Spectrophotometer – Color analysis	1st session / 2nd session / 3rd Session Color Change (ΔE*) 2.0 mm thickness: G1: 13.7 ± 3.7 / 2.7 ± 0.9 / 1.9 ± 0.2 G2: 9.1 ± 3.4 / 3.2 ± 0.8 / 2.4 ± 0.7 *3.5 mm thickness*: G1: 7.3 ± 0.8 / 1.4 ± 0.3 / 1.4 ± 0.2 G2: 7.6 ± 3.5 / 2.6 ± 1.1 / 2.1 ± 0.7	NS	The present study demonstrated that the whitening efficacy on the enamel surface did not depend on the bleaching agent's concentrations.	Positive
da Costa Soares *et al,* 2013 (Brazil)	*In vitro* (Bovine enamel)	G1: HP 35 % + CaGlu 2%: G2: HP 35 % n/per group: 30	The samples were fully covered with 0.05 ml of gel, forming a 1 mm thick layer. Applied for 40 min, then rinsed off with distilled water for 30 seconds. Two sessions were performed. Specimens were stored in daily replenished artificial saliva at 37°C.	Microhardness tester; Scanning electron microscopy (SEM)	Baseline / After 24 h / After 14 days Microhardness (VHN) G1: 355.7 ± 51.1 / 206.0 ± 53.2 / 198.0 ± 48.6 G2: 304.5 ± 36.6 / 311.6 ± 92.3 / 165.2 ± 45.7	G1: Calcium deposits could be observed after the bleaching treatment with calcium G2: Some depressions could be perceived samples previously bleached with both Whiteness HP	According to this in vitro study, 14 days after the bleaching treatment, both bleaching agents decreased the enamel microhardness and the values of initial microhardness. The gel containing calcium showed less loss of hardness.	Positive

Berger *et al,* 2012 (Brazil)	*In vitro* (Bovine enamel)	G1.1: HP 4 % + Calcium 0.05% G1.2: HP 7.5 % + ACP G2: HP 4 % n/per group: 17	G1.1 and G2: 0.1 ml of gel was applied daily for 8 h over 14 days at 37°C. G1.2: 0.1 ml of bleaching agent was applied twice daily for 30 min over 14 days. Slices were stored in artificial saliva at 37°C.	Universal testing machine: Elastic modulus (MPa)	24 h/7 days / 14 days Elastic modulus (MPa) G1.1: 2.5 ± 0.7 / 2.1 ± 0.4 / 2.7 ± 0.6 G1.2: 2.3 ± 0.8 / 2.2 ± 0.7 / 2.3 ± 1.0 G2: 2.0 ± 0.5 / 2.0 ± 0.4 / 2.5 ± 0.6	NS	The experimental groups showed similar post-treatment elastic modulus.	Null
Vasconcelos *et al,* 2012a (Brazil)	*In vitro* (Bovine enamel)	HP 7.5 % G1: HP 7.5 % + CPP-ACP, at three proportions (1.5 mL-1:1, 1.5 mL - 2:1, 2 mL-1:2) G2: HP 7.5 % CP 10% G1: CP 10%+ CPP-ACP G2: CP 10 % n/per group: 10	The samples were treated with 0.04 ml of bleaching gel for 7 days. For the HP groups, the treatment lasted 60 min per day, while for the CP bleaching groups, it was 4 h daily. The specimens were kept immersed in artificial saliva at 37C.	Microhardness tester; roughness tester; Scanning Electron Microscopy (SEM).	Baseline/ post-treatment Microhardness (VHN) HP 7.5% G1: 276.3 ± 40.8 / 321.8 ± 39.7 G2: 292.3 ± 35.2 / 306.9 ± 36.1 CP 10% G: 276.9 ± 33.8 / 332.1 ± 31.4 G2: 268.3 ± 32.7 / 261.7 ± 31.7 Roughness (Ra) HP 7.5%: G1: 0.072 ± 0.009 / G2: 0.07 ± 0.007 CP 10%: G1: 0.074 ±0.01 / G2: 0.07 ± 0.017	HP 7.5 % G1: The group presented images showing superficial deposition of granular structures G1/G2: The samples revealed slight morphological alterations characterized by small irregularities CP 10% G2: accentuated superficial alterations were found.	The bleaching gel containing CPP-ACP was able to prevent negative changes of hardness, roughness and morphology on bleached enamel.	Positive
Vasconcelos *et al,* 2012b (Brazil)	*In vitro* (Bovine enamel)	HP 7.5 % G1: HP 7.5 % + CPP-ACP at three proportions (1.5 mL-1:1, 1.5 mL - 2:1, 2 mL-1:2) G2: HP 7.5 % CP 10%	The samples were treated with 0.04 ml of bleaching gel for 7 days. For the HP groups, the treatment lasted 60 minutes per day, while for the CP bleaching groups, it was 4 hours daily.	Spectrophotometer	7 days / 14 days / 21 days Color Change (ΔE*) HP 7.5 % G1: 10.0 ± 4.0/ 11.9 ± 3.3/ 8.9 ± 2.2 G2: 10.1 ± 2.3/ 12.5 ± 2.3 /9.0 ± 1.4 CP 10% G1: 10.7 ± 4.3 / 13.7 ± 4.9 ± 10.7 ± 5.4	NS	The combination of CCP-ACP pastes and bleaching agents did not affect the efficacy of tooth whitening.	Null

