UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

ASTRID CAROLINA VALDIVIA TAPIA

ENXAGUATÓRIOS BUCAIS NÃO FLUORETADOS: POTENCIAL ANTIBACTERIANO E EROSIVO

NON-FLUORIDE MOUTHWASHES: ANTIBACTERIAL AND EROSIVE POTENTIAL

Piracicaba
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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de de Doutora em Odontologia, na Área de Cariologia,

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Orientador: Prof. Dr. ANTÔNIO PEDRO RICOMINI FILHO
Coorientador: Prof. Dr. JAIME APARECIDO CURY
Prof Dr. FRANK LIPPERT

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RESUMO

Enxaguatórios bucais não fluoretados (ENF) são comumente usados por pacientes por razones cosméticas e/ou terapêuticas, e eles têm diferentes composições e objetivos. Eles apresentam uma grande quantidade de ingredientes, podendo prevenir ou interferir o desenvolvimento de diferentes doenças da cavidade oral. O objetivo foi investigar os ENF existentes no mercado e seu efeito sobre biofilmes iniciais, bem como no potencial erosivo para esmalte e dentina. Foram desenvolvidos quatro estudos: I) Investigar a composição e as finalidades de ENF disponíveis de livre comercialização; II) Avaliar a eficácia de ENF em biofilme inicial de *Streptococcus mutans*; III) Avaliar o potencial erosivo de ENF em tecidos duros dentais, utilizando o protocolo da Organização Internacional de Padronização (ISO); IV) Investigar a perda de superfície de esmalte e dentina causada por ENF usando um modelo validado de ciclagem de pH erosivo. Diferentes metodologias foram desenvolvidas: I) Foram comprados diferentes ENF de supermercados, farmácias e lojas de variedades em Indianápolis, Indiana, EUA e realizado uma análise descritiva sobre as informações e formulações. Os produtos foram utilizados nos seguintes 3 estudos. II) Análise antibacteriana usando *S. mutans* UA159 em meio TSB suplementado com 1% de sacarose e contendo os enxaguatórios. Foram avaliadas as concentrações inibitórias mínima (CIM), planctônica e biofilme e a concentração bactericida mínima (CBM). Os dados analisados por ANOVA e teste de Tukey (α=5%). III) Foi realizada análise com conforme a ISO/FDIS 28888:2013. Este método quantificou as mudanças de pH (ΔpH) da solução de fosfato de cálcio uma vez que o enxaguatório bucal teste foi adicionado. Foi desenvolvida uma correlação entre o pH inicial e resultado do teste. IV) Finalmente foram selecionados 6 enxaguatórios a partir do estudo III e usados como tratamento em um modelo de ciclagem de pH de 5 dias (quatro tratamentos de 1 min de enxaguatório/dia) usando blocos de esmalte e dentina (n=8/grupo). A perfilometria a laser foi usada para determinar a perda de superfície (PS). Os dados analisados por ANOVA e teste de Tukey (α=5%). I) Foram identificados 81 ENF (30 terapêuticos). O objetivo terapêutico mais comum foi antiplaca/antigengivite (n=25/30%). A finalidade mais comum dos produtos cosméticos foi o clareamento (n=11). II) Em relação ao efeito em *S. mutans,* MIC, crescimento planctônico e biofilme de *S. mutans*, houve uma diminuição significativa para produtos com peróxido de hidrogênio e hexametafosfato de sódio. III) Os enxaguatórios apresentaram uma faixa de pH inicial de 3,00-9,47. Nenhum resultou em ΔpH superior ao padrão mais forte, e há uma correlação baixa entre pH inicial e o potencial erosivo. IV) Para a ciclagem de pH, nem todos os grupos apresentaram PS e este variou independente do pH do produto. Naqueles em que houve erosão, esta foi maior em dentina. Em conclusão os ENF variam consideravelmente em

sua composição e finalidade, muitos fornecendo apenas benefícios cosméticos. Entre os ENF terapêuticos, a maioria fornece benefícios antiplaca/antigengivite. Os produtos que contêm peróxido de hidrogênio apresentam maior efeito sobre biofilme inicial de *S. mutans*. Em relação ao potencial erosivo, alguns ENF apresentam pH ácido e podem causar perda mineral erosiva em esmalte e dentina, sendo os efeitos mais pronunciados na dentina.

ABSTRACT

Patients commonly use non-fluoride mouthwashes (FFM) for various cosmetic and/or therapeutic reasons and have different compositions and purposes. In addition, they have many ingredients that can intervene or affect different oral cavity diseases. Therefore, this project will aim to investigate the existing FFM in the market, its effect on biofilms, and its possible interaction with dental erosion. For this, four studies will be developed with different specific objectives: I) Investigate the composition and purposes of FFM available without a medical prescription. II) Evaluate the effectiveness of commercially available FFM on the initial biofilm of *Streptococcus mutans*. III) Evaluate the erosive potential of FFM in dental hard tissues using the International Organization for Standardization (ISO) protocol IV) Investigate enamel and dentin surface loss caused by FFM using a validated model of erosive pH cycling. For this investigation, different methodologies were combined: I) All different FFM were purchased from supermarkets, pharmacies, and variety stores in Indianapolis, Indiana, USA, and a descriptive analysis was carried out on their information and formulations. These products were the basis of the following three studies. II) Antibacterial analysis using *S. mutans* UA159 in TSB medium supplemented with 1% sucrose and containing mouthwashes. The minimum inhibitory concentrations (MIC), planktonic and biofilm, and the minimum bactericidal concentration (MBC). Data were analyzed by ANOVA and Tukey's test (α=5%). III). For this study, the ISO/FDIS 28888:2013 was used. This method measured the pH (ΔpH) changes of the calcium phosphate solution once the test mouthwash was added. A correlation was developed between the initial pH and the test result. IV) Six mouthwashes were selected from the study III and used as a treatment in a 5-day pH cycle (four treatments/1 min of mouthwash/day) using blocks of enamel and dentin from bovine incisors (n = 8 per group). Non-contact profilometry was used to determine surface loss (SL). Data were analyzed using one-way ANOVA. We identified 81 different FFM, of which 30 were therapeutic. The most common therapeutic objective was antiplaque/antigingivitis (n=25). The most common purpose of cosmetic products (n=51) was whitening (n=11). Regarding the effect on *S. mutans*, MIC, planktonic growth, and *S. mutans* biofilm, there was a significant decrease for products with hydrogen peroxide and sodium hexametaphosphate and products with cetylpyridine chloride. The mouthwashes had an initial pH range of 3.00-9.47. None of the rinses resulted in ΔpH higher than the strongest standard, and there is a low correlation between initial pH and erosive potential. For pH cycling, not all mouthwashes showed SL, and this varied regardless of the pH of the product. In those who had PS, it was higher in dentin. In conclusion, FFM varies considerably in their composition and purpose, many providing only cosmetic benefits. Among

therapeutic FFM, most provide antiplaque/antigingivitis benefits. Products containing hydrogen peroxide have a greater effect on the initial biofilm of *S. mutans*. Regarding the erosive potential, some FFM have an acidic pH and can cause erosive mineral loss in enamel and dentin, with the most pronounced effects on dentin.

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LISTA DE ABREVIATURAS E SIGLAS

ADA: *American Dental Asociation*

ANSI: *American National Standards Institute*

CMB: Concentração mínima bactericida

CMI: concentração mínima inibitória

CPC cloreto de cetilpiridínio

EOs: *Essential oils group*

ENF: enxaguatórios bucais não fluoretados

FDA: *Food and Drug Administration*

FFM: *fluoride free mouthwashes*

IN: Indianapolis

ISO: Organização Internacional de Padronização/ *International Organization for Standardization*

MBC: *minimum bactericidal concentration*

MIC: *minimum inhibitory concentrations*

OD: *optical density*

PE: plant extracts group

Rpm: revoluções por minuto

SD: *standard deviation*

SL: *surface loss*

TSBS: *Tryptic Soy broth supplemented with 1% sucrose*

uPVC: *Adhesive unplasticized polyvinyl chloride*

W: *whitening group*

ZC: *zinc chloride group*

ΔpH: diferença de pH

%: porcentagem

%SHL: *percentage of surface hardness loss*

°C: graus célsius

<: menor a

>: maior a

≤: menor o igual

$_{ABS}$: absorbância

g: gramas

h: hora

L: litros

M: molar

mg: miligramas

min: minutos

mL: mililitros

mm: milímetros

mm: milimolar

mm^2: milímetro quadrado

nm: nanômetros

ppm: partes por milhão

μL: microlitros

SUMÁRIO

1 INTRODUÇÃO

Os enxaguatórios bucais são produtos para higiene bucal, predominantemente utilizados após a escovação dos dentes. Existem diversos produtos disponíveis no mercado mundial [Radzki et al., 2022], e mais de 120 milhoes de pessoas nos EUA usam este tipo de produto. Eles podem ser divididos em duas categorias, como produtos cosméticos ou terapêuticos. Os enxaguatórios bucais cosméticos são frequentemente utilizados para fins que não são considerados clinicamente significativos, como refrescar o hálito. Diferentemente dos enxaguatórios bucais terapêuticos, os quais podem ser adquiridos como produto de livre comercialização ou por prescrição dependendo do ingrediente ativo (por exemplo clorexidina, somente com prescrição nos EUA) ou de sua concentração (por exemplo fluoreto, com prescrição apenas acima de 226 ppm de F nos EUA).

O termo terapêutico refere-se a "relativo à cura da doença". No entanto, não há clareza em relação ao uso do termo, pois os enxaguatórios bucais terapêuticos também têm sido considerados em relação aos benefícios cosméticos, como clareamento e mau hálito bucal, ao mesmo tempo em que abordam doenças bucais, como cárie dentária e gengivite, além de proporcionar alívio da dor, desbridamento oral e limpeza de feridas entre outros benefícios [ADA, 2021]. Existem também enxaguatórios bucais prévio a procedimentos que têm efeito bactericida e virucida, dependendo do ingrediente e da concentração [Mohd-Said et al., 2021; Chaudhary et al., 2021]. Em acréscimo, nos EUA os enxaguatórios bucais terapêuticos fornecem benefício anticárie ou mesmo antigengivite, mas não ambos. Isso ocorre apesar de não haver razão científica para um enxaguatório bucal possuir tal distinção, pois formulações contendo fluoreto de sódio (anticárie) e óleos essenciais (antigengivite) são compatíveis [Cortelli et al., 2012]. Esses enxaguatórios bucais não se restringem somente ao mercado norte americano, sendo comercializado em outros países.

Os enxaguatórios bucais anticárie (contêm fluoreto) são regulamentados pela *Food and Drug Administration* (FDA) dos EUA. Estes enxaguatórios fluoretados podem ser formulados em apenas duas concentrações (900 ou 226 ppm F), dependendo da frequência de uso (uma vez por semana ou duas vezes por dia), além de outros requisitos de formulação (por exemplo, faixa de pH, presença de ácido fosfórico/tampão de fosfato). Para a classificação de um enxaguatório bucal como não fluoretado (ENF) em terapêutico ou cosmético se tem como base a norma FDA [FDA 2003], a qual estabelece os compostos e concentrações que são consideradas terapêuticas. Os ENF estão se tornando cada vez mais populares entre os consumidores que procuram produtos alternativos para higiene bucal. Esses enxaguatórios bucais são projetados para fornecer muitos dos mesmos benefícios que os fluoretados, como refrescar o hálito e matar

bactérias, mas sem a adição de fluoreto. Há uma grande variedade de ENF disponível, sendo que muitos deles são regulamentados de maneira semelhante devido a suas alegações e ingredientes ativos. Por exemplo, alegações antiplaca/antigengivite são permitidas para ENF contendo cloreto de cetilpiridínio (CPC) em concentrações de 0,045-0,1%, enquanto aqueles contendo óleos essenciais devem ser formuladas na presença de 21,6-26,9% de álcool (FDA 2003). Vários outros ingredientes ativos são permitidos em ENF terapêutico de acordo com o FDA, como peróxido de hidrogênio (1,5%), como agente de desbridamento oral/limpeza de feridas/antisséptico, e mentol (0,04-2%) para alívio da dor oral [FDA, 2017].

Cárie dentária, erosão dentária e as doenças periodontais são doenças da cavidade bucal que têm aumentado ao longo dos anos [Jaeggi e Lussi, 2006; Salas et al., 2014, Kasesebaum et al., 2017]. A maioria das empresas comercializa produtos com o objetivo de controlar biofilme dental para prevenção e tratamento de cárie e doenças periodontais. Em um biofilme dental, *Streptococcus mutans* é uma bactéria associada ao desenvolvimento de cárie, sendo o biofilme formado na presença de sacarose também associada ao acúmulo de biofilme periodontopatogênico. Muitos dos enxaguatórios bucais antiplaca e antigengivite visam a morte bacteriana e sabe-se que a eficácia dos enxaguatórios com potencial antimicrobiano sobre *S. mutans* se mostra evidente em biofilmes iniciais [Dong et al., 2012; Batra et al. al., 2022; Takenaka et al., 2022]. Porém não se sabe o efeito antibacteriano de diferentes ENF, contendo diferentes princípios ativos em biofilmes iniciais.

Muitos dos produtos possuem diferentes ingredientes ativos, aromatizantes, detergentes, estabilizantes, corantes, entre outros, o que também poderia interferir no efeito de controle de formação de biofilme. Uma característica importante em enxaguatórios bucais, incluindo os ENF, é o pH das soluções, pois é um fator importante na erosão dentária. O desgaste dentário erosivo é uma condição que pode levar à perda da estrutura dentária devido à exposição a ácidos de origem não bacteriana, podendo ser de natureza extrínseca ou intrínseca [Schlueter et al., 2019]. Enxaguatórios bucais são agentes extrínsecos que podem apresentar potencial erosivo dependendo de fatores químicos, como pH, capacidade tampão e tipo de ácido presente na formulação [Zero, 1996; Lussi et al., 2011; Kanzow et al., 2016]. Em relação ao pH neste tipo de produto, verificou-se que existem no mercado produtos fluoretados com pH ácido [Valdivia-Tapia et al., 2021], e sabe-se que um pH baixo pode causar erosão [Zero, 1996; Lussi et al., 2021].

Em relação aos ENF não há informações específicas sobre o pH desses produtos, pois não é obrigatório descrevê-lo em suas embalagens. No entanto, a American Dental Association (ADA) afirma que os enxaguatórios bucais podem ser formulados com pH ácido, neutro ou

alcalino, com valores de pH permitidos variando de 3,0 a 10,5. Para enxaguatórios bucais com pH abaixo de 5,5, a *American National Standards Institute* (ANSI)/ADA *Standard 116* exige maior demonstração da segurança do produto, seja por meio de um teste de desmineralização, teste de erosão ou outros métodos apropriados [ADA, 2010, 2019].

Frente ao exposto, o objetivo dessa tese foi de investigar enxaguatórios bucais não fluoretados (ENF) comumente comercializado nos EUA, avaliando seu efeito antibacteriano e potencial erosivo. Para isso foram desenvolvidas quatro pesquisas com objetivos específicos: I) Investigar a composição e as finalidades de ENF de livre comercialização nos EUA; II) Avaliar a eficácia de ENF em biofilme inicial de *S. mutans*; III) Avaliar o potencial erosivo de ENF em tecidos duros dentais pela norma da ISO/FDIS 28888:2013); IV) Avaliar a perda de superfície de esmalte e dentina causada por ENF usando um modelo validado de ciclagem de pH para erosão.

2 ARTIGOS

2.1 Fluoride-free mouthwashes available over the counter in the US-an overview

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Short Title: Fluoride-free mouthwashes sold in the US.

Abstract

Background. Fluoride-free mouthwashes (FFM) are commonly used by patients and for a variety of cosmetic and/or therapeutic reasons. The purpose of this study was to investigate the composition and purposes of FFM available over-the-counter in the US.

Methods. This study utilized a convenience sample by purchasing all different FFM (not considering different flavors) from supermarkets, pharmacies, and variety stores in Indianapolis, Indiana, USA. The purposes and active ingredients were extracted from the bottle labels and ingredient listings and categorized. Only FFM with a 'drug facts' label as regulated by the US Food and Drug Administration were considered therapeutic.

Results. Eighty-one different FFM were identified, of which 30 were therapeutic. The most common therapeutic purpose was antiplaque/antigingivitis (n=25), followed by oral debriding/antiseptic/wound cleanser/pain reliever (n=5). Cetylpyridinium chloride (0.05-0.1%; n=15) and essential oils (0.092% eucalyptol/0.042% menthol/0.060% methyl salicylate/0.064% thymol; n=9) were the most used antiplaque/antigingivitis agents. The most common purpose of cosmetic products (n=51) was whitening (n=11), followed by bad breath management (n=6), pre-brushing (n=3), dry mouth (n=3), anti-tartar (n=2), and anti-sensitivity (n=1). The most used whitening agents were hydrogen peroxide and/or sodium hexametaphosphate (n=7). Among all FFM, 27 contained alcohol (≤26.9%). Not considering breath freshening, many FFM (n=35) do not appear to provide a clinically meaningful benefit.

Conclusions. FFM vary considerably in their composition and purpose with many providing only cosmetic benefits. Among the therapeutic FFM, most provide antiplaque/antigingivitis benefits.

Practical Implications. Dental professionals should consider the type of FFM used by their patients as part of their home-care instructions.

Key Words. Mouthwash, non-fluoride, oral hygiene, dentistry

Introduction

Mouthwashes are products for oral hygiene, are predominantly used after toothbrushing, and can be broadly divided into cosmetic and therapeutic ones. Cosmetic mouthwashes may be temporarily used for purposes that are not considered clinically meaningful, such as breath freshening. Therapeutic mouthwashes are available over-the-counter and by prescription, depending on the active ingredient (e.g., chlorhexidine – prescription only in the US) or its concentration (e.g., fluoride – prescription only above 226 ppm fluoride in the US). Therapeutic refers to 'relating to the healing of disease'. However, there is considerable confusion as therapeutic mouthwashes have also been considered in relation to cosmetic benefits, such as whitening and oral malodor, while also addressing oral diseases, such as dental caries and gingivitis, as well as affording pain relief, oral debriding, and wound cleansing among other benefits (ADA, 2021). There are also pre-procedural mouthwashes that have bactericidal and virucidal effects, depending on the ingredient and concentration (Mohd-Said et al., 2021; Chaudhary et al., 2021). To add to the incertitude, in the US, therapeutic mouthwashes either provide anticaries benefits or treat a different disease (e.g., gingivitis) but not both. This is despite there being no scientific reason for a mouthwash for such a distinction as, for example, sodium fluoride (anticaries) and essential oils (antigingivitis) are compatible (Cortelli et al., 2012). In fact, such mouthwashes are readily available in other countries.

Over-the-counter anticavity (i.e., fluoride-containing) mouthwashes are regulated by the US Food and Drug Administration (FDA), with only two fluoride concentrations being permissible (90 or 226 ppm) depending on the usage frequency (once or twice a day) in addition to other formulation requirements (e.g., pH range, presence of phosphoric acid/phosphate buffer). A much wider range of fluoride-free mouthwashes (FFM) are available, with many being regulated in a similar manner due to their claims and active ingredients. For example, antiplaque/antigingivitis claims are permissible for FFM containing cetylpyridinium chloride (CPC) at concentrations of 0.045-0.1%, while those containing essential oils must be formulated in the presence of 21.6-26.9% alcohol (FDA 2003). Several other active ingredients are permissible in therapeutic FFM according to the FDA: hydrogen peroxide (1.5%) as an oral debriding agent/wound cleanser/antiseptic, and menthol (0.04-2%) for oral pain relief (FDA, 2017).

To the authors' knowledge, no study has thus far investigated the commercial FFM landscape. Therefore, the purpose of this study was to investigate the composition and purposes of FFM available over-the-counter in the US.

Methods

Sampling procedure

The authors chose a convenience sample of FFM which were purchased in Indianapolis, IN, USA, between August 19-31, 2022. One bottle of each commercial brand was purchased, avoiding repeat purchases or mouthwashes with virtually identical lists of ingredients (same manufacturer) but varying only in flavor. Different variety stores, pharmacies, and supermarkets (Kroger, CVS, Walmart, Walgreens, Target, Dollar General, Dollar Tree, Fresh Thyme, Meijer, Aldi, and Whole Foods) were visited. Store brands (private labels) were purchased only from one store as they are highly likely identical in formulation. The authors did not sample FFM available from online retailers.

Data extraction and analysis

The information from the bottle labels (Appendix) was recorded as follows: a) commercial name; b) expiration date; c) lot n^{o}.; d) manufacturer; e) city and country of manufacture; f) list of ingredients; g) active ingredients and drug facts (if present); h) presence of alcohol and its concentration; i) purpose; j) used pre or post brushing; and k) usage recommendations. The purchase price was also recorded.

For the analysis and discussion, FFM were grouped based on their active ingredient(s) derived from the drug facts label (National archives, 2004), which then determined their primary purpose. Only those FFM bearing drug facts on their bottle labels were considered therapeutic. Cosmetic FFM were grouped by their claims and then by ingredient(s) that can be most likely attributed to the provided benefit. Breath freshening was not considered a unique benefit as all FFM provide some form of relief due to sensorial masking by virtue of being flavored. However, oral malodor reduction due to specific ingredients added to the formulation, such as zinc chloride or citrate, and sodium chlorite was considered.

