



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

ANA VITÓRIA PEREIRA FERNANDES

**Efeito de ciclotrifosfato de cálcio em géis clareadores
para uso profissional e técnica at-home**

Araçatuba – SP
2025

Ana Vitória Pereira Fernandes

ANA VITÓRIA PEREIRA FERNANDES

**Efeito de ciclotrifosfato de cálcio em géis clareadores
para uso profissional e técnica at-home**

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

Araçatuba – SP
2025

Catálogo na Publicação (CIP)

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

F363e Fernandes, Ana Vitória Pereira.
 Efeito de ciclotrifosfato de cálcio em géis clareadores para
 uso profissional e técnica at-home / Ana Vitória Pereira
 Fernandes. – Araçatuba, 2025
 78 f. : il.9

 Tese (Doutorado) – Universidade Estadual Paulista
 (UNESP), Faculdade de Odontologia, Araçatuba
 Orientador: Prof. Alberto Carlos Botazzo Delbem

 1. Clareamento dental 2. Esmalte dentário 3. Peróxido de
 hidrogênio 4. Eficácia 5. Fosfatos I. T.

Black D15
CDD 617.695

Claudio Hideo Matsumoto
CRB-8/5550

Dedicatória

Dedico esta tese

À minha família, alicerce firme e amoroso que sustentou cada passo da minha caminhada. À minha mãe, Thais, e ao meu pai, Silvio — por todo o colo, o carinho e o apoio que sempre ultrapassaram qualquer dimensão material. Com vocês, aprendi que o verdadeiro lar é onde mora a força, a ternura e a fé. Não poderia ter sonhado com uma base mais sólida ou um refúgio mais feliz. Ao meu companheiro, Felipe, por caminhar ao meu lado com generosidade, paciência e amor incondicional. Sua presença foi luz constante nos dias incertos e força nos momentos em que faltaram as minhas. E ao meu mentor, Gabriel, cuja sabedoria e generosidade moldaram não apenas minha formação acadêmica, mas também minha forma de enxergar a beleza da jornada. Obrigada por me ensinar que até os maiores desafios podem ser fontes de crescimento — e até mesmo de alegria.

Agradecimentos Especiais

Ao Universo,

A essa força maior, sábia e pulsante, que rege a vida com perfeição e propósito. Sou profundamente grata por ter sido guiada pelos ciclos da natureza, pelo Deus que existe dentro de cada um de nós, pelas sincronicidades que nunca foram acaso, e pelas leis invisíveis que operam com amor e equilíbrio. Ao Universo, por me lembrar que tudo o que emana retorna, e que pensamentos alinhados com gratidão, verdade e propósito são sementes de realizações. À energia que me envolveu nos momentos de silêncio, que sussurrou caminhos quando tudo parecia incerto, e que me sustentou quando minhas próprias forças se esgotavam. A essa Inteligência maior, que se manifesta em cada detalhe da criação, e que conspirou com sabedoria e tempo perfeito para que eu chegasse até aqui.

Aos meus pais,

Vocês sempre foram e continuam sendo a base da minha vida, os pilares sobre os quais construí minha trajetória. A minha mãe, **Thaís**, mulher mais forte e determinada que já conheci, minha eterna fonte de inspiração. Sua coragem em enfrentar a vida com alma livre, olhar vibrante e espírito empreendedor sempre me guiou como farol. Em mil vidas, eu escolheria nascer novamente de você. Um dia, você sonhou em ser doutora e hoje, de alguma forma, sinto que realizo esse sonho por nós duas. Todas as renúncias silenciosas, os medos enfrentados, os caminhos incertos que percorreu para me trazer ao mundo... tudo isso ecoa em mim com profunda gratidão. Mãe, se cheguei até aqui, foi porque caminhei sobre o chão firme que você construiu com tanto esforço. Foi você quem me ensinou que mesmo nas maiores dores há beleza, e mesmo nas maiores dúvidas, há sentido. Ao meu pai, **Silvio**, que sempre me ensinou a olhar a vida pelo lado mais leve e luminoso. Com sua calma serena e seu humor irreverente, você me mostrou que o essencial não está apenas nas conquistas, mas no prazer de percorrer o caminho. Foi com você que aprendi a valorizar os momentos mais simples e verdadeiros, uma boa conversa, uma risada inesperada, um domingo tranquilo. Sua forma única de enfrentar as adversidades, sempre com um sorriso no rosto e uma pitada de ironia, me ensinou que a vida não precisa ser pesada para ser profunda. A minha teimosia em trilhar o

meu próprio caminho e a tranquilidade com que administro as múltiplas faces da minha jornada, sei que herdei de você. Obrigada por acreditar em mim, por apoiar, ao lado da mami, cada um dos meus sonhos mais improváveis.

Ao primeiro e eterno lar,

À essa casa invisível que levo dentro de mim, feita de amor, afeto e raízes profundas. Ao lar que nasceu inesperadamente com a minha chegada ao mundo, e desde então, segue vivo, pulsante, no laço indissolúvel entre nós três: pai, mãe e filha. É desse lar que tiro minha força e a coragem com que enfrento a vida. É para ele que meus pensamentos sempre retornam quando preciso lembrar quem sou, quando preciso me lembrar de onde vim. Vocês, meus pais, são meu abrigo mais seguro, minha origem, minha direção e meu repouso sagrado. Se hoje consigo alcançar o céu, é porque, desde sempre, tive o chão mais firme e amoroso onde pousar. A vocês, minha eterna gratidão.

Aos meus avós e tios,

Cresci em meio a uma família pequena, simples em tamanho, mas imensa em afeto. Filha, neta e sobrinha única, fui moldada pelo cuidado de muitos corações generosos. Agradeço ao meu avô **Luís** (in memoriam), que foi mais que avô foi mestre dos primeiros passos, das primeiras palavras, do primeiro afeto consciente. Com você aprendi a caminhar pelo mundo com ternura e curiosidade. Obrigada por ter me dado tanto em tão pouco tempo. À minha avó **Miramar**, presença constante e coração que ora por mim mesmo à distância, meu agradecimento profundo. Seu amor e suas preces foram âncora silenciosa em muitos dos meus dias e disso, nunca me esquecerei. Aos meus tios, **Thiago e Vinícius**, que me acolheram com carinho leve e verdadeiro, obrigada por cada gesto de cuidado, por cada palavra de incentivo, por fazerem parte das memórias que aquecem minha história. Gratidão a essa família pequena no número, mas abundante em tudo o que realmente importa. Vocês são raízes silenciosas que sustentam o voo desta história que agora se escreve, sem vocês, ela jamais seria a mesma.

Ao meu amor, Felipe,

Você acreditou em mim até nos momentos em que eu mesma já não acreditava. Com o passar dos anos, à medida que nossa relação se aprofundava,

crescia também a admiração silenciosa que sinto por você e o incentivo diário que me impulsiona a ser uma mulher melhor, mais forte, mais inteira. Você esteve ao meu lado quando duvidava se seria capaz de conquistar uma pós-graduação, quando meus pensamentos se enchiam de incertezas... Em cada uma dessas fases, sua presença foi constante, firme, gentil, amorosa. Hoje, cinco anos depois, somos mais do que um casal: Somos lar. Um lar que construímos com respeito, entrega e cuidado mútuo, onde cada gesto seu revela a pureza da sua alma e a delicadeza com que você enxerga o mundo. Estar com você é me lembrar todos os dias de que o amor verdadeiro não sufoca, ele expande. Você desperta em mim o que há de melhor, me faz querer ser mais, por mim, por nós, por tudo que ainda vamos viver. Agradeço, de coração, por cada esforço em me ver feliz, por cada sonho que você acolhe como se fosse seu. Que a vida continue sendo generosa com a gente. Que nossos planos se realizem, que nossa parceria siga crescendo, e que o lar que estamos construindo seja, sempre, o nosso lugar seguro. Você é minha luz, ontem, hoje e todos os dias que virão.

Ao Gabriel Nunes, meu mentor e amigo,

Foi você quem acendeu, com generosidade e firmeza, a centelha que me guiou rumo ao universo acadêmico. Desde o nosso primeiro encontro, você enxergou em mim possibilidades que nem eu mesma ousava sonhar e, com palavras sempre certas, me encorajou a buscar tudo aquilo que morava no fundo do meu coração. Durante toda essa jornada, você foi bússola e farol. Me mostrou caminhos que eu jamais teria encontrado sozinha, me aconselhou com sabedoria, respeitou meu tempo, me impulsionou com doçura e verdade. Pensando no que seria melhor para mim sempre. Em todos esses anos vividos, nunca conheci alguém com tamanha generosidade, clareza de alma e dedicação à ciência como você. Como costumo brincar: você é um gênio. Mas, mais do que isso, é também coração, é presença, é inspiração. Você é, sem dúvida, a maior referência acadêmica que já tive. E mais do que isso: uma das maiores pessoas que a vida colocou no meu caminho. Agradeço profundamente por tudo o que você me ensinou dentro e fora do mundo acadêmico. Se hoje me vejo professora diante de uma turma, compartilhando o que sei com amor, é porque você acreditou que isso era possível antes mesmo que eu acreditasse. Cito seu nome sempre que posso e sempre com orgulho. Você merece o mundo, e eu estarei aqui para aplaudir cada uma das suas conquistas, de pé, com

o coração vibrando. Você é ciência. E é também o tipo de pessoa rara que faz da ciência um lugar mais humano, mais possível, mais cheio de alma. Gratidão eterna, Gabriel. Nada disso teria sido possível sem você.

Aos amigos que a vida me deu: Mirelle, Mariana, Bárbara, Beatriz, Larissa, Gabriele, Camila, Yasmín, João, Janaína, Natalia e Luciana

Agradeço de coração, por caminharem comigo ao longo desses anos, tantos momentos compartilhados, tantas memórias construídas com afeto, leveza e verdade. A amizade de vocês é abrigo nos dias nublados e celebração nos dias de sol. É riso que brota sem esforço, silêncio que acolhe sem julgamento, presença que permanece mesmo quando a distância insiste. Em cada fase da minha vida, houve um gesto, uma palavra ou um simples olhar que me lembrava: Eu não estava sozinha. E isso é tudo. Ter amigos assim, constantes, verdadeiros, cúmplices é um dos maiores presentes que a vida pode oferecer. A vocês, que me conhecem nas versões mais bonitas e também nas mais frágeis, meu amor, minha lealdade e meu eterno obrigada. Que a vida siga nos permitindo crescer juntos, rir juntos e encontrar, uns nos outros, sempre apoio e abrigo.

Às amigas da pós-graduação e alunas de iniciação científica: Renata Alves, Bianca Uehara, Lara Urzedo, Heloísa Mota

Agradeço imensamente pela colaboração na execução dos estudos realizados durante o doutorado. Durante esses anos, tive a oportunidade de conviver e trabalhar com mulheres incríveis que tornaram essa jornada mais leve e significativa. Agradeço a vocês pela parceria, pelo apoio mútuo e pelos momentos compartilhados ao longo dessa caminhada. Desejo muito sucesso a todas nós nas próximas etapas e que possamos levar adiante os aprendizados e conexões construídas aqui. Seguimos em frente, e sigo sempre à disposição no que estiver ao meu alcance.

Ao meu orientador Professor Alberto Delbem,

Meus sinceros agradecimentos pela orientação, apoio e acompanhamento durante todos os anos desta jornada acadêmica. Sua atuação como orientador foi

fundamental para o desenvolvimento deste trabalho e para a minha formação científica e profissional. Agradeço pelas oportunidades concedidas ao longo do percurso, pela confiança depositada em mim e pela liberdade que tive para explorar ideias, propor caminhos e buscar soluções de forma autônoma. Deixo aqui minha gratidão e reconhecimento por todo o apoio ao longo dessa importante etapa da minha trajetória.

A Professora Adriana Zavanelli,

Agradeço pelos momentos tão valiosos de convivência e aprendizado que compartilhamos ao longo do estágio de docência. Sua generosidade, dedicação e entusiasmo marcaram de forma especial essa etapa da minha formação. Muito além da técnica e do domínio acadêmico, você é uma inspiração enquanto mulher, profissional e ser humano. Admiro sua trajetória, sua determinação e, principalmente, a forma leve e acolhedora com que conduz tudo ao seu redor. Sua energia positiva e sua presença tão humana deixaram marcas bonitas nessa jornada. Muito obrigada, de coração!

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), pela concessão de bolsa de pesquisa - Código de Financiamento 001.

Ao Laboratório Multíusuário de Biotecnologia e Bioengenharia, por possibilitar o uso do sistema de microtomografia computadorizada (modelo 1272 SkyScan, Brucker).

Meu muito obrigada!

Epígrafe

“Nada floresce sem antes atravessar o silêncio do tempo, as noites de dúvida e as pausas que o universo, com sabedoria, impõe. Conquistar é mais do que vencer: é escutar a intuição, honrar os próprios ciclos e transformar as quedas em solo fértil. A mulher que se entrega ao conhecimento e caminha com coragem, mesmo em meio à incerteza, torna-se autora de si e coautora da criação. E quando desperta em sintonia com a energia que sustenta o cosmos, ela não apenas alcança, ela transborda, transforma e expande o mundo ao seu redor. Pois sonhar com verdade, pensar com clareza e sentir com propósito é já começar a moldar, no invisível, tudo aquilo que um dia se tornará realidade.”

Ana Vitória P. Fernandes

Resumo Geral

Fernandes, AVP. **Efeito do ciclofosfato de cálcio em géis clareadores para uso profissional e at-home**, 2025, 78f. Tese (Doutorado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba 2025.

RESUMO

O objetivo deste estudo foi sintetizar e caracterizar o ciclotrifosfato (trimetafosfato de sódio, “TMP”) com substituição total de sódio por cálcio (Ca^{2+}) (CaTMP); e analisar *in vitro* seu efeito em formulações clareadoras a base de peróxido de hidrogênio (H_2O_2) sobre os tecidos mineralizados na técnica in-office, at-home ou combinada. O ciclotrifosfato contendo cálcio foi sintetizado utilizando coluna para cromatografia e adição de sobrenadante de solução contendo hidróxido de cálcio e caracterizados por meio difração de raios X (DRX), microscopia eletrônica de varredura (MEV) e energia dispersiva de Raios X (EDX). **No artigo I:** Os géis clareadores foram aplicados sobre discos de dentes bovinos, de acordo com os grupos: 1) Controle negativo - água deionizada; 2) Gel clareador contendo H_2O_2 a 35% (Controle positivo); 3) Gel clareador contendo H_2O_2 a 35% + 1% CaTMP; 4) Gel clareador contendo H_2O_2 a 17,5%; 5) Gel clareador contendo H_2O_2 17,5% + 1% CaTMP. Os géis foram aplicados uma única vez, durante 3 sessões de 40 minutos/sessão, a cada 7 dias. Em seguida, foi avaliada a alteração de cor por espectrofotometria de reflexão quantificado (ΔE^* , ΔWI_D e ΔE_{00}), a difusão transamelodentinária de H_2O_2 e o conteúdo mineral do esmalte por Microtomografia computadorizada ($\text{gHAp} \times \text{cm}^{-3} \times \mu\text{m}$). Os resultados indicaram que os géis contendo 1% de CaTMP previniram a perda mineral e difusão transamelodentinária de H_2O_2 , e mostraram similar eficácia clareadora quando comparado aos géis homólogos convencionais. **No artigo II,** Os géis clareadores foram aplicados sobre discos de dentes bovinos, de acordo com os grupos: 1) Controle negativo - água deionizada; 2) Gel clareador contendo a 10% H_2O_2 (Controle positivo); 3) Gel clareador contendo H_2O_2 a 10% + 1% CaTMP; 4) Terapia clareadora combinada: uma sessão com gel clareador contendo H_2O_2 a 35% + Gel clareador contendo H_2O_2 a 10%; 5) Terapia clareadora combinada: uma sessão com gel clareador contendo H_2O_2 a 35% + Gel clareador contendo H_2O_2 a 10% + 1% CaTMP. O tratamento consistiu na aplicação dos géis clareadores da técnica caseira (10% H_2O_2 ou 10% H_2O_2 + 1% CaTMP) diariamente (40 min) durante 14 dias; nos grupos com terapia combinada foi feita uma aplicação única (40 min) com gel clareador a 35% H_2O_2 previamente ao protocolo da técnica caseira. Em seguida,

foi avaliada a alteração de cor por espectrofotometria de reflexão quantificado (ΔE^* , ΔWI_D e ΔE_{00}), microdureza, rugosidade, conteúdo mineral e morfologia do esmalte (MEV). A adição de 1% de CaTMP previniu a perda mineral e as alterações superficiais do esmalte em comparação aos protocolos convencionais de clareamento. A rugosidade superficial apresentou aumento significativo apenas no grupo combinado 35% HP/10% HP. Todos os tratamentos promoveram efeito clareador, com a técnica combinada (35% HP/10% HP) apresentando os maiores valores de ΔE e ΔE_{00} . A rugosidade superficial apresentou aumento significativo apenas no grupo combinado 35% HP/10% HP. **Conclusão Geral:** A incorporação de 1% de CaTMP em géis clareadores dentários reduz os efeitos adversos do clareamento nos tecidos dentários sem comprometer sua eficácia. Esse efeito foi observado em diferentes concentrações de peróxido de hidrogênio (HP) e técnicas de clareamento.

Palavras-Chave: Clareamento dental, Esmalte dentário, Peróxido de hidrogênio, Avaliação de eficácia, Fosfatos.

General Abstract

Fernandes, AVP. **Effect of calcium cyclophosphate in bleaching gels for professional and at-home use**, 2025, 78f. Thesis (Doctorate) - School of Dentistry, Universidade Estadual Paulista, Araçatuba 2025.

ABSTRACT

The objective of this study was to synthesize and characterize cyclotriphosphate (sodium trimetaphosphate, “TMP”) with total sodium substitution by calcium (Ca^{2+}) (CaTMP) and to analyze its *in vitro* effect in hydrogen peroxide (H_2O_2)-based whitening formulations on mineralized tissues using in-office, at-home, or combined techniques. Calcium-containing cyclotriphosphate was synthesized using a chromatography column and the addition of a supernatant solution containing calcium hydroxide. It was characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). ***In Article I:*** Whitening gels were applied to bovine tooth discs according to the following groups: (1) Negative control – deionized water; (2) Whitening gel containing 35% H_2O_2 (positive control); (3) Whitening gel containing 35% H_2O_2 + 1% CaTMP; (4) Whitening gel containing 17.5% H_2O_2 ; (5) Whitening gel containing 17.5% H_2O_2 + 1% CaTMP. The gels were applied once, over three sessions of 40 min per session, every seven days. Subsequently, color change was evaluated by reflectance spectrophotometry (ΔE^* , ΔWID , and ΔE_{00}), transamelodontinal diffusion of H_2O_2 , and enamel mineral content by micro-computed tomography ($\text{gHAp} \times \text{cm}^{-3} \times \mu\text{m}$). The results indicated that gels containing 1% CaTMP prevented mineral loss and transamelodontinal diffusion of H_2O_2 , while demonstrating similar whitening efficacy compared to conventional counterpart gels. ***In Article II:*** Whitening gels were applied to bovine tooth discs according to the following groups: (1) Negative control – deionized water; (2) Whitening gel containing 10% H_2O_2 (positive control); (3) Whitening gel containing 10% H_2O_2 + 1% CaTMP; (4) Combined whitening therapy: one session with a whitening gel containing 35% H_2O_2 + whitening gel containing 10% H_2O_2 ; (5) Combined whitening therapy: one session with a whitening gel containing 35% H_2O_2 + whitening gel containing % H_2O_2 + 1% CaTMP. The treatment involved daily application of at-home whitening gels (10% H_2O_2 or 10% H_2O_2 + 1% CaTMP) for 40 min over 14 days. In the combined therapy groups, a single 40-min application of 35% H_2O_2 whitening gel was performed before the

at-home protocol. Subsequently, color change was assessed using reflectance spectrophotometry (ΔE^* , ΔWID , and ΔE_{00}), as well as microhardness, roughness, mineral content, and morphology (SEM). The addition of 1% CaTMP prevented mineral loss and surface alterations of enamel compared to conventional whitening protocols. All treatments promoted a whitening effect, with the combined technique (35% HP/10% HP) showing the highest ΔE and ΔE_{00} values. A significant increase in surface roughness was observed only in the combined 35% HP/10% HP group. **General Conclusion:** The incorporation of 1% CaTMP in dental whitening gels reduces the adverse effects of bleaching on dental tissues without compromising its efficacy. This effect was observed across different hydrogen peroxide (HP) concentrations and whitening techniques.