		G1: CP 10%+ CPP-ACP G2: CP 10 % n/per group: 10	The specimens were kept immersed in artificial saliva at 37C.		G2: 13.9 ± 2.4 / 15.6 ± 3.3 / 12.0 ± 4.2			
Borges *et al,* 2011 (Brazil)	*In vitro* (Bovine enamel)	CP 10 % G1: CP 10% + CPP-ACP at proportion 1:1 G2: CP 10 % CP 16% G1: CP 16% + CPP-ACP at proportion 1:1 G2: CP 16 % n/per group: 10	The bleaching treatment was performed over 14 days. Every day, 0.2 mL maintained for eight hours After eight hours, the gel was removed from the enamel surface by placing it under running distilled water for 15 seconds. When the specimens were not in contact with the bleaching agents, they were immersed in artificial saliva kept at 37.8ºC, which was changed daily.	Microhardness tester Vita shade guide; spectrophotometer	Post-treatment Microhardness (VHN) CP 10% G1: 298.7 ± 36.4 /G2: 248.9 ± 39.0 CP 16% G1: 305.4 ± 40.2 /G2: 255.4 ± 38.9 ΔE: G1/G2 Color Change CP 10%: 11.9 ± 6.4 / 15.1 ± 6.5 CP 16%: 13.3 ± 4.5 / 16.7 ± 6.72 Baseline / Post-treatment Vita Shade CP 10% G1: 11.2 ± 3.62 / 5.3 ± 1.1 G2: 11.2 ± 1.9 / 2.7 ± 0.4 CP 16% G1: 11.6 ± 2.3 / 3.2 ± 0.7 G2: 11.12± 2.1 / 3.5 ± 0.9	NS	The use of CPP-ACP with 10% and 16% CPs increased postbleaching enamel microhardness and did not adversely affect bleaching efficacy.	Positive
Pinheiro *et al,* 2011 (Brazil)	*In vitro* (Human enamel)	G1: CP 16% + ACP G2: CP 16% n/per group: 5	The bleaching gels were applied for 4 hours per day over a period of 14 days. Between each session, the specimens were stored in distilled water at 37°C until the next application.	Microhardness test; Scanning electron microscopy (SEM)	Microhardness (KHN) G1: 311.0 ± 16.0 G2: 291.2 ± 12.9	G1: No evidence of demineralization. Enamel showed a uniform area of compressed crystals G2: Conventional bleaching gel caused an initial dissolution of the tissue, characterized by the presence of	The addition of calcium and phosphate to the whitening gel promoted enamel remineralization and did not alter the enamel structure.	Positive