Results

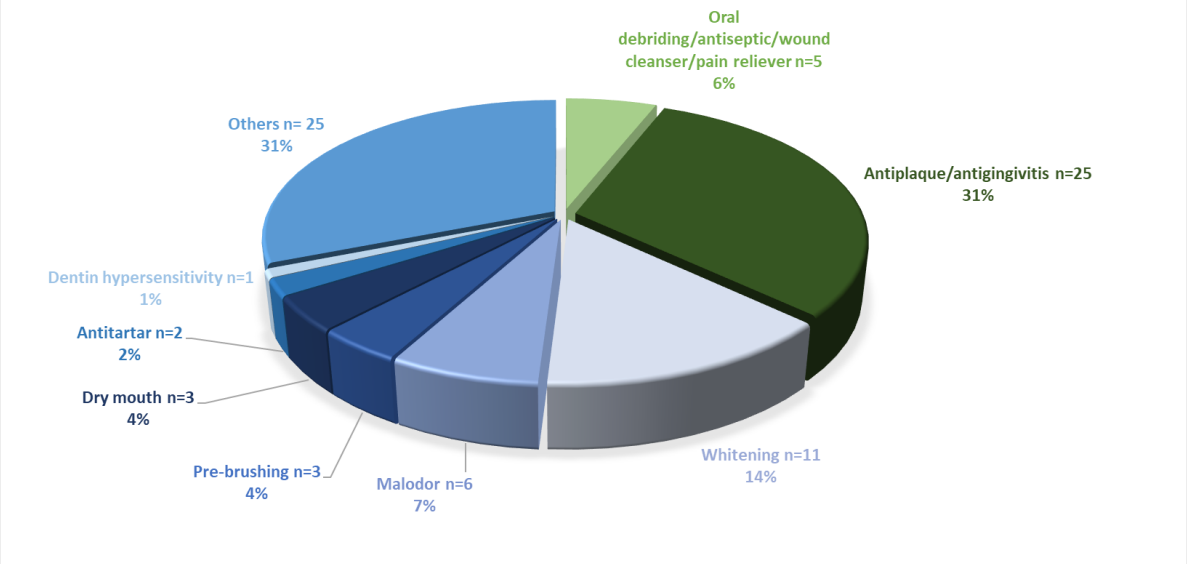

Figure: Fluoride-free mouthwashes **(**FFM) distribution by type and benefit: therapeutic (***bold/italic***) and cosmetic (not bold/not italic).

The complete list of FFM, their distinction, ingredients, and all other pertinent information can be found in the appendix. Eighty-one different FFM were identified, of which 30 were therapeutic (Figure). The most common therapeutic purpose was antiplaque/antigingivitis (n=25;31%, followed by oral debriding/antiseptic/wound cleanser/pain reliever (n=5;6%). CPC (0.05-0.1%; n=15;19%) and essential oils (0.092% eucalyptol/0.042% menthol/0.060% methyl salicylate/0.064% thymol; n=11%) were the most used antiplaque/antigingivitis agents. CPC concentrations varied: 0.05% (n=5;6%), 0.07% (n=7; 9%), 0.075% (n=2;2%), and 0.1% (n=1;1%). Other actives were: 0.2-1.0% menthol (oral pain relief), 1.5% hydrogen peroxide (oral debriding/antiseptic/wound cleanser, and 2.5% sodium bicarbonate (oral debriding/wound cleanser).

Of the products analyzed, a total of 51 (62%) were identified as cosmetic. The most common purpose of this group of products was whitening (n=11;14%), of which only three declared the concentration of the whitening agent (1.5% hydrogen peroxide), followed by bad breath management (n=6;7%), pre-brushing (n=3;4%), dry mouth (n=3;4%), anti-tartar (n=2;2%), and anti-sensitivity (n=1;1%). The most used whitening agents were hydrogen peroxide and/or sodium hexametaphosphate (n=11;14%). The agent for dentinal hypersensitivity management was dipotassium oxalate monohydrate. In addition, various plant extracts were utilized (e.g.,

Aloe barbadensis Leaf, Cinnamomum zeylanicum leaf oil, Mentha viridis leaf oil, Chamomilla recutita (Matricaria), Camellia sinensis leaf extract, Peppermint oil, among others; n=15. For fresh breath-related claims, (n=11), the agents utilized were sodium chlorite, zinc chloride, and zinc citrate.

Considering all FFM, n=25 (31%) contained alcohol in concentrations of 4.1 - 26.9% (<10%: n=8: 10-20%: n=9; >20%: n=4; four products did not disclose the alcohol concentration), with one of the products being a mouthwash concentrate (70% of alcohol), requiring dilution prior to use.

The recommended single usage volume varied between 20 mL to one fluid ounce, application times between 15-60 s, and the usage frequency between once to four times per day. Regarding age recommendations, n=20 (25%) warn against their use in children under 12 years of age, n=21 (26%) are only for children over six years of age, and one product (Listerine Sensitivity Zero alcohol) is only for anyone over 18 years of age. The cost per single-use ranged from US$ 0.02– 0.84/20 mL (20 mL is the recommended single dose of the best-selling brand Listerine), with estimated annual costs ranging between US$ 15 - 613, assuming twice daily application of 20 mL each.

Discussion

According to the American Dental Association (ADA), mouthwashes can be useful adjuncts to daily brushing and flossing in the maintenance of oral hygiene (ADA, 2023). The present study followed the ADA's differentiation between therapeutic and cosmetic mouthwashes. However, products were differentiated based on the drug facts label's presence (therapeutic) or absence (cosmetic) , on the bottle. This label is regulated by the FDA and is intended for over-the-counter medicines. It contains, among other information, the product's active ingredients and the purpose of the product. For cosmetic FFM, claims and corresponding ingredients were retrieved and compared. The information gathered for therapeutic and cosmetic FFM was then utilized to provide an overview of the over-the-counter FFM landscape in the US.

The present study highlighted that patients have considerable choice among FFM with the majority only providing cosmetic benefits. Broadly, only two types of therapeutic FFM were identified - antiplaque/antigingivitis and oral debriding/antiseptic/wound cleanser/pain reliever mouthwashes. Antiplaque/antigingivitis FFM can be further divided into their therapeutic agent(s) - CPC (0.05-0.1%), essential oils (0.092% eucalyptol/0.042% menthol/0.060% methyl salicylate/0.064% thymol), and aloe vera (20%). Substantial evidence supports the efficacy of

CPC and essential oils as antiplaque/antigingivitis agents (Gusolley, 2010; Figuero et al., 2020). CPC is a cationic surface-active agent with a broad antimicrobial spectrum, with the rapid killing of gram-positive pathogens (Pitten and Kramer, 2001). According to the FDA, FFM containing CPC at concentrations of 0.045-0.1% and chemical availability of at least 72% can be considered therapeutic as CPC is a quaternary compound inhibiting the first stages of biofilm formation (Schroeder et al., 1962). FFM containing a combination of essential oils in a hydroalcoholic formulation (21.6-26.9% alcohol) can also be efficacious in plaque/gingivitis prevention (Fine et al., 1985). Essential oils disrupt cell walls and cause the precipitation of cell proteins, while at lower concentrations, there is inactivation of essential enzymes (Ross et al., 1989). While there is ample evidence to support the use of CPC (Haps et al., 2008; Gunsolley et al., 2010; Van der Weiden et al., 2015; Langa et al., 2020) and essential oils (Stoeken et al., 2007; Van der Weiden et al., 2015; Gunsolley et al., 2010; Van Leeuwen et al., 2014; Figuero et al., 2019) as antiplaque/antigingivitis agents, there are sparse data on aloe vera. The FDA noted in its proposed rulemaking that "there are insufficient data to permit final classification of the safety and effectiveness of aloe vera as an over-the-counter antigingivitis/antiplaque ingredient" (FDA, 2003). Several studies have been conducted since. These studies generally favored aloe vera, although none of these studies involved US populations and were also considered of low quality (Al-Maweri et al., 2020; Tidke et al., 2022). Several cosmetic FFM were found to contain CPC or essential oils but did not state their concentrations. Regarding CPC, this is likely due to the use of clinically ineffective CPC concentrations or its poor chemical availability in the formulation. Concerning essential oils, this is due to the formulation not containing any alcohol. A recent study has, however, shown that an alcohol-free mouthwash containing essential oils can be as effective in preventing plaque/gingivitis compared to one containing alcohol (Lynch et al., 2018).

Some products without a stated concentration of hydrogen peroxide were intended for oral debridement, wound cleansing, or sometimes as an antiseptic. This agent in mouthwashes used alone is known not to consistently prevent plaque buildup, unlike when used as an adjunct to daily oral hygiene, where it has been found to reduce gingival redness (Hossainian et al., 2011). One FFM utilizes 2.5% sodium bicarbonate for oral wound cleansing and oral debriding. Sodium bicarbonate can have bactericidal effects. It is effective in the prevention and treatment of oral mucositis in patients with acute head and neck malignancy (Cabrera-Jaime et al., 2018; David and Shree, 2019; Mohammadi et al., 2022). Two FFM utilize menthol (0.2-1.0%) for oral pain relief. Although menthol has been used more as a flavoring agent rather than as an active ingredient, it has been proven to have considerable antimicrobial activity, it is considered

GRAS (Generally Regarded as Safe) by the FDA (Van Leeuwen et al., 2014; Freires et al., 2015).

One of the cosmetic products contains dipotassium oxalate monohydrate. This belongs to the family of oxalates, which are used in dentin hypersensitivity management because they can reduce the permeability of dentinal tubules. However, there is no strong evidence from clinical studies that demonstrate a consistent benefit (Lynch et al., 2018; Cunha-Cruz et al., 2011), oral debriding/antiseptic/wound cleanser/pain reliever mouthwashes

Whitening was the most common purpose of cosmetic FFM, which can be attributed to the presence of hydrogen peroxide and/or sodium hexametaphosphate. Two of the three pre-brushing rinses contain tetrasodium pyrophosphate and sodium lauryl sulfate. A review of clinical data on one of these products concluded that there is “some benefit in plaque and gingivitis reduction”, while questioning whether these benefits are clinically meaningful. The other product contains a wide range of plant extracts. A total of 15 FFM with plant extracts were identified, containing from one to more than 10 extracts. There has been a steady increase in the use and variety of plant extracts in oral care products, with the intention of naturally treating oral diseases and conditions. Plant extracts contain different classes of compounds, including polyphenols, essential oils, and alkaloids, thereby potentially affecting the control of oral biofilm-associated pathologies (Cardoso et al., 2021). Some plant extracts exert antimicrobial activity as broad-spectrum antibiotics, inhibiting microbial growth, reducing virulence factors, and presenting antibiofilm activity (Khoramian Tusi et al., 2020; Shaw and Wuest, 2020). However, there is currently little clinical evidence to support their use as therapeutic agents in mouthwashes.

Three FFM claimed to provide temporary relief from dry mouth (xerostomia). These products contain polymers (e.g., hydroxyethylcellulose) for the purpose of lubrication and hydration. Xerostomic patients often use a wide range of OTC and prescription products, incl. sprays, lozenges, sialogogues (drugs), and saliva substitutes.

Two anti-tartar FFM were identified. One product contains a wide range of plant extracts and essential oils, although there is no clinical evidence regarding a specific anti-tartar benefit for this formulation or any of the ingredients. The second product contains a zinc salt (chloride). There is evidence that zinc salts control plaque, reduce oral malodor by inhibiting volatile sulfur compounds, and reduce tartar formation by modifying/inhibiting crystal growth (Finney et al., 2003; Hall., 2003; Cvjetinovic et al., 2020; Rajendiran et al., 2021). Six FFM claim to provide relief from bad breath (malodor), which the ADA considers a therapeutic rather than a cosmetic benefit. Agents used to combat are zinc salts and sodium chlorite, with the latter producing

chlorine dioxide under acidic conditions, an effective antimicrobial compound that was shown to reduce oral malodor clinically (Lee et al., 2021). Several therapeutic FFM also contain zinc salts and most likely for malodor prevention.

A considerable number of FFM (n=25) contain alcohol (ethanol) and up to 26.9%. Alcohol is used as a solubilizer, stabilizer, preservative, to enhance the efficacy of anti-plaque agents, and for obtaining a distinctive flavor. Controversy exists, however, as comparative studies between alcohol-containing and non-alcoholic mouthwashes have mainly shown that alcohol content adds little to product efficacy (Werner and Seymour, 2009).

Lastly, FFM should not be recommended to patients whose primary concern is the prevention or management of dental caries. Rinsing with a FFM immediately after brushing with fluoride toothpaste has been shown to greatly reduce intra-oral fluoride retention and thereby negatively impacts the anticaries protection afforded by such toothpastes (Duckworth et al., 2009). For patients in need of both antiplaque/antigingivitis and anticaries benefits, FFM should be used in between periods of fluoride exposure rather than immediately following them.

This study had limitations as only OTC products and only those available in conventional retail stores were considered, with additional FFM being available exclusively online. Furthermore, regional differences in the availability of FFM may exist and not all 81 FFM are available in every store. The differentiation between therapeutic and cosmetic FFM was driven by the presence or absence of a drug label rather than whether there is irrefutable clinical evidence to support any of the claims made. Lastly, new products are being marketed, others are withdrawn or reformulated which suggests that repeats of a study of this kind may be warranted basis periodically.

Conclusion

A vast number of over-the-counter FFM are commercially available in the US, which vary considerably in their ingredients and purposes. Only 30 out of 81 FFM fulfilled the conditions to be considered therapeutic due to the presence of a drug label and the corresponding active ingredients. Antiplaque/antigingivitis FFM are the most common therapeutic mouthwashes, with strong clinical evidence supporting their use. However, some cosmetic products are also being considered therapeutic due to their supporting evidence for the relief of malodor, while many cosmetic FFM does not provide a clinically meaningful benefit. Therefore, dental professionals should consider the type of FFM used by their patients as part of their home-care instructions and educate their patients on how to distinguish between therapeutic and cosmetic FFM.

Ethics approval and consent to participate

"Not applicable"

Consent for publication

"Not applicable"

Availability of data and materials

Competing interests

"The authors declare that they have no competing interests."

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Authors' contributions

"ACVT and FL bought, analyzed, and reviewed the samples. ACVT, FL, APRF, and JAC authors contributed to writing the manuscript. All authors read and approved the final manuscript."

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References

Examples of the Vancouver reference style are shown below.

1. American Dental Association *(ADA). 2021. Department of Scientific Information, Evidence Synthesis & Translation Research, ADA Science & Research Institute, LLC.* https://www.ada.org/resources/research/science-and-research-institute/oral-health-topics/mouthrinse-mouthwash. [accessed April 1, 2023]
2. Mohd-Said S, Mohd-Dom TN, Suhaimi N, Rani H, McGra th C. Effectiveness of Pre-procedural Mouth Rinses in Reducing Aerosol Contamination During Periodontal Prophylaxis: A Systematic Review. Front Med (Lausanne) 2021;8:600769.
3. Chaudhary P, Melkonyan A, Meethil A, et al. Estimating salivary carriage of severe acute respiratory syndrome coronavirus 2 in nonsymptomatic people and efficacy of mouthrinse in reducing viral load: A randomized controlled trial. J Am Dent Assoc 2021;152(11):903-08.
4. Cortelli SC, Cortelli JR, Wu MM, Simmons K, Charles CA. Comparative antiplaque and antigingivitis efficacy of a multipurpose essential oil-containing mouthrinse and a cetylpyridinium chloride-containing

mouthrinse: A 6-month randomized clinical trial. Quintessence Int. 2012 Jul-Aug;43(7):e82-94. PMID: 22670258.

5. Food and Drug Administration (FDA). Department of Health and Human Services. 21 CFR Part 356 Oral Health Care Drug ProducIOverthe-Counter Human Use; Antigingivitis/ Antiplaque Drug Products; Establishment of a Monograph; Proposed Rules. Federal Register / Vol. 68, No. 103 / Thursday, May 29, 2003 / Proposed Rules. https://www.govinfo.gov/content/pkg/FR-2003-05-29/pdf/03-12783.pdf. [accessed April 1, 2023]
6. Food and Drug Administration (FDA). Oral Healthcare Drug Products: Anesthetic, Astringent, Antimicrobial, Debriding, Demulcent, Expectorant, Decongestant. 2017. https://www.fda.gov/drugs/historical-status-otc-rulemakings/rulemaking-history-otc-oral-healthcare-drug-products#oral [accessed April 1, 2023]
7. National archives and records administration. Code of Federal Regulation–s. Subpart C - Labeling Requirements for Over-the-Counter Drugs. Nomenclature changes to part 201 appear at 69 FR 13717, Mar. 24, 2004. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-201/subpart-C [accessed April 6, 2023]
8. American Dental Association (ADA). Mouthwash. MouthHealthy™. 2023. https://www.mouthhealthy.org/all-topics-a-z/mouthwash [accessed April 6, 2023]
9. Gunsolley JC. Clinical efficacy of antimicrobial mouthrinses. J Dent. 2010 Jun;38 Suppl 1:S6-10. doi: 10.1016/S0300-5712(10)70004-X. PMID: 20621242.
10. Figuero E, Roldán S, Serrano J, Escribano M, Martín C, Preshaw PM. Efficacy of adjunctive therapies in patients with gingival inflammation: A systematic review and meta-analysis. J Clin Periodontol. 2020 Jul;47 Suppl 22:125-143. doi: 10.1111/jcpe.13244. PMID: 31869441.
11. Pitten FA, Kramer A. Efficacy of cetylpyridinium chloride used as oropharyngeal antiseptic. Arzneimittelforschung. 2001;51(7):588-95. doi: 10.1055/s-0031-1300084. PMID: 11505791.
12. Schroeder HE, Marthaler TM, Mühlemann HR. Effects of some potential inhibitors on early calculus formation. Helv Odont Acta 1962; 6: 6–9.
13. Fine DH. Mouthrinses as adjuncts for plaque and gingivitis management. A status report for the American Journal of Dentistry. Am J Dent 1988;1:259-263.
14. Ross NM, Charles CH, Dills SS. Long-term effects of Listerine antiseptic on dental plaque and gingivitis. J Clin Dent. 1989 Spring;1(4):92-5. PMID: 2700894.
15. Haps S, Slot DE, Berchier CE, van der Weijden GA. The effect of cetylpyridinium chloride-containing mouth rinses as adjuncts to toothbrushing on plaque and parameters of gingival inflammation: a systematic review. Int J Dent Hyg 2008;6(4):290–303. 5.
16. Van der Weijden FA, Van der Sluijs E, Ciancio SG, Slot DE. Can Chemical Mouthwash Agents Achieve Plaque/Gingivitis Control? Dent Clin North Am. 2015 Oct;59(4):799-829. doi: 10.1016/j.cden.2015.06.002. PMID: 26427569.
17. Langa GPJ, Muniz FWMG, Costa RDSA, da Silveira TM, Rösing CK. The effect of cetylpyridinium chloride mouthrinse as adjunct to toothbrushing compared to placebo on interproximal plaque and gingival inflammation-a systematic review with meta-analyses. Clin Oral Investig. 2021 Feb;25(2):745-757. doi: 10.1007/s00784-020-03661-2. Epub 2020 Nov 13. PMID: 33185736.

18. Stoeken JE, Paraskevas S, van der Weijden GA. The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: a systematic review. Journal of Periodontology 2007;78(7):1218−28.
19. Van Leeuwen MP, Slot DE, Van der Weijden GA. The effect of an essential-oils mouthrinse as compared to a vehicle solution on plaque and gingival inflammation: a systematic review and meta-analysis. Int J Dent Hyg. 2014 Aug;12(3):160-7. doi: 10.1111/idh.12069. Epub 2014 Apr 10. PMID: 24720368.
20. Figuero E, Herrera D, Tobías A, Serrano J, Roldán S, Escribano M, Martín C. Efficacy of adjunctive anti-plaque chemical agents in managing gingivitis: A systematic review and network meta-analyses. J Clin Periodontol. 2019 Jul;46(7):723-739. doi: 10.1111/jcpe.13127. Epub 2019 May 31. PMID: 31058336.
21. Al-Maweri SA, Nassani MZ, Alaizari N, Kalakonda B, Al-Shamiri HM, Alhajj MN,et al.,. Efficacy of aloe vera mouthwash versus chlorhexidine on plaque and gingivitis: A systematic review. Int J Dent Hyg. 2020 Feb;18(1):44-51. doi: 10.1111/idh.12393. Epub 2019 Mar 25. PMID: 30829440.
22. Tidke S, Chhabra GK, Madhu PP, Reche A, Wazurkar S, Singi SR. The Effectiveness of Herbal Versus Non-Herbal Mouthwash for Periodontal Health: A Literature Review. Cureus. 2022 Aug 13;14(8):e27956. doi: 10.7759/cureus.27956. PMID: 36120261; PMCID: PMC9465625.
23. Lynch MC, Cortelli SC, McGuire JA, Zhang J, Ricci-Nittel D, Mordas CJ, Aquino DR, Cortelli JR. The effects of essential oil mouthrinses with or without alcohol on plaque and gingivitis: a randomized controlled clinical study. BMC Oral Health. 2018 Jan 10;18(1):6. doi: 10.1186/s12903-017-0454-6. PMID: 29321067; PMCID: PMC5763666.
24. Cunha-Cruz J, Stout JR, Heaton LJ, Wataha JC; Northwest PRECEDENT. Dentin hypersensitivity and oxalates: a systematic review. J Dent Res. 2011 Mar;90(3):304-10. doi: 10.1177/0022034510389179. Epub 2010 Dec 29. PMID: 21191127; PMCID: PMC3144108.
25. Lynch MC, Perfekt R, McGuire JA, Milleman J, Gallob J, Amini P, Milleman K. Potassium oxalate mouthrinse reduces dentinal hypersensitivity: A randomized controlled clinical study. J Am Dent Assoc. 2018 Jul;149(7):608-618. doi: 10.1016/j.adaj.2018.02.027. Epub 2018 May 2. PMID: 29728195.
26. Hossainian N, Slot DE, Afennich F, Van der Weijden GA. The effects of hydrogen peroxide mouthwashes on the prevention of plaque and gingival inflammation: a systematic review. Int J Dent Hyg. 2011 Aug;9(3):171-81. doi: 10.1111/j.1601-5037.2010.00492.x. Epub 2011 Jan 31. PMID: 21356027.
27. Cabrera-Jaime S, Martínez C, Ferro-García T, Giner-Boya P, Icart-Isern T, Estrada-Masllorens JM, Fernández-Ortega P. Efficacy of Plantago major, chlorhexidine 0.12% and sodium bicarbonate 5% solution in the treatment of oral mucositis in cancer patients with solid tumour: A feasibility randomised triple-blind phase III clinical trial. Eur J Oncol Nurs. 2018 Feb;32:40-47. doi: 10.1016/j.ejon.2017.11.006. Epub 2017 Dec 14. PMID: 29353631.
28. David, MPR, and Shree, MKT.Effectiveness of turmeric mouthwash and sodium bicarbonate mouthwash to reduce oral mucositis among patient undergoing radiation therapy. Scholars International Journal of Traditional and Complementary Medicine, . (2019). 2(7), 124-127Mohammadi F, Oshvandi K, Kamallan SR, Khazaei S, Ranjbar H, Ahmadi-Motamayel F, Gillespie M, Jenabi E, Vafaei SY. Effectiveness of sodium bicarbonate and zinc chloride mouthwashes in the treatment of oral mucositis and quality of life in patients with cancer under chemotherapy. Nurs Open. 2022 May;9(3):1602-1611. doi: 10.1002/nop2.1168. Epub 2022 Feb 16. PMID: 35170247; PMCID: PMC8994952.