Keywords: Dental bleaching, Dental enamel, Hydrogen peroxide, Efficacy evaluation, Phosphates.

Lista de Figuras

CAPÍTULO 1

Figure 1. Representative diagram of the study's experimental design. A. Enamel/Dentin Discs Preparation. B. Experimental Whitening Gels. C. Bleaching Protocol. D. Color Change Analysis E. Transamelodontinal Diffusion of H₂O₂. F. Mineral content of enamel and dentin

Figure 2. Scanning electron microscope (SEM) micrograph of the CaTMP samples (A) and corresponding EDS histogram (B).

Figure 3. Mean values (SD) of: A - Total color change (ΔE) by the Commission Internationale de l'Éclairage (CIE); B - Whitening index in dentistry (ΔWI_D); C - Color change by CIEDE2000 (ΔE_{00}), according to whitening gels and time of analysis (n = 10). Distinct lowercase letters indicate statistical differences between whitening gels at each time point and between time points for each gel (Student-Newman-Keuls; $p < 0.05$).

Figure 4. Mean values (SD) of trans-enamel-dentin diffusion of H₂O₂ according to the experimental groups. Distinct lowercase letters indicate statistical differences between whitening gels at each time point and between time points for each gel (Student-Newman-Keuls; $p < 0.05$).

Figure 5. Transversal profile of mineral concentration ($g_{HAP} \times cm^{-3} \times \mu m$) as a function of depth (μm) in different areas of dental enamel (sound, bleached) according to the experimental groups.

CAPÍTULO 2

Figure 1. Mean values (SD) of the alteration of: (A) lightness degree (ΔL^*), (B) green/red degree (Δa^*), (C) blue/yellow degree (Δb^*) by Comission Internationale de l'Eclairage (CIE) according to bleaching gels and moment of analysis ($n = 10$). Distinct superscript lowercase letters indicate statistical difference among the bleaching gels at each moment of analysis (Student-Newman-Keuls; $p < 0.05$).

Figure 2. Mean values ($\pm$ SD) of total color change (ΔE) (A), Whitening Index for Dentistry (ΔWI_D) (B), and total color change by CIEDE00 (ΔE_{00}) (C) according to the whitening gels and analysis time ($n = 10$). Distinct lowercase superscript letters indicate statistical differences between the whitening gels at each analysis time point (Student–Newman–Keuls; $p < 0.05$).

Figure 3. Mineral concentration profiles ($g_{HAp} \times cm^{-3}$) as a function of depth in enamel and dentin, according to experimental groups ($n = 10$).

Figure 4. Scanning electron microscopy (SEM) surface topography micrographs ($\times 5000$) and representative EDX of sound enamel and experimental groups: 10% HP, 35%HP/10% HP, 10% HP/CaTMP, and 35% HP/10% HP/CaTMP, respectively. The empty arrow indicates irregularities or depressions. The filled arrow indicates protruding interprismatic enamel.

Lista de Tabelas

CAPÍTULO 1

Table 1. Mean (SD) of the integrated area and percentage of mineral loss according to the hydrogen peroxide concentration (% H₂O₂), calcium trimetaphosphate concentration (% CaTMP), substrate (enamel and dentin), and whitening or non-whitening (healthy). Identical lowercase letters indicate no significant difference between the means of each variable based on the analysis (Student-Newman-Keuls; $p < 0.05$). SD represents the standard deviation of the means.

CAPÍTULO 2

Table 1. Mean values (SD) of roughness (Ra) and surface hardness (SH), percentage of surface hardness change (%SH) according to treatments ($n = 10$). Distinct superscript lowercase letters indicate statistical differences among bleaching gels in each variable (Student–Newman–Keuls test, $p < 0.001$). Different capital letters indicate the difference between the analysis moment (initial and final) for Ra and SH variables (Student-Newman-Keuls; $p < 0.05$).

Table 2. Mean values (SD) of integrated area of mineral loss according to the groups and substrate (enamel and dentin), and thickness after bleaching of the discs ($n = 10$)

Lista de Apêndices

Apêndice A: Referências bibliográficas da introdução geral.

Lista de Abreviaturas e Siglas

ANOVA Análise de Variância

°C Graus Celsius

Ca Cálcio

Ca⁺² Íon cálcio

CaNaTMP Trimetafosfato de Sódio e Cálcio

CaTMP Trimetafosfato de Cálcio

F Fluoreto

F⁻ Íon fluoreto

g Grama

h Hora

HF⁰ Fosfato de hidrogênio neutro

HP Peroxido de hidrogênio

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

1 INTRODUÇÃO GERAL	23
2 ARTIGO I	
2.1 RESUMO	29
2.2 INTRODUÇÃO	30
2.3 MATERIAIS E MÉTODOS	31
2.4 RESULTADOS	35
2.5 DISCUSSÃO	37
2.6 CONCLUSÃO	40
2.7 AGRADECIMENTOS	40
2.8 REFERÊNCIAS	40
3 ARTIGO II	
3.1 RESUMO	52
3.2 INTRODUÇÃO	53
3.3 MATERIAIS E MÉTODOS	54
3.4 RESULTADOS	59
3.5 DISCUSSÃO	60
3.6 CONCLUSÃO	65
3.7 AGRADECIMENTOS	65
3.8 REFERÊNCIAS	65
4 CONCLUSÃO GERAL	76
5 APÊNDICE	77

Introdução Geral

Ana Vitória Pereira Fernandes

1. Introdução Geral

Nos últimos anos, a estética dentária tem se tornado um foco crescente na odontologia, com a demanda por tratamentos eficazes para clareamento dental se intensificando consideravelmente (Sabbah, 2022). A busca por técnicas que proporcionem resultados estéticos satisfatórios sem comprometer a integridade dental levou ao aumento do uso de géis clareadores, especialmente aqueles à base de peróxido de hidrogênio (H_2O_2) (Aragão et al., 2024; Américo et al., 2023). Os tratamentos de clareamento podem ser realizados tanto no consultório quanto domiciliar (Aidos et al., 2024), com o uso de géis de H_2O_2 em concentrações mais baixas, principalmente na técnica domiciliar, sendo mais biocompatível e menos invasiva em comparação com o clareamento realizado em consultório, que utiliza concentrações mais altas de HP (Ludovichetti et al., 2024; Donassollo et al., 2021). Embora os tratamentos em consultório apresentem alta taxa de sucesso e efeito imediato nas primeiras sessões clareadoras, eles têm sido frequentemente associados a maiores efeitos adversos nos substratos dentários (Aidos et al., 2024; Américo et al., 2023), como a redução de microdureza do esmalte, perda mineral, aumento da rugosidade e alterações morfológicas (Gruba et al., 2023; Felipe Akabane et al., 2021). Além disso, os pacientes podem apresentar maior incidência de sensibilidade dentária devido ao alto poder de difusão do H_2O_2 que pode atingir o complexo dentino-pulpar (Aidos et al., 2024).

Estudos recentes têm buscado estratégias de minimizar esses efeitos adversos, ao mesmo tempo em que mantêm a eficácia estética dos tratamentos clareadores (De Oliveira Ribeiro et al., 2024; Martins et al., 2022). Uma das estratégias promissoras envolve a adição de agentes bioativos a composição do gel clareador, como o trimetafosfato de sódio (TMP) (Nunes et al., 2024; Gruba et al., 2023; Felipe Akabane et al., 2021; Dos Santos et al., 2020). O TMP é um composto cíclico que tem se mostrado eficaz na proteção do esmalte dental contra a desmineralização, e favorecer o processo de remineralização (Gruba et al., 2024; Oliveira et al., 2024). Estudos indicam que a incorporação de TMP em géis clareadores pode reduzir as alterações induzidas pelo peróxido de hidrogênio, como a diminuição da dureza do esmalte, alteração de rugosidade e morfologia do esmalte dentário (Nunes et al., 2024; Gruba et al., 2023; Júnior et al., 2021). No entanto, embora tenha apresentado efeito protetor, não preveniu integralmente essas alterações. Além disso, a eficácia do TMP é condicionada ao seu uso de forma combinada ao fluoreto de sódio, de modo que seu uso isolado apresenta efeito limitado.

Dessa forma, o advento da possibilidade de substituição iônica total dos íons sódio por

íons cálcio na estrutura do TMP poderia levar à formação de um composto mais reativo e inovador, o trimetafosfato de cálcio (CaTMP). Quando incorporado em géis clareadores, esse composto representaria um avanço significativo na busca por formulações mais biocompatíveis e seguras. A substituição total dos íons sódio por cálcio no TMP pode, potencialmente, aprimorar as propriedades protetoras desse agente, visto que o cálcio é um mineral essencial para a estrutura dos tecidos dentários duros. Dados preliminares (não publicados) indicam que a substituição parcial dos íons sódio por íons cálcio (CaNaTMP) demonstrou um efeito protetor considerável contra as alterações induzidas pelo clareamento, particularmente em géis com concentrações mais elevadas de H_2O_2 , usados na técnica de clareamento em consultório. Assim, supõe-se que a substituição total dos íons de sódio por cálcio possa resultar em um composto com maior capacidade preventiva, capaz de reduzir ou até prevenir as mudanças estruturais indesejadas causadas pelo clareamento, como perda mineral, difusão de HP e alterações morfológicas, sem comprometer a eficácia estética do tratamento.

Para preencher essa lacuna, seria relevante a realização de estudos *in vitro* que avaliem o impacto do CaTMP em géis de clareamento com alta concentração de H_2O_2 , como os de 35% e 17,5%, frequentemente usados nos tratamentos de consultório. Uma avaliação detalhada das propriedades mecânicas e físicas do esmalte, poderia ser realizada por meio de microtomografia computadorizada, que permite uma análise precisa da estrutura mineral dental. Além disso, o uso de amostras de esmalte nativo (sem polimento) para esses ensaios experimentais seria crucial, uma vez que essa abordagem mais fiel às condições clínicas pode proporcionar dados mais realistas sobre os efeitos dos tratamentos clareadores.

Além disso, seria relevante investigar o efeito da incorporação do CaTMP em géis com baixa concentração de H_2O_2 voltados para tratamentos de clareamento domiciliar. Géis com concentrações menores de H_2O_2 são reconhecidamente menos agressivos aos tecidos dentários (Donato et al., 2024; Grazioli et al., 2018), porém apresentam uma ação clareadora mais gradual, frequentemente exigindo um período prolongado de uso, o que pode impactar a adesão do paciente ao tratamento. Nesse contexto, seria interessante avaliar também os efeitos da técnica de clareamento combinada, que envolve um tratamento inicial no consultório seguido de um protocolo domiciliar. Essa abordagem busca acelerar os resultados estéticos, sendo uma alternativa promissora para contornar a necessidade de longo tempo de uso dos géis domiciliares. Embora essa técnica tenha sido investigada em ensaios clínicos (Zhong et al., 2023; Dourado Pinto et al., 2019; Bernadon et al., 2010), a literatura sobre os impactos dessa abordagem nos tecidos dentários em modelos

laboratoriais ainda é limitada, o que torna fundamental a análise *in vitro* dos efeitos dessa técnica para compreender melhor suas consequências sobre a saúde dental. Portanto, os objetivos dos estudos propostos incluem a avaliação da eficácia e segurança do CaTMP quando incorporado em géis clareadores de alta concentração de H₂O₂, realizados na técnica de consultório, além de investigar os efeitos desse agente em géis com baixa concentração de H₂O₂ para a técnica domiciliar. Outro aspecto relevante a ser investigado é a técnica de clareamento combinada, especialmente em relação às alterações que ela pode induzir nos tecidos dentários, sendo essa uma área ainda pouco explorada nos modelos experimentais existentes na literatura.

Objetivo Geral

Este estudo teve como objetivo avaliar, em modelos *in vitro*, novas formulações de géis clareadores à base de peróxido de hidrogênio (H_2O_2) contendo trimetafosfato de cálcio, obtido por substituição química total dos íons sódio por íons cálcio (CaTMP). O foco foi analisar o impacto dessas formulações nas alterações induzidas pelo processo clareador nos substratos dentários, em diferentes protocolos de clareamento, incluindo as técnicas em consultório (*in-office*), caseiro (*at-home*) e/ou combinada.

Artigo I

2. Total calcium ionic replacement in sodium trimetaphosphate and its incorporation into dental whitening gels: a novel strategy for in-office treatment

Ana Vitória Pereira Fernandes¹, Gabriel Pereira Nunes¹, Lara Oliveira Ribeiro Urzedo¹, Priscila Toninatto Alves de Toledo², Renata de Oliveira Alves¹, Tamires Passadori Martins¹, Alberto Carlos Botazzo Delbem¹

¹ Department of Preventive and Restorative Dentistry, Araçatuba School of Dentistry, São Paulo State University - UNESP, Araçatuba, SP, Brazil.

²Department of Operative Dentistry, Endodontics, and Dental Materials, Bauru School of Dentistry, University of São Paulo-USP, Bauru, SP, Brazil.

Corresponding author:

Alberto Carlos Botazzo Delbem

São Paulo State University (UNESP), School of Dentistry, Araçatuba Department of Preventive and Restorative Dentistry

Rua José Bonifácio 1193 16015-050 – Araçatuba, SP - Brazil Tel.

+55 18 3636 3314 Fax +55 18 3636 3332

Email: alberto.delbem@unesp.br

*Artigo publico no periódico *Journal of Dentistry*:
<https://doi.org/10.1016/j.jdent.2025.105861>

2.1 Abstract

Objectives: The aim of this study was to synthesize a cyclophosphate with total sodium replacement (CaTMP) and to evaluate, *in vitro*, its incorporation into hydrogen peroxide (HP) whitening gels.

Materials and Methods: CaTMP was synthesized through column chromatography using ion-exchange resin and calcium hydroxide solution. Bovine enamel/dentin discs ($n = 150$) were divided into five groups: 1) negative control (no treatment), 2) 35% HP whitening gel (positive control), 3) 35% HP + 1% CaTMP, 4) 17.5% HP, and 5) 17.5% HP + 1% CaTMP. Whitening gels were applied weekly for three 40-minute sessions. Color change was measured using ΔE_{ab} , ΔWI_D , and ΔE_{00} . Transamelodontinal HP diffusion was determined, as well the mineral content of dental substrates by μ -CT ($g_{HAp} \times cm^{-3} \times \mu m$). Data were analyzed with ANOVA and Student-Newman-Keuls test ($p < 0.05$).

Results: All whitening gels caused significant color changes ($p < 0.001$), with no differences between groups in ΔE , ΔE_{00} , and ΔWI_D ($p > 0.05$). The highest transamelodontinal HP diffusion occurred in the 35% HP group ($p < 0.001$), but adding 1% CaTMP reduced this diffusion in both HP concentrations ($p < 0.05$), with a greater reduction in the 17.5% HP gel. Conventional gels (17.5% and 35% HP) caused greater mineral loss ($g_{HAp} \times cm^{-3} \times \mu m$), while CaTMP protected against these changes.

Conclusions: Incorporating 1% CaTMP into whitening gels (17.5% and 35% HP) significantly reduced HP diffusion and preserved the mineral content of enamel and dentin, while providing whitening results similar to conventional gels.

Clinical relevance: CaTMP may act as a protective agent against peroxide-induced oxidative damage in dental tissues. While these findings are based on *in vitro* data, they support a promising direction for future translational research aimed at enhancing the safety and effectiveness of in-office whitening procedures.

Keywords: Tooth whitening, Enamel, Hydrogen peroxide, Calcium, Phosphates.

2.2 Introduction

Dental discoloration remains one of the most prevalent clinical conditions that prompts patients to seek aesthetic interventions [1]. Whitening techniques that adopt conservative approaches for treating discolored teeth are often preferred, aiming to minimize alterations to healthy dental structure [2]. The widespread adoption of both at-home and in-office whitening methods is attributed to their efficacy, conservative approach, simplicity, and relatively low cost compared to other aesthetic procedures [3,4].

Despite its aesthetic benefits, several studies have highlighted adverse effects associated with the use of whitening gels [5-7]. Dental whitening occurs due to the low molecular weight of hydrogen peroxide (HP), which allows its diffusion through the enamel and dentin [4,5]. During this process, HP releases reactive oxygen species (ROS) that interact with loosely bound compounds, leading to the formation of toxic molecules in the dental substrate [5,8]. These chemical alterations have been linked to decreased enamel strength, mineral loss, and the formation of surface depressions and porosities [2,5,8,9]. Additionally, due to its high transamelodentinal diffusion capacity, HP may reach the pulp tissue, frequently leading to dental sensitivity [3,10,11].

To minimize these adverse effects, alternative protocols have been investigated to enhance the biocompatibility and safety of whitening treatments [9,12,13]. One promising approach involves the incorporation of sodium trimetaphosphate (TMP), a cyclic polyphosphate recognized for its ability to reduce demineralization and promote remineralization by interacting with hydroxyapatite and enhancing mineral deposition [14,15]. When added to whitening gels, TMP has demonstrated the potential to mitigate enamel damage; however, its protective action is significantly dependent on the presence of sodium fluoride (NaF) [16,17]. Even under these conditions, complete prevention of bleaching-induced enamel alterations has not been consistently achieved [16-18].

Given these limitations, a preliminary study [19] explored the partial substitution of sodium ions by calcium ions in the TMP structure, resulting in calcium-sodium trimetaphosphate (CaNaTMP). The rationale for this modification is based on the premise that calcium enrichment may enhance the material's bioactivity, increasing its affinity for dental tissues and promoting the formation of more stable calcium phosphate deposits [20,21]. Whitening gels containing 1% of this synthesized salt were effective in reducing mineral loss, hydrogen HP diffusion, and topographical alterations in dental enamel, without compromising whitening efficacy. However, despite these benefits and the absence of fluoride, the formulation

did not fully prevent the adverse effects associated with bleaching. Additionally, the study assessed enamel mineral content using X-ray microtomography (μ -CT); however, prior surface polishing of the specimens may have compromised the primary enamel layer, potentially influenced the results, and not reflecting actual clinical conditions

Therefore, it is hypothesized that a complete substitution of sodium ions by calcium ions in the TMP structure could enhance the protective effects already observed, offering greater reactivity and bioactivity to benefit dental tissues. In light of this, the aim of this study was to synthesize and characterize calcium trimetaphosphate (CaTMP) with total replacement of sodium ions by calcium ions, and to evaluate, *in vitro*, the influence of adding 1% CaTMP to HP-based whitening formulations at concentrations of 35% and 17.5% on whitening efficacy, transamelodentinal diffusion of HP, and enamel mineral content. Accordingly, three independent null hypotheses were established: (1) the addition of CaTMP to the bleaching gels does not affect whitening efficacy; (2) the experimental formulations do not alter the transamelodentinal diffusion of HP; and (3) there are no effects on the mineral content of enamel and dentin.

2.3. Material and Methods

2.3.1. Synthesis and Characterization of CaTMP

The calcium-substituted TMP compound (CaTMP) was synthesized by replacing sodium ions with calcium in particulate NaTMP. To begin, 6 g of sodium tripolyphosphate 40 mL of deionized water, resulting in a solution with a pH of 6.01. This solution was then passed through a 2 cm $\times$ 30 cm glass column containing a strong acid cation exchange resin (PUROLITE C100H, Purolite®, Philadelphia, PA, USA) to exchange sodium ions for calcium ions at a flow rate of 0.5 mL/min. The pH of the effluent was measured at 0.77 after passing through the column. To adjust the pH back to a value similar to that of the original NaTMP solution (around 5.90), 4.39 g of calcium hydroxide ($\text{Ca}(\text{OH})_2$, Code HC09743RA, Exôdo, Sumaré, SP, Brazil) dissolved in 100 mL of deionized water was added to the filtrate, bringing the final pH to 6.02. The solution was then placed in an oven at 37°C for 24 h to allow for precipitation of the CaTMP, following the method outlined by Griffith et al. 1962 [22]. Afterward, the precipitate was separated by filtration using quantitative filter paper (Quanty JP42, J Prolab, São José dos Pinhais, PR, Brazil) and a Buchner funnel under vacuum (-600 mmHg). The collected CaTMP was dried in an oven at 70°C for 24 h, then ground in a ball mill (Planetary Micro Mill PULVERISETTE 7 classic line, Fritsch GmbH, Idar-Oberstein,

Germany) for 5 min to homogenize the powder.