						cracks between prismatic and interprismatic enamel.		
Cavalli, *et al,* 2011 (Brazil)	*In vitro* (Human enamel)	G1: CP 10 % + Calcium 0.2% G2: CP 10% n/per group: 10	The bleaching treatment consisted of the application of 0.01 g of the gel to exposed enamel for 6 h per day, over 14 days. Samples were stored at 37ºC during bleaching.	FT-Raman spectroscopy; microhardness tester; polarized light microscopy.	Mineral Loss (Δ Z) G1: 583.2 ± 483.3 G2: 1311.6 ± 507.5 Lesion Depth (um) G1: 12.75 ± 2.6 G2: 39.19 ± 3.6 Mean Mineral Volume (%) Depth (μm) 20 / 40 μm G1: 72.6 ± 9.7 / 80.5 ± 6.8 G2: 69.7 ± 9.2 / 85.3 ± 12.4	Polarized light microscopy: Mineral loss was evident in the bleached groups, with the CP 10% group showing the most significant demineralized area. However, even Group 1 displayed some level of depth in injury. Group 2 exhibited a consistent zone of demineralization.	The addition of Ca to home-applied bleaching agents were able to minimize the adverse effects of carbamide peroxide on enamel surface and subsurface demineralization, as well as lesion depth progression, in vitro.	Positive
Abreu *et al,* 2011 (Brazil)	*In vitro* (Human enamel)	HP 7.5 % G1: HP 7.5 % + ACP G2: HP 7.5% HP 9.5 % G1: HP 9.5 % + ACP G2: HP 9.5 % n/per group: 10	A calibrated syringe was used to place 0.02 mL of each treatment agent on the specimens. The individual mold was placed over the specimen and kept there for 30 minutes a day. This application protocol was performed daily for 3 weeks	Microhardness and roughness testers	Baseline / 7 days / 14 days / 21 days Microhardness (KHN) HP 7.5 % G1: 337.7 ± 24.4 / 333.1 ± 43.5 / 320.7 ± 28.8 / 343.2 ± 35.3 G2: 321.8 ± 65.6 / 311.0 ± 43.1 / 334.8 ± 46.4 / 322.2 ± 63.9 HP 9.5 % G1: 315.2 ± 52.1 / 323.7 ± 45.5 / 336.0 ± 45.2 / 319.3 ± 43.0 G2: 300.8 ± 46.1 / 320.3 ± 38.2 / 285.8 ± 40.9 / 317.8 ± 57.4 Surface Roughness (Ra) HP 7.5 % G1: 1.2±0.1 /1.2±0.1 /1.2±0.1 /1.2±0.1 G2: 1.2±0.1 /1.3±0.1/1.3±0.1/1.3 ± 0.1 HP 9.5 % G1:1.2±0.1/1.3±0.0/1.2±0.1/1.2±0.1	NS –	The addition of ACP to the whitening gel reduces the loss of hardness and surface roughness.	Positive