29. Hasturk H, Nunn M, Warbington M, Van Dyke TE. Efficacy of a fluoridated hydrogen peroxide-based mouthrinse for the treatment of gingivitis: a randomized clinical trial. J Periodontol. 2004 Jan;75(1):57-65. doi: 10.1902/jop.2004.75.1.57. PMID: 15025217.
30. Angelillo IF, Nobile CG, Pavia M. Evaluation of the effectiveness of a pre-brushing rinse in plaque removal: a meta-analysis. J Clin Periodontol. 2002 Apr;29(4):301-9. doi: 10.1034/j.1600-051x.2002.290405.x. PMID: 11966927.
31. Cardoso VFDS, Amaral Roppa RH, Antunes C, Silva Moraes AN, Santi L, Konrath EL. Efficacy of medicinal plant extracts as dental and periodontal antibiofilm agents: A systematic review of randomized clinical trials. J Ethnopharmacol. 2021 Dec 5;281:114541. doi: 10.1016/j.jep.2021.114541. Epub 2021 Aug 18. PMID: 34416298.
32. Khoramian Tusi S, Jafari A, Marashi SMA, Faramarzi Niknam S, Farid M, Ansari M. The effect of antimicrobial activity of Teucrium Polium on Oral *Streptococcus mutans*: a randomized cross-over clinical trial study. BMC Oral Health. 2020 May 1;20(1):130. doi: 10.1186/s12903-020-01116-4. PMID: 32357889; PMCID: PMC7195746.
33. Shaw E, Wuest WM. Virulence attenuating combination therapy: a potential multi-target synergy approach to treat Pseudomonas aeruginosa infections in cystic fibrosis patients. RSC Med Chem. 2020 Feb 19;11(3):358-369. doi: 10.1039/c9md00566h. PMID: 33479641; PMCID: PMC7580779.
34. Jose A, Siddiqi M, Cronin M, DiLauro TS, Bosma ML. A randomized clinical trial in subjects with dry mouth evaluating subjective perceptions of an experimental oral gel, an oral rinse and a mouth spray compared to water. Am J Dent. 2016 Feb;29(1):58-64. PMID: 27093778.
35. Finney, M.; Walker, J.T.; Marsh, P.D.; Brading, M.G. Antimicrobial effects of a novel Triclosan/zinc citrate dentifrice against mixed culture oral biofilms. *Int. Dent. J.* **2003**, *53* (Suppl. S1), 371–378.
36. Hall PJ, Green AK, Horay CP, de Brabander S, Beasley TJ, Cromwell VJ, Holt JS, Savage DJ. Plaque antibacterial levels following controlled food intake and use of a toothpaste containing 2% zinc citrate and 0.3% Triclosan. Int Dent J. 2003 Dec;53(6 Suppl 1):379-84. doi: 10.1111/j.1875-595x.2003.tb00913.x. PMID: 14725382.
37. Cvjetinovic A, Ramseier CA, Salvi GE, Laugisch O. Chemische Zusätze in Zahnpasten zur Hemmung der Zahnsteinbildung. Eine Literaturübersicht [Chemical additives in toothpastes to inhibit calculus formation]. Swiss Dent J. 2020 Jun 15;130(6):503-513. German. PMID: 32512986.
38. Rajendiran M, Trivedi HM, Chen D, Gajendrareddy P, Chen L. Recent Development of Active Ingredients in Mouthwashes and Toothpastes for Periodontal Diseases. Molecules. 2021 Apr 1;26(7):2001. doi 10.3390/molecules26072001. PMID: 33916013; PMCID: PMC8037529
39. Lee SS, Suprono MS, Stephens J, Withers SA, Oyoyo U, Li Y. A randomized, blinded, clinical investigation of breath odor reduction efficacy of a stabilized chlorine-dioxide containing flavored mouthwash. Am J Dent. 2021 Aug;34(4):195-200. PMID: 34370911.
40. Freires IA, Denny C, Benso B, de Alencar SM, Rosalen PL. Antibacterial Activity of Essential Oils and Their Isolated Constituents against Cariogenic Bacteria: A Systematic Review. Molecules. 2015 Apr 22;20(4):7329-58. doi 10.3390/molecules20047329. PMID: 25911964; PMCID: PMC6272492.
41. Werner CW, Seymour RA. Are alcohol containing mouthwashes safe? Br Dent J. 2009 Nov 28;207(10): E19; discussion 488-9. doi: 10.1038/sj.bdj.2009.1014. PMID: 19942865.

42. Duckworth RM, Maguire A, Omid N, Steen IN, McCracken GI, Zohoori FV. Effect of rinsing with mouthwashes after brushing with a fluoridated toothpaste on salivary fluoride concentration. Caries Res. 2009;43(5):391-6. doi: 10.1159/000239753. Epub 2009 Sep 16. PMID: 19776570.

Appendix: Complete information of the samples, their ingredients and purpose.

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Biotène dry mouth oral rinse	Feb-25	2C053C	GSK consumer healthcare	Warren, NJ, 07059. Canada	7.49	Kroger	–	Water, glycerin, xylitol, sorbitol, propylene glycol, poloxamer 407, sodium benzoate, hydroxyethylcellulose, methylparab, propylparaben, flavor, sodium phosphate, disodium phosphate	–	Dry mouth	Cosmetic		Fresh mint	30s rinse with 15 mL, <5 times per day	Children 12 years and under. Not for use unless directed by a healthcare	White
Oral B Dry mouth	Dec-23	203351782A 16:51	Procter & Gamble	Cincinnati, OH 4502. USA	11.79	CVS	Cetylpyridinium Chloride	Flavor, glycerin, propylene glycol, xylitol, cellulose gum, sodium hyaluronate, poloxamer 407, sodium benzoate, cetylpyridinium chloride, benzoic acid	–	Dry mouth	Cosmetic	Pos	Immediate dry mouth symptom relief for up to 6 h	30s rinse with 15 mL, <5 times per day	Dont use children < 12 years of age	White
Ultimate Essential MouthCare. Eco dent	Mar-24	2C68	ECO–DENT	Twin Lakes, WI 53181. USA	9.99	Whole Foods	Sodium Bicarbonate 2.5%	Purified water, glycerin,polysorbate 20, aloe Barbadensis leaf juice, menthol, mentha piperita oil, stevia rebaudiana extract, echinacea purpurea extract, citrus aurantium dulcis oil, citrus limon oil, lavandula angustifolia oil, ricinus communis seed oil, hydrastis canadensis leaf extract, pimpinella anisum fruit oil, pelargonium graveolens oil, rosmarinus officialis leaf/stem oil, hamamelis virginiana water, citrus aurantium bergamia peel oil, citrus paradisi seed extract, foeniculum vulgare oil, eugenia carypophyllus flower oil, ocimum basilicum oil, rosewater concentrate, ubiquinone	–	Oral Wound Cleanse/Oral Debriding Agent	Therapeuthic	Pos	Dayly rinse and oral wound cleanser oral debriding agent	1 capful 30s	Adults and children 12 years and older: use as directed above. Children under 12: supervised use. Children under 2 years. Do not use.	Blue
Plax sofmint flavor mouthwash	Elab 2017	09722A	Johnson & Johnson Consumer INC	Markham, Canada L3R5L2	5.97	Walmart	tetrasodium pyrophosphate	Water, sorbitol, alcohol (8.6%), tetrasodium pyrophosphate, sodium benzoate, benzoic acid, sodium lauryl sulfate, poloxamer 407, flavor, xanthan gum, sodium saccharin, blue 1, yellow 5	Alcohol 8.6 % v/v	Prebrushing	Cosmetic	PRE	Rinse before brushing	30 s rinse with 20 mL, twice a day	Dont use children < 12 years of age	Green
Pre Brush Dental Rinse	Jan-23	47612	CVS Pharmacy, Inc	Woonsocket, RI 02895 USA	4.99	CVS	tetrasodium pyrophosphate	Water, glycerin and/or sorbitol, alcohol 8.7%, tetrasodium pyrophosphate, benzoic acid, flavor, poloxamer 407, sodium benzoate, sodium lauryl sulfate, sodium saccharine, xanthan gum, FD&C blue nº 1, FD&C yellow nº 5	Alcohol 8.7%	Prebrushing	Cosmetic	PRE	Compare to the ingredients in Palx Advanced formula plaque loosening Rinse	20 mL aprox 30 s pre-brushing	6 years. Do not use.	Blue
Desert Essence Prebiotic Plant based brushing rinse Mint	–	21231AG	Desert Essence	Hauppauge, NY 11788. USA	9.29	Whole Foods	–	Water, glycerin, polysorbate 80, perilla pcymoides seed extract, cichorium intybus root extract, hydrolyzed pea protein, inulin, glycyrhiza glabra root extract, quillaja saponaria bark extract, quisetum arvense leaf extract, glutathione, mentha piperita flower/leaf/stem oil, mentha arvensis steam/leaf oil,mentha viridis leaf oil, anthemis nobilis flower oil, echinacea angustifolia extract, zinc citrate, salvia officinalis oil, commiphora myrha oil, calcium ascorbate, ascorbic acid, aloe barbadensis leaf juice, calndula officinalis flower oil, hydrastis canadensis extract, melaleuca alternifolia leaf oil. hamamelis virginiana	–	Prebrushing	Cosmetic	PRE and Pos	Prebiotic Plant based brushing rinse Mint with Perilla seed	15 mL 30s	Children under 6 years: Keep out of reach	Brown

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Crest 3D white brilliance	Elab 2020	21515395UB	Procter & Gamble	Cincinnati, OH 4502. USA	6.99	Kroger	sodium hexametaphosphate	Water, glycerin, sodium hexametaphosphate, poloxamer 407, sodium benzoate, sodium lauryl sulfate, flavor, phosphoric acid, sodium saccharin, sucrallose, red 33, green 3	–	Whitening	Cosmetic	No information	Great tarting whitenin, guaranteed	60 s with 20 mL, twici a day	Keep out of reach of children under 6 years of age	White
Crest Bacteria Blast	Elab 2020	21995395RF	Procter & Gamble	Cincinnati, OH 4502. USA	497	Walmart	hydrogen peroxide, sodium hexametaphosphate	Water, glycerin, alcohol (5wt%), hydrogen peroxide, sodium hexametaphosphate, poloxamer 407, flavor, sodium citrate, sodium saccharin,	Alcohol 5% wt%	whitening	Cosmetic	Pos	Kills millions of bd breath bacteria	60 s rinse with 15 mL, twice a day	Dont use children < 12 years of age	White
Clean mint Withening alcohol free rinse	Jul-25	573124	Procter & Gamble	Cincinnati, OH 4502. USA	5.29	Kroger	Hydrogen peroxide, sodium hexametaphosphate	Water, glycerin, hydrogen peroxide, propylene glycol, sodium hexametaphosphate, poloxamer 407, sodium citrate, flavor, PEG-40, Hydrognated castor oil, soddium saccharine, citric acid.	–	whitening	Cosmetic	No information	Withening alcohol free rinse	30s rinse with 15 mL, twice a day	Dont use children < 12 years of age	White
Splendid white whitening mouth rinse alcohol free Up&Up	–	5724415	Target Corp	MN 55403. USA	3.69	Target	Hydrogen peroxide, sodium hexametaphosphate	Water, glycerin, hydrogen peroxide, propylene glycol, sodium hexametaphosphate, poloxamer 407, sodium citrate, flavor, PEG-40, Hydrognated castor oil, sodium saccharine, citric acid.	–	Whitening	Cosmetic	Pos	Compare to Crest 3D White	60 s rinse with 15 mL, twice a day	Keep out of reach of children under 12 years of age	White
ARC turn up the bright	Aug-23	127051782A	Procter & Gamble	Cincinnati, OH 4502. USA	5.99	Target	Hydrogen peroxide, sodium hexametaphosphate	Water, glycerin, propylee glycol, hydrogen peroxide, sodium hexametaphosphate, poloxamer 407, flavor, sodium citrate, sodium saccharin, citric acid, sucralose	–	whitening	Cosmetic	Pos	Enamel Safe	60 s rinse with 15 mL, twice a day	Dont use children < 12 years of age	Bottle is black with bright purple
Thera Breath Dentist formulated withening fresh breath	Jun-24	CM21730220	Dr. Harold Katz. LLC	Los Angeles, CA. 90038. USA	8.72	Walmart		Water, glycerin,polysorbate 20, sodium benzoate, PVP, Natural mint flavor, papain, D-limonene, menthol, glucose oxidase		whitening	Cosmetic		Protects teeth from staining for 24 hours	Rinse 2 min with one capeful, wait 30 min for eating or drinking	Dont use children < 12 years of age	Shiney
Lumineux Oral essentials	Feb-24	22B22A1	Oral Essentials, Inc.	Beverly Hills, CA 90210	9.99	Fresh Thyme	–	Organic Aloe Barbadensis (Aloe Vera) Leaf Juice, Xylitol, Purified water, dead sea salt, gautheria procumbens leaf oil, cocos nucifera oil, citrus limon peel oil, salvia officinalis oil, mentha ciridis leaf oil, Organic mentha piperita leaf oil, ocimum basilicum oil, Eugenia Caryophyllus flower Oil.	–	Whitening	Cosmetic	Pos	Whitening without the sensitivity	1 capfoul 2/3 times for day for at least 60 seconds	–	White
Desert Essence Tea Tree Oil Whitening Plus Mouthwash	–	22069BG	Desert Essence	Hauppauge, NY 11788. USA	8.39	Whole Foods	–	Purified water, glycerin, polysorbate-80, Phyllostachis Bambusoides Juice, Mentha Viridis Leaf oil, Equisetum Arvense Leaf extract, Gaultheria Procumbens Leaf oil, Eco-Harves Melaleuca Alternifolia leaf oil, Zinc Citrate, Calcium absorbate, ascorbic acid, hamamelis virginiana extract, Stevia Rebaudiana Leaf/stem power	–	whitening	Cosmetic	–	Tree Oil Whitening Plus Mouthwash	15 mL 30s	Adults and children 6 years and older: use as directed above. Children under 6 years.Keep out of reach	White
Jason Healthy powersmile brightening	Elab 2020	2113D1BL	The Hain Celestial Group, Inc	Lake Sucess, NY 11042. USA	7.99	Whole Foods	–	Water, glycerin, polysorbate 20, mentha piperita oil, aloe barbadensis leaf juice, calendula officinalis flower extract, carica papaya fruit extract, citrus grandis seed extract, echinacea angustifolia extract, hamamelis virginiana water, hydrastis canadensis extract, perilla ocymoides seed extract, ascorbic acid,	–	whitening	Cosmetic	Pos	Fresh breath mouthwash with calcium ascorbate	One part of mouthwash + one part of water before the use. 20-30s	Children under 2 years: consult s dentist	White

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Guru Nanda Oxyburst Whitening Technology	Jan-24	_0252224LL1	Guru Nanda LLC	Buena Park, CA 90620	3.69	Target	hydrogen peroxide, calcium lactate	Purified water, glycerin, xylitol, sorbitol, hydrogen peroxide, sodium benzoate, aloe vera, sodium bicarbonate, Peg-40 hydrogenated castor oil, sodium lauroyl sarcosinate, sodium chloride, peppermint oil, stevia, cardamom oil, clove oil, calcium lactate, vitamin E, Vitamin D, spearmint oil, fenne oil, tea tree oil, oregano oil, jasmine oil, menthol, citric acid	–	whitening	Cosmetic	–	100% pure essential oils combine hydrogen peroxide for safe and effective oral care	15 mL 60s	Keep out of reach of children under 12 years of age	White
Colgate Optic White High Impact White Advanced	Sep-23	2081US561F	Colgate Palmolive CO	New Orleans, LA 70129.USA	16.5	Krager	Hydrogen Peroxide	Water, Glycerin, Propylene Glycol, Sorbitol, Hydrogen Peroxide, Polysorbate 20, Sodium Acrylates/Methacryloylethyl Phosphate Copolymer, Phosphoric Acid, Citric Acid, Flavor, PVM/MA Copolymer, Sodium Saccharin.	–	Whitening	Cosmetic	Pos	Actively whitens & seals out new stains Enamel Safe/Freshens breath	15 mL 60 s	> 12 years of age	White
Family Wellness Blue mint Mouthwash	–	202375A 14:35	Midwood Brands, LLC	Chesapeake, VA 23320. Canada	1	Family Dollar	Cetylpyridinium Chloride	Water, alcohol (12.25 wt%), glycerin and/or sorbitol, polysorbate 80 and/or polysorbate 20, flavor, sodium saccharin, sodium benzoate, cetylpiridinium chloride, benzoic acid, blue 1	12.25%		Cosmetic	Pos	Helps kill germs that cause bad breath freshens breath	–	–	Blue
Listerin edition coconut & lime blend	Elab 2021	00722CA	Johnson & Johnson Consumer INC	Canada	6.47	Walmart	–	Water, sorbitol, propylene glycol, poloxamer 407, lauramidopropyl betaine, eucalyptol, benzoic acid, sodium benzoate, flavor, methyl salicylate, thymol, sucralose, menthol, sodium saccharin, yellow 10, green 3	–		Cosmetic	Pos	Botanically inspired flavor bend neutralizes bad breath germs	30 s rinse with 20 mL, twice a day	Dont use children < 12 years of age	light green
Crest Scope Classic Classique	–	21305395UA	Procter & Gamble	Cincinnati, OH 4502. USA	5.49	Kroger	Cetylpyridinium Chloride	Water/EAU, alcohol (15wt%), glycerin, flavor, polysorbate 80, sodium saccharin, sodium benzoate, cetylpyridinium chloride, benzoic acid, blue 1. yellow 5	Alcohol 15% wt%		cosmetic	Pos	Mouthwash rince-bouche	30 s rinse with 30 mL, twice a day	6-12 year supervise use. > 12 years withouth supervise	Green
Mint dry mouth oral rinse	Jun-25	574783	Procter & Gamble	Cincinnati, OH 4502. USA	3.79	Kroger		Water, glycerin, xylitol, sorbitol, propylene glycol, poloxamer 407, sodium benzoate, hydroxyethylcellulose, hydroxiaceatophenone, 1,2-hexanediol, caprylyl glycol, flavor, sodium phosphate, disodium phosphate	–		Cosmetic	No information	Scoothes and hydrates dry mouth	30s rinse with 15 mL, <5 times per day	Dont use children < 12 years of age	White
Oral B Breath Purify	–	1228C793A1	Procter & Gamble	Cincinnati, OH 4502. USA	7.99	Walgreens	sodium chlorite	Water, Peg-40, hydrogenated castor oil, sodium chlorite, tetrasodium EDTA, sodium benzoate , sodium bicarbonate, flavor, Illicium Verum (Anise) fruit/seed oil, glycerin, sodium carbonate	–		Cosmetic	Pre	Special Care Oral Rinse	15 mL 60s before eating, twice a day	Dont use children < 12 years of age	White
ARC fresh breath mouth rinse	Nov-23	136151782A 03:44	Procter & Gamble	Cincinnati, OH 4502. USA	6.99	Target	Cetylpyridinium Chloride, zinc lactate	Water, glycerin, flavor, cetylpiridinium chloride, zinc lactate, methylparabe, sodium saccharin, sucralose, propylparabem, poloxamer 407	–		Cosmetic	Pos	Fights Bad breath	30 s rinse with 20 mL, twice a day	Dont use children < 6 years of age	Bottle is black with bright pink
Mouthwash up&up	–	568103	Target Corp	MN 55403. USA	1.79	Target	Cetylpyridinium Chloride	Water, alcohol 15 wt%, glycerin, flavor, polysorbate 80, sodium saccharin, sodium benzoate, cetylpiridinium chloride, benzoic acid. blue 1. yellow 5	Alcohol 15% wt%		cosmetic	Pos	Compare to Scope Original mint	30 s rinse with 20 mL, twice a day	Children 6 years supervised use.	Green
Thera Breath dentist formulated fresh breath oral rinse	Jun-25	CM21740510	Dr. Harold Katz. LLC	Los Angeles, CA. 90038. USA	8.99	Kroger	sodium chlorite	Water, glycerin, PEG-40, hydrogenated caster oil, citric acid, sodium hydroxide, pepper mint oil,menthol, sodium chlorite, citrus limon peel oil, sodium benzoate, sucralose, xylitol, sodium bicarbonate.	–		Cosmetic	–	Fresh breath	1 min with one capful. Gargle with another capful for 30 s to clean your	Keep out of reach of children	Blue