The composite was characterized for crystallinity using X-ray diffraction (XRD) with a Shimadzu XRD 6000 diffractometer, employing CuK α radiation at 30 kV and 30 mA, within a $5^\circ \leq 2\theta \leq 85^\circ$ range and a scan rate of 0.2° per min. The morphology of the CaTMP particles was examined via scanning electron microscopy (SEM) at 20 kV and a magnification of 3,000 $\times$. The chemical composition of the samples was analyzed using energy dispersive X-ray spectroscopy (EDS) with a spatial resolution of approximately 2 μ m, focusing on the elements phosphorus (P), oxygen (O), sodium (Na), and calcium (Ca).

2.3.2. Preparation, Selection, and Distribution of Samples for Testing

The sample size for each group was initially based on a prior study [17] and subsequently confirmed by an a priori power analysis performed using SigmaPlot software (version 12.0). Considering the primary outcomes, surface hardness (mean difference = 9, SD = 4) and cross-sectional hardness (mean difference = 3200, SD = 1600), the calculated effect sizes were 2.25 and 2.0, respectively. The power analysis indicated that, with an α error of 5% and a power of 90%, a minimum of 7 and 9 specimens per group would be required for surface and cross-sectional hardness, respectively. Therefore, the use of 10 enamel/dentin discs per group was considered appropriate to ensure statistical validity. Bovine lower central incisors were sectioned using a bench drill, obtaining discs with a diameter of 5.7 mm from the middle region of the vestibular surface of each tooth. Manual rotary movements were applied at each granulation step to achieve a standardized enamel/dentin thickness of 2.3 mm, simulating the thickness of lower incisors ([Fig. 1A](#)). The dentin surfaces were first polished using an automatic polishing machine (Vector-Phoenix Beta, Buehler, Lake Bluff, IL, USA), applying a pressure of 4 lbs and a rotation speed of 200 rpm. Silicon carbide paper discs, cooled with water, were used for this process, starting with grit sizes 320 and 600 (Extex Corp., Enfield, CT, IL, USA), followed by final polishing with a fabric disc (EXTEC® Multi-Cloth™, Extex Corp., CT, IL, USA) moistened with a 0.25 μ diamond suspension (EXTEC I Permanent Diamond Suspensions, Extex Corp., Enfield, CT, IL, USA) [16]. The study involved 150 bovine incisors, divided into three groups of 50 samples each. One group (10 samples per subgroup) was used for color change evaluation, another for analysis of transamelodentinal diffusion of HP, and the third for mineral content analysis of enamel and dentin using μ -CT.

2.3.3. Aesthetic Efficacy Analysis

2.3.3.1. Preparation and Pigmentation of Enamel/Dentin Discs

The discs selected for analysis underwent an initial color assessment based on the parameters defined by the International Commission on Illumination (CIE). A UV-Visible Reflection Spectrophotometer (Model UV-2450, Shimadzu, Kyoto, Japan) was used, configured for the CIE Lab* color system [9]. Color measurements were performed against a standardized gray background to minimize external influences. Discs with Lab* values closest to the average, within a 5% margin of error, were selected. The discs were then stored in microtubes (Kasvi K6-0150, 1.5 mL, São José dos Pinhais, PR, Brazil), each containing 1 mL of black tea infusion at room temperature for the pigmentation process. The infusion was prepared using 1.6 g of black tea (Chá Matte Leão, Curitiba, PR, Brazil) per 100 mL of deionized water, which was heated to boiling (100 °C) prior to infusion. The tea was steeped for 5 min before use [17]. Pigmentation was monitored over 6 days, with the infusion being replaced daily. After removal from the tea solution, the specimens were rinsed with deionized water for 30 s and gently dried with absorbent paper prior to analysis. Then, the color values of the discs were recorded again using the CIE Lab* system [16].

2.3.3.2. Composition and Treatment with Whitening Gel

The whitening gel formulations consisted of a thickening agent (12% Carbopol) and HP at concentrations of 17.5% and 35%. In test groups, 1% CaTMP was added (concentration selected based on the optimal results from a previous study [19]). Additionally, glycerin, water (q.s.p.), and NaOH were included to adjust the gel's pH to approximately 7.0. Five experimental groups were established for the study: 1) Negative control – deionized water; 2) Whitening gel with 35% HP (positive control); 3) Whitening gel with 35% HP + 1% CaTMP; 4) Whitening gel with 17.5% HP; 5) Whitening gel with 17.5% HP + 1% CaTMP ([Fig. 1B](#)).

After the pigmentation process, the whitening gels were applied to the enamel surface (0.04 mL) for 40 min using a dispensing syringe and a microbrush (KG Sorensen, Cotia, SP, Brazil). The whitening protocol included 3 sessions, with a 7-day interval between each. After each application, the gels were removed with gauze, and the discs were rinsed with deionized water for 30 s ([Fig. 1C](#)). Between whitening sessions, the discs were stored in individual plastic containers containing 2 mL of artificial saliva, consisting of 1.5 mmol/L $\text{Ca}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$, 0.9 mmol/L $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and 150 mmol/L KCl in a 0.1 mol/L sodium cacodylate buffer, pH 7.0 [9], at 37 °C, which was changed daily.

2.3.3.3. Color Measurement

Color evaluation was performed 48 h after each whitening session and 14 days after the

completion of the treatment, aiming to assess the color stabilization over time. Color changes were quantified using the ΔE and ΔE_{00} indices, in addition to the whitening index (ΔW_{ID}). To calculate these differences, the absolute variations (Δ) of the color coordinates (L^* , a^* , b^*) at different time points (post-pigmentation, post-whitening, and 14 days after the end of treatment) were compared to the initial values (Fig. 1D). The variation in ΔL^* was used to indicate changes in brightness (positive values indicate increased brightness, while negative values indicate darkening), Δa^* reflects changes in the red-green component (positive for redder and more negative for greener), and Δb^* represents changes in the yellow-blue component (positive for more yellow and negative for bluer). The total color change was determined using the formula: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. Additionally, the dental whiteness index (ΔW_{ID}) was calculated using the equation: $\Delta W_{ID} = 0.511L^* - 2.324a^* - 1.100b^*$. For the ΔE_{00} calculation, the CIEDE2000 system was applied, following the formula: $E_{00} = [(\Delta L/K_L \times S_L) + (\Delta C/K_C \times S_C)^2 + (\Delta H/K_H \times S_H)^2 + R_T \times (\Delta C/K_C \times S_C) \times (\Delta H/K_H \times S_H)]^{0.5}$, where ΔL^* , ΔC^* , and ΔH^* represent the differences in brightness, chroma, and hue, respectively, and R_T describes the interaction between chroma and hue in the blue region. The weighting functions for luminance, chroma, and hue are represented by S_L , S_C , and S_H , while K_L , K_C , and K_H are the parametric factors, all set to 1. The perceptibility (PT) and acceptability (AT) thresholds were defined as 50:50%, with ΔE_{00} thresholds at 0.81 (PT) and 2.7 (AT) and ΔW_{ID} thresholds at 0.72 (PT) and 2.60 (AT) [23].

2.3.4. Determination of Transamelodentinal Diffusion of HP

Enamel/dentin discs ($n = 10$ per group) were placed in the upper compartment of an artificial pulp chamber (APC) using silicone rings and sealed with pink wax No. 7 (Wilson®, Polidental, Cotia, SP, Brazil), as described by Trindade et al., 2009 [24]. The lower compartments of the APCs, containing the dentin surface, were immersed 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) [16]. The samples were maintained in a humidified environment at 37 °C, and the whitening gels were applied once, following the procedure described earlier. To assess the amount of HP present in the buffer solution, the colorimetric method of [25] was employed. After 40 min of gel application, 1 mL of crystal violet (0.5 mg/mL in HCl, Sigma Chemical Co., St. Louis, MO, USA), 50 μ L of horseradish peroxidase enzyme (1 mg/mL in water, Sigma Chemical Co., St. Louis, MO, USA), and 3 mL of deionized water were added [16]. A standard curve of HP concentrations (0.5–5.0 μ g/mL) was constructed under the same conditions applied

to the samples. Absorbance readings were taken using a spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) at a wavelength of 596 nm (Fig. 1E).

2.3.5. Mineral Content of Enamel and Dentin by X-ray microtomography (μ -CT)

The 50 enamel/dentin discs were divided into two areas: 1) control area (unexposed) and 2) bleached area, with half of the enamel surface covered by acid-resistant varnish (nail polish – Risqué Ltd., SP, Brazil), preventing contact with the proposed treatment groups. After completing the whitening treatment, as described earlier, the mineral content of the dental substrates was analyzed using a high-resolution microtomograph (SkyScan1272, Bruker- μ -CT, Kontich, Belgium) and the Nrecon algorithmic reconstruction software, to determine the mineral content of the dental substrates ($g_{HAp} \times cm^{-3}$) in 3D. The μ -CT was operated at 125 kV, 0.26 mA, with a 1 mm aluminum filter, spatial resolution of 1.5 μm , rotation step of 0.600°, and frame averaging set to 2 [16,26]. Reconstruction was performed using isotropic voxels of 1 μm (volume elements) (Fig. 1F).

The analyses were based on mineral concentration, calculated from the linear attenuation coefficient [16,26]. Differential mineral concentration profiles were constructed for each enamel/dentin disc: the profile of the treated (exposed) area was subtracted from the profile of the healthy (sound) area. The integrated area of the differential profiles was then calculated using the trapezoidal rule (GraphPad Prism®, version 7.00, San Diego, CA, USA), resulting in the integrated mineral concentration (ΔIMC , $g_{HAp} \times cm^{-3} \times \mu m$) [16,26].

2.3.6. Statistical Analysis

The statistical analysis was performed using Sigmaplot® for Windows, version 12.0, with a significance level of 5%. For the color change data, the different types of whitening gels and evaluation times were treated as factors, while the analyzed variables were ΔE , ΔWI_D , and ΔE_{00} . Regarding the HP transamelodentinal diffusion and the mineral content ($g_{HAp} \times cm^{-3} \times \mu m$) of the dental substrates, the obtained values were considered as dependent variables, with the types of whitening gels as the factor. The collected data were first evaluated for normality using the Shapiro-Wilk test and for homoscedasticity with the Levene test. Subsequently, analysis of variance (ANOVA) was performed, followed by the Student-Newman-Keuls multiple comparison test.

2.4. Results

2.4.1. CaTMP Characterization

SEM images revealed large, uniform, and homogeneous crystals for NaTMP (Fig. 2A), while CaTMP exhibited smaller, irregular granular crystals (Fig. 2B). EDS analysis confirmed the presence of oxygen (O), calcium (Ca), and phosphorus (P) with no detectable sodium (Na) peak in the CaTMP sample (Fig. 2B). The elemental weight composition of CaTMP were as follows: O (53.96%), P (22.33%), and Ca (23.5%).

2.4.2. Aesthetic Effect

The whitening agents evaluated caused a significant change in ΔE after the whitening sessions ($p < 0.001$) in enamel previously stained. All treatments showed similar whitening efficacy, regardless of the evaluation period ($p > 0.05$). The color change was progressive and continuous from the first whitening session (Fig. 3A). In terms of ΔWI_D , all groups induced gradual and continuous color changes after the whitening sessions, making the samples whiter ($p < 0.001$) (Fig. 3B). No differences in ΔWI_D were observed between groups during any whitening application or throughout the whitening sessions ($p > 0.05$), with all gels maintaining a stable chromatic effect 14 days after the treatment.

For color changes assessed by ΔE_{00} , the whitening protocols produced clinically perceptible color changes ($\Delta E_{00} > 0.8$), with all experimental gels showing a similar whitening effect, regardless of the time of analysis ($p > 0.05$) (Fig. 3C). Overall, the different HP concentrations resulted in a similar whitening effect in both analyses ($p > 0.05$). Furthermore, the addition of CaTMP did not interfere with the aesthetic effect compared to the corresponding gels without the salt, regardless of the HP concentration evaluated ($p > 0.05$).

2.4.3. Transamelodontinal Diffusion Analysis

Regarding transamelodontinal diffusion, the highest HP were observed in specimens whitened with 35% HP ($p < 0.05$; Fig. 4). However, the presence of CaTMP in the gels resulted in a significant reduction in HP diffusion compared to their conventional counterparts ($p < 0.001$). A dose-dependent reduction in HP diffusion was observed with increasing HP concentrations in the gels. The gel containing 17.5% HP and 1% CaTMP exhibited the lowest HP transamelodontinal diffusion among the whitening groups ($p < 0.001$).

2.4.4. Mineral Content of Enamel and Dentin

The enamel and dentin treated with conventional gels containing 17.5% and 35% HP exhibited a significantly lower integrated area ($g_{HAP} \times cm^{-3} \times \mu m$) compared to their respective untreated regions ($p < 0.05$), indicating mineral loss in the dental tissues after conventional whitening protocols ($p < 0.05$; Table 1; Fig. 5). However, the dental tissues exposed to

whitening agents containing CaTMP showed an integrated area ($g_{HAP} \times cm^{-3} \times \mu m$) similar to that of the untreated regions, with no statistically significant differences ($p > 0.05$; Table 1; Fig. 5).

Regarding the treatments with CaTMP, only the gel containing 17.5% HP and 1% CaTMP resulted in a mineral loss in enamel, which was significantly lower compared to gels without CaTMP ($p < 0.05$). Additionally, no mineral loss was observed in dentin in the groups treated with gels containing CaTMP. On the other hand, treatment with the gel containing 35% HP and CaTMP promoted a mineral gain ($g_{HAP} \times cm^{-3}$) of 0.3% in enamel and 0.5% in dentin. In short, similar trends were observed in both enamel and dentin, with a reduction in the integrated area and mineral loss in treatments with conventional gels, compared to gels containing CaTMP, regardless of the HP concentration ($p < 0.05$; Table 1; Fig. 5).

2.5. Discussion

The effectiveness and safety of treatment are crucial aspects when selecting a whitening technique or agent. In this context, the addition of bioactive compounds with remineralizing properties to the whitening gel has emerged as a promising approach to minimize changes in dental substrates and clinically reduce dental sensitivity [11, 18, 27, 28]. In the present study, the results suggest that the addition of CaTMP to the whitening gel maintains bleaching efficacy while reducing the transamelodentinal diffusion of HP and providing a protective effect against mineral loss in dental substrates, even at higher concentrations of HP. Thus, the null hypothesis regarding the whitening effect was accepted, while the null hypotheses related to mineral concentration and transamelodentinal diffusion were rejected.

The inclusion of CaTMP in HP-based whitening gels did not significantly alter the bleaching effectiveness. This finding aligns with previous studies showing that the addition of different phosphate-based salt – such as sodium trimetaphosphate, sodium hexametaphosphate, and calcium glycerophosphate – does not impair gel performance [17,28,29]. Despite chemical differences in molecular structure and ionic composition, these salts serve comparable functional roles in dental applications, mainly by modulating mineral interactions and mitigating demineralization. Although the compound examined in this study (CaTMP) contains calcium instead of sodium, this ionic substitution is not intended to enhance the bleaching effect. Therefore, the lack of impact on whitening outcomes was anticipated, as phosphate salts are primarily designed to attenuate adverse effects on dental tissues by modulating mineral dynamics, rather than directly contributing to the whitening process.

The whitening protocol, performed in three sessions, showed the greatest whitening effect

after the first application, with a decrease in intensity in subsequent sessions. This pattern can be explained by the higher redox activity of HP during the initial application, which acts directly on the enamel pigments. Although the gel with 35% HP showed more pronounced whitening initially, its effectiveness was similar to that of the 17.5% gel in the following sessions, indicating that multiple sessions with a lower concentration can achieve comparable results throughout the treatment [16]. Furthermore, the free radicals generated by 35% HP promote more intense whitening at the beginning, but the intensity of the effect tends to decrease in subsequent sessions [9,28]. After 14 days following the end of the treatment, the whitening results remained stable, regardless of the gel used, with the different chromatic methodologies showing similar effectiveness. This suggests that, while the addition of CaTMP and the concentration of HP do not substantially alter the whitening effect, they may provide benefits in terms of mineral protection for the teeth during treatment.

Recent studies show that, in addition to the aesthetic effect caused by the generation of active oxygen and free radicals, such as perhydroxyl radicals, tooth whitening can cause alterations in dental substrates [8,9,30]. The decomposition of HP releases hydrogen ions (H^+), creating an acidic environment that can damage both the surface and the subsurface of the teeth [9,16]. Although the whitening products used in this study have a neutral pH, consecutive sessions with high concentrations of HP can induce structural changes in the enamel, leading to mineral loss and compromising its integrity. Therefore, evaluating the mechanical properties of enamel is a common strategy to investigate the effects of these treatments on tooth structure. In the present study, it was observed that the use of conventional HP-based gels, regardless of concentration, significantly affected the mineral concentration of both enamel and dentin. However, when the whitening gels were supplemented with CaTMP, no mineral loss was observed, suggesting that this bioactive compound can protect the mineral integrity of teeth, reducing the transamelodentinal diffusion of HP, and potentially attenuating the adverse effects of whitening, even when high concentrations of HP are used.

The ability of gels containing CaTMP to protect against dental demineralization is linked to the unique properties of this salt. Studies suggest that TMP acts as a protective barrier by reducing the diffusion of acids and preventing deep softening of dental substrates, which assists preserve the integrity of enamel and dentin [9,14,31,32]. The favorable results observed with gels containing CaTMP can also be attributed to TMP's ability to increase anionic sites, which electrostatically attract Ca^{2+} ions. This process forms crystallization nuclei and apatite precipitation, altering selective permeability and ion diffusion in dental substrates [32,33].

Although the combination of TMP with fluoride has also shown efficacy in whitening gels

in previous studies [9,16,17], the present study indicates that the substitution of sodium ions with calcium ions in the TMP molecule is sufficient to provide protective effects, without the need for fluoride. The replacement of Na^+ with Ca^{2+} in TMP molecules creates a reticular structure, where each calcium atom is bound to two TMP molecules [33]. This alteration in bonding imparts to TMP the ability to reduce acid penetration into enamel and dentin, protecting against mineral loss during tooth whitening sessions. Additionally, the negatively charged TMP layer formed on enamel facilitates calcium phosphate deposition, contributing to the reduction of enamel dissolution in acidic environments [16,33]. These results confirm that CaTMP, due to its specific properties, can be effective in protecting against demineralization, even without the association with fluoride.

In this study, mineral loss was evaluated using μ -CT, a non-destructive technique offering high-resolution, three-dimensional analysis without the distortions from physical sectioning. The use of native enamel surfaces is particularly relevant, as they more accurately reflect clinical conditions compared to polished enamel. While polished surfaces are commonly used for mechanical and de/remineralization studies, they may not represent the natural enamel found in the clinical situation. Our findings underscore the importance of using native enamel in whitening studies, and the μ -CT method ensures precise, clinically relevant data by maintaining the integrity of the sample throughout the analysis [34-38].

While mineral loss associated with whitening treatments is generally reported as minimal, it remains a clinically relevant parameter to evaluate. Even minor changes in enamel mineral content or structure can influence surface integrity, increase susceptibility to staining, and impact long-term dental health, especially with repeated use or high concentrations of whitening agents [16,19]. Furthermore, variability in findings regarding mineral loss exists, depending on factors such as formulation, exposure time, and substrate type (enamel vs. dentin). Therefore, assessing mineral loss in this study contributed to a more comprehensive evaluation of the whitening protocol's safety profile.

In an overall assessment of the results, it is possible to observe the positive and significant impact of CaTMP in the different whitening formulations evaluated. Although the trans-amelodentinal diffusion of HP was lower for the gel containing 17.5% HP and CaTMP, this difference does not seem to be sufficiently significant to indicate less aggression to the dental tissues when compared to the gel with 35% HP and CaTMP. This is supported by the similarity in aesthetic effects and changes in mineral content, regardless of the dental substrate evaluated. In short, despite the reduction in HP diffusion, the oxidative capacity of the gels with CaTMP appears to be equivalent, regardless of the HP concentration, resulting in consistent aesthetic

and protective effects on dental tissues. Thus, the addition of CaTMP to whitening gels provides greater safety during use, even in gels with high concentrations of HP, which is particularly relevant in light of the growing reports of adverse effects associated with the use of highly concentrated agents.