					G2:1.2±0.1/1.2±0.1/1.3±0.1/1.3±0.0			
Cavalli, *et al,* 2010 (Brazil)	*In vitro* (Human enamel)	G1: CP 10 % + Calcium 0.2 % G2: CP 10% n/per group: 10	Application 0.01 g of the gel to exposed enamel for 6 h per day, over 14 days. Samples were stored at 37ºC during bleaching.	Surface microhardness Calcium and fluoride concentration.	Microhardness (KHN) G1: ~ 440 ± 56 G2: ~ 270 ± 90 Calcium / fluoride concentration (ppm) G1: ~250 / ~5 G2: ~100 / ~1	NS	Ca experimental bleaching agents promoted microhardness values that were superior to those of the nonenhanced (CP).	Positive
Borges *et al,* 2009 (Brazil)	*In vtro (*Human enamel)	G1: HP 35% + Calcium G2: HP 35% n/per group: 20	The bleaching agents applied in one 30 min session and changed every 10 min.	Microhardness tester	Surface / Subsurface Microhardness (VNH) G1: 402.2 ± 40.8 / 389.6 ± 37.2 G2: 302.3 ± 29.2 / 304.1 ± 43.5	NS	The bleaching gel with calcium increased the microhardness of bleached enamel.	Positive
da Costa et *al,* 2007 (Brazil)	*In vitro (*Human enamel)	G1: CP 10 % + ACP G2: CP 10% n/per group: 10	Application of 1 mm layer of the bleaching gel for eight hours and kept in a humid atmosphere at 37°C. The teeth were bleached for 21 consecutive days.	Microhardness tester	Baseline/ 14d / 2d / 35d Microhardness (KHN) G1: 399.2 ± 18.7 / 314.5 ± 21.4 / 283.8 ± 22.2 / 319.1 ± 11.2 G2: 391.4 ± 33.3 / 342.8 ± 24.8 / 298.5 ± 21.6 / 294.5 ± 32.2	NS	The inclusion of ACP in the whitening gel showed similar effects to the group that solely received CP.	Null
Gianini *et al,* 2006 (Brazil)	*In vitro (*Human enamel)	G1: CP 10 % + Calcium 0.5 % G2: CP 10% n/per group: 11	Bleaching gel applied for 8 hours/day for 14 days. During the treatment period specimens were kept in 100% relative humidity at 37 ºC.	Cyanoacrylate glue and universal testing Machine; scanning eletron microscope (SEM).	Artificial Saliva/ Relative Humidity Tensile strength of enamel (MPa) G1: 35.6 ± 5.0 / 32.0 ± 8.5 G2: 23.2 ± 6.5 / 24.8 ± 2.9	The calcium group exhibited a smoother appearance of the enamel compared to those treated solely with 10% CP, suggesting reduced porosity.	The whitening gel containing calcium demonstrated a comparable effect to the control group.	Positive
Oliveira *et al,* 2005 (Brazil)	*In vitro (*Human enamel)	G1.1: CP 10 % + Calcium 0.05 % G1.2: CP 10 % + Calcium 0.1 % G2: CP 10%	The enamel blocks from the experimental groups were exposed to one daily application of bleaching agents for	Microhardness tester	Baseline / 7d / 14d / 21d Microhardness (KHN) G1.1: 268.2 ± 39.2 / 111.9 ± 55.8 / 112. 2 ± 46.2 / 117. 9 ± 48.2 G1.2: 284.2 ± 40.7 / 139.3 ± 66.0 / 150.0 ± 70.2 / 145.4 ± 79.9	NS	CP bleaching gel containing f calcium did not avoid mineral loss resulting from the whitening	Null

		n/per group: 14	6 h during 14 consecutive days.		G2: 266.4 ± 42.3 / 114.5 ± 49.8 / 127.6 ± 52.7 / 130.5 ± 42.2		procedures. However, the addition of calcium can be beneficial on prevention of greater mineral change.	

Abbreviations ACP: Amorphous calcium phosphate; AFM: Atomic force microscopy; ARI: Adhesive remnant index; CaGP: Calcium glycerophosphate; CP: Carbamide peroxide; CPP-ACP Casein Phosphopeptide -Amorphous Calcium Phosphate (CCP-ACP); EDX:Energy-Dispersive X-Ray Spectroscopy; HP: Hydrogen peroxide; KHN: Knoop hardneess number; MPa: megapascal ; NS: not studied ; Ra: Roughness average; SEM: Scanning electronmicroscopy; VNH: Vickers hardness number