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Thera Breath Dentist formulated fresh breath	May-25	PA2125012	Dr. Harold Katz. LLC	Los Angeles, CA. 90038. USA	7.69	Target	sodium chlorite	Water, Peg-40, hydrogenated castor oil, sodium chlorite, tetrasodium EDTA, sodium benzoate , sodium bicarbonate, mentha piperita (peppermint) oil, sodium hydroxide			Cosmetic		Fights bad breath for 24 hours	1 min with one capful. Gargle with another capful for 30 s to clean your throat. Twice a day. Wait 5 minutes before ating	Keep out of reach of children	Green
Smart mouth Original	May-26	11053122	Triumph pharmaceuticals inc.	St Louis, Missouri 63141.USA	10.99	Kroger	sodium chlorite, zinc chloride	Solution 1: Purified water, sodium benzoate, sodium chlorite and benzoic acid. Solution 2: Purified water, glycerin, poloxamer 407, propylene glycol, poloxamer 124, zinc chloride, flavor, sodium benzoate, benzoic acid, sodium saccharin, sodium chloride, benzyl alcohol, D&C yellow № 10, FD&C blue №1	–		Cosmetic	–	All day confidence	30s rinse with 20 mL, <5 times per day	Adults and children 12 years and older: use as directed above. Children 6 years to under 12: supervised use. Children under 6 years. Do not use.	Blue an Green
Sea salt oral rinse	Mar-25	322072	CVS Pharmacy, Inc	Woonsocket, RI 02895 USA	12.99	CVS		Water, sea salt, xylitol, natural flavor, lysozyme, menthol, potassium sorbate, sodium benzoate, poloxamer 407	–		Cosmetic	–	Promotes good oral hygiene	20 mL aprox 30 s pre-brushing		White
Tom`s Natural Fluoride-free Wicked fresh	Feb-24	2056US561F	Tom's of Maine, Inc	Kennebunk, ME 04043. USA	3.98	Walmart	zinc citrate	Water, glycerin, sorbitol, Aloe barbadensis leaf juice (organic), propanediol, cylitol, natural flavor, benzoic acid, zinc citrate, menthol, sodium hydroxide	–		Cosmetic	–	Clinically proven fresh breath long-lasting alcohol free	1 min. Any time of day	–	White
Hello Activated Charcoal	Feb-24	2038US3010	Hello Products LLC	Montclair, NJ 07042. USA	5.99	Target		Puridied water, vegetable glycerin (soother and moisturizes), polysorbate 80 (emulsifier), flavor (yum), xylitol (aweetener), erythritol (sweetener), poloxamer 407 (emulsifier), charcoal powder (freshens breath), cocos nucifera (coconut) oil (soothes and moisturizes), sodium benzoate (maintains stability), melaleuca alternifolia (tea tree) leaf oil (freshens breath)	–		Cosmetic	–	Epic freshening, rocks tastebuds, moisturizes mouth	30 s rinse with 20 mL	–	Black
Swan Alcohol free Mouthwash	–	551687	Vi-Jon, Inc	St Louis, Missouri 63114.USA	1	Dollar General	–	Water, sorbitol, propylene glycol, poloxamer 407, flavor, benzoic acid, sodium benzoate, sodium saccharin, blue 1	–		Cosmetic	–	Refreshing mouth rinse, hepls mouth fell fresh and clean	30 ml (1FL OZ) 30 s	Do not use with patients on mechanical ventilators or patients suffering from cystic fibrosis	Blue
PerCara Mouthwash & Gargle Refresh mint	–	2206683F	Podium Brands	Winter Park, FL. Canada.	1	Dollar General	cetylpiridinium chloride	Water, alcohol, sorbitol, polysorbate 20, flavor, cetylpiridinium chloride, sodium saccharine, sodium benzoate, benzoic acid, blue 1, yellow 5	Has, but does not describe the amount		Cosmetic	Pre/Pos brushing	After meals and before social engagements	30 ml 30s	Keep out of reach of children. Children under 6 years. Do not use.	Green
Lavoris Freash Breath Mouthwash	–	2217448	Podium Brands	Winter Park, FL. Canada.	1	Dollar General	zinc chloride	Water, alcohol, glycerin/sorbitol, flavor, poloxamer 407, polysorbate 20, dosium saccharin, zinc chloride, citric acid, blue 1	Has, but does not describe the amount		Cosmetic	–	Clinically proven fresh breath with natural peppermint, top rated, great tasting, long lasting	30 s rinse with 20 mL, twice a day	Keep out of reach of children	Blue

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Spry Oral Rinse (Dental defense system)	Mar-25	22073A	Xlear Inc.	American Fork UT 84003. USA	7.49	Fresh Thyme	–	Purified Water, Xylitol, grain alcohol (ethanol), coolmint flavor (blend of natural flavors), vegetable glycerin, calcium glycerophosphate, aloe vera, Marigold, Chamomile, Echinacea, Olive Leaf, Thyme	Has, but does not describe the amount		Cosmetic	Pos	Dental defense system	60 s with a half capful	–	White
Perio Brite Complete Oral Care Natural Mouthwash	Feb-26	215055	Nature's Answer	NY 11788. USA	10.29	Fresh Thyme	–	Water, glycerin, xylitol, Bio-Saponns (Yucca schidigera Root extract, quillaja saponaria Root extract, Dioscorea villosa (Wild Yam) Tuber Extract, Smilax aristolochiifolia Root Extract. Mentha piperita (peppermint) oil, Calendula officinalis flower extract, echinacea purpurea flower/leaf/Steam Extract, Chamomilla recutita (Matricaria), flower extract, Phytoplenolin (Centipeda cunninghamii extract), Centella asiatica Etract (Gotu Kola), Origanum vulgare Leaf oil (oregano), zanthoxylum americanum bark extract (Prickly Ash Bark), Lavandula angustifolia (Lavender) oil, Thymol, Eugenia caryophyllus (clove) bud oil, Folic Acid, Olea Europaea (Olive) Leaf extract, thymus vulgaris (Thymel) flower/Leaf oil, Eucalyptus globulus Leaf Oil, Cinnamomum zeylanicum Bark Oil, Citrus Paradisi (Grapefruit) Seed Extract, Juglans nigra (Black Walnut) Shell Extract, Ubiquinone (CoQ10), Camellia Sinensis Leaf Extract (Green Tea), Rosmarinus Officinalis (Rosemary) Leaf Oil, Aloe barbadensis Leaf juice (Aloe Vera), Citrus	–		Cosmetic	Pos	Natural Mouthwash	2 capful 30-60s twice a day	Do not use if you are sensitive or allergic to any ingredient	Brown
Bubble Gum Kid's Spry Mouth wash	Jul-24	22016B	Xclear	American Fork, UT 84003	7.49	Fresh Thyme	Calcium Glycerophosphate	Purified Water, xylitol, vegetable glycerin, erythritol, Echinacea Purpurea, Chamomile, Olive Leaf, Marigold, Thyme, Oat Beta Glucan, Aloe Vera, Calcium Glycerophosphate, Cocamidopropyl betaine, Honeysuckle, Natural flavors, Color Stabilizer, Natural Plant Coloring	–		Cosmetic	Pos	Spry alcohol-free Mouth Wash rinses the hard to reach places, ensuring your child's mouth is fresh and clean withouth the unconfortable burn	half capful, 60 s twice a day	–	Pink
HPM Hydrogen Peroxide Mouthwash	Apr-24	1070219	Seychelles Organics, Inc	Bowling Green, FL 33834. USA	8.99	Fresh Thyme	Hydrogen peroxide	Water, hydrogen Peroxide, alcohol, Thymus sephyllum (White thyme) Leaf Oil, Eucalyptol, Menthol, Natural Wintergreen flavor	Has, but does not describe the amount		Cosmetic	Pos	Natural flavors	1 min 10 mL/ 4 times daily (no more than 7 days)	Adults and children 12 years and older: use as directed above. Children under 12: supervised use. Children under 2 years. Do not use.	Brown
BR rinse. Organic Mouthwash	Elab 02/2021	DA29	Raw Essentials Living Foods, LLC	San Rafael, CA. USA	6.49	Fresh Thyme	hydrogen peroxide	Aloe leaf juice, purified water, hydrogen peroxide, acacia, wintergreen oil, xanthan gum, peppermint oil, rosemary leaf oil, eucalyptus leaf oil, lemon peel oil, cinnamon leaf oil (organic ingredient) Aloe leaf juice, purified water, hydrogen	–		Cosmetic	PRE	Wither teeth, fresher breath	Mouthful for 60 s	Children under 12 years. Consult a doctor or dentist	White

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
BR rinse. Organic Mouthwash Peppermint	Elab 02/2022	DG66	Raw Essentials Living Foods, LLC	San Rafael, CA. USA	14.29	Whole Foods	hydrogen peroxide	Aloe leaf juice, purified water, hydrogen peroxide, acacia, peppermint oil, rosemary leaf oil, eucalyptus leaf oil, lemon peel oil, clove bud oil (eugenia caryophyllus), cinnamon leaf oil (organic ingredient)	–		Cosmetic	PRE	Wither teeth, fresher breath, Kill bad	Mouthful for 60 s	Children under 12 years. Consult a doctor or dentist	White
Tea Tree therapy Mouth Wash	Mar-25	798	Tea Tree therapy, Inc	Ventura, CA 93003	11.99	Fresh Thyme	–	Deionized Water, sorbitol, tea tree oil, (oil of Melaleuca alternifolia) Natural Mint flavor, citric acid, sodium citrate	–		Cosmetic	Pos	Natural fresh flavor	Rinse for 30 s	Children under 3 years. Do not use.	White
CloSYS	Nov-24	1308A 22-11103 08:35	Rowpar Pharmaceuticals, Inc	Scittsdale, AZ 85260. USA	14.99	CVS	stabilized chloride dioxide	Purified Water, Cloralstan (stabilized chloride dioxide), trisodium phosphate, citric acid, flavor, sucralose	–		Cosmetic	–	Sensitive Rinse	20 mL , 30-45s Try no eat or drink for 20 min	Safe 2+ years old with supervision of adult	White
Desert Essence Tea Tree OilSpearmint	–	21163AG	Desert Essence	Hauppauge, NY 11788. USA	8.39	Whole Foods	–	Water, glycerin, polysorbate 80, Eco-Harvest Melaleuca Alternifolia leaf oil, Aloe barbadensis Leaf, juice, Mentha Viridis leaf oil, Hamamelis Virginiana extract, Ascorbic acid, calcium ascorbate, citric acid	–		Cosmetic	–	Spearmint	15 mL 30s	Adults and children 6 years and older: use as directed above. Children under 6 years.Keep out of reach	White
Jason Halthy mouth Tartar Control cinnamon clove	Elab 2020	2070D1BL	The Hain Celestial Group, Inc	Lake Sucess, NY 11042. USA	15.98	Whole Foods	–	Water, glycerin, polysorbate 20, cinnamon zeylanicum bark oil, eugenia caryophyllus flower oil, melaleuca alternifolia leaf oil, melia azadirachta seed oil, Aloe barbadensis leaf juice, calendula officinalis flower extract, citrus grandis seed extract, echinacea purpurea extract, hamamelis virginiana water, hydrastis canadensis extract, melissa officinalis leaf extract, perilla ocymoides seed extract, ascorbic acid, calcium ascorbate, menthol, sodium bicarboante, sea salt, cinnamal. eugenol	–	antitartar	Cosmetic	Pos	Fresh breath mouthwash with tea tree oil	One part of mouthwash + one part of water before the use. 20-30s	Children under 2 years: consult s dentist	White
Jason Halthy mouth Fresh breath cinnamon	Elab 2022	2159F1BL	The Hain Celestial Group, Inc	Lake Sucess, NY 11042. USA	7.99	Whole Foods	–	Water, glycerin, cinnamomum zeylanicum leaf oil, mentha piperita oil, citric acid, polysorbate 20, benzyl alcohol, potassium sorbate, sodium benzoate	–		Cosmetic	Pos	Fresh breath mouthwash cinnamon	One part of mouthwash + one part of water before the use. 20-30s	Children under 2 years: consult s dentist	White
Tom´s Sea Salt	Feb-24	2052US561F	Tom's of Maine, Inc	Kennebunk, ME 04043. USA	5.79	Whole Foods	zinc citrate	Water, glycerin, sorbitol, Aloe barbadensis leaf juice, propanediol, xylitol, sodium chloride, aroma/flavor (natural), zinc citrate, benzoic acid, menthol, sodium hydroxide	–		Cosmetic	Pos	Natural, fluride free, refreshing mint	60 s. Any times	–	White
Dr. Tichenor's All natural Peppermint Mouthwash Concentrate	–	C13HKK	DR. G.H. Tichenor Antiseptic Company	New Orleans, LA 70129.USA	8.79	Walgreens	–	Alcohol (70%), peppermint oil, arnica, sacccharum carbonate, USP purified water	Alcohol 70%		Cosmetic	–	Kills bad breath germs long lasting & refreshing	Dilute with 5 parts water and use as needed to provide a long lasting minty breath refreshment	–	Caramel
Crest Scope mouthwash rince-bouche	–	13515395UA	Procter & Gamble	Toronto, ON M5W 1C5 DSP IA 10. Canada	5	Family Dollar	Cetylpyridinium Chloride	Water, alcohol 13wt%, glycerin, flavor, polysorbate 80, sodium saccharin, sodium enzoate, cetylpiridinium Chloride, Benzoic Acid, blue 1 F.C.F, yellow 5/tartrazine	13%%		Cosmetic	–	Outlast up to une haleine fraiche 5x longer fresh feeling breath plus long temps	20 mL 30 s twice a day	Keep out of reach of children	Green

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Swan Mouthwash fresh mint	–	553112	Vi-Jon, Inc	St. Louis, MO 63114. USA	3.35	Family Dollar	Cetylpyridinium Chloride	Water, alcohol 15 wt%, glycerin, flavor, polysorbate 80, sodium saccharin, sodium benzoate, cetylpiridinium chloride, benzoic acid, blue 1, yellow 5	15.00%		Cosmetic	–	Compare ingredients of Scope	21 mL 30 s	Keep out of reach of children	Green
Close.UP Mouthwash with Calcium cinnamon	Jun-23	2116515CC1	Ranir, LLC	MI 49512. USA	1	Family Dollar	Cetylpyridinium Chloride, zinc gluconate, calcium lactate	Purified water, glycerin, poloxamer 407, sodium benzoate, xylitol, flavor, sodium saccharin, menthol, cetylpiridinium chloride, zinc gluconate, citric acid, calcium lactate, D&C red 33, FD&C Red 40	–		Cosmetic	–	With Calcium	20 mL 30 s	Keep out of reach of children	Red
Jason Total Protection sea salt mouthrinse	–	2040H1BL	The Hain Celestial Group, Inc	Lake Sucess, NY 11042. USA	7.99	Whole Foods	–	Water, sea salt, glycerin, sorbitol, mentha piperita oil, camellia sinensis leaf extract, citrus grandis seed extract, hamamelis virginiana extract, salvia officinalis leaf extract, ascorbic acid, citric acid, mentho, polysorbate 20, sodium bicarbonate, benzoic acid, potassium sorbate, sodium benzoate	–		Cosmetic	Pos	Fresh breath & complete oral Hygiene Cool mint	20-30s twice a day	Children under 2 years: consult s dentist	White
The Natural Dentist Healthy gums	Mar-24	_0742	Recive Personal Products Company	Madison, NJ 07940. USA	6.78	Walmart	Aloe Vera (20%)	Purified Wate, Vegetable Glycerin, Echinacea, Goldenseal, Calendula, Citric Acid, Polysorbate 80, Natural Flavors (contains cinnamon oil) Grapefruit seed extract, potassium citrate, copper chlorophyllin Color	–	Antiplaque Antigingivitis	Therapeuthic	–	20 % Aloe vera	30 s rinse with 20 mL, twice a day	Adults and children 12 years and older: use as directed above. Children 6 years to under 12: supervised use. Children under 6 years. Consult a doctor or	White
Crest Pro-Health Bacteria Guard	Apr-24	21995395RB	Procter & Gamble	Cincinnati, OH 4502. USA	5.79	Kroger	Cetylpyridinium Chloride 0.05%	Water, alcohol (15wt%), propylene glycol, flavor, poloxamer 407, sucralose, benzoic acid, sodium benzoate, blue 1, yellow 6	Alcohol 15% wt%	Antiplaque Antigingivitis	Therapeuthic	Pos	Kills 99% of bacteria	30 s rinse with 20 mL, twice a day	6-12 year supervise use. > 12 years withouth supervise	Blue
Crest Pro-Health Intense	Mar-24	21715395RD	Procter & Gamble	Cincinnati, OH 4502. USA	4.97	Walmart	Cetylpyridinium Chloride 0.05%	Water, alcohol (15wt%), propylene glycol, flavor, poloxamer 407, sucralose, benzoic acid, sodium benzoate, blue 1, yellow 6	Alcohol 15% wt%	Antiplaque Antigingivitis	Therapeuthic	Pos	99% Germ Kill	30 s rinse with 20 mL, twice a day	Dont use children < 6 years of age	Blue/green
Thera breath periodontist formulated Healthy gums oral rinse	Elab 2022	PERLB00075R11	Dr. Harold Katz. LLC	Los Angeles, CA. 90038. USA	10.29	CVS	Cetylpyridinium Chloride 0.05%	Water, glycerin, poloxamer 407, flavor, sucralose		Antiplaque Antigingivitis	Therapeuthic		Fights gingivitis for 24 hours	30 s rinse with 20 mL, twice a day	Children 6 years to under 12: supervised use. Children under 6 years. Do not use.	Blue
Smarth mouth Clinical zinc activated oral rinse	Apr-25	A11040822	Triumph pharmaceuticals inc.	St Louis, Missouri 63141.USA	13.99	CVS	Cetylpyridinium Chloride 0.05%	Solution 1: Purified water, sodium benzoate, sodium chlorite and benzoic acid. Solution 2: Purified water, glycerin, poloxamer 407, propylene glycol, poloxamer 124, zinc chloride, flavor, sodium benzoate, benzoic acid, sodium saccharin, sodium chloride, benzyl alcohol, D&C yellow № 10, FD&C blue №1	–	Antiplaque Antigingivitis	Therapeuthic	–	Bad breath prevention with a morning and evening rinse	30s rinse with 20 mL, <5 times per day	Adults and children 12 years and older: use as directed above. Children 6 years to under 12: supervised use. Children under 6 years. Do not use. Adults and	Blue and Green

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Cepacol ntibacterial multi - protection mouthwash	Jul-23	KT1189841173 7	Reckitt Benckiser	Parsippany, NJ 07054-0224. USA	6.99	Walgreens	Cetylpyridinium Chloride 0.05%	Purified water, alcohol 14% v/v, glycerin sodium phosphate dibasic, eucalyptus oil, polysorbate 80, methyl salicylate, cinnamon oil, peppermint oil, saccharin sodium, sodium phosphate monobasic anhydrous, menthol, edetate disodium , FD&C yellow #5	Alcohol 14%	Antiplaque Antigingivitis	Therapeuthic	Pos	Reduces plaque, freshens breath	20 mL 30 s twice a day	Adults and children 12 years and older: use as directed above. Children 6 years to under 12: supervised use. Children under 6 years. Do not use.	Yellow
Crest Pro-Health Clean Mint multiprotection	Apr-25	21985395RA	Procter & Gamble	Cincinnati, OH 4502. USA	6.99	Kroger	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, poloxamer 407, sodium saccharin, methylparaben, sucralose, propylparaben, blue 1	–	Antiplaque Antigingivitis	Therapeuthic	Pos	Reduce Plaque Bacteria, Helps prevent Gngivitis, freshens breath	30 s rinse with 20 mL, twice a day	6-12 year supervise use. > 12 years withouth supervise	Blue
Crest GUM and Breath Purify	Jul-23	13055395RD	Procter & Gamble	Cincinnati, OH 4502. USA	6.99	Kroger	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, zinc lactate, methylparaben, sodium saccharin, sucralose, prpylparaben, poloxamer 407, blue1	–	Antiplaque Antigingivitis	Therapeuthic	Pos	Reduces Gum Swelling & Redness, Protects Agains gingivitis at the gum line	30 s rinse with 20 mL, twice a day	6-12 year supervise use. > 12 years withouth supervise	Blue
Parodontax active gum health Mint	Apr-24	567777	GSK consumer healthcare	Warren, NJ, 07059. Canada	3.79	CVS	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, poloxamer 188, sodium saccharin, propylene glycol, sodium benzoate, sucralose, benzoic acid	Alcohol	Antiplaque Antigingivitis	Therapeuthic	Pos	Kills 99% of plaque bacteria	30 s rinse with 20 mL, twice a day	Children 6 years to under 12: supervised use. Children under 6 years. Do not use.	Blue
Parodontax active gum health Clear mint	Dec-23	550645	GSK consumer healthcare	Warren, NJ, 07059. Canada	7.59	CVS	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, poloxamer 188, sodium saccharin, propylene glycol, sodium benzoate, sucralose, benzoic acid	–	Antiplaque Antigingivitis	Therapeuthic	Pos	Targets bacteria & forms an antibaterial shield	30 s rinse with 20 mL, twice a day	Children 6 years to under 12: supervised use. Children under 6 years. Do not use.	White
Multi-action alcohol free. Antiseptic mouth rinse	Jun-24	_0569886	Target Corp	Mpls., MN 55403. USA	3.69	Target	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, poloxamer 188, sodium saccharin, propylene glycol, sodium benzoate, sucralose, benzoic acid, blue 1	–	Antiplaque Antigingivitis	Therapeuthic	Pos	Compare to active plaque and gingivitis helps reove germs that cause bad breath	20 mL 30 s twice a day	Keep out of reach of children under 6 years of age	Blue
Oral B Gum Detoxify	Feb-24	20675151782A 15:11	Procter & Gamble	Cincinnati, OH 4502. USA	8.99	Kroger	Cetylpyridinium Chloride 0.07%, zinc lactate	Flavor, glycerin, methylparaben, poloxamer 407, propylparaben, sodium saccharin, sucralose, water, zinc lactate	–	Antiplaque Antigingivitis	Therapeuthic	Pos	Special care oral rinse, 24-hour gingivitis protection	30 s rinse with 20 mL, twice a day	Keep out of reach of children under 6 years of age	White
Crest all fresh no stress Scope All day	Nov-23	20555395UA	Procter & Gamble	Cincinnati, OH 4502. USA	5.65	Family Dollar	Cetylpyridinium Chloride 0.07%, zinc lactate	Water, glycerin, flavor, zinc lactate, methylparaben, sodium saccharin, sucralose, propylparaben, poloxamer 407	–	Antiplaque Antigingivitis	Therapeuthic	Pos	24 Hr breath protection Kill 99% of bad breath bacteria	20 mL 30 s twice a day	Keep out of reach of children under 6 years of age	White
Colgate Total Whole Mouth Health	Jan-24	2165US561F	Colgate Palmolive CO	New York NY 10022. USA	4.99	Kroger	Cetylpyridinium Chloride 0.075%	Water, glycerin, propylene glycol, sorbitol, poloxamer 407, flavor, potassium sorbate, citric acid, sodium saccharine, blue 1	–	Antiplaque Antigingivitis	Therapeuthic	Pos	12 hr protection	30 s rinse with 20 mL, twice a day	6-12 year supervise use. > 12 years withouth supervise Adults and	Blue