Despite the promising findings, this study presents inherent limitations associated with its *in vitro* design. The use of artificially pigmented bovine teeth, although common due to their availability and structural similarity to human enamel, does not fully replicate human dental tissues in terms of microstructure, permeability, and mineral composition. However, the extrapolation of these results to a clinical setting is not direct, as factors such as patient behavior, the presence of human saliva, and intra-oral variations can influence the response to treatments in real-life scenarios. Another limitation lies in the use of an artificial pulp chamber for transamelodontinal diffusion analysis, which does not replicate the complex defense mechanisms of human pulp tissue, such as vascular flow, pulp pressure, and enzymatic activity, potentially limiting the clinical relevance of the findings. Additionally, it was not possible to test the effects of the gel on other types of dental pigmentation, nor to assess the potential reduction in dental sensitivity or other biological effects. To move forward, future clinical studies are crucial to test the efficacy and safety of whitening gels with CaTMP under real-world conditions, considering their biocompatibility with the dentin-pulp complex and the long-term effects. Such investigations could pave the way for the development of whitening treatments that are not only effective but also safer, with fewer risks of adverse effects, benefiting both the aesthetics and the dental health of patients undergoing in-office treatments.

2.6. Conclusion

Based on the data obtained and considering the limitations of the experimental model, it can be concluded that the addition of 1% CaTMP to whitening gels containing 17.5% and 35% HP significantly reduces the transamelodontinal diffusion of HP, protects against mineral loss in dental substrates, and maintains the whitening efficacy of the gels.

2.3 Acknowledgments

This study was financially supported by the Coordination of Higher Education Personnel (CAPES, Finance Code 88887.817416/2023-00) - concession of a scholarship to the first author.

2.8. References

1. M. A. Goulart, A. M. Condessa, J. B. Hilgert, F. N. Hugo, R. K. Celeste, Concerns about dental aesthetics are associated with oral health related quality of life in Southern Brazilian adults. *Ciencia &*

- saude coletiva, 23(11) (2018) 3957–3964. <https://doi.org/10.1590/1413-812320182311.24172016>
2. R. Alkahtani, S. Stone, M. German, P. Waterhouse, A review on dental whitening, *J. Dent.* 100 (2020) 103423. <https://doi.org/10.1016/j.jdent.2020.103423>.
 3. B.M. Maran, C. Bersezio, J. Martin, M.W. Favoreto, M. Rezende, L. Vallejo-Izquierdo, A. Reis, A.D. Loguercio, E. Fernandez, The influence of dental bleaching on patient's quality of life: A multistudy analysis of aesthetic and psychosocial outcomes, *J. Dent.* 151 (2024) 105397. <https://doi.org/10.1016/j.jdent.2024.105397>.
 4. A. Joiner, W. Luo, Tooth colour and whiteness: A review, *J. Dent.* 67S (2017) S3–S10. <https://doi.org/10.1016/j.jdent.2017.09.006>.
 5. W.A.B. Aragão, V.S. Chemelo, C.M. Alencar, C.M. Silva, S. Pessanha, A. Reis, R.D. Souza-Rodrigues, R.R. Lima, Biological action of bleaching agents on tooth structure: A review, *Histol. Histopathol.* 39 (2024) 1229–1243. <https://doi.org/10.14670/HH-18-726>.
 6. L. Fiorillo, L. Laino, R. De Stefano, C. D'Amico, S. Bocchieri, G. Amoroso, G. Isola, G. Cervino, Dental whitening gels: Strengths and weaknesses of an increasingly used method, *Gels* 5 (2019) 35. <https://doi.org/10.3390/gels5030035>.
 7. M.Q. Alqahtani, Tooth-bleaching procedures and their controversial effects: A literature review, *Saudi Dent. J.* 26 (2014) 33–46. <https://doi.org/10.1016/j.sdentj.2014.02.002>.
 8. H.M. Ellfallah, L.E. Bertassoni, N. Charadram, C. Rathsam, M.V. Swain, Effect of tooth bleaching agents on protein content and mechanical properties of dental enamel, *Acta Biomater.* 20 (2015) 120–128. <https://doi.org/10.1016/j.actbio.2015.03.035>.
 9. A.S. Gruba, G.P. Nunes, M.T. Marques, M. Danelon, R.O. Alves, P.T.A. de Toledo, A.L.F. Briso, A.C.B. Delbem, Influence of bleaching gels formulated with nano-sized sodium trimetaphosphate and fluoride on the physicochemical, mechanical, and morphological properties of dental enamel, *J. Dent.* 139 (2023) 104743. <https://doi.org/10.1016/j.jdent.2023.104743>.
 10. C.A. Costa, H. Riehl, J.F. Kina, N.T. Sacono, J. Hebling, Human pulp responses to in-office tooth bleaching, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 109 (2010) e59–e64. <https://doi.org/10.1016/j.tripleo.2009.12.002>.
 11. B. Gümüştaş, B. Dikmen, Effectiveness of remineralization agents on the prevention of dental bleaching induced sensitivity: A randomized clinical trial, *Int. J. Dent. Hyg.* 20 (2022) 650–657. <https://doi.org/10.1111/idh.12524>.
 12. K. Pintado-Palomino, O. Peitl Filho, E.D. Zanutto, C. Tirapelli, A clinical, randomized, controlled study on the use of desensitizing agents during tooth bleaching, *J. Dent.* 43 (2015) 1099–1105. <https://doi.org/10.1016/j.jdent.2015.07.002>. Erratum in: *J. Dent.* 62 (2017) 98. <https://doi.org/10.1016/j.jdent.2017.05.019>.
 13. D.H. Kim, J. Bae, J.H. Heo, C.H. Park, E.B. Kim, J.H. Lee, Nanoparticles as next-generation tooth-whitening agents: Progress and perspectives, *ACS Nano* 16 (2022) 10042–10065. <https://doi.org/10.1021/acsnano.2c01412>.
 14. G.P. Nunes, M. Danelon, J.P. Pessan, L.C. Capalbo, N.A.N. Junior, A.A. Matos, J.A.S. Souza, M.A.R. Buzalaf, A.C.B. Delbem, Fluoride and trimetaphosphate association as a novel approach for

- remineralization and antiproteolytic activity in dentin tissue, *Arch. Oral Biol.* 142 (2022) 105508. <https://doi.org/10.1016/j.archoralbio.2022.105508>.
15. N.G. Emerenciano, A.C.B. Delbem, J.P. Pessan, G.P. Nunes, F.N. Souza Neto, E.R. de Camargo, M. Danelon, In situ effect of fluoride toothpaste supplemented with nano-sized sodium trimetaphosphate on enamel demineralization prevention and biofilm composition, *Arch. Oral Biol.* 96 (2018) 223–229. <https://doi.org/10.1016/j.archoralbio.2018.09.019>.
 16. G.P. Nunes, M.T. Marques, P.T.A. de Toledo, R.O. Alves, T.P. Martins, A.C.B. Delbem, Effect of a novel low-concentration hydrogen peroxide bleaching gel containing nano-sized sodium trimetaphosphate and fluoride, *J. Dent.* 150 (2024) 105330. <https://doi.org/10.1016/j.jdent.2024.105330>.
 17. S.T.F. Akabane, M. Danelon, G.P. Nunes, A.S. Gruba, C.A. de Souza-Costa, C. de O. Duque, M. de O. Gallinari, A.L.F. Briso, A.C.B. Delbem, Evaluation of the aesthetic effect, enamel microhardness and trans-amelodentinal cytotoxicity of a new bleaching agent for professional use containing trimetaphosphate and fluoride, *J. Mech. Behav. Biomed. Mater.* 114 (2021) 104225. <https://doi.org/10.1016/j.jmbbm.2020.104225>
 18. R.A.T.P. Júnior, M. Danelon, J.P. Pessan, N.G. Emerenciano, R.F. Cunha, M.S. Shinohara, A.C.B. Delbem, Effect of daily use of fluoridated dentifrice and bleaching gels containing calcium, fluoride, or trimetaphosphate on enamel hardness: An in vitro study, *Clin. Oral Investig.* 25 (2021) 883–889. <https://doi.org/10.1007/s00784-020-03375-5>.
 19. G.P. Nunes, G.F. Batista, P.T.A. de Toledo, T.P. Martins, R.O. Alves, A.V.P. Fernandes, A.C.B. Delbem, Synthesis and application of calcium cyclotriphosphate in bleaching formulations: Effects on dental enamel properties, *J. Dent.* 105614 (2025). <https://doi.org/10.1016/j.jdent.2025.105614>.
 20. G.P. Nunes, R. de Oliveira Alves, G.R. Peres, M.H.F. Ragghianti, P.T.A. de Toledo, A.H. Dos Reis Prado, C. Ferreira-Baptista, A.C.B. Delbem, Protective role of calcium-based agents in dental bleaching gels: insights from a systematic review and meta-analysis of clinical and laboratory evidence. *Clinical oral investigations*, 29 (2025). 180. <https://doi.org/10.1007/s00784-025-06265-w>
 21. J. Jeong, J.H. Kim, J.H. Shim, N.S. Hwang, C.Y. Heo, Bioactive calcium phosphate materials and applications in bone regeneration. *Biomater Res.* 23 (2019) 4. doi:10.1186/s40824-018-0149-3
 22. E.J. Griffith, The structure of salt sodium calcium metaphosphate ($\text{Na}_4\text{CaP}_6\text{O}_{18}$), *Inorg. Chem.* 1 (1962) 962.
 23. R.D. Paravina, M.M. Pérez, R. Ghinea, Acceptability and Perceptibility Thresholds in Dentistry: A Comprehensive Review of Clinical and Research Applications, *J. Esthet. Restor. Dent.* 31 (2019) 103–112. <https://doi.org/10.1111/jerd.12465>.
 24. F.Z. Trindade, A.P. Ribeiro, N.T. Sacono, C.F. Oliveira, F.C. Lessa, J. Hebling, C.A. Costa, Trans-Enamel and Trans-Dentinal Cytotoxic Effects of a 35% H_2O_2 Bleaching Gel on Cultured Odontoblast Cell Lines after Consecutive Applications, *Int. Endod. J.* 42 (2009) 516–524. <https://doi.org/10.1111/j.1365-2591.2009.01544.x>.
 25. H.A. Mottola, B.E. Simpson, G. Gorin, Absorptiometric Determination of Hydrogen Peroxide in Submicrogram Amounts with Leuco Crystal Violet and Peroxidase as Catalyst, *Anal. Chem.* 42 (1970)

410–411.

26. G. Dalpasquale, A.C.B. Delbem, J.P. Pessan, G.P. Nunes, L.F. Gorup, F.N.S. Neto, E.R. de Camargo, M. Danelon, Effect of the Addition of Nano-Sized Sodium Hexametaphosphate to Fluoride Toothpastes on Tooth Demineralization: An In Vitro Study, *Clin. Oral Investig.* 21 (2017) 1821–1827. <https://doi.org/10.1007/s00784-017-2093-3>. Erratum in: *Clin. Oral Investig.* 21 (2017) 1829. <https://doi.org/10.1007/s00784-017-2116-0>.
27. M. Melo, R. Fioresta, J.L. Sanz, M.P. Pecci-Lloret, C. Llena, Effect of Highly Concentrated Bleaching Gels on Enamel Microhardness and Superficial Morphology, and the Recovery Action of Four Remineralizing Agents, *BMC Oral Health* 22 (2022) 645. <https://doi.org/10.1186/s12903-022-02693-2>.
28. N.A.N. Júnior, G.P. Nunes, A.S. Gruba, M. Danelon, L.M.A.V. da Silva, G.F. de Farias Batista, A.L.F. Briso, A.C.B. Delbem, Evaluation of Bleaching Efficacy, Microhardness, and Trans-Amelodentinal Diffusion of a Novel Bleaching Agent for an In-Office Technique Containing Hexametaphosphate and Fluoride, *Clin. Oral Investig.* 26 (2022) 5071–5078. <https://doi.org/10.1007/s00784-022-04480-3>.
29. J.G. Amaral, J.P. Pessan, J.A.S. Souza, J.C.S.S. Moraes, A.C.B. Delbem, Cyclotriphosphate Associated to Fluoride Increases Hydroxyapatite Resistance to Acid Attack, *J. Biomed. Mater. Res. B Appl. Biomater.* 106 (2018) 2553–2564. <https://doi.org/10.1002/jbm.b.34072>.
30. M. Sulieman, M. Addy, J.S. Rees, Development and Evaluation of a Method In Vitro to Study the Effectiveness of Tooth Bleaching, *J. Dent.* 31 (2003) 415–422. [https://doi.org/10.1016/s0300-5712\(03\)00069-1](https://doi.org/10.1016/s0300-5712(03)00069-1)
31. C.O. Favretto, A.C.B. Delbem, J.C.S.S. Moraes, E.R. de Camargo, P.T.A. de Toledo, D. Pedrini, Dentinal Tubule Obliteration Using Toothpastes Containing Sodium Trimetaphosphate Microparticles or Nanoparticles, *Clin. Oral Investig.* 22 (2018) 3021–3029. <https://doi.org/10.1007/s00784-018-2384-3>.
32. E.K.P. Nalin, M. Danelon, E.S. da Silva, T.Y. Hosida, J.P. Pessan, A.C.B. Delbem, Surface Free Energy, Interaction, and Adsorption of Calcium and Phosphate to Enamel Treated with Trimetaphosphate and Glycerophosphate, *Caries Res.* 55 (2021) 496–504. <https://doi.org/10.1159/000518943>.
33. L.P. Pancote, M.M. Manarelli, M. Danelon, A.C. Delbem, Effect of Fluoride Gels Supplemented with Sodium Trimetaphosphate on Enamel Erosion and Abrasion: An In Vitro Study, *Arch. Oral Biol.* 59 (2014) 336–340. <https://doi.org/10.1016/j.archoralbio.2013.12.007>.
34. V.T. Pelá, M.A.R. Buzalaf, S.H. Niemeyer, et al., Acquired Pellicle Engineering with Proteins/Peptides: Mechanism of Action on Native Human Enamel Surface, *J. Dent.* 107 (2021) 103612. <https://doi.org/10.1016/j.jdent.2021.103612>.
35. T. Attin, F.J. Wegehaupt, Methods for Assessment of Dental Erosion, *Monogr. Oral Sci.* 25 (2014) 123–142. <https://doi.org/10.1159/000360355>.
36. C. Nobrega, G.P. Nunes, B.Y. de Paiva Buischi, N.C. Kajimoto, A.C.B. Delbem, In Vitro Assessment of Dental Erosion Caused by Clear Aligners, *J. Mech. Behav. Biomed. Mater.* 152 (2024) 106390. <https://doi.org/10.1016/j.jmbbm.2024.106390>.
37. H. Hamba, T. Nikaido, A. Sadr, S. Nakashima, J. Tagami, Enamel Lesion Parameter Correlations

- between Polychromatic Micro-CT and TMR, J. Dent. Res. 91 (2012) 586–591. <https://doi.org/10.1177/0022034512444127>.
38. V.M. Soviero, S.C. Leal, R.C. Silva, R.B. Azevedo, Validity of MicroCT for In Vitro Detection of Proximal Carious Lesions in Primary Molars, J. Dent. 40 (2012) 35–40. <https://doi.org/10.1016/j.jdent.2011.09.002>.

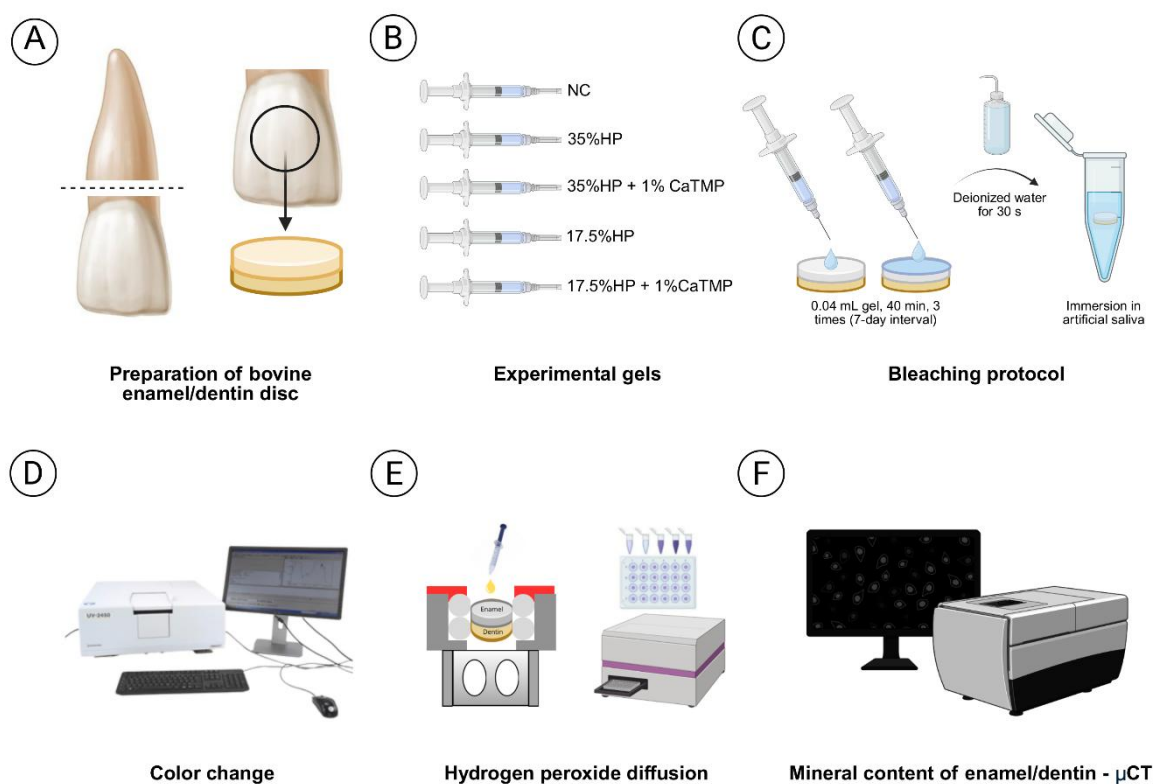


Figure 1. Representative diagram of the study's experimental design. A. Enamel/Dentin Discs Preparation. B. Experimental Whitening Gels. C. Bleaching Protocol. D. Color Change Analysis E. Transamelodentinal Diffusion of H_2O_2 . F. Mineral content of enamel and dentin

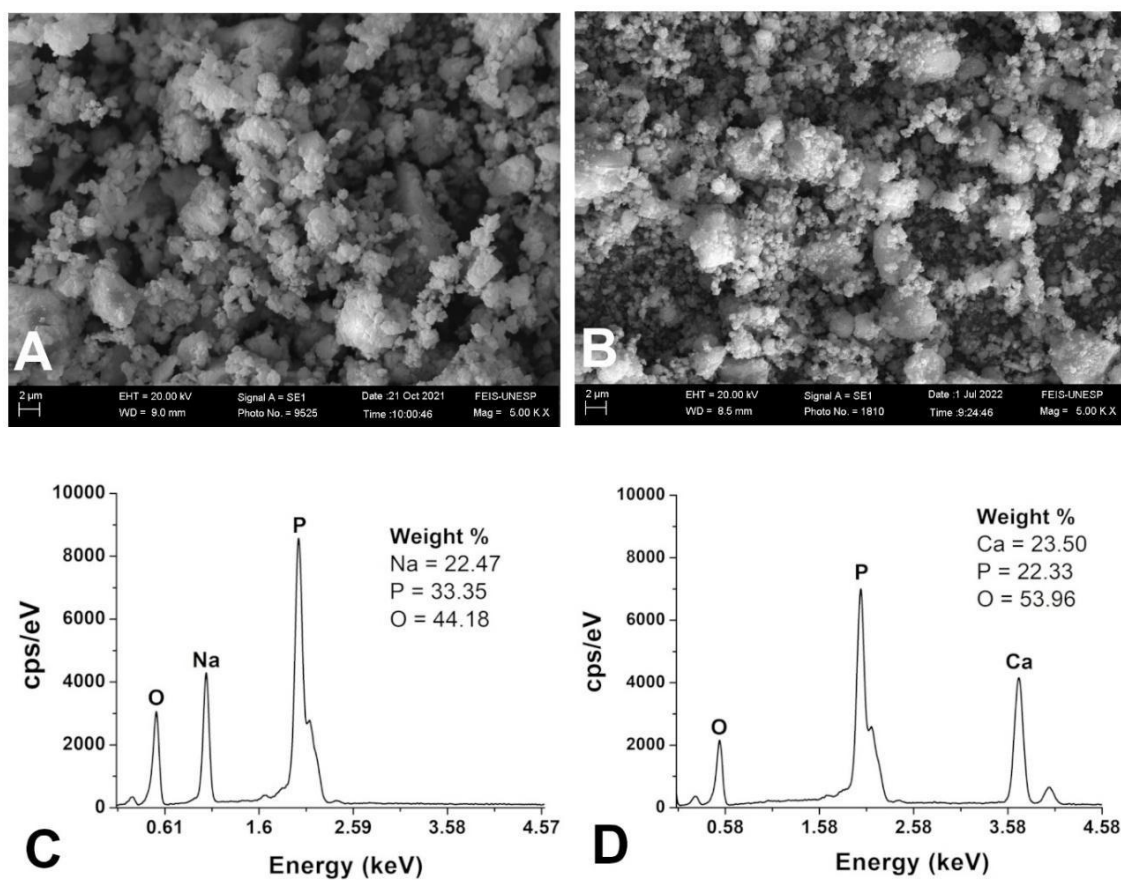


Figure 2. Scanning Electron Microscopy (SEM) images of NaTMP (A) and CaTMP (B) samples. (C) Energy-dispersive X-ray spectroscopy (EDX) spectra of NaTMP (C) and CaTMP (D) compounds.