Table 3. Critical appraisal of *in vitro* included studies

	Was the aim of the study clearly stated?	Was the sample size justified?	Was the assignment to treatment groups truly random?	Were those assessing the outcomes blind to the treatment allocation?	Were control and treatment groups comparable at entry?	Were groups treated identically other than for the named interventions?	Was the experimental protocol clearly described?	Were outcomes measured in the same way for all groups?	Were outcomes measured in a reliable way?	Was appropriate statistical analysis used?	Total score
Centenaro et al., 2024	1	1	1	0	1	1	1	1	1	1	9
Garcia et al., 2024	1	0	1	0	1	1	1	1	1	1	8
Balkaya et al., 2024	1	0	1	0	1	1	1	1	1	1	8
Dos Anjos et al., 2024	1	0	1	0	1	1	1	1	1	1	8
Ortiz et al., 2024	1	1	1	0	1	1	1	1	1	1	9
Gruba et al., 2023	1	1	1	0	1	1	1	1	1	1	9
Ortiz et al., 2023	1	1	1	0	1	1	1	1	1	1	9
da Silva et al., 2023	1	1	1	0	1	1	1	1	1	1	9
Júnior et al., 2022	1	1	1	0	1	1	1	1	1	1	9
Yang et al., 2022	1	1	1	0	1	1	1	1	1	1	9
Pini et al., 2022	1	1	1	0	1	1	1	1	1	1	9
Júnior et al., 2021	1	0	1	0	1	1	1	1	1	1	8
Felipe Akabane et al., 2021	1	1	1	0	1	1	1	1	1	1	9
Limas et al., 2020	1	1	0	0	1	1	1	1	1	1	8
Vieira et al., 2020	1	0	1	0	1	1	1	1	1	1	8
Barbosa et al., 2020	1	1	1	1	1	1	1	1	1	1	10
Moura et al., 2019	1	0	1	0	1	1	1	1	1	1	8
Torres et al., 2019	1	1	1	0	1	1	1	1	1	1	9

Kutuk et al., 2019	1	0	1	0	1	1	1	1	1	1	8
Cavalli et al., 2018	1	0	1	0	1	1	1	1	1	1	8
Furlan et al., 2017	1	0	1	0	1	1	1	1	1	1	8
Moreira et al., 2017	1	0	1	0	1	1	1	1	1	1	8
Santos et al., 2016	1	0	1	0	1	1	1	1	1	1	8
Borges et al., 2016	1	0	1	0	1	1	1	1	1	1	8
Khoroushi et al., 2016	1	0	1	0	1	1	1	1	1	1	8
Moraes et al., 2015	1	0	1	0	1	1	1	1	1	1	8
Khoroushi et al., 2015	1	0	1	0	1	1	1	1	1	1	8
Basting et al., 2015	1	0	1	0	1	1	1	1	1	1	8
Mena-Serrano et al., 2015	1	0	1	0	1	1	1	1	1	1	8
Rauen et al., 2015	1	0	1	0	1	1	1	1	1	1	8
Pizani et al., 2015	1	0	1	0	1	1	1	1	1	1	8
Sasaki et al., 2015	1	0	1	0	1	1	1	1	1	1	8
Alexandrino et al., 2014	1	0	1	1	1	1	1	1	1	1	9
Borges et al., 2014	1	0	1	0	1	1	1	1	1	1	8
Borges et al., 2013	1	1	1	0	1	0	1	1	1	1	8
Machado et al., 2013	1	0	1	0	1	1	1	1	1	1	8
D'arce et al., 2013	1	0	1	0	1	1	1	1	1	1	8
Da Costa Soares et al., 2013	1	0	0	0	1	1	1	1	1	1	7
Berger et al., 2012	1	0	1	0	1	0	1	1	1	1	7
Vasconcelos et al., 2012a	1	0	1	0	1	1	1	1	1	1	8
Vasconcelos et al., 2012b	1	0	1	0	1	1	1	1	1	1	8
Borges et al., 2011	1	0	1	0	1	1	1	1	1	1	8
Pinheiro et al., 2011	1	0	0	0	1	1	1	1	1	1	8
Cavalli et al., 2011	1	0	1	0	1	1	1	1	1	1	8
Abreu et al., 2011	1	0	1	0	1	1	1	1	1	1	8
Cavalli et al., 2010	1	1	1	0	1	1	1	1	1	1	9
Borges t al., 2009	1	0	1	0	1	1	1	1	1	1	8