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Hello Peace out, plaque	Jun-24	2158US3014	Hello Products LLC	Montclair, NJ 07042. USA	4.99	Target	Cetylpyridinium Chloride 0.075%	Water, glycerin, aloe barbadensis leaf juice, polysorbate 80, erythritol, xylitol, poloxamer 407, flavor, cocos nucifera (coconut) oil (certified organic), sodium benzoate, citric acid, rebaudiside A (certified organic), melaleuca aiternifoil (tea tree) leaf oil	–	Antiplaque Antigingivitis	Therapeuthic	–	Promotes healthy gums, freshens like crazy, no alcohol, no brainer	30 s rinse with 20 mL, twice a day	Adults and children 12 years and older: use as directed above. Children 6 years to under 12: supervised use. Children under 6 years.	White
Crest Pro-Health Clinical	Aug-23	13135395R0	Procter & Gamble	Cincinnati, OH 4502. USA	8.99	CVS	Cetylpyridinium Chloride 0.1%, Hydrogen peroxide	Water, glycerin, Hydrogen peroxide, flavor, sucralose, poloxamer 407	–	Antiplaque Antigingivitis	Therapeuthic	Pos	Deep cleant mint	30 s rinse with 20 mL, twice a day	Do not use. Keep out of reach of children under 12 years of age	White
Listerine Sensitivity Zero alcohol	Feb-24	07622CC	Johnson & Johnson Consumer INC	Made in Canada	7.99	CVS	dipotassium oxalate monohydrate	Water, sorbitol, propylene glycol, dipotassium oxalate monohydrate, flavor, phosphoric acid, ploxamer, sodium benzoate, sodium methyl cocoyl taurate, sodium laurylsulfate, sodium saccharin, sucralose	–	Sensitivity	Therapeuthic	Pos	For 24-hour relief (with conitnued use) from painful tooth sensitivity	10 mL, 60 s, twice daily	Dont use <18 years of age	White
Listerine Freshburst	Mar-24	0972LZ	Johnson & Johnson Consumer INC	Skilman NJ 08558. USA	6.49	Kroger	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (21.6% v/v), sorbitol, poloxamer 407, benzoic acid, sodium saccharin, falvor, sodium benzoate, Yellow 10, green 3	21.6% v/v	Antiplaque Antigingivitis	Therapeuthic	No information	For a fresher & Cleaner Mouth than Brushing Alone	30 s rinse with 20 mL, twice a day	Dont use children < 12 years of age	Green
Listerine Original	Apr-24	1452LZ	Johnson & Johnson Consumer INC	Skilman NJ 08558. USA	6.49	Kroger	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (26.9 % v/v), poloxamer 407, benzoic acid sodium benzoate, Caramel	Alcohol 26.9 % v/v	Antiplaque Antigingivitis	Therapeuthic	No information	For a fresher & Cleaner Mouth than Brushing Alone	30 s rinse with 20 mL, twice a day	Dont use children < 12 years of age	Caramel
Antiseptic Mouthwash antigingivits antiplaque	May-24	569175	Target Corp	MN 55403. USA	3.69	Target	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (26.9 % v/v), poloxamer 407, benzoic acid sodium benzoate, Caramel	Alcohol 26.9 % v/v	Antiplaque Antigingivitis	Therapeuthic	No information	Compare to Listerine Antiseptic Original	30 s rinse with 20 mL, twice a day	Keep out of reach of children	Caramel
Antiseptic Mouthwash antigingivits antiplaque	Feb-24	560307	CVS Pharmacy, Inc	Woonsocket, RI 02895 USA	2.99	CVS	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (21.6% v/v), sorbitol, flavor, poloxamer 407, benzoic acid, sodium saccharin, sodium benzoate, FD&C green nº3	21.6% v/v	Antiplaque Antigingivitis	Therapeuthic	Pos	Compare to the active ingredients in listerine Cool mint Antiseptic	30 s rinse with 20 mL, twice a day	Childer under 12 years of age consult a dentist or doctor	Blue
Antiseptic Mouthwash up &up	Jul-24	574928	Target Corp	Mpls., MN 55403. USA	3.69	Target	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, alcohol 21.6%, sorbitol solution, flavor, poloxamer 407, benzoic acid, sodium saccharin, sodium citrate, D&C yellon nº 10, FD&C green nº 3	21.6% v/v	Antiplaque Antigingivitis	Therapeuthic	–	Compare to Listerine Antiseptic Fresh Burst	30 s rinse with 20 mL	Adults and children 12 years and older: use as directed above. Children under 12 years.	Green
Swan Antiseptic Mouth rinse Spring Mint	Apr-24	565177	Vi-Jon, Inc	Smyrnam TN 37167. USA	3.35	Family Dollar	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (21.6% v/v), sorbitol, flavor, poloxamer 407, benzoic acid, sodium saccharin, sodium citrate, D&C yellow nº 10, FD&C green nº3	Alcohol 21.6%	Antiplaque Antigingivitis	Therapeuthic	Pos	Compare to acctive ingredients in FreshBurst Listerine	20 mL 30 s twice a day	Consult a doctor or Children under 12 years. Consult a doctor or dentist	Green

Name	Exp Date	Lot	Manufacturer	City and Country of manufacture	Price	Shop place	Ingredients		Alcohol	Purpouse	smetic/ Therapeu	Pre/Pos brushing	Aditional information	Recomendation 1	Recomendation 2	Color
							Active	Other								
Advanced Mouth Rinse	Apr-24	_0565166	Walgreen Co	Deerfield, IL 60015. USA	3.79	Walgreens	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%), zinc chloride	Water, Alcohol (21.6% v/v), sorbitol, flavor, poloxamer 407, benzoic acid, zinc chloride, sodium benzoate, sucralose, sodium saccharin, green nº3	Alcohol 21.6%	Antiplaque Antigingivitis	Therapeuthic	Pos	Premium tartar control antiseptic	20 mL 30 s twice a day	Keep out of reach of children	Blue
Listerine Ultra Clean Zero alcohol	Elab 2019	1102LZ	Johnson & Johnson Consumer INC	Skilman NJ 08558. USA	10.49	CVS	Eucaliptol, Menthol, Methyl Salicylate, Thymol	Water, sorbitol, propylene glycol, poloxamer 407, flavor, eucaliptol, zinc chloride,benzoic acid,sodium benzoate, lauramidoprpyl betaine, methyl salicylate. Thymol, menthol, sodium saccharin, sucralose,blue 1, red 33, green 3	_	antitartar	Cosmetic	Pos	For up to a 3x longer lasting clean feeling vs brushing alone	>12 yeras of age: 30 s rinse with 20 mL, twice a day. Children 6-11 years: 10 mL 30 s	Dont use children <6 years of age	Light blue
Colgate Peroxyl	Jun-24	2175US561F	Colgate Palmolive CO	New York NY 10022. USA	10.99	Kroger	Hydrogen peroxide 1.5% /W/v)	Water, sorbitol, propylene glycol, poloxamer 338, polysorbate 20, flavor, sodium saccharin, FD&C blue no.1	_	Oral debriding agent/oral wound cleanser	Therapeuthic	No information	Mouth sore rinse	1 min 10 mL/ 4 times daily (no more than 7 days)	Children under 2 year of age_ consult a dentist or physician. Children under 12 years should	Blue
Oral B Mouth Sore	Feb-24	208351782A 05:11	Procter & Gamble	Cincinnati, OH 4502. USA	7.99	Walgreens	Hydrogen peroxide 1.5% /W/v), sodium hexametaphosphtate	Citric acid, flavor, glycerin, poloxamer 407, propylene glycol, sodium citrate, sodium hexametaphosphtate, sodium saccharin, sucralose, water	_	Oral debriding agent/oral wound cleanser	Therapeuthic	Pos	Special Care Oral Rinse	20 mL 60s 4 tomes daily	Do not use this product for more than 7 days. Children 6 years to under 12: supervised use. Children under 6 years. Do not use.	White
Orajel 2x Mouth Sores Rinse Medicated	Jun-24	KK216801	Church & Dwight Co., Inc	Ewing, NJ 08628	1.79	Target	Hydrogen peroxide 1.5% Menthol 1%	Alcohol 4.1% (by volume), disodium EDTA, FD&C blue nº1, methyl salicylate, phosphoric acid, poloxamer 338, polysorbate 20, sodium saccharine, sorbitol, water	4.10%	Oral debriding agent/oral antiseptic/ Oral pain reliever	Therapeuthic	_	Efective Pain Relief	1 min 10 mL/ 4 times daily (no more than 7 days)	Adults and children 12 years and older: use as directed above. Children under 12: supervised use. Children under 2 years. Do not use.	White
Smart mouth Mouth Sore zinc activatted oral rinse	Mar-24	M11030422	Triumph pharmaceuticals inc.	St Louis, Missouri 63141.USA	9.84	Walmart	Menthol 0.2% (When mixed directed), sodium chlorite, zinc chloride	Solution 1: Purified water, sodium benzoate, sodium chlorite and benzoic acid. Solution 2: Purified water, sorbitol, poloxamer 407, propylene glycol, poloxamer 124, zinc chloride, flavor, sodium benzyl alcohol, sodium saccharin, speppermint oil, benzoic acid, sodium chloride, , D&C yellow Nº 10, FD&C blue Nº1	_	Oral Pain Reliever	Therapeuthic	_		60s 20 mL, 4 times iin a day	Adults and children 12 years and older: use as directed above. Children 6 years to under 12: supervised use. Children under 6 years. Do not use	Blue and Green

2.2 Antimicrobial effect of fluoride-free mouthwashes on *Streptococcus mutans* biofilm

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Abstract

Objective: To evaluate the efficacy of commercially available, fluoride-free mouthwashes sold in Indianapolis, IN, on *Streptococcus mutans* biofilm.

Materials and methods: Eighty-one different mouthwashes were purchased in Indianapolis, IN. A culture of *S. mutans* UA159 was incubated with the mouthwashes in three dilutions (1:3, 1:6, and 1:12), prepared in Tryptic Soy broth supplemented with 1% sucrose. The minimum inhibitory concentrations (MIC), planktonic, and biofilm growth were evaluated. In addition, the growth for minimum bactericidal concentration (MBC) was evaluated using 5 μL and incubated in blood agar. For the analysis of the results, the mouthwashes were separated into 6 groups according to their active ingredients (cetylpyridinium chloride/CPC, n=25; essential oils/EOs, n=10; whitening/W, n=12; Plant extracts, n=15; zinc chloride/ZC, n=3; others/O, n=16). ANOVA following of Tukey test was performed (p=0.05).

Results: Regarding MIC, planktonic, and biofilm growth of *S. mutans*, there was a significant decrease for W and CPC groups. For the EOs group, all products of the W groups had more inhibition in the *S. mutans* biofilm compared to the CPC group. For ZC, PE, and O there were different effects within the same group, presenting a large variability.

Conclusion: The mouthwashes demonstrated some effect on S. *mutans* biofilm, especially in the 1:3 dilution, and W and CPC groups have a more significant effect on *S. mutans* biofilm.

Clinical relevance: *S. mutans* is an important bacterium in dental caries and periodontal diseases. Our study showed that non-fluoridated mouthwashes have an effect in the initial stages of the formation of biofilm.

Keywords: Mouthwash, *S. mutans,* Dental caries, Biofilm, antibacterial

Introduction

Mouthrinses are widely used as an additional oral hygiene substance, and there are several products available on the worldwide market (Radzki et al., 2022). While mouthrinses are a heavily utilized oral care vehicle with over 120 million mouthwash users in the United States. These can be classified based on their application, therapeutics, or cosmetic products. Therapeutic mouthwashes are available both over the counter and by prescription, depending on the formulation, and may help control biofilm, gingivitis, bad breath, dental caries (ADA, 2021), and dental erosion (Abdelwahed et al., 2019). Cosmetic products have an objective intended to cleanse, beautify, promote attractiveness, or alter the appearance without the presence of drugs that have therapeutic purposes. Furthermore, some products may not clearly fall under one definition or the other. Therefore, another consideration in classifying a product is the ‘‘intended use’’ of the product, which is largely dependent on the claims made for the product and the accompanying labeling.

The most of product have as objective the control of dental plaque para la prevention and treatment of caries and periodontal diseases. However, some products may not clearly fall under one definition or the other. Many types of mouthrinse active ingredients have been evaluated for their plaque-reducing effectiveness and ability to reduce *Streptococcus mutans*, including chlorhexidine, essential oils, triclosan, cetylpyridinium chloride, sodium dodecyl sulphate, and various metal ions (tin, zinc, copper) (Subramaniam and Nandan, 2011). However, the evidence supporting the effectiveness of antiplaque agents in preventing dental caries, except for chlorhexidine, is very limited (Zero 2006).

Chlorhexidine is the only ingredient that does not present controversy in its effect (Zero 2006); it is considered the gold standard antimicrobial, maximizing its efficacy and reducing its adverse effects when its properties and limitations are known. (Jones 2000). Essential oils and Cetylpyridinium chloride mouthwashes are the next extensively studied; however, these present some controversies in their uses (Stoeken et al., 2007; Haps et al., 2008; Val Leeuwen et al., 2011). These and other ingredients have the ability to reduce the accumulation of dental biofilm, supported by available scientific evidence (Gunsolley, 2006; 2010). Mouthwashes can suppress or reduce bacterial load; however, these are intended to suppress bacterial adhesion during the initial stages of dental biofilm formation and not for mature biofilms (Takenaka et al., 2022).
In a dental biofilm, *Streptococcus mutans* is a fundamental bacterium in dental caries and periodontal disease. The effectiveness of mouthwashes with antimicrobial potential on this bacterium is more evident in the primary stages (Dong et al., 2012; Batra et al., 2022; Takenaka et al., 2022). Some have membrane disruption as a mechanism of action (Jones, 1997), while

others have membrane disruption and the inactivation of essential enzymes (Stoeken et al., 2007), disrupting the transport of nutrients across the cell wall (Paraskevas et al., 2008) among other effects. All having as objective a bactericidal effect.

In the market, there is a high quantity of types of mouthwash products, with different activities and combinations of ingredients, which increases year after year. As these are products for daily use within reach of the majority of the population, with indications in many cases of plaque control, it is necessary to assess these on *S. mutans*. For this reason, the aim was to evaluate the efficacy of commercially available, fluoride-free mouthwashes sold in Indianapolis, IN, on initial *Streptococcus mutans biofilm*.

Material and Methods:

Experimental design

An in vitro study was performed using eighty-one different types of fluoride-free mouthwash sold in Indianapolis, IN, USA (Appendix 1). A 24-h culture of *S. mutans* UA159 in microtiter plates was treated with the mouthwashes in three different dilutions (1:3, 1:6, and 1:12), prepared in Tryptic Soy broth supplemented with 1% sucrose (TSBS). The minimum inhibitory concentrations (MIC), planktonic, and biofilm growth were evaluated using a spectrophotometer. In addition, the growth for minimum bactericidal concentration (MBC) was evaluated using 5 μL of each culture incubated for 48 h in blood agar. For the analysis of the results, the mouthwashes were separated into 6 groups according to their active ingredients. Additional Confocal laser scanning microscopy was performed for appreciate the different effects.

Preparation of the samples

Eighty-one different mouthwashes were purchased in Indianapolis, Indiana, USA. Three different serial dilutions (1:3, 1:6, and 1:12) in tryptic soy broth supplemented with 1% sucrose (TSBS). Two controls were prepared, of negative control was only with TSBS, and the positive control was 0.12% chlorhexidine (Chlorhexidine digluconate solution, 20% water, Sigma) prepared in TSBS.

MIC, MBC, Planktonic, and Biofilm

A 16 h culture of *S. mutans* UA159 (ATCC 700610) was grown in TSB at 37 °C in 5% CO_2. The samples were prepared in quadruplicate and repeated three different times. An aliquot of 10 μL of a 16 h culture of *S. mutans* in TSB was added to 190 μL of each mouthwash dilution and incubated at 37 °C, 5% CO_2 for 24 h in sterile 96-well flat-bottom microtiter plates (Fisher Scientific, Newark, DE, USA). The MIC was determined by the concentration where there was an obvious clear-cut decrease in the absorbance. The optical density (OD) values of the bacterial cultures were measured at 595 nm (no shake) in a spectrophotometer (SpectraMax 190; Molecular Devices, Sunnyvale, CA, USA). After the MIC determination, 5 μL of each dilution of the different mouthwashes was added to a blood agar plate and incubated for 48h at 37 °C, 5% CO_2 for the MBC determination. Using the rest of the sample, the unbound planktonic cells (120 μL) were aspirated and transferred to a new 96-well plate. The OD at 595 nm (shake) was determined to calculate the effect on planktonic cells. The remaining planktonic cells were removed from the biofilm microtiter plate wells (leaving attached biofilm), and 200 μL of 10% formaldehyde was added to each well for 30min to fix the cells. After 30min, the formaldehyde was removed, and the biofilm cells were washed three times with deionized water. Two hundred μL of 0.5% crystal violet dye was added to each well, and the cells were stained for 30 min. The wells were rinsed three times, and 200 μL of 2-isopropanol was placed into each well for 1 h to lyse the cells and extract the crystal violet. The plates were read in a spectrophotometer at 490 nm (shake) to measure biofilm formation (Huang and Gregory, 2012).

Confocal laser scanning microscopy:

A 16 h culture of *S. mutans* UA159 was grown in TSB at 37 °C in 5% CO_2. Were selected one mouthwash with a low effect and 1 with a higher effect for the analysis. The samples were prepared in quadruplicate and repeated three different times. Ten μL of a 16 h culture of *S. mutans* in TSB was added to 190 μL of each mouthwash dilution and incubated at 37 °C, 5% CO_2 for 24 h in sterile 96-well flat-bottom microtiter plates. The contents were aspirated and pipetted into a microcentrifuge tube. The samples were centrifuged for 5 min at 13000 *g*. The supernatant was discarded, and the precipitate was added to 1.5 mL of deionized water, being shaken until the sample was homogenized. Were added 3 μL of nucleic acid stain (Banas et al., 2007; Molecular Probes Inc.), and then incubated in the dark for 10 min. Five μL of each sample was added to a glass plate to be observed under the Confocal laser scanning microscopy to 40x.

Statistical Analysis:

Each experiment was repeated three times, and the mouthwashes were separated into 6 groups according to their active ingredients (cetylpyridinium chloride/CPC, n=25; essential oils/EOs, n=10; whitening/W (hydrogen peroxide or sodium hexametaphosphate), n=12; Plants extracts /PE, n=15; zinc chloride/ZC, n=3; and others/O, n=16). For MIC, planktonic and biofilm statistical analyses were performed, and for MBC, a descriptive analysis was performed, classifying the results in <1:3, <1:6, <1:12, or >1:12. Two-way ANOVA was performed to compare the effects of mouthwash exposure (each dilution individually) and their interaction on MIC, planktonic, and biofilm. Were compared different dilutions with the negative control. Also, was performed a comparison of these six groups to each other.

Results:

All Eighty-one different brands of mouthwashes were the validity period declared by the manufacturer. The active ingredients found were CPC (n=25); EOs (n=10); W (n=12), ZC (n=3), PE (n=15); and OR (n=16). The CPC concentrations ranged from 0.05% - 0.1% (9 products did not state concentration). About the EOs, only one of the 10 products did not declare the concentration; the other 9 were described on the packaging (Eucalyptol; 0.092%, Menthol 0.042%, Methyl Salicylate 0.060%, and Thymol 0.064%) and this contained alcohol in their composition. For the ZC, it does not describe the concentration. About PE, a great variety was found, and those classified as others had sea salt, charcoal powder, and sodium bicarbonate, among others. Of all the rinses, 32.1% contained alcohol in concentrations of 4.1 - 26.9%, and four products said they contained alcohol but did not declare the concentration. The different purposes found were Antiplaque/antigingivitic (33.3%), products for bad breath (24.7%), whitening (9.9%), and others for freshness, oral care, brushing, and dry mouth, among others. About the type of the product, 63% are sold as cosmetic products, and only 8.6% (n=7) describe being Pre-brushing.

For the MIC (Table 1A) analysis, the group in decreasing order in the first 1:3 dilution, the one that presented a greater effect than the rest of the groups was the W group ($0.0084 \pm 0.0129_{ABS}$) The W group presented statistical differences with all the other groups (p-value <0.001 – 0.0196). The second group with the best effect was CPC ($0.0455 \pm 0.0625_{ABS}$). The CPC group presented a difference from all the groups except the ZC group (p-value = 0.6481). The next group was EOs ($0.0637 \pm 0.0829_{ABS}$). The EOs group presented a statistical difference with all except group O (p value=0.3728). In the PE, ZC and O groups presented a lower effect (0.1101 ± 0.1056; 0.1073 ± 0.1516; $0.10504 \pm 0.1076_{ABS}$, respectively). For the OI group, no

statistical difference was found with the O group (p value=0.3728). And for ZC only, there was no difference with the CPC group. The negative control presented a low effect of 0.5384 ± 0.0518 (p-value <0.0001). For planktonic analysis (Table 1B), values between 0.0231-0.2841_{ABS} were found; 0.0669-0.2293_{ABS}; 0.0743 – 0.1972_{ABS} for 1:3, 1:6, and 1:12 dilution respectively.