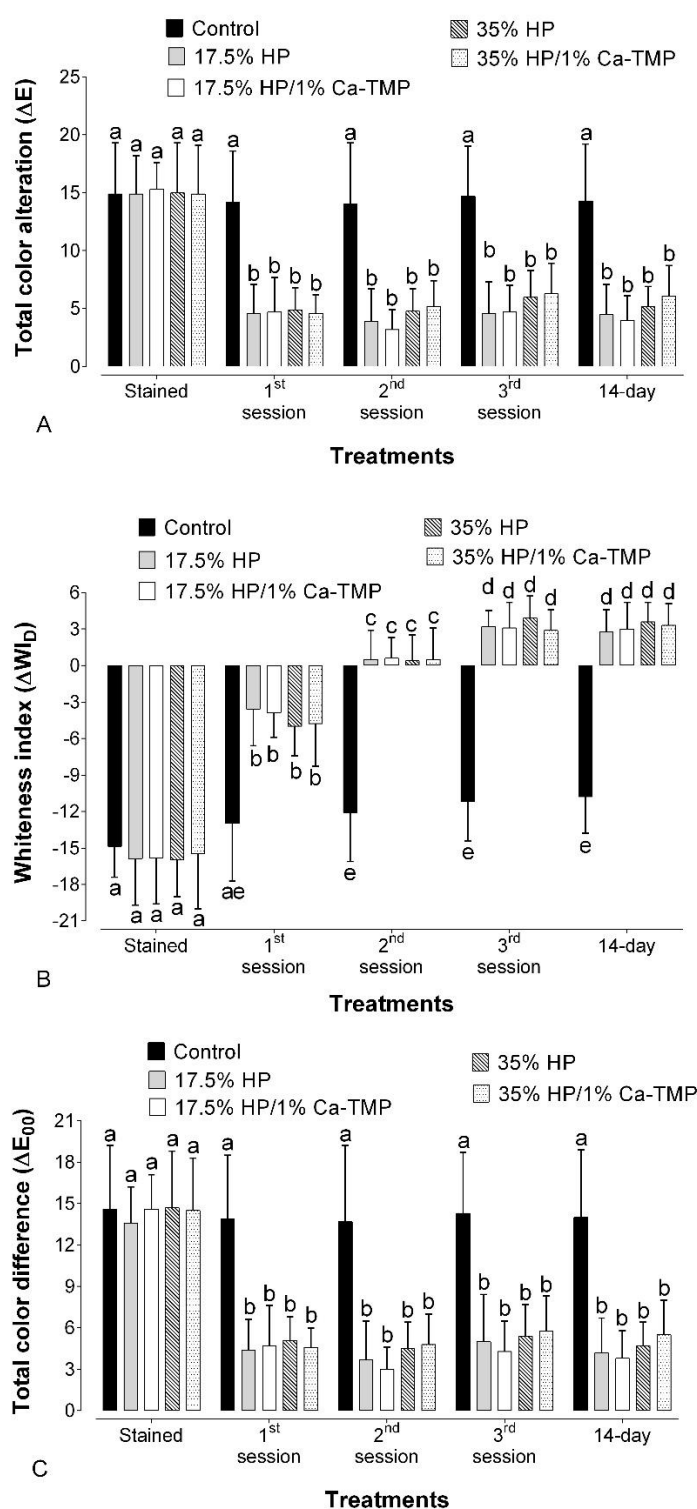


Figure 3. Mean values (SD) of: A - Total color change (ΔE) by the Commission Internationale de l'Éclairage (CIE); B - Whiteness index in dentistry (ΔWI_D); C - Color change by CIEDE2000 (ΔE_{00}), according to whitening gels and time of analysis (n = 10). Distinct lowercase letters indicate statistical differences between whitening gels at each time point and between time points for each gel (Student-Newman-Keuls; $p < 0.05$).

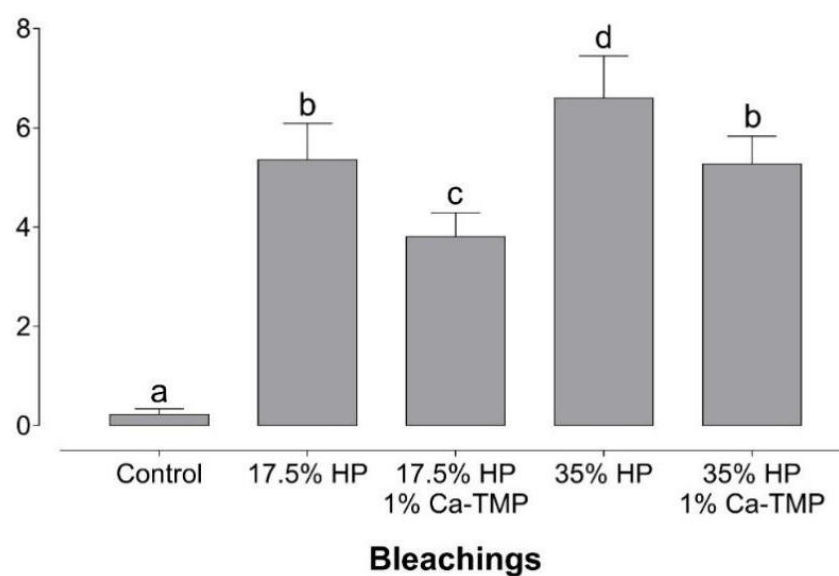


Figure 4. Mean values (SD) of transamelodentinal diffusion of HP according to the experimental groups. Distinct lowercase letters indicate statistical differences between whitening gels at each time point and between time points for each gel (Student-Newman-Keuls; $p < 0.05$).

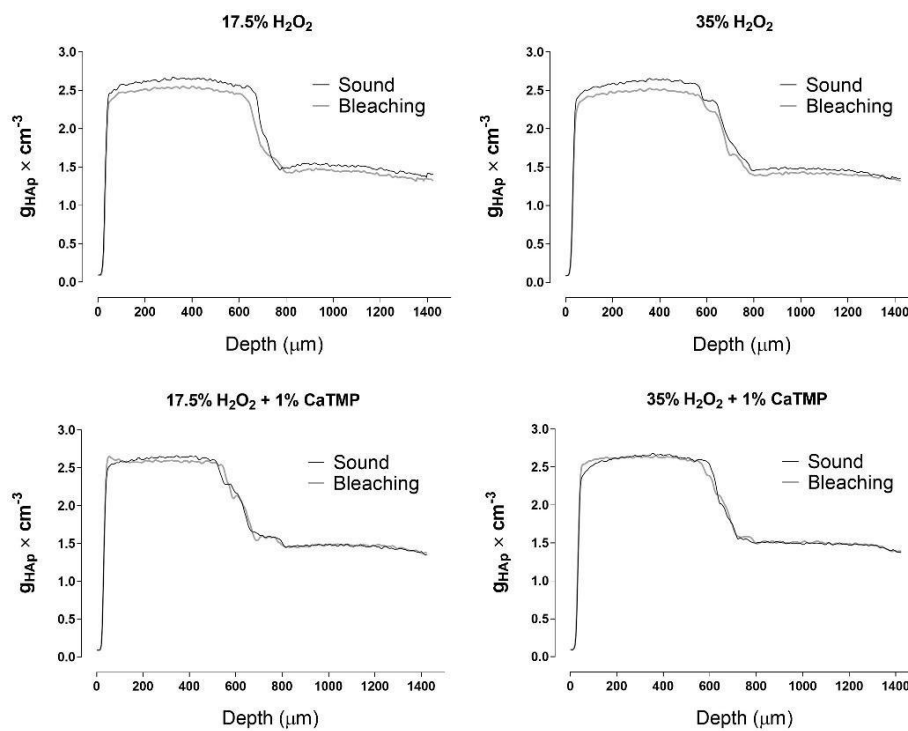


Figure 5. Transversal profile of mineral concentration ($g_{HAp} \times cm^{-3} \times \mu m$) as a function of depth (μm) in different areas of dental enamel (sound, bleached) according to the experimental groups

Table 1. Mean (SD) of the integrated area and percentage of mineral loss according to the hydrogen peroxide concentration (% H₂O₂), calcium trimetaphosphate concentration (% CaTMP), substrate (enamel and dentin), and whitening or non-whitening (sound).

% HP	% CaTMP	Integrated area (g _{HAp} × cm ⁻³ × μm)				% Mineral Loss (g _{HAp} × cm ⁻³ × μm)	
		Sound		Bleached			
		Enamel	Dentin	Enamel	Dentin	Enamel	Dentin
17.5%	no-CaTMP	1348.0 ^a (18.0)	775.5 ^b (41.8)	1291.7 ^c (9.7)	739.5 ^b (39.2)	-4.1 ^a (1.3)	-4.6 ^a (1.8)
	1% CaTMP	1341.9 ^a (20.2)	755.4 ^b (26.9)	1332.9 ^a (14.8)	758.4 ^b (26.6)	-1.0 ^b (0.6)	0.4 ^b (1.4)
35%	no-CaTMP	1335.7 ^a (19.0)	771.2 ^b (64.6)	1273.6 ^c (28.8)	741.7 ^b (59.5)	-4.7 ^a (1.1)	-4.1 ^a (1.7)
	1% CaTMP	1343.4 ^a (17.9)	762.8 ^b (17.5)	1346.1 ^a (8.2)	768.4 ^b (31.6)	0.3 ^b (1.1)	0.5 ^b (2.2)

Identical lowercase letters indicate no significant difference between the means of each variable based on the analysis (Student-Newman-Keuls; $p < 0.05$). SD represents the standard deviation of the means.

*Artigo II***3. Enhancing protection in at-home and combined bleaching techniques: the effect of calcium trimetaphosphate-enriched whitening gels on hard dental substrates**

Ana Vitória Pereira Fernandes¹, Gabriel Pereira Nunes¹, Heloísa Caroline da Mota¹, Priscila Toninatto Alves de Toledo², Tamires Passadori Martins¹, Renata de Oliveira Alves¹, Alberto Carlos Botazzo Delbem¹

¹Department of Preventive and Restorative Dentistry, Araçatuba School of Dentistry, São Paulo State University - UNESP, Araçatuba, SP, Brazil.

²Department of Operative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, São Paulo University (USP), Bauru, São Paulo, Brazil

Corresponding author:

Alberto Carlos Botazzo Delbem

São Paulo State University (UNESP), School of Dentistry, Araçatuba Department
of Preventive and Restorative Dentistry

Rua José Bonifácio 1193 16015-050 – Araçatuba, SP - Brazil Tel.

+55 18 3636 3314 Fax +55 18 3636 3332

Email: alberto.delbem@unesp.br

3.1 Abstract

Aim: This *in vitro* study aimed to evaluate the effects of incorporating calcium trimetaphosphate (CaTMP) into whitening gels containing 10% hydrogen peroxide (HP) on the aesthetic outcomes, microhardness, roughness, enamel morphology, both in isolated at-home use and in a combined in-office and at-home treatment protocol.

Methods: Bovine enamel/dentin discs were assigned to five groups and subjected to various whitening treatments: a negative control (no treatment), 10% HP, 10% HP with 1% CaTMP, combined 35% HP and 10% HP, and combined 35% HP and 10% HP with 1% CaTMP. The treatments were applied for 14 days (40 min daily). For the combined therapy groups, a single 40-minute application of 35% HP whitening gel was applied before the home whitening protocol. The discs were then analyzed for color change (ΔE , ΔW_{ID} , and ΔE_{00}), surface microhardness, roughness, mineral content, and morphology and chemical composition using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), respectively. Data were analyzed using ANOVA followed by the Student-Newman-Keuls test ($p < 0.05$).

Results: All whitening treatments produced color changes ($p < 0.05$), with the combined technique (35% HP/10% HP) resulting in a more pronounced effect on ΔE and ΔE_{00} . Surface roughness increased only in the combined 35% HP/10% HP group ($P > 0.05$). Enamel hardness significantly decreased in all groups, but CaTMP reduced this loss. Mineral content in CaTMP-treated groups was similar to the control. The presence of CaTMP also led to fewer surface alterations compared to conventional whitening groups.

Conclusions: The addition of 1% CaTMP to 10% HP whitening gels did not influence the whitening effect, and showed protective effects on the integrity of dental substrates, especially in combined bleaching protocol. It reduced surface roughness and morphological changes, and minimized mineral loss. These findings suggest that CaTMP can improve the safety of bleaching treatments on dental hard tissues.

Key-words: Tooth whitening, Hydrogen peroxide, calcium, dental enamel, phosphates, in-office, at-home bleaching.

3.2 Introduction

In recent decades, society has markedly focused its attention on beauty and appearance, leading to a significant rise in the demand for interventions aimed at enhancing the smile (Kettle et al., 2024). This growing interest in aesthetic treatments has driven the search for dental whitening procedures (Butera et al., 2024). In this context, hydrogen peroxide (HP)-based gels are widely used in whitening therapy, being applied through different methods, concentrations, and dosages (Alkahtani et al., 2020). Regarding the techniques, dental whitening can be performed in-office or at home (Zhong et al., 2023; Alkahtani et al., 2020). In-office whitening generally involves the prescription of gels with high concentrations of HP, while at-home therapy predominantly relies on the daily use of whitening gels in low concentrations, through customized trays (Aidos et al., 2024).

Considering that high concentrations of HP can result in deleterious effects on the dental structure (Donato et al., 2024; Nunes et al., 2024; Donassollo et al., 2021), at-home whitening has been regarded as a method with greater biocompatibility for dental tissues in the treatment of vital teeth (Donato et al., 2024; Pontes et al., 2020; Maran et al., 2020). Furthermore, this technique presents advantages such as reduced time spent in the dental office, increased safety, personalization of results, and lower cost (Cordeiro et al., 2019; Donassollo et al., 2021; Fioresta et al., 2023). Despite the beneficial effects and the use of less concentrated gels, continuous exposure to HP-based agents is associated with a reduction in the enamel microhardness, mineral loss, increased surface roughness, and morphological alterations of this substrate (Nunes et al., 2024; Abu-Saq Al Yami et al., 2020). Additionally, the high diffusion of HP and the cumulative effects over the course of therapy can lead to pulp inflammation, clinically manifested as tooth sensitivity (Zhong et al., 2023; Dourado Pinto et al., 2019).

In this context, alternatives to traditional dosages, such as the incorporation of bioactive agents into whitening gels, have been explored (Dascanio et al., 2024; Nunes et al., 2024; Kury et al., 2022). The addition of sodium trimetaphosphate (TMP) in gels with both high (Gruba et al., 2023; Akabane et al., 2021) and low concentrations of HP (Nunes et al., 2024; Dos Santos et al., 2020) has shown protective effects against the alterations in dental substrates induced by whitening. However, it was not able to completely prevent such alterations, and its effect is limited to the synergy with the combined use of sodium fluoride. Recent studies have demonstrated that partial or complete replacement of sodium ions with calcium ions in the TMP molecule reduced the enamel changes resulting from dental whitening in gels applied in the in-office technique (Nunes et al., 2025; Fernandes et al., 2025). However, the possibility of a

complete substitution of the mentioned ions (CaTMP) remains unexplored for optimizing at-home bleaching gels.

Moreover, as previously reported, gels bleaching with lower concentrations of HP are also recognized to have greater biocompatibility with dental tissues (Donato et al., 2024; Maran et al., 2020; Pontes et al., 2020). In this context, the incorporation of CaTMP into at-home bleaching gels may represent a promising strategy for developing formulations that combine higher biocompatibility, reduced enamel demineralization, and enhanced safety during treatment. In clinical practice, it is important to bear in mind that the at-home technique presents challenges related to patient adherence (Pavani et al., 2023; Zhong et al., 2023). Such resistance can be attributed to the inherent characteristics of the whitening protocol, such as the need for daily self-application, the time spent using the trays, and a longer period required to perceive a change in color (aesthetic effect) (Alkahtani et al., 2020; Pavani et al., 2023). Due to these considerations, the adoption of a combined (mixed) therapy, integrating both at-home whitening and in-office treatment (with an initial whitening session), has become increasingly prescribed (Bernardon et al., 2010, Dourado Pinto et al., 2019; Zhong et al., 2023). This approach aims to provide a faster whitening effect than that achieved solely through at-home therapy (Bernardon et al., 2010; Dourado Pinto et al., 2019).

Although clinical studies have demonstrated that combining dental whitening techniques accelerates color change within the first week of treatment (Bernardon et al., 2011; Dourado Pinto et al., 2019), it can also result in higher reports of tooth sensitivity (Zhong et al., 2023; Machado et al., 2016). However, little is known about the enamel changes induced by whitening, as laboratory studies on this topic are still scarce. Therefore, this *in vitro* study evaluated the effect of adding calcium trimetaphosphate (CaTMP) to whitening gels containing 10% HP, both in the isolated use of the at-home technique and the combined technique (with one in-office session), on aesthetic effect, microhardness, roughness, mineral content, and enamel morphology. The null hypothesis of this study is that there is no difference in the whitening effect on microhardness, roughness, mineral content, morphology of enamel exposed to whitening therapy with CaTMP-containing gels when compared to conventional gels, evaluated in both the at-home and combined whitening techniques.

3.3 Materials and Methods

3.3.1 Experimental design

To conduct the study, TMP compounds with total sodium substitution for calcium

(CaTMP) were synthesized using a strong acid cation resin column. Bovine enamel/dentin discs were subsequently obtained. The sample size per group ($n=10$ discs per group) was determined based on a previous study (Nunes et al., 2024), where the primary outcomes included color change, surface and cross-sectional hardness. The mean differences between groups were 10, 14 and 2200, respectively, with standard deviations of 4, 6 and 1400, respectively. An α error of 5% and a β error of 10% were adopted for statistical analysis. Consequently, 50 discs were designated for color change analysis, and 50 for hardness, roughness, and SEM/EDS analyses. The discs were randomly allocated to five groups according to the treatment regimens: I) Negative control - deionized water; II) Whitening gel containing 10% HP (positive control); III) Whitening gel containing 10% HP + 1% CaTMP; IV) Combined whitening therapy: one session with whitening gel containing 35% HP + Whitening gel containing 10% HP; V) Combined whitening therapy: one session with whitening gel containing 35% HP + Whitening gel containing 10% HP + 1% CaTMP. The treatment protocol involved daily application of the home technique whitening gels (10% HP or 10% HP + 1% CaTMP) for 40 min over 14 days (groups II and III). For the combined therapy groups (IV and V), a single 40-min application of 35% HP whitening gel was made before the home technique protocol. The discs were stored in artificial saliva between treatment sessions, which was replaced daily. After completing the treatment protocol, the total color change, surface hardness and roughness, and enamel morphology and chemical composition were evaluated.