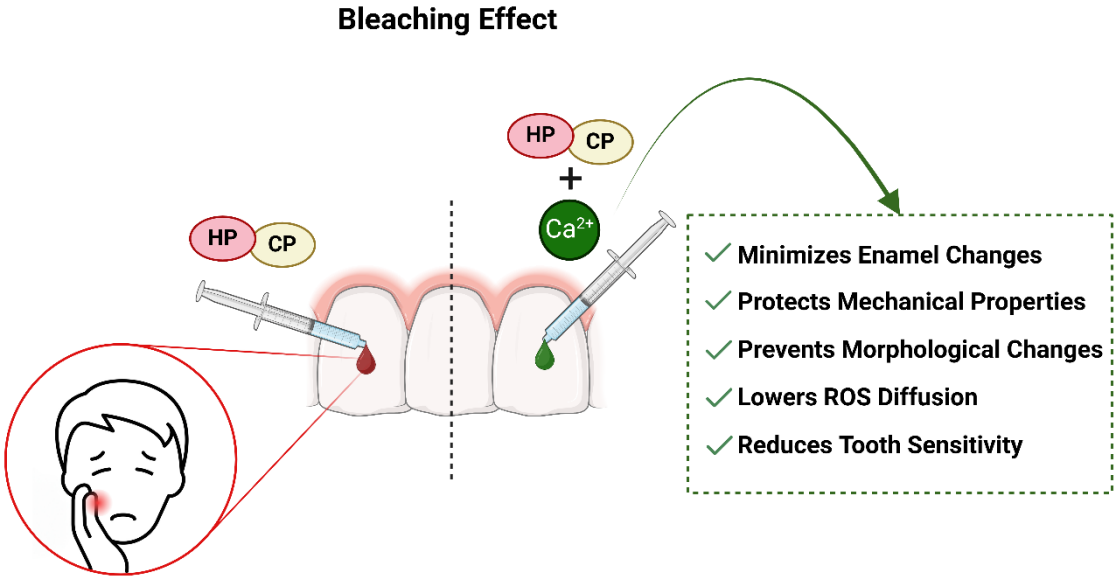
da Costa et al., 2007	1	0	1	0	1	1	1	1	1	1	8
Giannini et al., 2006	1	0	1	0	1	1	1	1	1	1	8
Oliveira et al., 2005	1	0	1	0	1	1	1	1	1	1	8