For Biofilm results (Table 1C), the best result in the 1:3 dilution was for the W group with 0.0194± 0.0040ABS; only it did not present a statistical difference with the ZC group (p-value = 0.8833). The CPC group with 0.0447 ± 0.0140ABS alone did not present a statistical difference with ZC (p value=0.5449) and EOs group (p value=0.9580). The EOS group (0.0638 ± 0.0090 $_{ABS}$) did not present a statistical difference with the ZC group (p value=0.2756) and with the CPC group. The PE group presented a statistical difference with ZC (p value=0.0144) and O (p value = 0.0670) groups. The O group and the Negative control presented statistical differences with all the other groups and between them.

In the evaluation of the reduction percentage in MIC analysis (Fig 1) of the treatments when compared with the negative control, numerically, the W group presents a greater reduction (98.2% ± 1.9). The second-best result was for the CPC group (90.1% ± 12.9), followed by the EOS group (86.2% ± 8.8). Finally, there is O, ZC and PE group (77.1 ± 23.3; 76.7% ± 38.8; 76.2% ± 20.4, respectively) Regarding the percentage of reduction on *S. mutans* biofilms (Fig 2), the highest percentage is from the W group (97.5 % ± 2.4), following for the CPC group (94.2% ± 10.9), EOS group (91.8% ± 11.01) and finally are, O (76.8% ± 25.4), PE (74.8% ± 22.1), and ZC (71.0.% ± 50.1) group.

The Results of Two-way ANOVA statistical analyses performed to compare the effects of mouthwash exposure (between the dilution of each mouthwash) and their interaction on MIC, planktonic, and biofilm, and MBC and percentage of reduction of values compared with the negative control, and the statistical difference in the first dilution (1:3) of each mouthwash with other for the MIC and biofilm analysis are in appendix 2 and 3.

Table 1: MIC (1A), planktonic (1B), and biofilm (1C) statistical analyses compared different each group with other and negative control.

A

MIC																		
	1 : 3							1 : 6							1 : 12						
Group	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others
Negative Control	0.5384 ± 0.0518	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5384 ± 0.0518	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5384 ± 0.0518	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CPC	0.0455 ± 0.0625	-	<.0001	<.0001	<.0001	0.6481	<.0001	0.0815 ± 0.0833	-	<.0001	<.0001	<.0001	0.021	<.0001	0.0736 ± 0.0700	-	<.0001	<.0001	<.0001	0.0042	<.0001
Essential Oils	0.0637 ± 0.0829	-	-	<.0001	0.0023	0.0107	0.3728	0.2530 ± 0.0860	-	-	<.0001	<.0001	0.0001	0.0354	0.3904 ± 0.0517	-	-	<.0001	<.0001	<.0001	<.0001
Whitening	0.0082 ± 0.0129	-	-	-	<.0001	0.0196	<.0001	0.0101 ± 0.0114	-	-	-	<.0001	<.0001	<.0001	0.0123 ± 0.0124	-	-	-	<.0001	<.0001	<.0001
Organic ingredients	0.1101 ± 0.1056	-	-	-	-	0.0196	0.3728	0.1824 ± 0.1194	-	-	-	-	0.0037	0.234	0.2492 ± 0.1198	-	-	-	-	0.0022	0.1143
Zinc Chloride	0.1073 ± 0.1516	-	-	-	-	-	0.0316	0.1278 ± 0.1661	-	-	-	-	-	0.0001	0.1558 ± 0.1460	-	-	-	-	-	0.0042
Others	0.1054 ± 0.1076	-	-	-	-	-	-	0.2043 ± 0.1589	-	-	-	-	-	-	0.2579 ± 0.1558	-	-	-	-	-	-

B

Planktonic																					
	1 : 3							1 : 6							1 : 12						
Group	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others
Negative Control	0.1296 ± 0.0750	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1296 ± 0.0750	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1296 ± 0.0750	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CPC	0.2841 ± 0.5149	-	0.0005	<.0001	<.0001	<.0001	<.0001	0.2293 ± 0.4795	-	0.0004	<.0001	<.0001	0.0007	0.0037	0.1972 ± 0.4543	-	0.0012	<.0001	<.0001	0.0061	0.0005
Essential Oils	0.0731 ± 0.2550	-	-	<.0001	<.0001	<.0001	<.0001	0.1505 ± 0.3433	-	-	<.0001	<.0001	<.0001	<.0001	0.1544 ± 0.4101	-	-	<.0001	<.0001	<.0001	<.0001
Whitening	0.2103 ± 0.4921	-	-	-	0.9649	0.7305	0.0024	0.1299 ± 0.3867	-	-	-	<.0001	0.038	<.0001	0.1666 ± 0.4411	-	-	-	0.0005	0.0017	<.0001
Organic ingredients	0.0997 ± 0.3214	-	-	-	-	0.6042	0.0004	0.1210 ± 0.3686	-	-	-	-	0.5192	0.001	0.0743 ± 0.2870	-	-	-	-	0.5845	<.0001
Zinc Chloride	0.0231 ± 0.0644	-	-	-	-	-	0.1013	0.0669 ± 0.1887	-	-	-	-	-	0.015	0.1722 ± 0.5262	-	-	-	-	-	0.0285
Others	0.1306 ± 0.3920	-	-	-	-	-	-	0.1541 ± 0.4218	-	-	-	-	-	-	0.0800 ± 0.2594	-	-	-	-	-	-

C

Biofilm																					
	1 : 3							1 : 6							1 : 12						
Group	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others	Mean; SD	CPC	Essential Oils	Whitening	Plant Extracts	Zinc Chloride	Others
Negative Control	0.7994 ± 0.1849	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7994 ± 0.1849	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7994 ± 0.1849	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CPC	0.0447 ± 0.0140	-	0.958	0.01	<.0001	0.5449	<.0001	0.0727 ± 0.1482	-	<.0001	<.0001	<.0001	0.0912	<.0001	0.0698 ± 0.1726	-	<.0001	<.0001	<.0001	<.0001	<.0001
Essential Oils	0.0638 ± 0.0090	-	-	0.031	<.0001	0.2756	<.0001	0.4053 ± 0.2165	-	-	<.0001	0.2606	0.0006	0.1041	0.6756 ± 0.2643	-	-	<.0001	<.0001	<.0001	<.0001
Whitening	0.0194 ± 0.0040	-	-	-	<.0001	0.8833	<.0001	0.0162 ± 0.0214	-	-	-	<.0001	0.383	<.0001	0.0263 ± 0.0508	-	-	-	<.0001	<.0001	<.0001
Organic ingredients	0.1953 ± 0.1190	-	-	-	-	0.0144	0.067	0.3576 ± 0.2352	-	-	-	-	0.0015	0.4537	0.4995 ± 0.2685	-	-	-	-	0.0014	0.1237
Zinc Chloride	0.2253 ± 0.000	-	-	-	-	-	0.067	0.2492 ± 0.3571	-	-	-	-	-	0.0183	0.3004 ± 0.3013	-	-	-	-	-	0.0708
Others	0.1795 ± 0.2029	-	-	-	-	-	-	0.3531 ± 0.3106	-	-	-	-	-	-	0.4699 ± 0.3447	-	-	-	-	-	-

Table 2: Minimum Bactericidal Concentration (MBC) of all products from each of the 6 groups (CPC: cetylpyridinium chloride; EOs: essential oils; W: whitening; PE: plant extract; ZC: zinc chloride; and O: Others).

CPC		EOs		W		OI		ZC		O	
Code	MBC	Code	MBC	Code	MBC	Code	MBC	Code	MBC	Code	MBC
R	>1:12	BM	<1:3	BJ	>1:12	AW	<1:3	CA	>1:12	BF	>1:12
E	>1:12	O	<1:3	BD	>1:12	BT	>1:12	BZ	>1:12	H	<1:3
G	>1:12	BP	<1:3	BE	>1:12	U	>1:12	AE	<1:3	BL	>1:12
V	>1:12	I	<1:3	BH	>1:12	AU	<1:6			K	>1:12
Y	>1:12	BQ	<1:6	AY	>1:12	M	<1:6			AZ	<1:3
F	>1:12	AB	<1:3	C	>1:12	Q	<1:3			BK	<1:3
CB	>1:12	AI	<1:3	BU	>1:12	AM	<1:3			BG	>1:12
CC	>1:12	AL	<1:3	BR	>1:12	AK	<1:3			J	<1:6
BS	>1:12	BO	<1:3	BC	>1:12	T	<1:3			AG	>1:12
AR	>1:12	AD	<1:3	X	>1:12	AX	<1:3			AA	<1:3
AC	>1:12			D	>1:12	BA	<1:3			L	<1:6
AQ	>1:12			AJ	>1:12	AT	<1:3			W	<1:3
BV	>1:12					AV	<1:3			BB	<1:3
BI	>1:12					BY	<1:3			S	<1:3
Z	>1:12					BW	<1:3			BX	<1:3
A	>1:12									B	<1:3
P	>1:12										
AN	>1:12										
AO	>1:12										
N	>1:12										
AP	>1:12										
BN	>1:12										
AS	>1:12										
AH	>1:12										
AF	<1:3										

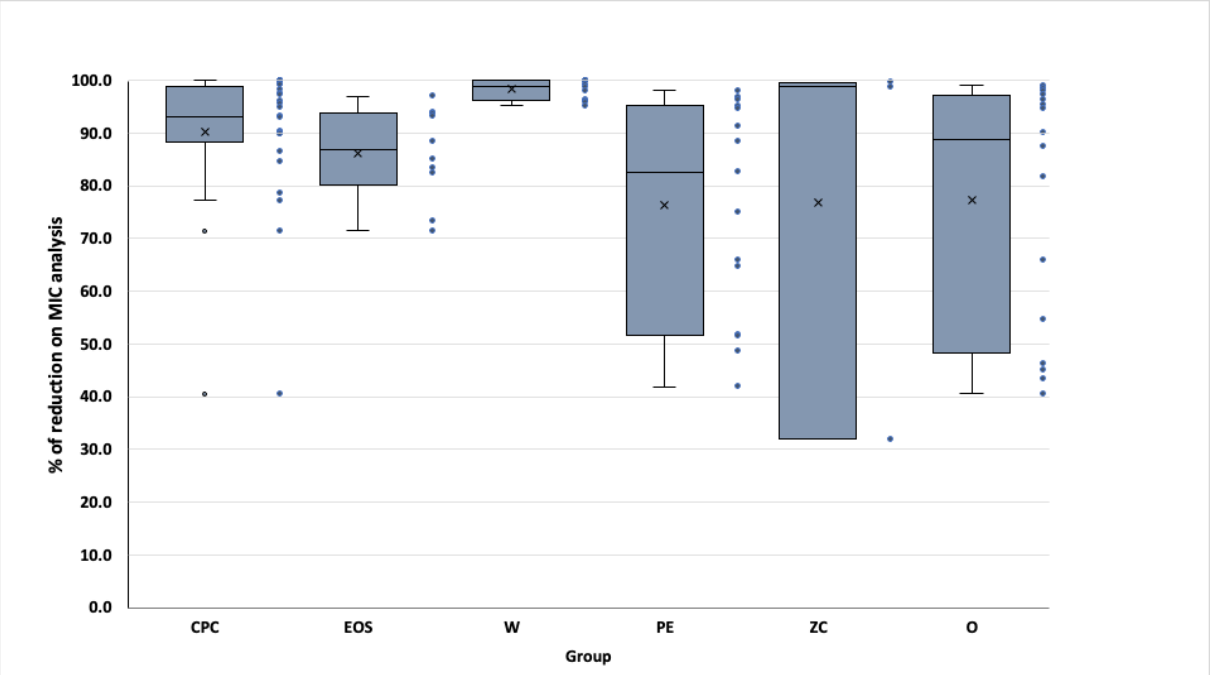

Figure 1: Percentage (%) of reduction on MIC analysis of the different groups of treatments in de minimum inhibitory concentrations (MIC).

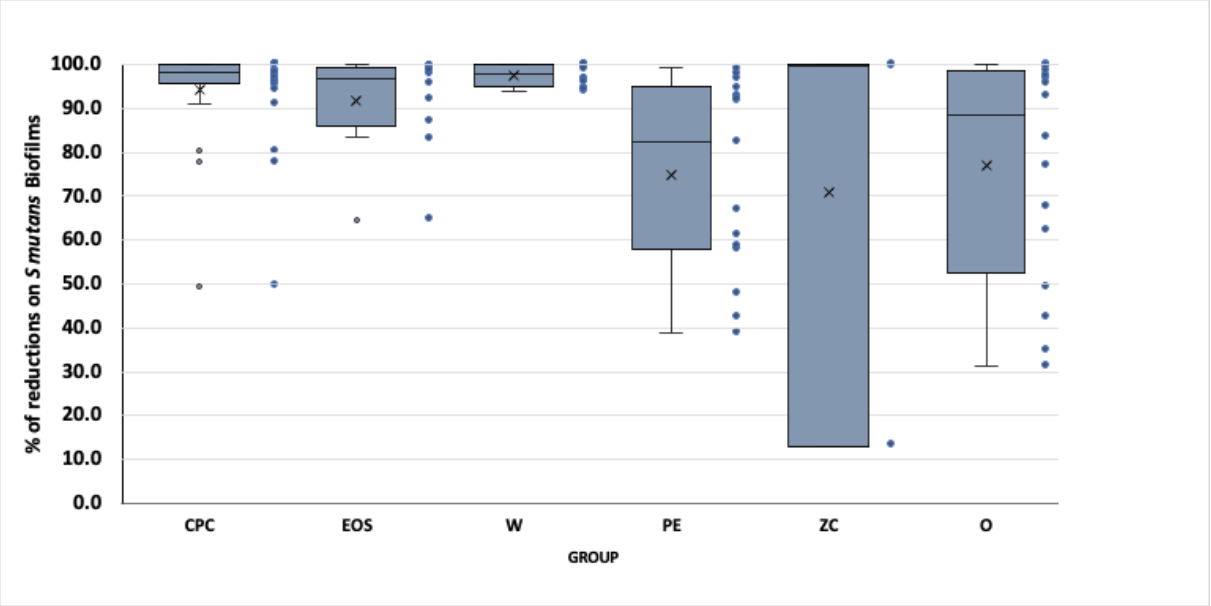

Figure 2: Percentage (%) of reduction on *S. mutans* biofilms of the different groups of treatments in de minimum inhibitory concentrations (MIC).

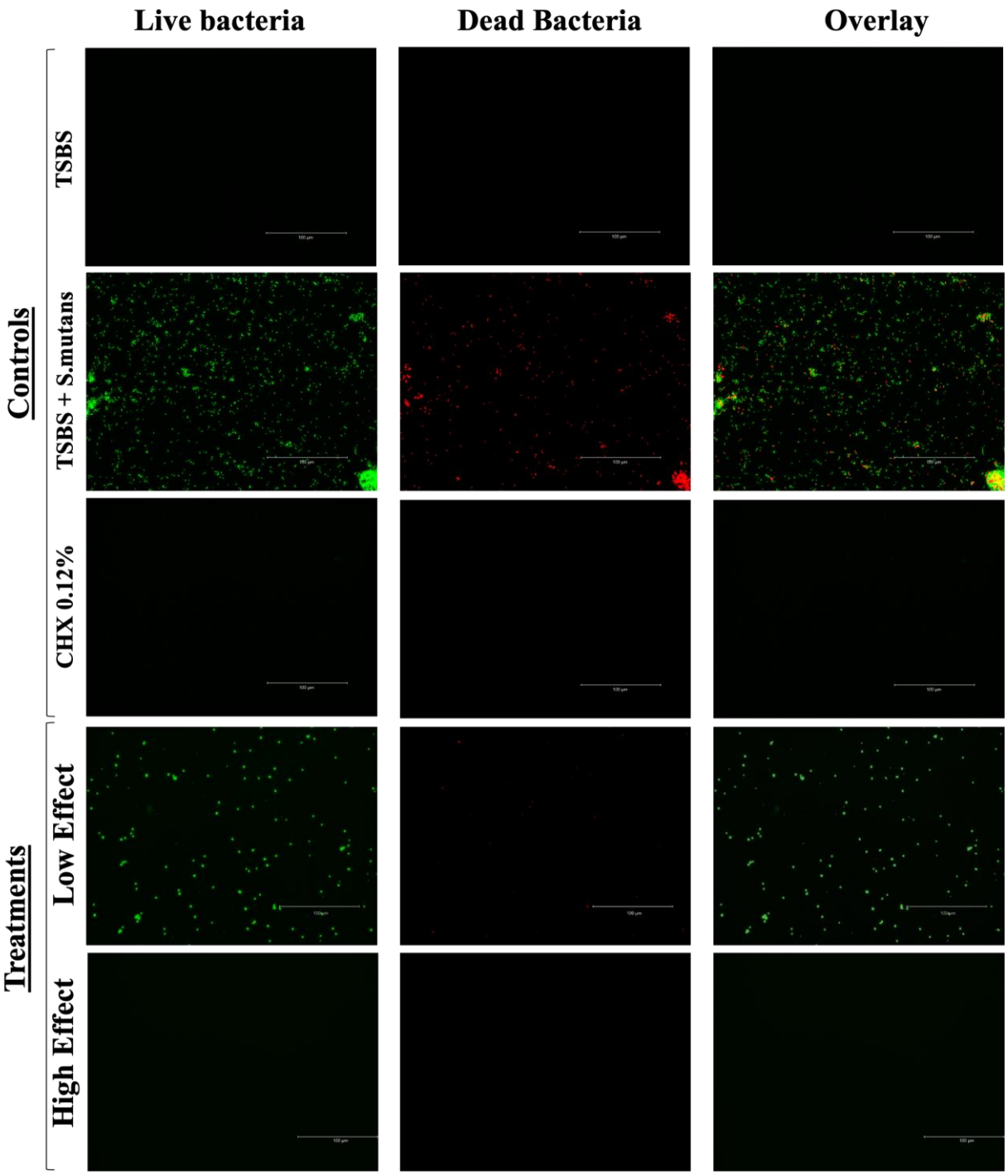

Figure 3: Confocal laser scanning microscopy for comparison between controls (only medium; TSBS, negative control; medium + bacteria; TSBS + *S. mutans*, positive control; CHX 0.12%) and treatments (one with low and one with high effect)

Discussion:

There is a wide variety of non-fluoride mouthwashes on the market, which contain different active ingredients that have specific objectives, among which are to reduce the number of bacteria in the mouth, antigingivitic/antiplaque, whitening, sensitivity, etc., and many indicate that they have some antimicrobial effect. Our study evaluated all non-fluoride mouthwashes found in Indianapolis, IN. With the aim of testing its effectiveness on initial *S. mutans* biofilm. considered as normal flora species in the oral cavity but fundamentally in dental caries and periodontal disease. The effectiveness of different mouthwashes with antimicrobial potential on *S. mutans* has been found to be clearer in immature biofilms or in the first stages of formation (Dong et al., 2012; Batra et al., 2022; Takenaka et al., 2022).

It is known that Chlorhexidine continues to be the gold standard as an antimicrobial, it is effective in different bacteria, and it is a cationic biguanide with broad antibacterial activity, low mammalian toxicity, and a strong affinity for binding to skin and mucous membranes and has a wide spectrum of activity encompassing gram-positive and gram-negative bacteria (Denton, 1991). Its antimicrobial activity is of the membrane-active type, used to describe an antimicrobial agent that damages the inner (cytoplasmic). As we can observe in figure 1, chlorhexidine at 0.12% does not allow the growth of *S. mutans* biofilm. This agent is antimicrobial, and it is the only one that does not present any controversy with its use (Zero 2006), as long as one considers how to maximize the effect and reduce the adverse effects (Jones, 2000).

Of the commercial products analyzed, those that presented hydrogen peroxide and sodium hexametaphosphate were grouped as the W group. It presented the best result for both MIC (1:3 dilution $0.0084 \pm 0.0129_{ABS}$) and biofilm (1:3 dilution $0.0194 \pm 0.0040_{ABS}$), with a statistically significant difference from all other groups, being corroborated with the MBC tests (>1:12). It is proven that hydrogen peroxide (H_2O_2) has an antimicrobial effect on Gram-positive and Gram-negative bacteria (Brown et al., 1947). Several factors are necessary for the antimicrobial effect of H_2O_2 to occur. Concentration and length of exposure are the most critical factors. To maximize the effect and reduce side effects, it is appropriate to use concentrations ≤1.5% (Hossainian et al., 2011). Thus, of the 10 products that claimed to contain H_2O_2, only 3 reported the concentration (1.5%), and even so, all had a greater effect on *S. mutans* than the other products (p-value <0.001 – 0.0196). However, when compared with 0.12% Chlorhexidine digluconate, it was much less effective in reducing incomplete. For the other ingredient the sodium, hexametaphosphate could increase outer membrane cell permeability (Shibata and Morioka 1982), and in concentrations of 1%, it would have a considerable effect on *S. mutans*

biofilm (Hosida et al., 2021). Although the 3 products with this compound have a high effect, their concentration is not declared on the product label.