3.3.2 CaTMP Synthesis

Calcium trimetaphosphate (CaTMP) was synthesized as described by Fernandes et al. (2025), by fully replacing sodium ions in sodium trimetaphosphate (NaTMP) using a cation exchange resin. A NaTMP solution was passed through a PUROLITE C100H (Purolite®, Philadelphia, PA, USA) resin column, and the acidic effluent was neutralized with calcium hydroxide to restore the pH. The mixture was incubated at 37 °C for 24 h to allow CaTMP precipitation, which was then filtered, dried at 70 °C, and homogenized using a ball mill.

3.3.3 Preparation of Enamel/Dentin Discs

Initially, each bovine lower central incisor was sectioned into discs with a diameter of 5.7 mm, extracted from the central region of the vestibular surface using a bench drill. To adjust the final thickness of the discs to 3.7 mm, consisting of 1.3 mm of enamel and 2.4 mm of dentin (± 0.2 mm), the process involved polishing using an automatic machine (Vector-Phoenix Beta, Buehler, Lake Bluff, IL, USA) with 4 lbs of pressure and 200 rpm. Silicon carbide paper discs

with grades 320 and 600 were used, cooled with water (Exttec Corp., Enfield, CT, IL, USA), followed by final polishing with a cloth disc (EXTEC® Multi-Cloth™, Exttec Corp., CT, IL, USA) moistened with a 0.25 µm diamond suspension (EXTEC I Permanent Diamond Suspensions, Exttec Corp., Enfield, CT, IL, USA). The thickness was measured using a digital caliper (model 500-144B, Mitutoyo Sul América Ltda, SP, Brazil) (Gruba et al., 2023).

3.3.4 Formulation of Whitening Gels and Whitening Protocol

The gel base consisted of a thickener (12% Carbopol) and whitening agents (10% and 35% HP). The formulation also included glycerin, water (q.s.p.), and NaOH, sufficient to adjust the gel's pH to approximately 7.0. Depending on the experimental group, either 10% or 35% HP was added. The CaTMP at 1% was incorporated only into the 10% HP gel. The whitening gels (10% HP) were applied daily to the enamel surface (0.04 mL) for 40 min using a dispensing syringe and a microbrush for 14 days. In the combined treatment protocols, the professional-use gel (35% HP) was applied on the first day for 40 min before applying the at-home gels. After the treatment time, the gels were removed with gauze, followed by a 30 s rinse with deionized water to eliminate any residual gel. Between treatments, the samples were individually stored in containers with 2 mL of artificial saliva, which was replaced daily throughout the experiment.

3.3.5 Experiment for Color Change Analysis

3.3.5.1 Sample Selection and Pigmentation

After obtaining the discs, an initial color reading was performed based on the International Commission on Illumination (CIE) guidelines, which allow the specification of color perceptions in three-dimensional models. For this, a UV-Visible Reflection Spectrophotometer (Model UV2450, Shimadzu, Kyoto, Japan) was used, adopting the CIE Lab color system (Suliman et al., 2003; Akabane et al., 2021; Júnior et al., 2022). Using the obtained Lab values, the average of the entire sample was calculated, selecting the 100 dental discs with the Lab* values closest to the average, within a tolerance of 5%.

For the pigmentation process, enamel/dentin discs were stored in Eppendorf-type microtubes containing 1 mL of black tea infusion at room temperature. The solution was prepared with 1.6 g of black tea (Chá Matte Leão, Curitiba, PR, Brazil) for every 100 mL of distilled or deionized water (Gruba et al., 2023; Júnior et al., 2022; Suliman et al., 2003). The procedure was carried out over 6 days, with the solution being replaced daily. Throughout the process, the tubes containing the samples and infusion were kept at room temperature. After completing the pigmentation process, a new selection of samples was made, totaling 50 samples (n=10 per group), following the same protocol described earlier.

3.3.5.2 Color Measurement

The color of the specimens was evaluated using the UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan), with measurements taken after the discs were stored in artificial saliva for 24 h to avoid dehydration. The readings followed the CIE Lab* model, which represents color in three dimensions: "L" for lightness (ranging from 0 to 100), "a" for red (positive) and green (negative), and "b" for yellow (positive) and blue (negative) (Sulieman et al., 2003; Gruba et al., 2024). To standardize the position of the specimens during the readings, black silicone supports (diameter: 5.7 mm; thickness: 3.5 mm) were used. The analyses were conducted at different time points: T₀ (initial), T₁ (after pigmentation), T₂ (24 h after completion of whitening), and T₃ (14 days post-treatment to assess color stabilization). Color changes were calculated using the following formulas: ΔE (CIE Lab*) = $[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$; ΔWI_D (Whiteness Index) = $0.511\Delta L - 2.324\Delta a - 1.100\Delta b$; and ΔE_{00} (CIEDE2000): $(\Delta L/KL \times SL) + (\Delta C/KC \times SC)^2 + (\Delta H/KH \times SH)^2 + RT \times (\Delta C/KC \times SC) \times (\Delta H/KH \times SH)^{0.5} [(\Delta L/KLS + (\Delta C/KCSC)^2 + (\Delta H/KHSH)^2 + RT (\Delta C/KCSC) (\Delta H/KHSH))]^{1/2}$.

3.3.6 Surface Microhardness and Roughness Analyses

The surface of the enamel/dentin discs was leveled and polished to remove approximately 120 μ m from the outer enamel layer. After polishing, initial surface hardness (SH) readings were taken using a microhardness tester (Micromet 5114, Buehler, Lake Bluff, IL, USA) with a diamond Knoop indenter, applying a static vertical load of 25 g for 10 s. Microhardness and roughness (Ra) analyses were performed at the following time points: T₀ (initial reading) and T₁ (after whitening treatment). Microhardness was measured at 5 surface impressions, with 100 μ m spacing between indentations in the horizontal direction, using the microhardness tester (Akabane et al., 2021). The discs were randomly assigned to five experimental groups (n = 10), as determined by Microsoft Excel software (Microsoft Corporation, USA). After the whitening treatment, a new set of 5 impressions was made, 100 μ m apart from the initial impressions, to assess the final surface hardness (SHF) (Gruba et al., 2023). Surface roughness (Ra) was evaluated using a profilometer (model SJ-401, Mitutoyo, Kawasaki, Japan), with a 2 mm radius, constant speed of 0.1 mm/s, 5 N load, and a cutoff value of 0.25 mm. The average roughness (Ra) was calculated from the arithmetic mean of the absolute distances of the roughness profile relative to the centerline, within a measurement length of 0.25 mm. Three Ra measurements were made at distinct locations on each sample: parallel, oblique, and perpendicular (Gruba et al., 2023).

3.3.7 Mineral Content of Dental Substrates Assessed by X-ray Microtomography (μ CT)

Mineral content of dental substrates was assessed using a high-resolution micro computed tomography system (SkyScan 1272, Bruker MicroCT, Kontich, Belgium). Three-dimensional mineral concentration ($\text{g}_{\text{HAp}} \times \text{cm}^{-3}$) was determined using reconstruction algorithms from the NRecon software. The scanning parameters included a voltage of 80 kV, current of 125 μA , 1 mm aluminum filter, spatial resolution of 4 μm , rotation step of 0.600° , and frame averaging set to 2. Image reconstruction was performed with NRecon software (version 1.6.10.2, Bruker), applying level 3 smoothing, ring artifact correction at level 9, and 60% beam hardening correction (Nunes et al., 2025). After reconstruction, two-dimensional coronal slices were generated using Data Viewer software (version 1.5.1.2, Bruker). The stacked slices were then analyzed in ImageJ software (version 1.47, National Institute of Mental Health, Bethesda, MD, USA) to create mineral concentration profiles ($\text{g}_{\text{HAp}} \times \text{cm}^{-3}$) as a function of depth (μm). Quantitative analysis was based on a calibration curve constructed using mineral reference standards at concentrations of 0, 0.25, and 0.75 $\text{g}_{\text{HAp}} \times \text{cm}^{-3}$ (Nunes et al., 2025; Fernandes et al., 2025).

The mean enamel thickness of the samples was $481.0 \pm 10.7 \mu\text{m}$, ranging from 460.0 to 500.0 μm . To standardize the evaluation of mineral content across the full enamel thickness, measurements were limited to a depth of 0 to 460 μm . The same thickness (460 μm) was adopted for dentin analysis, covering the region between 620 and 1080 μm in depth. Additionally, in enamel, a specific analysis from 0 to 52 μm was performed to assess mineral content near the surface, allowing correlation with surface microhardness data.

3.3.8 Enamel Morphology and Surface Chemical Composition Analysis Using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX)

Enamel discs ($n=3$) of each group were analyzed using SEM and EDX. SEM was performed to assess the morphology of the enamel discs after bleaching treatments. SEM images were acquired using a Philips XL-30 FEG. All discs underwent a gradual dehydration process in an ascending series of ethanol concentrations (50%, 60%, 70%, 80%, and 100%) and were then coated with gold (Quorum - Q150T E). The discs were subsequently examined using SEM (Carl Zeiss, EVO ILS15, Carl Zeiss NTS LTD, Germany) at 20 kV and magnification of 5000x. EDX (XL 30 SEM, Philips, Netherlands) was used to analyze the chemical compositions on the surface of the enamel discs (Gruba et al., 2023).

3.3.9 Statistical Analysis

Statistical analysis was carried out using Sigmaplot® version 12.0 for Windows, with a

significance level set at 5%. For the color change, microhardness, and roughness data, the different whitening gels and time points were considered as factors of variation, with the CIE L, a, b*, ΔE , ΔWID , and E_{00} , SHF, Ra, and $gHAp \times cm^{-3}$ parameters treated as variables. The data were analyzed using analysis of variance (ANOVA), followed by the Student-Newman-Keuls multiple comparison test.

3.4 Results

Staining of the samples in black tea infusion resulted in a significant decrease in luminosity ($p < 0.001$) and increases in red ($p < 0.001$) and yellow ($p < 0.001$) hues, as shown in Figure 1. Following the whitening treatment protocol, there was a notable increase in luminosity ($p < 0.001$), a reduction in redness ($p < 0.05$), and a shift toward a more bluish hue ($p < 0.001$). However, no statistically significant differences were observed between the groups exposed to the whitening agents for the variables ΔL^* , Δa^* , and Δb^* ($p > 0.05$) (Fig. 1). No statistically significant differences were observed between the groups exposed to the whitening agents for any of the variables analyzed ($p > 0.05$). Regarding the Whiteness Index for Dentistry (ΔWID), all the whitening groups showed similar results, regardless of whether they used the simple or combined home technique, or whether the 10% HP gel contained CaTMP (Fig. 2B). For the ΔE (Figure 2A) and ΔE_{00} (Figure 2C) parameters, all whitening therapies caused similar color changes, except for the group that used the combined technique (35% HP/10% HP/1%), which showed a greater color change in these parameters. Additionally, the addition of 1% CaTMP to the 10% HP gels did not affect the whitening performance in the simple technique; however, in the combined technique, a lower effect was observed compared to the combined technique using conventional gels, based on the ΔE and ΔE_{00} parameters (Fig 2A and 2C).

After the whitening treatment, the experimental groups did not show an increase in enamel surface roughness, except for the group that used the combined technique (35% HP/10% HP), which exhibited a significant change in roughness. However, the addition of 1% CaTMP to the 10% HP gel in the combined technique prevented the increase in roughness, maintaining the integrity of the enamel surface in this treatment protocol (Table 1). Regarding enamel microhardness, all whitening treatments led to a significant reduction in enamel hardness (SHF: $p < 0.001$; Table 1). The greatest hardness loss was observed in the 35% HP/10% and 10% HP groups, respectively. The combined technique resulted in a greater loss of hardness compared to the simple technique (%SH: $p < 0.05$). The addition of CaTMP to the 10% HP gel minimized the loss of hardness, both in the simple home treatment (~37%) and in the combined technique (~53%), compared to the corresponding treatments using conventional gels.

For the analysis of mineral concentration by micro-CT, regarding the dental enamel substrate, in both evaluated depths (0–460 μm and 0–52 μm), only the groups subjected to conventional bleaching treatments (10% HP and 10% HP/35% HP) exhibited significantly lower mineral concentrations compared to the control group (Table 2). In contrast, both bleaching protocols incorporating CaTMP-containing gels resulted in enamel mineral concentrations similar to the non-bleached control (Table 2). For dentin, a similar response pattern was observed; however, no statistically significant differences were found between the bleached groups, regardless of the presence of CaTMP in the gels. The mineral concentration profiles demonstrated that treatments with CaTMP-containing gels resulted in higher mineral content values ($\text{gHAp} \times \text{cm}^{-3}$) at all analyzed depths in both dental substrates when compared to their respective formulations without CaTMP (Fig. 3).

The dental enamel surfaces not exposed to any whitening treatment (sound enamel) appeared uniform or with minimal irregularities, with a Ca/P ratio of 1.64 (Fig. 4). Samples subjected to the whitening protocols exhibited moderate morphological alterations and surface irregularities in the enamel, particularly in the 10% HP and 35% HP/10% HP groups, which showed Ca/P ratios of 1.72 and 1.71, respectively (Fig. 4). Pronounced exposure of enamel prisms and protrusion of interprismatic enamel were observed mainly in the groups treated without 1% CaTMP, especially in the 35% HP/10% HP group. On the other hand, the groups treated with CaTMP-containing gels (10% HP/CaTMP and 35% HP/10% HP/CaTMP) showed fewer surface alterations compared to the conventional bleaching groups. Notably, the 35% HP/10% HP/CaTMP group presented a Ca/P ratio of 1.66 and an enamel surface morphology closely resembling that of the control group (Fig. 4).

3.5 Discussion

The success and safety of tooth whitening treatments are crucial factors when selecting an adequate method or product. Although the at-home technique typically uses gels with lower concentrations of whitening agents, it still presents challenges related to the need for safer protocols to ensure patient well-being (Zhong et al., 2023). This *in vitro* study aimed to assess the influence of adding 1% CaTMP to a 10% HP whitening gel on aesthetic outcomes, enamel microhardness, roughness, morphology, both with the at-home technique and a combined technique involving one in-office session. The findings shown that the inclusion of CaTMP in the whitening gel effectively minimized mineral loss, surface roughness, and morphological changes in the enamel without compromising the aesthetic effect in the at-home technique. In the combined technique, CaTMP demonstrated a significant color change in stained teeth,

highlighting its ability to protect enamel while ensuring a satisfactory aesthetic result. Based on these results, the null hypothesis concerning the whitening effect was accepted, while the null hypotheses related to changes in hardness, roughness, mineral content, and enamel morphology were rejected.

Despite the remarkable effectiveness of peroxide-based whitening gels, both in-office and at-home treatments, concerns regarding their potential adverse effects on dental structure remain common (Aidar et al., 2025; Gruba et al., 2023; Elfallah et al., 2015). In this study, the addition of CaTMP in a 10% HP whitening gel was investigated as a strategy to develop more biocompatible and less aggressive at-home whitening methods. During the diffusion of the whitening agent into the enamel prisms, the degradation products of HP react with organic and inorganic molecules, potentially affecting the mechanical properties of the enamel (Jiang et al., 2008). The results highlight the potential of CaTMP to reduce common adverse effects in conventional whitening treatments, such as mineral loss, increased roughness, and morphological changes in the enamel. The observed mineral loss can be attributed to the release of H^+ ions from HP, leading to structural changes in the enamel. Therefore, changes in the roughness and mineral component of the enamel induced by HP free radicals have been extensively documented in several studies (Nunes et al., 2025; Nunes et al., 2024; Gruba et al., 2023; Junior et al., 2022; Prette-Junior, 2021). In the present study, it was observed that only the group that used combined techniques with conventional gels showed a significant change in roughness. However, all interventions resulted in a significant reduction in the microhardness of the enamel surface after the whitening treatment.

Changes in enamel roughness following exposure to bleaching agents have also been associated with the HP concentration in the whitening product (Nunes et al., 2025; Nunes et al., 2024). Since the at-home bleaching technique typically employs gels with lower HP concentrations (~6–16%), roughness alterations are not frequently reported, unlike the in-office procedure, which utilizes higher concentrations (35–40% HP (Nunes et al., 2024; Gruba et al., 2023; Sürmelioğlu et al., 2021; Pinto et al., 2004). In the present study, only the conventional combined technique led to changes in roughness. Thus, it is plausible to infer that the absence of an effect on enamel roughness is related to the low HP concentration (10%) used in the simple at-home technique. However, when combined with an in-office session, the oxidative potential and ionic dissociation may be enhanced, leading to alterations in enamel roughness and morphology. SEM analysis reinforced the roughness findings by revealing distinct surface alterations among the treatment groups. While the control enamel showed a smooth and uniform morphology, conventional bleaching protocols (10% HP and 35% HP/10% HP) resulted in

visible surface roughening, including enamel prism exposure and interprismatic degradation—features commonly associated with oxidative damage. In contrast, gels containing CaTMP effectively preserved enamel morphology, even under the combined bleaching regimen, showing surfaces more similar to the untreated control.

Moreover, the present findings corroborate previous studies reporting significant reductions in enamel surface hardness following HP bleaching protocols (Nunes et al., 2025; Nunes et al., 2024; Garcia et al., 2024; Dos Santos et al., 2020; Pinto et al., 2004). However, in the groups treated with CaTMP-containing gels, this reduction was considerably attenuated compared to HP-only formulations, in both single and combined bleaching approaches. These outcomes are consistent with prior evidence indicating that TMP incorporation into bleaching agents, even at low concentrations, minimizes deleterious effects on enamel (Nunes et al., 2024; Gruba et al., 2023; Dos Santos et al., 2020; Akabane et al., 2021). The complete replacement of sodium with calcium ions in the TMP structure likely enhances these effects by improving enamel reactivity and promoting mineral retention. In addition, Micro-CT analysis further supports these findings, showing that CaTMP-based bleaching gels preserved enamel mineral concentration at both superficial (0–52 μm) and deeper (0–460 μm) layers, with values comparable to the unbleached control. In contrast, conventional bleaching groups (10% HP and 10%/35% HP) exhibited significant mineral loss. In dentin, while intergroup differences did not reach statistical significance, a consistent trend of higher mineral values was observed in CaTMP-treated samples across all depths. These outcomes suggest that CaTMP contributes to the preservation of the mineral matrix, likely by reducing ionic diffusion and forming a protective, negatively charged $\text{TMP-Ca}^{2+}\text{-PO}_4^{3-}$ layer on the dental surface. This layer may interact with reactive species from HP dissociation (HO_2^- , OH^- , H^+), modulating acid permeability and promoting the retention or redeposition of calcium and phosphate ions (Nalin et al., 2021; Amaral et al., 2018). Altogether, these results support the hypothesis that the calcium replacement in TMP enhances its protective effects during bleaching procedures, contributing to the maintenance of both mechanical integrity and mineral density of dental tissues.

Considering the characteristics of the bleaching agents used, all the experimental gels were adjusted to a pH of 7.0 to prevent enamel demineralization that could be induced by the acidity of the whitening gels, as suggested by Xu et al. (2011). Maintaining a neutral pH is crucial because the acidic nature of these agents can promote the dissolution of the mineral content in enamel, compromising its structural integrity. The 14-day treatment protocol adopted reflects common practice in at-home tooth whitening treatments. Literature data indicate that prolonged

exposure to whitening agents may result in more significant damage to the enamel, particularly due to an increased risk of surface degradation and changes in mineral composition (Nunes et al., 2025; Cvikl et al., 2016; Sasaki et al., 2015). Although the literature includes longer treatment protocols (Moura et al., 2019; Sasaki et al., 2015; De Abreu et al., 2011), such extensions may compromise enamel health, becoming a long-term risk factor for dental integrity.