0: not reported or reported but inadequate, 1: reported and adequate

Supplementary File 1. Search strategies used for the electronic databases

Search Strategy
PUBMED/MEDLINE: 1265 (http://www.ncbi.nlm.nih.gov/pubmed)
(((Tooth Discoloration) OR (tooth staining) OR (discolored tooth) OR (Tooth whitening) OR (Tooth Bleaching) OR (Bleaching, tooth) OR (Teeth Whitening) OR (Whitening, Teeth) OR (Whitening, Tooth) OR (Teeth Bleaching) OR (Bleaching, Teeth) OR (Tooth bleaching agents) OR (Carbamide peroxide) OR (Hydrogen peroxide) OR (at-home) OR (in-office)) AND ((Dental Enamel) OR (Enamel) OR (Enamels) OR (Dental Enamels) OR (Enamels Dental) OR (Enamel,Dental) OR (Tooth Sensitivity) OR (Sensitivities, Tooth) OR (Sensitivity, Tooth) OR (Tooth Sensitivities) OR (Dental sensitivity) OR (sensitivity) OR (pain) OR (Hypersensitivity))) AND ((Calcium) OR (Calcium gluconate) OR (Calcium Phosphate) OR (Calcium Polyphosphate) OR (Calcium Silicate) OR (Amorphous Calcium Phosphate) OR (Calcium lactate) OR (Calcium-based) OR (Calcium phosphate) OR (Casein phosphopeptide-amorphous calcium phosphate) OR (ACP) OR (CPP-ACP) OR (CPP-ACPF))
SCOPUS: 1363 (http://www.scopus.com)
(TITLE-ABS-KEY ((tooth AND discoloration) OR (tooth AND staining) OR (discolored AND tooth) OR (tooth AND whitening) OR (tooth AND bleaching) OR (bleaching, AND tooth) OR (teeth AND whitening) OR (whitening, AND teeth) OR (whitening, AND tooth) OR (teeth AND bleaching) OR (bleaching, AND teeth) OR (tooth AND bleaching AND agents) OR (carbamide AND peroxide) OR (hydrogen AND peroxide) OR (at-home) OR (in-office)) AND TITLE-ABS-KEY ((dental AND enamel) OR (enamel) OR (enamels) OR (dental AND enamels) OR (enamels AND dental) OR (enamel,dental) OR (tooth AND sensitivity) OR (sensitivities, AND tooth) OR (sensitivity, AND tooth) OR (tooth AND sensitivities) OR (dental AND sensitivity) OR (sensitivity) OR (pain) OR (hypersensitivity)) AND TITLE-ABS-KEY ((calcium) OR (calcium AND gluconate) OR (calcium AND phosphate) OR (calcium AND polyphosphate) OR (calcium AND silicate) OR (amorphous AND calcium AND phosphate) OR (calcium AND lactate) OR (calcium-based) OR (calcium AND phosphate) OR (casein AND phosphopeptide-amorphous AND calcium AND phosphate) OR (acp) OR (cpp-acp) OR (cpp-acpf)))
EMBASE: 786 (https://www.embase.com)
('tooth discoloration'/exp OR 'tooth discoloration' OR (('tooth'/exp OR tooth) AND ('discoloration'/exp OR discoloration)) OR 'tooth staining'/exp OR 'tooth staining' OR (('tooth'/exp OR tooth) AND ('staining'/exp OR staining)) OR 'discolored tooth' OR (discolored AND ('tooth'/exp OR tooth)) OR 'tooth whitening'/exp OR 'tooth whitening' OR (('tooth'/exp OR tooth) AND whitening) OR 'tooth bleaching'/exp OR 'tooth bleaching' OR (('tooth'/exp OR tooth) AND ('bleaching'/exp OR bleaching)) OR 'bleaching, tooth' OR (('bleaching,'/exp OR bleaching,) AND ('tooth'/exp OR tooth)) OR 'teeth whitening'/exp OR 'teeth whitening' OR (('teeth'/exp OR teeth) AND whitening) OR 'whitening, teeth' OR (whitening, AND ('teeth'/exp OR teeth)) OR 'whitening, tooth' OR (whitening, AND ('tooth'/exp OR tooth)) OR 'teeth bleaching' OR (('teeth'/exp OR teeth) AND ('bleaching'/exp OR bleaching)) OR 'bleaching, teeth' OR (('bleaching,'/exp OR bleaching,) AND ('teeth'/exp OR teeth)) OR 'tooth bleaching agents'/exp OR 'tooth bleaching agents' OR (('tooth'/exp OR tooth) AND ('bleaching'/exp OR bleaching) AND agents) OR 'carbamide peroxide'/exp OR 'carbamide peroxide' OR (('carbamide'/exp OR carbamide) AND ('peroxide'/exp OR peroxide)) OR 'hydrogen peroxide'/exp OR 'hydrogen peroxide' OR (('hydrogen'/exp OR