The products that contain CPC present a small but significant additional benefit in reducing to reduce the accumulation of dental biofilm (Haps, et al., 2008). This is produced for the quaternary compound of CPC that inhibits the forming of biofilm in the first stages (Schroeder et al, 1962). Also, CPC is a cationic surface-active agent and has a broad antimicrobial spectrum, with the rapid killing of gram-positive pathogens (Pitten and Kramer, 2001). This is consistent with the results found in our studio (1:3 dilution, MIC: $0.0455 \pm 0.0625_{ABS}$; Biofilm: $0.0447 \pm 0.0140_{ABS}$). Formulations with high bioavailable CPC are associated with greater biological activity and therefore suggest an increased probability of clinical efficiency (Versteeg et al., 2010). *S. mutans*, being a gram-positive bacterium, is for this reduced in the presence of CPC products. Although there is a controversy that these tend to have an increased clinical effect when used as adjuncts to mechanical oral hygiene regimens (Barnett, 2003). However, in addition, or by itself, it is considered that CPC mouthwashes promote changes in oral microbial structure and/or reductions in community diversity that favor the resolution of dysbiosis and re-establishment of a health-compatible microbial community (do Amaral et al., 2022), being recognized and recommended by the ADA as effective in plaque and gingivitis (ADA, 2021). Researchers also, though, have found comparable performance between chlorhexidine and CPC as a preprocedural rinse in reducing bacterial load in aerosols. (Mauri, et al., 2019).

The products with EOs, (thymol, eucalyptol, menthol, methyl salicylate) presented a lower effect than W and CPC group for MIC ($0.0637 \pm 0.0829_{ABS}$) and less than W group and equal to CPC group and greater than the remainder of the other groups, for Biofilm analyzes ($0.0638 \pm 0.0090_{ABS}$). Its lower effectiveness when compared to the W and CPC group is corroborated by the MBC results where one product was <1:6 and the rest <1:3. There is strong evidence to support the efficacy of these agents as anti-plaque, anti-gingivitis agents (Gusolley 2010), provides an additional benefit about plaque and gingivitis reduction (Stoken et al., 2007). EOs are used in an over-the-counter mouthwash containing a fixed formula of 2-phenol related EOs, thymol 0.064% and eucalyptol 0.092%, mixed with menthol 0.042% and methyl salicylate 0.060% in alcohol vehicle (Fine et al., 1985). The mechanisms of action of EOs against bacteria are complex. At high concentrations, there is disruption of the cell wall and precipitation of cell proteins, while at lower concentrations, there is inactivation of essential enzymes (Ross et al., 1989). Bacteria are prevented from aggregating with Gram-positive bacteria, and bacterial multiplication is slow. (Fine, 1988; Ouhayoun, 2003).

The products with ZC as the main ingredient were only 3; thus, two presented a high effect in terms of MIC reduction (76.7% ± 38.8), and biofilm reduction (71.0.% ± 50.1), and one presented a low effect, so its SD is high. It is known that ZC is used as an antimicrobial since it has multiple inhibitory effects on the activities of intact bacterial cells, such as glycolysis and can improve the proton permeability of bacterial cell membranes (Phan et al., 2004). It is a concentration-dependent product, since in high concentrations it can inhibit the formation of biofilm and tartar, but in low concentrations, no effect has been found (Lynch 2011; Almoudi et al., 2018). The problem with this group may be that these products do not declare the concentration, and there may be variations within the group that are reflected in the results.
Regarding the PE and O group, the results are varied since the ingredients are not uniform (*Mentha piperita* oil, *Calendula officinalis* flower extract, *Echinacea purpurea* flower/leaf/Steam Extract, *Chamomilla recutita* flower extract, Phytoplenolin, among others). There are products with one plant extract (in addition to other ingredients such as solvents, dyes, detergents, etc.) as products that have more than 10. It is known that there are plants that have some antimicrobial effect, but having a large number of extracts, recognizing who is attributes the bactericidal/bacteriostatic effect is difficult. The same happens with the group of others where we have, Sodium Chloride, Sodium Bicarbonate, sodium lauryl sulfate, charcoal power, Sea Salt, among others.

These groups, without considering the case of the ZC group, present the highest SD for MIC (PE 77.1 ± 23.3 and 0 76.2% ± 20.4) and for Biofilm (PE 76.8% ± 25.4 and PE 74.8% ± 22.1). They also presented the lowest reduction when compared to the other groups of chemical agents. It was important to analyze these products due to the quantity and variety available in the market. Plant extracts represent 18.5% of all the products analyzed and 19.8%, the majority of which are marketed for presenting an anti-plaque effect, anti-gingivitis, bad odor, diseases caused by bacteria, among others.

For reasons of a clearer reading interpretation of the results, the information of all the mouthwashes separately is described in appendix 1, including the code of these to make the comparison between all the dilutions of each product and between all of them. When the 3 dilutions were compared with each other (1:3/1:6, 1:3/1:12 and 1:6/1:12), different results were observed. Furthermore, in general, the 1:3 dilution was more effective than the 1:6, and in some cases, 1:3 was equal to the 1:6 dilution, and in a few cases, the 3 dilutions had a similar effect.
For a better analysis of the results, in appendix 2 are the means: SD of each dilution and the p values of each comparison. Regarding the comparison between the 1:3 dilution of all the products, in appendix 3, we can find the values of the percentage of reduction of values

compared with the negative control and the statistical difference in each mouthwash with other for the MIC and biofilm analysis, describing the mouthwashes (code) with which they presented a statistical difference (p-value <0.05) here all the results are independent and the data extensive. So, each rinse presents a different behavior.

Mouthwashes are an element of individual oral hygiene. They do not need a dental or medical prescription; it is within reach of most of the population. Companies sell different products on the market, being able to find a large quantity at various points of commerce, many with the aim of reducing the bacterial load, reducing, or helping in the control of periodontal diseases.

Our results verified that mouthrinses are effective in the first stages of the biofilms formation of *S. mutans* bacteria. Being those that present whitening agents the ones that present the best results (MIC: 98.2% ± 1.9 of reduction), followed by those CPC (90.1% ± 12.9), EOs, (86.2% ± 8.8), and finally ZC, plant extracts and other ingredients. In addition to the first 4 are products that present more benefits than secondary effects since they are in an adequate concentration. This study is relevant because *S. mutans* is an important bacterium for dental caries and periodontal diseases, and if people maintain adequate biofilm control by avoiding tartar formation and using mouthwashes, these would reinforce biofilm control (Dong et al., 2012; Batra et al., 2022; Takenaka et al., 2022). Despite our study being in vitro and using only an initial mono-species biofilm, our results are promising and of clinical relevance. It also opens the possibility of carrying out *in situ/in vivo* tests.

Conclusions:

The mouthwashes demonstrated some effect on S. *mutans* biofilm, especially in the lowest dilution (1:3), and products containing Whitening agents (Hydrogen peroxide/ Sodium hexametaphosphate) and CPC have a more significant effect on *S. mutans* biofilm.

Conflict of interest:

The authors declare no conflict of interest.

References

1. Abdelwahed AG, Temirek MM, Hassan FM. Antierosive Effect of Topical Fluorides: A Systematic Review and Meta-Analysis of *In Situ* Studies. Open Access Maced J Med Sci. 2019 May 12;7(9):1523-1530. doi: 10.3889/oamjms.2019.291.

2. Almoudi MM, Hussein AS, Abu Hassan MI, Mohamad Zain N. A systematic review on antibacterial activity of zinc against *Streptococcus mutans*. Saudi Dent J. 2018 Oct;30(4):283-291. doi: 10.1016/j.sdentj.2018.06.003.
3. American Dental Association *(ADA). 2021. Department of Scientific Information, Evidence Synthesis & Translation Research, ADA Science & Research Institute, LLC.* https://www.ada.org/resources/research/science-and-research-institute/oral-health-topics/mouthrinse-mouthwash
4. Barnett ML. The role of therapeutic antimicrobial mouthrinses in clinical practice. Control of supragingival plaque and gingivitis. J Am Dent Assoc 2003; 134: 699–701.
5. Batra C, Alalshaikh M, Gregory RL, Windsor LJ, Blanchard SB, Hamada Y. An in vitro comparison of four antibacterial agents with and without nicotine and their effects on human gingival fibroblasts. J Periodontol. 2022 Feb;93(2):e24-e33. doi: 10.1002/JPER.21-0262.
6. Brown EA, Krabek W, Skiffington R. Glycerite of hydrogen peroxide: I. Comparison of its bacteriotoxic with that of mercurial solutions. J Bacteriol 1947; 53: 793–799.
7. Denton GW. Chlorhexidine. In: Block SS, ed. Disinfection, Sterilization and preservation. 4th edn. Philadelphia: Lea and Febiger, 1991: 274-289.
8. do Amaral GCLS, Hassan MA, Sloniak MC, Pannuti CM, Romito GA, Villar CC. Effects of antimicrobial mouthwashes on the human oral microbiome: Systematic review of controlled clinical trials. Int J Dent Hyg. 2023 Feb;21(1):128-140. doi: 10.1111/idh.12617.
9. Dong L, Tong Z, Linghu D, Lin Y, Tao R, Liu J, Tian Y, Ni L. Effects of sub-minimum inhibitory concentrations of antimicrobial agents on *Streptococcus mutans* biofilm formation. Int J Antimicrob Agents. 2012 May;39(5):390-5. doi: 10.1016/j.ijantimicag.2012.01.009.
10. Fine DH, Letizia J, Mandel ID. The effect of rinsing with Listerine antiseptic on the properties of developing dental plaque. J Clin Periodontol 1985;12:660-666.
11. Fine DH. Mouthrinses as adjuncts for plaque and gingivitis management. A status report for the American Journal of Dentistry. Am J Dent 1988;1:259-263.
12. Gunsolley, J.C. A Meta-Analysis of Six-Month Studies of Antiplaque and Antigingivitis Agents. J. Am. Dent. Assoc. 2006, 137, 1649–1657. [CrossRef]
13. Gunsolley, J.C. Clinical Efficacy of Antimicrobial Mouthrinses. J. Dent. 2010, 38 (Suppl. S1), S6–S10.
14. Haps, S.; Slot, D.E.; Berchier, C.E.; van der Weijden, G.A. The Effect of Cetylpyridinium Chloride-Containing Mouth Rinses as Adjuncts to Toothbrushing on Plaque and Parameters of Gingival Inflammation: A Systematic Review. Int. J. Dent. Hyg. 2008, 6, 290–303.
15. Hosida TY, Pessan JP, Cavazana TP, Sampaio C, Monteiro DR, Botazzo Delbem AC. Effect of sodium hexametaphosphate and fluoride on dual-species biofilms of *Candida albicans* and *Streptococcus mutans*. Biofouling. 2021 Oct-Nov;37(9-10):939-948. doi: 10.1080/08927014.2021.1916816.

16. Hossainian N, Slot DE, Afennich F, Van der Weijden GA. The effects of hydrogen peroxide mouthwashes on the prevention of plaque and gingival inflammation: a systematic review. Int J Dent Hyg. 2011 Aug;9(3):171-81. doi: 10.1111/j.1601-5037.2010.00492.x.
17. Huang R, Li M, Gregory RL. Effect of nicotine on growth and metabolism of *Streptococcus mutans*. Eur J Oral Sci. 2012;120(4):319–25.
18. Jones CG. Chlorhexidine: is it still the gold standard? Periodontol 2000. 1997 Oct;15:55-62. doi: 10.1111/j.1600-0757.1997.tb00105.x.
19. Lynch, R.J.M., 2011. Zinc in the mouth, its interactions with dental enamel and possible effects on caries; a review of the literature. Int. Dent. J. 61, 46–54.
20. Marui VC, Souto MLS, Rovai ES, et al. Efficacy of preprocedural mouthrinses in the reduction of microorganisms in aerosol: A systematic review. J Am Dent Assoc 2019;150(12):1015-26.e1.
21. Ouhayoun JP. Penetrating the plaque biofilm: Impact of essential oil mouthwash. J Clin Periodontol 2003; 30(Suppl. 5):10-12
22. Paraskevas, S.; Rosema, N.A.M.; Versteeg, P.; Van der Velden, U.; Van der Weijden, G.A. Chlorine Dioxide and Chlorhexidine Mouthrinses Compared in a 3-Day Plaque Accumulation Model. J. Periodontol. 2008, 79, 1395–1400.
23. Phan, T.-N., Buckner, T., Sheng, J., Baldeck, J.D., Marquis, R.E., 2004. Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. Oral Microbiol. Immunol. 19, 31–38.
24. Pitten FA, Kramer A. Efficacy of cetylpyridinium chloride used as oropharyngeal antiseptic. Arzneim Forsch/Drug Res 2001; 51: 588–595.
25. Radzki D, Wilhelm-Węglarz M, Pruska K, Kusiak A, Ordyniec-Kwaśnica I. A Fresh Look at Mouthwashes-What Is Inside and What Is It For? Int J Environ Res Public Health. 2022 Mar 25;19(7):3926. doi: 10.3390/ijerph19073926.
26. Ross NM, Charles CH, Dills SS. Long-term effects of Listerine antiseptic on dental plaque and gingivitis. J Clin Dent 1989;1:92-95.
27. Schroeder HE, Marthaler TM, Muhlemann HR. Effects of some potential inhibitors on early calculus formation. Helv Odont Acta 1962; 6: 6–9.
28. Shibata H, Morioka T. Antibacterial action of condensed phosphates on the bacterium *Streptococcus mutans* and experimental caries in the hamster. Arch Oral Biol. 1982;27(10):809-16. doi: 10.1016/0003-9969(82)90034-6.
29. Stoeken, J.E.; Paraskevas, S.; van der Weijden, G.A. The Long-Term Effect of a Mouthrinse Containing Essential Oils on Dental Plaque and Gingivitis: A Systematic Review. J. Periodontol. 2007, 78, 1218–1228.
30. Subramaniam P, Nandan N. Effect of xylitol, sodium fluoride and triclosan containing mouth rinse on *Streptococcus mutans*. Contemp Clin Dent. 2011;2(4):287.

31. Takenaka S, Sotozono M, Ohkura N, Noiri Y. Evidence on the Use of Mouthwash for the Control of Supragingival Biofilm and Its Potential Adverse Effects. Antibiotics (Basel). 2022 May 28;11(6):727. doi: 10.3390/antibiotics11060727.
32. van Leeuwen, M.P.; Slot, D.E.; van der Weijden, G.A. Essential Oils Compared to Chlorhexidine with Respect to Plaque and Parameters of Gingival Inflammation: A Systematic Review. J. Periodontol. 2011, 82, 174–194
33. Versteeg PA, Rosema NA, Hoenderdos NL, et al. The plaque inhibitory effect of a CPC mouthrinse in a 3-day plaque accumulation model – a cross-over study. Int J Dent Hyg 2010;8:269–75.
34. Zero DT. Dentifrices, mouthwashes, and remineralization/caries arrestment strategies. BMC Oral Health. 2006 Jun 15;6 Suppl 1(Suppl 1):S9. doi: 10.1186/1472-6831-6-S1-S9.

Appendix 1: Complete information of the samples, their ingredients and purpose.

Code	Name	Ingredients		Alcohol	Purpouse
		Active	Other		
A	Close.UP Mouthwash with Calcium cinnamon	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (21.6% v/v), sorbitol, poloxamer 407, benzoic acid, sodium saccharin, falvor, sodium benzoate, Yellow 10, green 3	21.6% v/v	Antiplaque Antigingivitis
B	Sea salt oral rinse	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (26.9 % v/v), poloxamer 407, benzoic acid sodium benzoate, Caramel	Alcohol 26.9 % v/v	Antiplaque Antigingivitis
C	Colgate Optic White High Impact White Advanced	–	Water, sorbitol, propylene glycol, poloxamer 407, lauramidopropyl betaine, eucalyptol, benzoic acid, sodium benzoate, flavor, methyl salicylate, thymol, sucralose, menthol, sodium saccharin, yellow 10, green 3	–	Bad Breath
D	Colgate Peroxyl	Eucaliptol, Menthol, Methyl Salicylate, Thymol	Water, sorbitol, propylene glycol, poloxamer 407, flavor, eucaliptol, zinc chloride,benzoic acid,sodium benzoate, lauramidoprpyl betaine, methyl salicylate. Thymol, menthol, sodium saccharin, sucralose,blue 1, red 33, green 3	–	Antiplaque Antigingivitis
E	Oral B Gum Detoxify	–	Water, sorbitol, propylene glycol, dipotassium oxalate monohydrate, flavor, phosphoric acid, ploxamer, sodium benzoate, sodium methyl cocoyl taurate, sodium laurylsulfate, sodium saccharin, sucralose	–	Sensibility
F	Crest Pro-Health Bacteria Guard	–	Water, sorbitol, alcohol (8.6%), tetrasodium pyrophosphate, sodium benzoate, benzoic acid, sodium lauryl sulfate, poloxamer 407, flavor, xanthan gum, sodium saccharin, blue 1, yellow 5	Alcohol 8.6 % v/v	Prebrushing
G	Crest Pro-Health Clinical	Cetylpyridinium Chloride 0.075%	Water, glycerin, propylene glycol, sorbitol, poloxamer 407, flavor, potassium sorbate, citric acid, sodium saccharine, blue 1	–	Antiplaque Antigingivitis
H	Ultimate Essential MouthCare. Eco dent	Hydrogen peroxide 1.5% /W/v)	Water, sorbitol, propylene glycol, poloxamer 338, polysorbate 20, flavor, sodium saccharin, FD&C blue no.1	–	Oral debriding agent/oral wound cleanser
I	Listerine Freshburst	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, poloxamer 407, sodium saccharin, methylparaben, sucralose, propylparaben, blue 1	–	Antiplaque Antigingivitis
J	Pre Brush Dental Rinse	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, zinc lactate, methylparaben, sodium saccharin, sucralose, prpylparaben, poloxamer 407, blue1	–	Antiplaque Antigingivitis/ bad breath
K	Listerin edition coconut & lime blend	–	Water, glycerin, sodium hexametaphosphate, poloxamer 407, sodium benzoate, sodium lauryl sulfate, flavor, phosphoric acid, sodium saccharin, sucrallose, red 33, green 3	–	Whitening mouthwash
L	Oral B Breath Purify	Cetylpyridinium Chloride 0.05%	Water, alcohol (15wt%), propylene glycol, flavor, poloxamer 407, sucralose, benzoic acid, sodium benzoate, blue 1, yellow 6	Alcohol 15% wt%	Antiplaque Antigingivitis
M	Thera Breath Dentist formulated withening fresh breath	–	Water, glycerin, alcohol (5wt%), hydrogen peroxide, sodium hexametaphosphate, poloxamer 407, flavor, sodium citrate, sodium saccharin, citric acid, sucralose	Alcohol 5% wt%	Bad breath bacteria
N	Colgate Total Whole Mouth Health	Cetylpyridinium Chloride 0.05%	Water, alcohol (15wt%), propylene glycol, flavor, poloxamer 407, sucralose, benzoic acid, sodium benzoate, blue 1, yellow 6	Alcohol 15% wt%	Antiplaque Antigingivitis
O	Listerine Ultra Clean Zero alcohol	Cetylpyridinium Chloride 0.1%	Water, glycerin, Hhydrogen peroxide, flavor, sucralose, poloxamer 407	–	Antiplaque Antigingivitis
P	Cepacol Antibacterial multi -protection mouthwash	Cetylpyridinium Chloride	Water/EAU, alcohol (15wt%), glycerin, flavor, polysorbate 80, sodium saccharin, sodium benzoate, cetylpyridinium chloride, benzoic acid, blue 1. yellow 5	Alcohol 15% wt%	Antiplaque Antigingivitis
Q	Perio Brite Complete Oral Care Natural Mouthwash	Hydrogen peroxide	Water, glycerin, hydrogen peroxide, propylene glycol, sodium hexametaphosphate, poloxamer 407, sodium citrate, flavor, PEG-40, Hydrognated castor oil, soddium saccharine, citric acid.	–	Whitens surface of teeth
R	Crest Pro-Health Intense	–	Water, glycerin, xylitol, sorbitol, propylene glycol, poloxamer 407, sodium benzoate, hydroxyethylcellulose, hydroxiaceatophenone, 1,2-hexanediol, caprylyl glycol, flavor, sodium phosphate, disodium phosphate	–	–
S	Hello Activated Charcoal	–	Water, Peg-40, hydrogenated castor oil, sodium chlorite, tetrasodium EDTA, sodium benzoate , sodium bicarbonate, flavor, Illicium Verum (Anise) fruit/seed oil, glycerin, sodium carbonate	–	Bad Breath
T	Jason Halthy mouth Tartar Control cinnamon clove	Hydrogen peroxide 1.5% /W/v)	Citric acid, flavor, glycerin, poloxamer 407, propylene glycol, sodium citrate, sodium hexametaphosphtate, sodium saccharin, sucralose, water	–	Whitening mouth rinse
U	Thera Breath Dentist formulated fresh breath	Cetylpyridinium Chloride 0.07%	Flavor, glycerin, methylparaben, poloxamer 407, propylparaben, sodium saccharin, sucralose, water, zinc lactate	–	Antiplaque Antigingivitis
V	Parodontax active gum health Mint	Cetylpyridinium Chloride	Flavor, glycerin, propylene glycol, xylitol, cellulose gum, sodium hyaluronate, poloxamer 407, sodium benzoate, , cetylpyridinium chloride, benzoic acid	–	Dry mouth
W	Biotène dry mouth oral rinse	Cetylpyridinium Chloride	Water, glycerin, flavor, cetylpiridinium chloride, zinc lactate, methylparabe, sodium saccharin, sucralose, propylparabem, poloxamer 407	–	Bad Breath
X	ARC turn up the bright	Cetylpyridinium Chloride	Water, alcohol 15 wt%, glycerin, flavor, polysorbate 80, sodium saccharin, soium benzoate, cetylpiridinium chloride, benzoic acid, blue 1, yellow 5	Alcohol 15% wt%	Antiplaque Antigingivitis
Y	Crest Pro-Health Clean Mint multiprotection	Hydrogen peroxide	Water, glycerin, hydrogen peroxide, propylene glycol, sodium hexametaphosphate, poloxamer 407, sodium citrate, flavor, PEG-40, Hydrognated castor oil, soddium saccharine, citric acid.	–	Whitening mouth rinse
Z	Mouthwash up&up	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (26.9 % v/v), poloxamer 407, benzoic acid sodium benzoate, Caramel	Alcohol 26.9 % v/v	Antiplaque Antigingivitis
AA	Dr. Tichenor's All natural Peppermint Mouthwash Concentrate	–	Water, glycerin, propylee glycol, hydrogen peroxide, sodium hexametaphosphate, poloxamer 407, flavor, sodium citrate, sodium saccharin, citric acid, sucralose	–	Teeth Whitening
AB	Antiseptic Mouthwash antigingivits antiplaque	Sodium Cholirde	Water, glycerin, PEG-40, hydrogenated caster oil, citric acid, sodium hydroxide, pepper mint oil,menthol, sodium chlorite, citrus limon peel oil, sodium benzoate, sucralose, xylitol, sodium bicarbonate.	–	Bad Breath
AC	Hello Peace out, plaque	Cetylpyridinium Chloride 0.05%	Water, glycerin, poloxamer 407, flavor, sucralose		Antiplaque Antigingivitis
AD	Listerine Original	–	Water, glycerin,polysorbate 20, sodium benzoate, PVP, Natural mint flavor, papain, D-limonene, menthol, glucose oxidase		Fresh breath
AE	Lavoris Freash Breath Mouthwash	–	Water, Peg-40, hydrogenated castor oil, sodium chlorite, tetrasodium EDTA, sodium benzoate , sodium bicarbonate, mentha piperita (peppermint) oil, sodium hydroxide		Fresh breath
AF	Oral B Dry mouth	–	Water, glycerin, xylitol, sorbitol, propylene glycol, poloxamer 407, sodium benzoate, hydroxyethylcellulose, methylparab, propylparaben, flavor, sodium phosphate, disodium phosphate	–	Dry mouth brad
AG	Plax sofmint flavor mouthwash	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, poloxamer 188, sodium saccharin, propylene glycol, sodium benzoate, sucralose, benzoic acid	Alcohol	Antiplaque Antigingivitis
AH	ARC fresh breath mouth rinse	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, poloxamer 188, sodium saccharin, propylene glycol, sodium benzoate, sucralose, benzoic acid	–	Antiplaque Antigingivitis
AI	Antiseptic Mouthwash up &up	–	Solution 1: Purified water, sodium benzoate, sodium chloride and benzoic acid. Solution 2: Purified water, glycerin, poloxamer 407, propylene glycol, poloxamer 124, zinc chloride, flavor, sodium benzoate, benzoic acid, sodium saccharin, sodium chloride, benzyl alcohol, D&C yellow Nº 10, FD&C blue Nº1	–	Bad breath prevention
AJ	Oral B Mouth Sore	Cetylpyridinium Chloride 0.05%	Solution 1: Purified water, sodium benzoate, sodium chloride and benzoic acid. Solution 2: Purified water, glycerin, poloxamer 407, propylene glycol, poloxamer 124, zinc chloride, flavor, sodium benzoate, benzoic acid, sodium saccharin, sodium chloride, benzyl alcohol, D&C yellow Nº 10, FD&C blue Nº1	–	Antiplaque Antigingivitis
AK	Tom`s Natural Fluoride-free Wicked fresh	Menthol 0.2% (When mixed directed)	Solution 1: Purified water, sodium benzoate, sodium chloride and benzoic acid. Solution 2: Purified water, sorbitol, poloxamer 407, propylene glycol, poloxamer 124, zinc chloride, flavor, sodium benzyl alcohol, sodium saccharin, speppermint oil, benzoic acid, sodium chloride, , D&C yellow Nº 10, FD&C blue Nº1	–	Oral Pain Reliever
AL	Listerine Cool Mint	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (21.6% v/v), sorbitol, flavor, poloxamer 407, benzoic acid, sodium saccharin, sodium benzoate, FD&C green nº3	21.6% v/v	Antiplaque Antigingivitis
AM	The Natural Dentist Healthy gums	–	Water, glycerin and/or sorbitol, alcohol 8.7%, tetrasodium pyrophosphate, benzoic acid, flavor, poloxamer 407, sodium benzoate, sodium laurylsulfate, sodium saccharine, xanthan gum, FD&C blue nº 1, FD&C yellow nº 5	Alcohol 8.7%	–
AN	Thera breath periodontist formulated Healthy gums oral rinse	–	Water, sea salt, xylitol, natural flavor, lysozyme, menthol, potassium sorbate, sodium benzoate, poloxamer 407	–	–
AO	Oral B Dry mouth	Aloe Vera (20%)	Purified Wate, Vegetable Glycerin, Echinacea, Goldenseal, Calendula, Citric Acid, Polysorbate 80, Natural Flavors (contains cinnamon oil) Grapefruit seed extract, potassium citrate, copper chlorophyllin Color	–	Antiplaque Antigingivitis
AP	Swan Mouthwash fresh mint	–	Water, glycerin, sorbitol, Aloe barbadensis leaf juice (organic), propanediol, cylitol, natural flavor, benzoic acid, zinc citrate, menthol, sodium hydroxide	–	Bad Breath (prevent)
AQ	Crest Scope mouthwash rince-bouche	Cetylpyridinium Chloride 0.075%	Water, glycerin, aloe barbadensis leaf juice, polysorbate 80, erythritol, xylitol, poloxamer 407, flavor, cocos nucifera (coconut) oil (certified organic), sodium benzoate, citric acid, rebaudiside A (certified organic), melaleuca aiternifoil (tea tree) leaf oil	–	Antiplaque Antigingivitis
AR	Family Wellness Blue mint Mouthwash	–	Puridied water, vegetable glycerin (soother and moisturizes), polysorbate 80 (emulsifier), flavor (yum), xylitol (aweetener), erythritol (sweetener), poloxamer 407 (emulsifier), charcoal powder (freshens breath), cocos nucifera (coconut) oil (soothes and moisturizes), sodium benzoate (maintains stability), melaleuca alternifolia (tea tree) leaf oil (freshens breath)	–	Natural fresh
AS	Crest all fresh no stress Scope All day	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, alcohol 21.6%, sorbitol solution, flavor, poloxamer 407, benzoic acid, sodium saccharin, sodium citrate, D&C yellon nº 10, FD&C green nº 3	21.6% v/v	Antiplaque Antigingivitis
AT	Desert Essence Tea Tree OilSpearmint	Hydrogen peroxide 1.5% Menthol 1%	Alcohol 4.1% (by volume), disodium EDTA, FD&C blue nº1, methyl salicylate, phosphoric acid, poloxamer 338, polysorbate 20, sodium saccharine, sorbitol, water	4.10%	Oral debriding agent/oral antiseptic/ Oral pain reliever