In color change analysis, after the staining procedure, the whitening treatments were effective in enhancing the appearance of the enamel, as demonstrated by the increase in luminosity (brightness) and the reduction in redness and yellowing. Furthermore, no statistically significant differences were observed between the bleached groups in terms of ΔL^* , Δa^* , and Δb^* values (Figure 2). This indicates that regardless of the whitening agent used or the technique applied, all treatments resulted in similar outcomes when measured using the CIELAB parameters (ΔL^* , Δa^* , and Δb^*). Moreover, it was observed that the addition of CaTMP did not affect any of the different chromatic metrics (ΔE , ΔWI_D and ΔE_{00}) in the conventional at-home technique (Figure 3). In the combined technique, however, a difference was noted only in the ΔE and ΔE_{00} parameters (Fig. 3A and 3C). Despite this, the change in color was still significant and perceptible to the naked eye ($\Delta E > 3.3$, $\Delta WI_D: 0.72$ and $\Delta E > 1.8$), which suggests that the addition of CaTMP does not inhibit the whitening process in a clinically relevant way. Furthermore, for the whitening index in dentistry (ΔWI_D), all treatments, including those with CaTMP, showed similar effects, regardless of whether the technique was simple or combined. Although the combined technique (35%HP/10%HP group) demonstrated a more pronounced whitening effect at the end of the treatment, the results suggest that this benefit may not necessarily be advantageous when considering other important parameters, such as the ΔWI_D , which showed similar effects across the different whitening interventions evaluated. In general, it is worth noting that, although gels containing CaTMP were tested for stability and color recovery after 14 days of whitening (all parameters), their efficacy still needs to be clinically evaluated with a longer follow-up period.

From a clinical standpoint, it should be emphasized that the treatment is likely to be more expensive, as it requires an additional clinical session at the dental office. Additionally, the combined technique (35%HP/10%HP) induced more significant alterations in the enamel, such as mineral loss, increased roughness, and changes in topography, which could be detrimental to the integrity of the dental tissues in the long term. It is important to note that these findings are supported by results from previous clinical studies that evaluated the use of combined techniques, which reported accelerated initial aesthetic effects but no significant long-term

differences (Dourado Pinto et al., 2019), and also reported greater dental sensitivity associated with the use of such techniques (Zhong et al., 2023; Machado et al., 2016).

That said, it's worth considering that, for patients who struggle to adhere to home treatment, the combined technique, when paired with a home gel containing CaTMP, may serve as an effective alternative to prevent or minimize enamel changes. This protocol appears to offer significant potential for faster whitening in the early days of treatment, which may increase patient satisfaction by providing a more noticeable aesthetic effect in less time. Additionally, reducing the overall treatment time for the home gel could contribute to a more comfortable, cost-effective, and safe treatment experience. Given the potential positive impact of protocols with gels containing CaTMP for home treatment adherence, it would be valuable for future clinical trials to explore these aspects in greater depth. Future studies could consider the efficacy of the protocol in different patient profiles, as well as evaluate potential long-term adverse effects, ensuring a balanced approach between rapid aesthetic results and biocompatibility with dental tissues.

In general, it is important to interpret the data obtained in this study with caution, as an *in vitro* model was used to evaluate clinical aesthetic procedures performed on vital teeth. It should be considered that, under real physiological conditions, vital teeth may present a variety of distinct characteristics. As with other dental interventions, discolorations and ongoing whitening processes may occur within the complex dynamics of the oral cavity. Moreover, it is important to mention that other factors, such as the frequency and duration of treatment, as well as the individual clinical characteristics of the patient, may also influence the effects on dental structure (Chemin et al., 2018; Sundfeld et al., 2014). In addition, it is also crucial to highlight that there are differences between the simulated laboratory conditions and the actual aspects of the oral cavity in its real context. It should also be noted that the samples analyzed were bovine in origin, and clinical characteristics were not reproduced in this model, such as the use of artificial saliva, the absence of daily fluoride treatments (such as toothpaste use), and the abrasion effects caused by tooth brushing.

Although the study demonstrated the effectiveness of the whitening gels, regardless of the presence of CaTMP, the clinical benefits, as well as the long-term color maintenance, still need to be further investigated. Understanding how dental whitening agents affect enamel properties is crucial for developing whitening protocols that achieve satisfactory aesthetic results without compromising the integrity of dental tissues. In this regard, the scientific evidence obtained in this research regarding the CaTMP whitening gel is promising. This formulation presents an interesting alternative to be explored in future clinical studies, with the potential for developing

home and/or combined whitening therapies that are both aesthetically effective and biologically compatible with the dentin-pulp complex. The aim would be to effectively minimize the side effects associated with dental whitening, ensuring greater safety for the procedure.

3.6 Conclusion

The incorporation of 1% CaTMP to the 10% HP whitening gel proved effective in reducing mineral loss, changes in roughness, and preserving the morphology of dental enamel, both in the at-home and combined whitening techniques. Although it did not affect the whitening effect in the simple at-home technique, it resulted in a reduced chromatic change in the combined approach. Nonetheless, the overall aesthetic outcome demonstrated a significant color change post-whitening, highlighting the treatment's ability to balance enamel protection with desirable esthetic results.

3.7 Acknowledgments

This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 88887.817416/2023-00) and the São Paulo Research Foundation (FAPESP, Grant #2022/15847-8), with scholarships awarded to the first and third authors, respectively.

3.8 References

1. Abu-Saq Al Yami A, Al Qahtani S, Shokair N, Al Ghamdi M, Al Bouni R. Effect of home and in-office bleaching systems on the nanomechanical properties of tooth enamel. *Saudi Dent J*. 2020;32(7):343-348. doi:10.1016/j.sdentj.2019.10.007
2. Aidar KMS, Cintra LTA, Ferreira MCB, et al. Comparative Analysis Between Strip and Gels Indicated for at Home Bleaching: Analysis of Color Alteration, Roughness and Microhardness of Dental Enamel. *J Esthet Restor Dent*. Published online January 17, 2025. doi:10.1111/jerd.13412
3. Aidos M, Marto CM, Amaro I, et al. Comparison of in-office and at-home bleaching techniques: An umbrella review of efficacy and post-operative sensitivity [published correction appears in *Heliyon*. 2024 Nov 01;10(24):e39823. doi: 10.1016/j.heliyon.2024.e39823.]. *Heliyon*. 2024;10(3):e25833. doi:10.1016/j.heliyon.2024.e25833
4. Felipe Akabane ST, Danelon M, Nunes GP, et al. Evaluation of the aesthetic effect, enamel microhardness and trans-amelodentinal cytotoxicity of a new bleaching agent for professional use containing trimetaphosphate and fluoride. *J Mech Behav Biomed Mater*. 2021;114:104225. doi:10.1016/j.jmbbm.2020.104225

5. Alkahtani R, Stone S, German M, Waterhouse P. A review on dental whitening. *J Dent.* 2020;100:103423. doi:10.1016/j.jdent.2020.103423
6. Amaral JG, Pessan JP, Souza JAS, Moraes JCS, Delbem ACB. Cyclotriphosphate associated to fluoride increases hydroxyapatite resistance to acid attack. *J Biomed Mater Res Part B* 2018; doi: 10.1002/jbm.b.34072.
7. Bernardon JK, Sartori N, Ballarin A, Perdigão J, Lopes GC, Baratieri LN. Clinical performance of vital bleaching techniques. *Oper Dent.* 2010;35(1):3-10. doi:10.2341/09-008CR
8. Butera A, Maiorani C, Rederlene G, Checchi S, Nardi GM. Evaluation of the Effectiveness of Different Types of Professional Tooth Whitening: A Systematic Review. *Bioengineering (Basel).* 2024;11(12):1178. doi: 10.3390/bioengineering11121178.
9. Chemin K, Rezende M, Milan FM, Dantas TB, Gomes KDN, Kossatz S. Clinical Evaluation of 10% Hydrogen Peroxide on Tooth Sensitivity and Effectiveness in at Home Dental Bleaching. *J Contemp Dent Pract.* 2018;19(11):1376-1380.
10. Cvikl B, Lussi A, Moritz A, Flury S. Enamel Surface Changes After Exposure to Bleaching Gels Containing Carbamide Peroxide or Hydrogen Peroxide. *Oper Dent.* 2016;41(1):E39-E47. doi:10.2341/15-010-L
11. Cordeiro D, Toda C, Hanan S, et al. Clinical Evaluation of Different Delivery Methods of At-Home Bleaching Gels Composed of 10% Hydrogen Peroxide. *Oper Dent.* 2019;44(1):13-23. doi:10.2341/17- 174-C
12. Dascanio R, de Oliveira Ribeiro RA, Coelho CSS, Souza MT, Kury M, Zanutto ED, de Souza Costa CA, Cavalli V. Effectiveness and safety of biosilicate-enhanced bleaching gels on enamel with early erosion lesion. *J Esthet Restor Dent.* 2024;36(10):1412-1425. doi: 10.1111/jerd.13271.
13. De Abreu DR, Sasaki RT, Amaral FL, Flório FM, Basting RT. Effect of home-use and in-office bleaching agents containing hydrogen peroxide associated with amorphous calcium phosphate on enamel microhardness and surface roughness. *J Esthet Restor Dent.* 2011;23(3):158-168. doi:10.1111/j.1708-8240.2010.00394.x.
14. Donato MV, Dos Reis-Prado AH, Abreu LG, de Arantes LC, Goto J, Chaves HGDS, Cintra LTA, Briso ALF, Peixoto IFDC, Benetti F. Influence of dental bleaching on the pulp tissue: A systematic review of in vivo studies. *Int Endod J.* 2024;57(6):630-654. doi: 10.1111/iej.14061.
15. Donassollo SH, Donassollo TA, Coser S, et al. Triple-blinded randomized clinical trial comparing efficacy and tooth sensitivity of in-office and at-home bleaching techniques. *J Appl Oral Sci.* 2021;29:e20200794. Published 2021 Oct 1. doi:10.1590/1678-7757-2020-0794
16. Dos Santos ALE, Delbem ACB, Danelon M, Marcon LN, Shinohara MS. Evaluation of new compositions of 10% hydrogen peroxide-based bleaching agents containing trimetaphosphate and fluoride on enamel demineralization. *Eur J Oral Sci.* 2020;128(5):450-456. doi:10.1111/eos.12735.
17. Dourado Pinto AV, Carlos NR, Amaral FLBD, França FMG, Turssi CP, Basting RT. At-home, in-office and combined dental bleaching techniques using hydrogen peroxide: Randomized clinical trial evaluation of effectiveness, clinical parameters and enamel mineral content. *Am J Dent.*

- 2019;32(3):124-132.
18. Elfallah HM, Bertassoni LE, Charadram N, Rathsam C, Swain MV. Effect of tooth bleaching agents on protein content and mechanical properties of dental enamel. *Acta Biomater.* 2015;20:120-128. doi:10.1016/j.actbio.2015.03.035
 19. Fernandes AVP, Nunes GP, Urzedo LOR, et al. Total sodium replacement by calcium in trimetaphosphate and its incorporation into dental whitening gels: A novel strategy for in-office treatment. *J Dent.* 2025;160:105861. doi:10.1016/j.jdent.2025.105861
 20. Fioresta R, Melo M, Forner L, Sanz JL. Prognosis in home dental bleaching: a systematic review. *Clin Oral Investig.* 2023 Jul;27(7):3347-3361. doi: 10.1007/s00784-023-05069-0.
 21. Garcia KG, Nunes GP, Delbem A, et al. Inorganic Phosphate Effect in a Hydrogen Peroxide-based Bleaching Agent: Physicochemical, Mechanical, and Morphological Properties of Dental Enamel [published correction appears in *Oper Dent.* 2024 Nov 1;49(6):750. doi: 10.2341/1559-2863-49-6-750.]. *Oper Dent.* 2024;49(4):465-474. doi:10.2341/24-002-L
 22. Gruba AS, Nunes GP, Marques MT, et al. Influence of bleaching gels formulated with nano-sized sodium trimetaphosphate and fluoride on the physicochemical, mechanical, and morphological properties of dental enamel. *J Dent.* 2023;139:104743. doi:10.1016/j.jdent.2023.104743.
 23. Jiang T, Ma X, Wang Y, et al. Investigation of the effects of 30% hydrogen peroxide on human tooth enamel by Raman scattering and laser-induced fluorescence. *J Biomed Opt.* 2008;13(1):014019. doi:10.1117/1.2870114.
 24. Júnior NAN, Nunes GP, Gruba AS, et al. Evaluation of bleaching efficacy, microhardness, and trans- amelodentinal diffusion of a novel bleaching agent for an in-office technique containing hexametaphosphate and fluoride. *Clin Oral Investig.* 2022;10.1007/s00784-022-04480-3. doi:10.1007/s00784-022-04480-3
 25. Kettle J, Warren L. Capturing the smile: Exploring embodied and social acts of smiling. *Sociol Health Illn.* 2024;46(8):1626-1646. doi:10.1111/1467-9566.13815
 26. Kury M, Hiers RD, Zhao YD, Picolo MZD, Hsieh J, Khajotia SS, Esteban Florez FL, Cavalli V. Novel Experimental In-Office Bleaching Gels Containing Co-Doped Titanium Dioxide Nanoparticles. *Nanomaterials (Basel).* 2022;12(17):2995. doi: 10.3390/nano12172995
 27. Machado LS, Anchieta RB, dos Santos PH, et al. Clinical Comparison of At-Home and In-Office Dental Bleaching Procedures: A Randomized Trial of a Split-Mouth Design. *Int J Periodontics Restorative Dent.* 2016;36(2):251-260. doi:10.11607/prd.2383
 28. Maran BM, Matos TP, de Castro ADS, Vochikovski L, Amadori AL, Loguercio AD, Reis A, Berger SB. In-office bleaching with low/medium vs. high concentrate hydrogen peroxide: A systematic review and meta-analysis. *J Dent.* 2020;103:103499. doi: 10.1016/j.jdent.2020.103499.
 29. Moura CW, Catelan A, Zanatta RF, et al. Effects of bleaching using 10% carbamide peroxide with calcium or amorphous calcium phosphate on enamel mineral content and hardness *Acta Odontol Latinoam.* 2019;32(3):126-132.
 30. Nalin EKP, Danelon M, da Silva ES, Hosida TY, Pessan JP, Delbem ACB. Surface Free Energy,

- Interaction, and Adsorption of Calcium and Phosphate to Enamel Treated with Trimetaphosphate and Glycerophosphate. *Caries Res.* 2021;55(5):496-504. doi:10.1159/000518943.
31. Nunes GP, Batista GF, Toledo PTA, Martins TP, Alves RO, Fernandes AVP, Delbem ACB. Synthesis and application of calcium cyclotriphosphate in bleaching formulations: effects on dental enamel properties, *Journal of Dentistry* 2025; 105614, <https://doi.org/10.1016/j.jdent.2025.105614>.
 32. Nunes GP, Marques MT, de Toledo PTA, Alves RO, Martins TP, Delbem ACB. Effect of a novel low- concentration hydrogen peroxide bleaching gel containing nano-sized sodium trimetaphosphate and fluoride. *J Dent.* 2024 Nov;150:105330. doi: 10.1016/j.jdent.2024.105330.
 33. Pavani CC, Fagundes TC, Sundfeld D, et al. Influence of daily usage times on patients' compliance during at-home bleaching: a randomized clinical trial. *J Appl Oral Sci.* 2023;31:e20230181. Published 2023 Oct 9. doi:10.1590/1678-7757-2023-0181
 34. Pinto CF, Oliveira Rd, Cavalli V, Giannini M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. *Braz Oral Res.* 2004;18(4):306-311. doi:10.1590/s1806- 83242004000400006.
 35. Prette-Junior, R.A.T., Danelon, M., Pessan, J.P. et al. Effect of daily use of fluoridated dentifrice and bleaching gels containing calcium, fluoride, or trimetaphosphate on enamel hardness: an in vitro study. *Clin Oral Invest* 2021; 25:883–889.
 36. Pontes M, Gomes J, Lemos C, Leão RS, Moraes S, Vasconcelos B, Pellizzer EP. Effect of Bleaching Gel Concentration on Tooth Color and Sensitivity: A Systematic Review and Meta-analysis. *Oper Dent.* 2020; 45(3):265-275. DOI: 10.2341/17-376-L.
 37. Sulieman M, Addy M, Rees JS. Development and evaluation of a method in vitro to study the effectiveness of toothbleaching. *J Dent* 2003;31:415-422.
 38. Sundfeld RH, Sundfeld Neto D, Machado LS, et al. Dental bleaching with a 10% hydrogen peroxide product: a six-month clinical observation. *Indian J Dent Res.* 2014;25(1):4-8. doi:10.4103/0970-9290.131046
 39. Sürmelioglu D, Özçetin HK, Özdemir ZM, Yavuz SA, Aydın U. Effectiveness and SEM-EDX analysis following bleaching with an experimental bleaching gel containing titanium dioxide and/or chitosan. *Odontology.* 2021;109(1):114-123. doi:10.1007/s10266-020-00526-8
 40. Xu B, Li Q, Wang Y. Effects of pH values of hydrogen peroxide bleaching agents on enamel surface properties. *Oper Dent.* 2011;36(5):554-562. doi:10.2341/11-045-1
 41. Zhong BJ, Yang S, Hong DW, Cheng YL, Attin T, Yu H. The Efficacy of At-home, In-office, and Combined Bleaching Regimens: A Randomized Controlled Clinical Trial. *Oper Dent.* 2023;48(3):E71- E80. doi: 10.2341/22-099-C. PMID: 37079917.

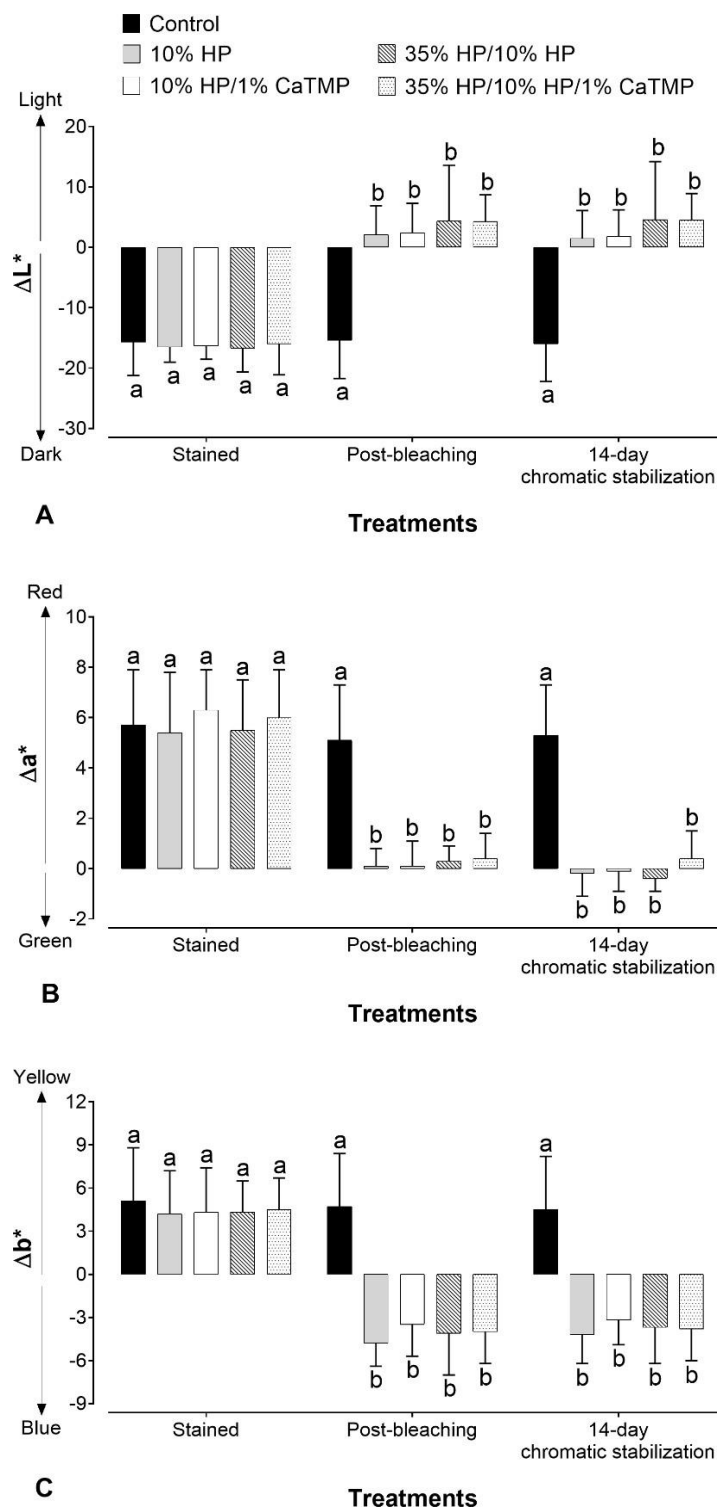


Figure 1. Mean values (SD) of the alteration of: (A) lightness degree (ΔL^*), (B) green/red degree (Δa^*), (C) blue/yellow degree (Δb^*) by Commission Internationale de l'Eclairage (CIE) according to bleaching gels and moment of analysis ($n = 10$). Distinct superscript lowercase letters indicate statistical difference among the bleaching gels at each moment of analysis (Student-Newman-Keuls; $p < 0.05$).