hydrogen) AND ('peroxide'/exp OR peroxide)) OR 'at home' OR 'in office') AND ('dental enamel':ti,ab,kw OR enamel:ti,ab,kw OR enamels:ti,ab,kw OR 'dental enamels':ti,ab,kw OR 'enamels dental':ti,ab,kw OR enamel,dental:ti,ab,kw OR 'tooth sensitivity':ti,ab,kw OR 'sensitivities, tooth':ti,ab,kw OR 'sensitivity, tooth':ti,ab,kw OR 'tooth sensitivities':ti,ab,kw OR 'dental sensitivity':ti,ab,kw OR sensitivity:ti,ab,kw OR pain:ti,ab,kw OR hypersensitivity:ti,ab,kw) AND (calcium:ti,ab,kw OR 'calcium gluconate':ti,ab,kw OR 'calcium polyphosphate':ti,ab,kw OR 'calcium silicate':ti,ab,kw OR 'amorphous calcium phosphate':ti,ab,kw OR 'calcium lactate':ti,ab,kw OR 'calcium based':ti,ab,kw OR 'calcium phosphate':ti,ab,kw OR 'casein phosphopeptide-amorphous calcium phosphate':ti,ab,kw OR acp:ti,ab,kw OR 'cpp acp':ti,ab,kw OR 'cpp acpf':ti,ab,kw) AND [embase]/lim
WEB OF SCIENCE: 803 (https://clarivate.com/webofsciencegroup/solutions/web-of-science-core-collection)
(Tooth Discoloration) OR (tooth staining) OR (discolored tooth) OR (Tooth whitening) OR (Tooth Bleaching) OR (Bleaching, tooth) OR (Teeth Whitening) OR (Whitening, Teeth) OR (Whitening, Tooth) OR (Teeth Bleaching) OR (Bleaching, Teeth) OR (Tooth bleaching agents) OR (Carbamide peroxide) OR (Hydrogen peroxide) OR (at-home) OR (in-office) (Topic) AND (Dental Enamel) OR (Enamel) OR (Enamels) OR (Dental Enamels) OR (Enamels Dental) OR (Enamel,Dental) OR (Tooth Sensitivity) OR (Sensitivities, Tooth) OR (Sensitivity, Tooth) OR (Tooth Sensitivities) OR (Dental sensitivity) OR (sensitivity) OR (pain) OR (Hypersensitivity) (Topic) AND (Calcium) OR (Calcium gluconate) OR (Calcium Phosphate) OR (Calcium Polyphosphate) OR (Calcium Silicate) OR (Amorphous Calcium Phosphate) OR (Calcium lactate) OR (Calcium-based) OR (Calcium phosphate) OR (Casein phosphopeptide-amorphous calcium phosphate) OR (ACP) OR (CPP-ACP) OR (CPP-ACPF)
LIBRARY COCHRANE: 68 (https://www.cochranelibrary.com)
(Tooth Discoloration) OR (tooth staining) OR (discolored tooth) OR (Tooth whitening) OR (Tooth Bleaching) OR (Bleaching, tooth) OR (Teeth Whitening) OR (Whitening, Teeth) OR (Whitening, Tooth) OR (Teeth Bleaching) OR (Bleaching, Teeth) OR (Tooth bleaching agents) OR (Carbamide peroxide) OR (Hydrogen peroxide) OR (at-home) OR (in-office) in Title Abstract Keyword AND (Dental Enamel) OR (Enamel) OR (Enamels) OR (Dental Enamels) OR (Enamels Dental) OR (Enamel,Dental) OR (Tooth Sensitivity) OR (Sensitivities, Tooth) OR (Sensitivity, Tooth) OR (Tooth Sensitivities) OR (Dental sensitivity) OR (sensitivity) OR (pain) OR (Hypersensitivity) in Title Abstract Keyword AND (Calcium) OR (Calcium gluconate) OR (Calcium Phosphate) OR (Calcium Polyphosphate) OR (Calcium Silicate) OR (Amorphous Calcium Phosphate) OR (Calcium lactate) OR (Calcium-based) OR (Calcium phosphate) OR (Casein phosphopeptide-amorphous calcium phosphate) OR (ACP) OR (CPP-ACP) OR (CPP-ACPF) in Title Abstract Keyword

Graphical Abstract



Conclusão Geral

5. Conclusão Geral

A incorporação de TMPnano e fluoreto, CaNaTMP e outros agentes à base de cálcio em géis clareadores com H_2O_2 demonstrou ser uma estratégia eficaz para reduzir os danos estruturais ao esmalte e os efeitos adversos induzidos pelo procedimento clareador. Esses agentes não comprometem a eficácia estética, representando uma abordagem promissora para otimizar a segurança e os resultados do clareamento dental, proporcionando uma alternativa segura e eficaz para tratamento estético dentário.

Apêndices

APÊNCICE A. Referências bibliográficas da Introdução Geral

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