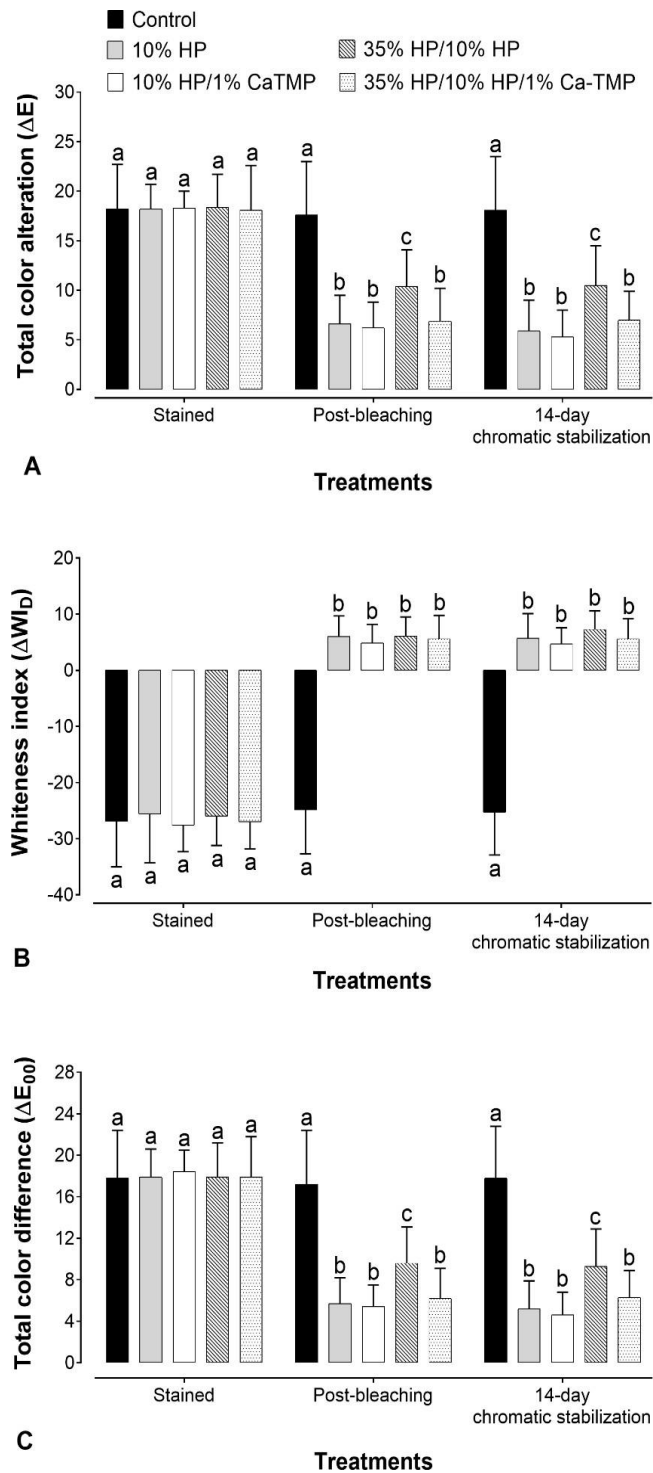
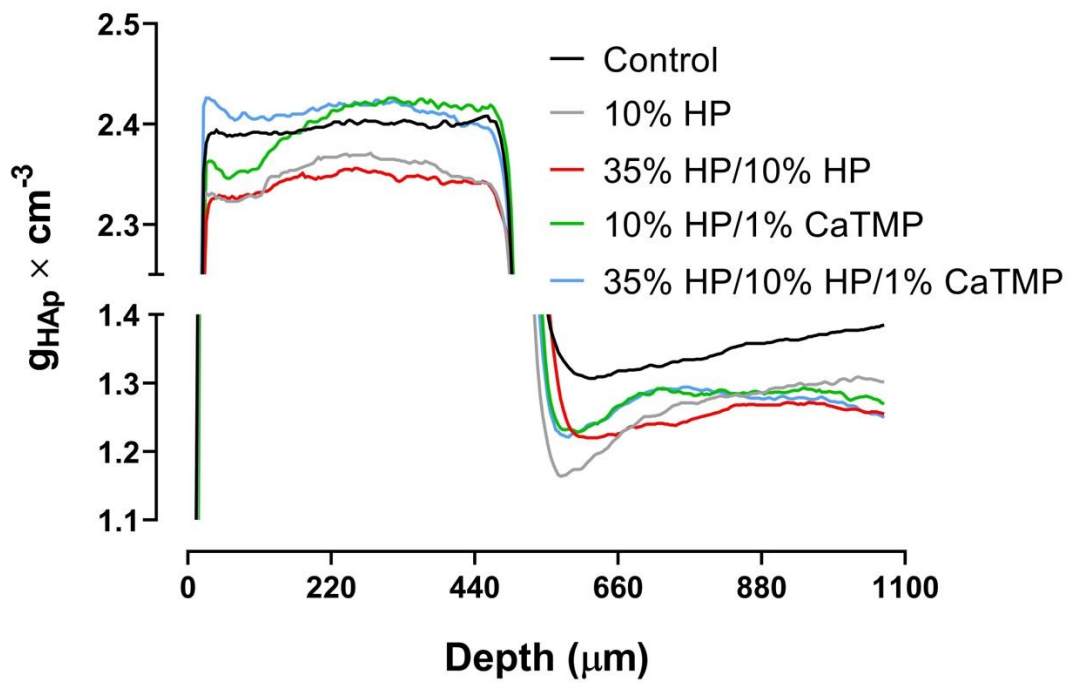


Figure 2. Mean values ($\pm$ SD) of total color change (ΔE) (A), Whitening Index for Dentistry (ΔWI_D) (B), and total color change by CIEDE00 (ΔE_{00}) (C) according to the whitening gels and analysis time ($n = 10$). Distinct lowercase superscript letters indicate statistical differences between the whitening gels at each analysis time point (Student–Newman–Keuls; $p < 0.05$).

Figure 3. Mineral concentration profiles ($g_{\text{HAP}} \times \text{cm}^{-3}$) as a function of depth in enamel and dentin, according to experimental groups (n = 10)



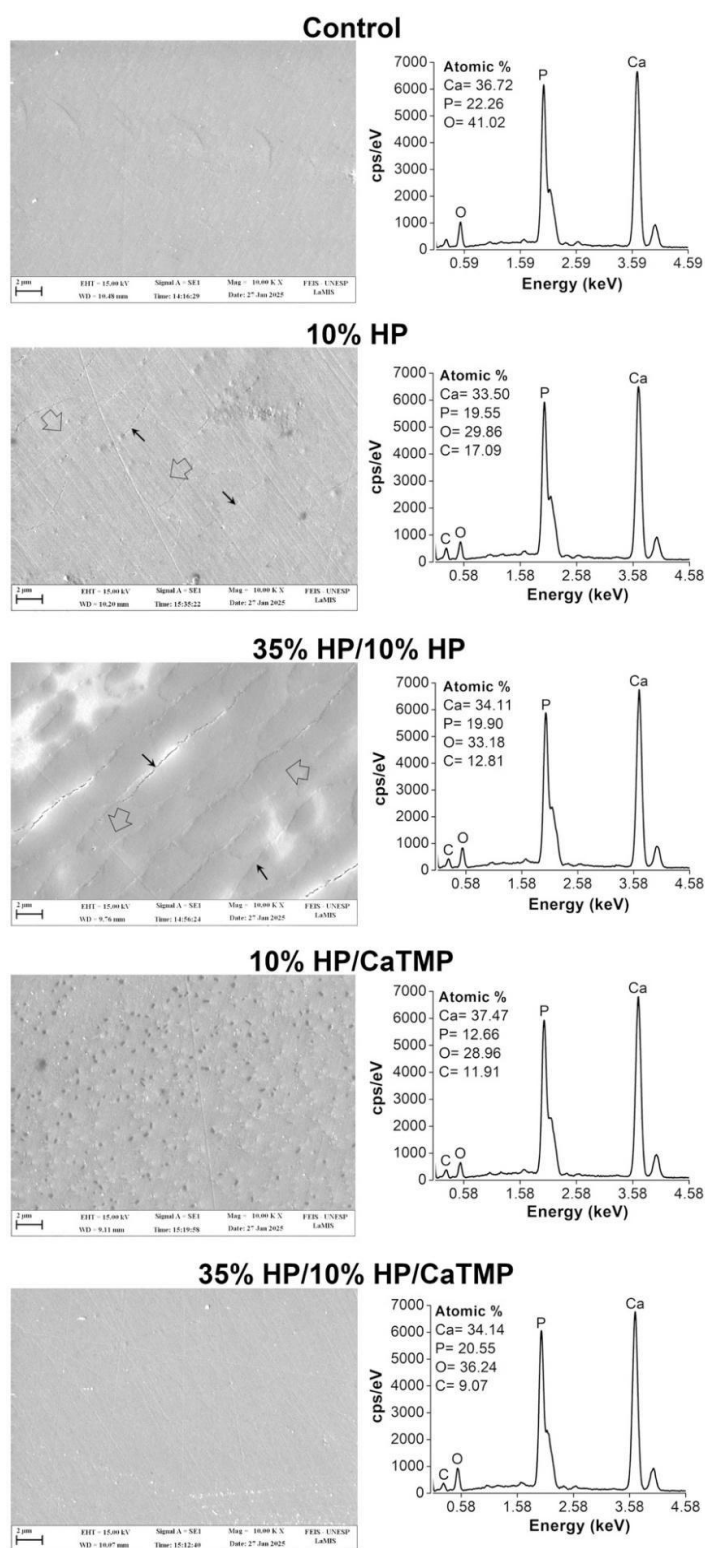


Figure 4. Scanning electron microscopy (SEM) surface topography micrographs ($\times 5000$) and representative EDX of sound enamel and experimental groups: 10% HP, 35%HP/10% HP, 10% HP/CaTMP, and 35% HP/10% HP/CaTMP, respectively. The empty arrow indicates irregularities or depressions. The filled arrow indicates protruding interprismatic enamel.

Table 1. Mean values (SD) of roughness (Ra) and surface hardness (SH), percentage of surface hardness change (%SH) according to treatments (n = 10)

Treatments	Variables				
	Ra		SH		%SH
	Initial	Final	Initial	Final	
Control	0.029 ^{aA}	0.030 ^{aA}	366.0 ^{aA}	367.1 ^{aA}	0.3 ^a
	(0.007)	(0.006)	(5.9)	(5.6)	(0.5)
10% HP	0.029 ^{aA}	0.035 ^{aA}	365.8 ^{aA}	245.9 ^{bB}	-32.8 ^b
	(0.008)	(0.006)	(5.2)	(8.7)	(2.3)
10% HP/1% CaTMP	0.028 ^{aA}	0.036 ^{aA}	365.9 ^{aA}	289.8 ^{cB}	-20.8 ^c
	(0.007)	(0.006)	(5.3)	(9.1)	(2.7)
35% HP/10% HP	0.030 ^{aA}	0.051 ^{bB}	365.9 ^{aA}	232.8 ^{dB}	-36.4 ^d
	(0.010)	(0.020)	(5.7)	(12.9)	(3.9)
35% HP/10% HP/1% CaTMP	0.029 ^{aA}	0.033 ^{aA}	365.7 ^{aA}	302.6 ^{eA}	-17.2 ^e
	(0.011)	(0.012)	(5.8)	(9.7)	(2.4)

Distinct superscript lowercase letters indicate statistical differences among bleaching gels in each variable (Student–Newman–Keuls test, $p < 0.001$). Different capital letters indicate the difference between the analysis moment (initial and final) for Ra and SH variables (Student–Newman–Keuls; $p < 0.05$).

Table 2. Mean values (SD) of integrated area of mineral concentration according to the groups and substrate (enamel and dentin), and thickness after bleaching of the discs (n = 10)

Treatments	Integrated area ($\text{g}_{\text{HAP}} \times \text{cm}^{-3} \times \mu\text{m}$)		
	Enamel		Dentin
	0 – 460 μm	0 – 52 μm	620 – 1080 μm
Control	1102.8 ^A (7.9)	124.3 ^{AC} (0.8)	615.9 ^A (26.3)
10% HP	1081.3 ^B (14.2)	120.5 ^B (2.1)	572.9 ^B (25.4)
35% HP/10% HP	1077.1 ^B (10.3)	120.6 ^B (1.4)	573.8 ^B (17.0)
10% HP/1%CaTMP	1104.2 ^A (7.3)	122.8 ^C (1.6)	591.9 ^{AB} (28.6)
35% HP/10% HP/1%CaTMP	1109.2 ^A (6.1)	125.6 ^A (2.6)	599.5 ^{AB} (24.3)

Distinct capital letters indicate difference among average for each variable according to analysis (Student-Newman-Keuls; $p < 0.05$). SD stands for the standard deviation of means.

Conclusão Geral

4. Conclusão Geral

A incorporação de CaTMP a 1% em géis clareadores dentários, com diferentes concentrações de peróxido de hidrogênio, atenua de maneira eficaz as alterações nos tecidos dentários causadas pelo clareamento, sem comprometer a eficácia do procedimento. Esse efeito foi observado tanto em géis com alta concentração de HP, utilizados na técnica de consultório, quanto em géis com concentrações mais baixas, aplicados na técnica caseira, incluindo também a combinação das técnicas. A presença de CaTMP reduz a difusão transamelodentinária do HP, diminui a perda mineral, minimiza a alteração da rugosidade da superfície e preserva a morfologia do esmalte. Esses benefícios destacam o potencial da estratégia para aumentar a segurança e biocompatibilidade do clareamento dentário.

Apêndices

5. Apêndice A. Referências bibliográficas da Introdução Geral

- Aidos M, Marto CM, Amaro I, et al. Comparison of in-office and at-home bleaching techniques: An umbrella review of efficacy and post-operative sensitivity [published correction appears in Heliyon. 2024 Nov 01;10(24):e39823. doi: 10.1016/j.heliyon.2024.e39823.]. Heliyon. 2024;10(3):e25833. Published 2024 Feb 3. doi:10.1016/j.heliyon.2024.e25833
- Américo MA, Portella FF, Zimmer R, et al. Effect of a Toothpaste for Sensitive Teeth on the Sensitivity and Effectiveness of In-office Dental Bleaching: A Randomized Clinical Trial. Oper Dent. 2023;48(6):627-637. doi:10.2341/23-009-C
- Aragão WAB, Chemelo VS, Alencar CM, et al. Biological action of bleaching agents on tooth structure: A review. Histol Histopathol. 2024;39(10):1229-1243. doi:10.14670/HH-18-726
- Bernardon JK, Sartori N, Ballarin A, Perdigão J, Lopes GC, Baratieri LN. Clinical performance of vital bleaching techniques. Oper Dent. 2010;35(1):3-10. doi:10.2341/09-008CR
- de Oliveira Ribeiro RA, Peruchi V, Sores IPM, et al. Combined catalytic strategies applied to in office tooth bleaching: whitening efficacy, cytotoxicity, and gene expression of human dental pulp cells in a 3D culture model. Clin Oral Investig. 2024;28(12):669. doi:10.1007/s00784-024-06069-4
- Donato MV, Dos Reis-Prado AH, Abreu LG, de Arantes LC, Goto J, Chaves HGDS, Cintra LTA, Briso ALF, Peixoto IFDC, Benetti F. Influence of dental bleaching on the pulp tissue: A systematic review of in vivo studies. Int Endod J. 2024;57(6):630-654. doi: 10.1111/iej.14061.
- Donassollo SH, Donassollo TA, Coser S, et al. Triple-blinded randomized clinical trial comparing efficacy and tooth sensitivity of in-office and at-home bleaching techniques. J Appl Oral Sci. 2021;29:e20200794. doi:10.1590/1678-7757-2020-0794
- Dos Santos ALE, Delbem ACB, Danelon M, Marcon LN, Shinohara MS. Evaluation of new compositions of 10% hydrogen peroxide-based bleaching agents containing trimetaphosphate and fluoride on enamel demineralization. Eur J Oral Sci. 2020;128(5):450-456. doi:10.1111/eos.12735.
- Dourado Pinto AV, Carlos NR, Amaral FLBD, França FMG, Turssi CP, Basting RT. At-home, in-office and combined dental bleaching techniques using hydrogen peroxide: Randomized clinical trial evaluation of effectiveness, clinical parameters and enamel mineral content. Am J Dent. 2019;32(3):124- 132.
- Felipe Akabane ST, Danelon M, Nunes GP, et al. Evaluation of the aesthetic effect, enamel microhardness and trans-amelodentinal cytotoxicity of a new bleaching agent for professional use containing trimetaphosphate and fluoride. J Mech Behav Biomed Mater. 2021;114:104225. doi:10.1016/j.jmbbm.2020.104225
- Grazioli G, Valente LL, Isolan CP, Pinheiro HA, Duarte CG, Münchow EA. Bleaching and enamel surface interactions resulting from the use of highly-concentrated bleaching gels. Arch Oral Biol.

2018;87:157-162. doi:10.1016/j.archoralbio.2017.12.026

Gruba AS, Nunes GP, Danelon M, et al. The use of mouthwash containing trimetaphosphate as an adjunct therapy to fluoridated toothpaste reduces enamel demineralization. *J Dent*. 2024;145:104966. doi:10.1016/j.jdent.2024.104966

Gruba AS, Nunes GP, Marques MT, et al. Influence of bleaching gels formulated with nano-sized sodium trimetaphosphate and fluoride on the physicochemical, mechanical, and morphological properties of dental enamel. *J Dent*. 2023;139:104743. doi:10.1016/j.jdent.2023.104743.

Júnior RATP, Danelon M, Pessan JP, et al. Effect of daily use of fluoridated dentifrice and bleaching gels containing calcium, fluoride, or trimetaphosphate on enamel hardness: an in vitro study. *Clin Oral Investig*. 2021;25(3):883-889. doi:10.1007/s00784-020-03375-5

Ludovichetti FS, Zerman N, Stellini E, Zambon G, Mazzoleni S, Zuccon A. Dental bleaching: patient perception and satisfaction. *Minerva Dent Oral Sci*. 2024;73(4):217-223. doi:10.23736/S2724-6329.24.04950-7

Martins BV, Dias MF, de Oliveira Ribeiro RA, Leite MLAES, Hebling J, de Souza Costa CA. Innovative strategy for in-office tooth bleaching using violet LED and biopolymers as H_2O_2 catalysts. *Photodiagnosis Photodyn Ther*. 2022;38:102886. doi:10.1016/j.pdpdt.2022.102886

Nunes GP, Marques MT, de Toledo PTA, Alves RO, Martins TP, Delbem ACB. Effect of a novel low-concentration hydrogen peroxide bleaching gel containing nano-sized sodium trimetaphosphate and fluoride. *J Dent*. 2024;150:105330. doi:10.1016/j.jdent.2024.105330

Oliveira MAF, Gonçalves FMC, Delbem ACB, Fernandes GLP, Cannon ML, Danelon M. Effect of treatment with phosphate, casein phosphopeptide and fluoride on the remineralization: in vitro study. *Braz Oral Res*. 2024;38:e036. doi:10.1590/1807-3107bor-2024.vol38.0036

Sabbah A. Smile Analysis: Diagnosis and Treatment Planning. *Dent Clin North Am*. 2022;66(3):307-341. doi:10.1016/j.cden.2022.03.001

Zhong BJ, Yang S, Hong DW, Cheng YL, Attin T, Yu H. The Efficacy of At-home, In-office, and Combined Bleaching Regimens: A Randomized Controlled Clinical Trial. *Oper Dent*. 2023;48(3):E71- E80. doi: 10.2341/22-099-C. PMID: 37079917.