AU	Jason Healthy powersmile brightening	–	Water, sorbitol, propylene glycol, poloxamer 407, flavor, benzoic acid, sodium benzoate, sodium saccharin, blue 1	–	Refreshing Mouth rinse
AV	Lumineux Oral essentials	cetylpiridinium chloride	Water, alcohol, sorbitol, polysorbate 20, flavor, cetylpiridinium chloride, sodium saccharine, sodium benzoate, benzoic acid, blue 1, yellow 5	Has, but does not describe the amount	Refreshing
AW	Jason Total Protection sea salt mouthrinse	–	Water, alcohol, glycerin/sorbitol, flavor, poloxamer 407, polysorbate 20, dosium saccharin, zinc chloride, citric acid, blue 1	Has, but does not describe the amount	Proven to freshen breath
AX	Tea Tree therapy Mouth Wash	–	Purified Water, Xylitol, grain alcohol (ethanol), coolmint flavor (blend of natural flavors), vegetable glycerin, calcium glycerophosphate, aloe vera, Marigold, Chamomile, Echinacea, Olive Leaf, Thyme	Has, but does not describe the amount	Help keep the mouth and teeeth refreshing clean
AY	HPM Hydrogen Peroxide Mouthwash	–	Water, glycerin, xylitol, Bio-Saponns (Yucca schidigera Root extract, quillaja saponaria Root extract, Dioscorea villosa (Wild Yam) Tuber Extract, Smilax aristolochiifolia Root Extract. Mentha piperita (peppermint) oil, Calendula officinalis flower extract, echinacea purpurea flower/leaf/Steam Extract, Chamomilla recutita (Matricaria), flower extract, Phytoplenolin (Centipeda cunninghamii extract), Centella asiatica Etract (Gotu Kola), Origanum vulgare Leaf oil (oregano), zanthoxylum americanum bark extract (Prickly Ash Bark), Lavandula angustifolia (Lavender) oil, Thymol, Eugenia caryophyllus (clove) bud oil, Folic Acid, Olea Europaea (Olive) Leaf extract, thymus vulgaris (Thymel) flower/Leaf oil, Eucalyptus globulus Leaf Oil, Cinnamomum zeylanicum Bark Oil, Citrus Paradisi (Grapefruit) Seed Extract, Juglans nigra (Black Walnut) Shell Extract, Ubiquinone (CoQ10), Camellia Sinensis Leaf Extract (Green Tea), Rosmarinus Officinalis (Rosemary) Leaf Oil, Aloe barbadensis Leaf juice (Aloe Vera), Citrus Linon (Lemon) Peel Oil, Hydrastis Canadensis (Goldensea) Extract, Cinnamomum cassia Bark Extract...	–	Complete Oral Care
AZ	Bubble Gum Kid's Spry Mouth wash	–	Purified Water, xylitol, vegetable glycerin, erythritol, Echinacea Purpurea, Chamomile, Olive Leaf, Marigold, Thyme, Oat Beta Glucan, Aloe Vera, Calcium Glycerophosphate, Cocamidopropyl betaine, Honeysuckle, Natural flavors, Color Stabilizer, Natural Plant Coloring	–	Fresh and Clean
BA	Jason Healthy powersmile brightening	Hydrogen peroxide	Water, hydrogen Peroxide, alcohol, Thymus sephyllum (White thyme) Leaf Oil, Eucalyptol, Menthol, Natural Wintergreen flavor	Has, but does not describe the amount	Fresh breath
BB	Swan Alcohol free Mouthwash	–	Aloe leaf juice, purified water, hydrogen peroxide, acacia, wintergreen oil, xanthan gum, peppermint oil, rosemary leaf oil, eucalyptus leaf oil, lemon peel oil, cinnamon leaf oil (organic ingredient)	–	Brushing Rinse
BC	Orajel 2x Mouth Sores Rinse Medicated	–	Aloe leaf juice, purified water, hydrogen peroxide, acacia, peppermint oil, rosemary leaf oil, eucalyptus leaf oil, lemon peel oil, clove bud oil (eugenia caryophyllus), cinnamon leaf oil (organic ingredient)	–	Brushing Rinse
BD	Crest Bacteria Blast	–	Organic Aloe Barbadensis (Aloe Vera) Leaf Juice, Xylitol, Purified water, dead sea salt, gautheria procumbens leaf oil, cocos nucifera oil, citrus limon peel oil, salvia officinalis oil, mentha ciridis leaf oil, Organic mentha piperita leaf oil, ocimum basilicum oil, Eugenia Caryophyllus flower Oil.	–	Whitening mouthwash
BE	Clean mint Withening alcohol free rinse	–	Deionized Water, sorbitol, tea tree oil, (oil of Melaleuca alternifolia) Natural Mint flavor, citric acid, sodium citrate	–	Clean and refresher
BF	Thera Breath dentist formulated fresh breath oral rinse	–	Purified Water, Cloralstan (stabilized chloride dioxide), trisodium phosphate, citric acid, flavor, sucralose	–	Bad Breath
BG	Listerine Sensitivity Zero alcohol	–	Purified water, glycerin, polysorbate-80, Phyllostachis Bambusoides Juice, Mentha Viridis Leaf oil, Equisetum Arvense Leaf extract, Gaultheria Procumbens Leaf oil, Eco-Harves Melaleuca Alternifolia leaf oil, Zinc Citrate, Calcium absorbate, ascorbic acid, hamamelis virginiana extract, Stevia Rebaudiana Leaf/stem power	–	Whitening mouthwash
BH	Splendid white whitening mouth rinse alcohol free Up&Up	–	Water, glycerin, polysorbate 80, Eco-Harvest Melaleuca Alternifolia leaf oil, Aloe barbadensis Leaf, juice, Mentha Viridis leaf oil, Hamamelis Virginiana extract, Ascorbic acid, calcium ascorbate, citric acid	–	Feel clean and extra fresh
BI	Crest Scope Classic	Sodium Bicarbonate 2.5%	Purified water, glycerin,polysorbate 20, aloe Barbadensis leaf juice, menthol, mentha piperita oil, stevia rebaudiana extract, echinacea purpurea extract, citrus aurantium dulcis oil, citrus limon oil, lavandula angustifolia oil, ricinus communis seed oil, hydrastis canadensis leaf extract, pimpinella anisum fruit oil, pelargonium graveolens oil, rosmarinus officialis leaf/stem oil, hamamelis virginiana water, citrus aurantium bergamia peel oil, citrus paradisi seed extract, foeniculum vulgare oil, eugenia carypophyllus flower oil, ocimum basilicum oil, rosewater concentrate, ubiquinone	–	Oral Wound Cleanse/Oral Debriding Agent
BJ	BR rinse. Organic Mouthwash	–	Water, glycerin, polysorbate 20, cinnamon zeylanicum bark oil, eugenia caryophyllus flower oil, melaleuca alternifolia leaf oil, melia azadirachta seed oil, Aloe barbadensis leaf juice, calendula officinalis flower extract, citrus grandis seed extract, echinacea purpurea extract, hamamelis virginiana water, hydrastis canadensis extract, melissa officinalis leaf extract, perilla ocymoides seed extract, ascorbic acid, calcium ascorbate, menthol, sodium bicarboante, sea salt, cinnamal, eugenol	–	Fresh breath mouthwash
BK	Tom´s Sea Salt	–	Water, glycerin, cinnamomum zeylanicum leaf oil, mentha piperita oil, citric acid, polysorbate 20, benzyl alcohol, potassium sorbate, sodium benzoate	–	Fresh breath
BL	CloSYS	–	Water, glycerin, polysorbate 20, mentha piperita oil, aloe barbadensis leaf juice, calendula officinalis flower extract, carica papaya fruit extract, citrus grandis seed extract, echinacea angustifolia extract, hamamelis virginiana water, hydrastis canadensis extract, perilla ocymoides seed extract, ascorbic acid, calcium ascorbate, menthol, sea salt, sodium bicarbonate, xylitol	–	Brightening
BM	Antiseptic Mouthwash antigingivits antiplaque	–	Water, glycerin, sorbitol, Aloe barbadensis leaf juice, propanediol, xylitol, sodium chloride, aroma/flavor (natural), zinc citrate, benzoic acid, menthol, sodium hydroxide	–	Bad breath bacteria
BN	Crest GUM and Breath Purify	–	Water, glycerin, polysorbate 80, perilla pcymoides seed extract, cichorium intybus root extract, hydrolyzed pea protein, inulin, glycyrhiza glabra root extract, quillaja saponaria bark extract, quisetum arvense leaf extract, glutathione, mentha piperita flower/leaf/stem oil, mentha arvensis steam/leaf oil,mentha viridis leaf oil, anthemis nobilis flower oil, echinacea angustifolia extract, zinc citrate, salvia officinalis oil, commiphora myrha oil, calcium ascorbate, ascorbic acid, aloe barbadensis leaf juice, calndula officinalis flower oil, hydrastis canadensis extract, melaleuca alternifolia leaf oil. hamamelis virginiana extract, menthol, maltodextrin, xylitol phytic acid, citric acid	–	Brushing Rinse
BO	Swan Antiseptic mouth rinse Original	Cetylpyridinium Chloride 0.05%	Purified water, alcohol 14% v/v, glycerin sodium phosphate dibasic, eucalyptus oil, polysorbate 80, methyl salicylate, cinnamon oil, peppermint oil, saccharin sodium, sodium phosphate monobasic anhydrous, menthol, edetate disodium , FD&C yellow #5	Alcohol 14%	Antiplaque Antigingivitis
BP	Swan Antiseptic Mouth rinse Spring Mint	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (21.6% v/v), sorbitol, flavor, poloxamer 407, benzoic acid, zinc chloride, sodium benzoate, sucralose, sodium saccharin, green nº3	Alcohol 21.6%	Antiplaque Antigingivitis
BQ	Advanced Mouth Rinse	–	Alcohol (70%), peppermint oil, arnica, saccharum carbonate, USP purified water	Alcohol 70%	Bad Breath
BR	Crest 3D white brilliance	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (21.6% v/v), sorbitol, flavor, poloxamer 407, benzoic acid, sodium saccharin, sodium citrate, D&C yellow nº 10, FD&C green nº3	Alcohol 21.6%	Antiplaque Antigingivitis
BS	Multi-action alcohol free. Antiseptic mouth rinse	Cetylpyridinium Chloride	Water, alcohol 13wt%, glycerin, flavor, polysorbate 80, sodium saccharin, sodium enzoate, cetylpiridinium Chloride, Benzoic Acid, blue 1 F.C.F, yellow 5/tartrazine	13%%	Fresh breath and Bad breath
BT	BR rinse. Organic Mouthwash	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, zinc lactate, methylparaben, sodium saccharin, sucralose, propylparaben, poloxamer 407	–	Bad Breath
BU	Guru Nanda Oxyburst Whitening Technology	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (21.6% v/v), sorbitol, flavor, poloxamer 407, benzoic acid, sodium saccharin, sodium benzoate, FD&C green nº3	21.60%	Antiplaque Antigingivitis
BV	PerCara Mouthwash & Gargle Refresh mint	Cetylpyridinium Chloride	Water, alcohol (12.25 wt%), glycerin and/or sorbitol, polysorbate 80 and/or polysorbate 20, flavor, sodium saccharin, sodium benzoate, cetylpiridinium chloride, benzoic acid, blue 1	12.25%	Clean and fresher breath
BW	Spry Oral Rinse (Dental defense system)	Eucaliptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%) Thymol (0.064%)	Water, Alcohol (26.9 % v/v), poloxamer 407, benzoic acid sodium benzoate, Caramel	26.90%	Antiplaque Antigingivitis
BX	Desert Essence Tea Tree Oil Whitening Plus Mouthwash	Cetylpyridinium Chloride	Water, alcohol 15 wt%, glycerin, flavor, polysorbate 80, sodium saccharin, soium benzoate, cetylpiridinium chloride, benzoic acid, blue 1, yellow 5	15.00%	Bad Breath
BY	Desert Essence Prebiotic Plant based brushing rinse Mint	Cetylpyridinium Chloride	Purified water, glycerin, poloxamer 407, sodium benzoate, xylitol, flavor, sodium saccharin, menthol, cetylpiridinium chloride, zinc gluconate, citric acid, calcium lactate, D&C red 33, FD&C Red 40	–	Clean and fresher breath
BZ	Smart mouth Mouth Sore zinc activatted oral rinse	Cetylpyridinium Chloride 0.07%	Water, glycerin, flavor, poloxamer 188, sodium saccharin, propylene glycol, sodium benzoate, sucralose, benzoic acid, blue 1	–	Antiplaque Antigingivitis
CA	Smart mouth Original	hydrogen peroxide	Purified water, glycerin, xylitol, sorbitol, hydrogen peroxide, sodium benzoate, aloe vera, sodium bicarbonate, Peg-40 hydrogenated castor oil, sodium lauroyl sarcosinate, sodium chloride, peppermint oil, stevia, cardamom oil, clove oil, calcium lactate, vitamin E, Vitamin D, spearmint oil, fenne oil, tea tree oil, oregano oil, jasmine oil, menthol, citric acid	–	Oral Care
CB	Smarth mouth Clinical zinc activated oral rinse	–	Water, sea salt, glycerin, sorbitol, mentha piperita oil, camellia sinensis leaf extract, citrus grandis seed extract, hamamelis virginiana extract, salvia officinalis leaf extract, ascorbic acid, citric acid, mentho, polysorbate 20, sodium bicarbonate, benzoic acid, potassium sorbate, sodium benzoate	–	Fresh breath & complete oral Hygiene
CC	Parodontax active gum health Clear mint	–	Water, Glycerin, Propylene Glycol, Sorbitol, Hydrogen Peroxide, Polysorbate 20, Sodium Acrylates/Methacryloylethyl Phosphate Copolymer, Phosphoric Acid, Citric Acid, Flavor, PVM/MA Copolymer, Sodium Saccharin	–	Whitening mouthwash

Appendix 2: One-way ANOVA statistical analyses performed to compare the effects of mouthwash exposure (between the dilution of each mouthwash) and their interaction on MIC, planktonic, and biofilm.

		MIC						Planktonic						Biofilm					
		1:3	1:6	1:12	p value 1:3 vs 1:6 dilution	p value 1:12 vs 1: dilution	p value 1:6 vs 1:12 dilution	1:3	1:6	1:12	p value 1:3 vs 1:6 dilution	p value 1:12 vs 1: dilution	p value 1:6 vs 1:12 dilution	1:3	1:6	1:12	p value 1:3 vs 1:6 dilution	p value 1:12 vs 1: dilution	p value 1:6 vs 1:12 dilution
A	Close.UP Mouthwash with Calcium Cinnamon	0.0452 ± 0.0122	0.0330 ± 0.0138	0.0266 ± 0.0140	0.0357	0.0336	0.0414	0.2053 ± 0.4310	0.2038 ± 0.4227	0.1147 ± 0.3924	1.0000	0.3223	0.1503	0.0123 ± 0.0103	0.0130 ± 0.0134	0.0109 ± 0.0120	0.9585	0.6553	0.6915
B	Sea Salt Oral Rinse	0.2740 ± 0.0135	0.3848 ± 0.0367	0.3870 ± 0.0339	0.0001	0.0001	0.6413	0.3744 ± 0.5779	0.3020 ± 0.5996	0.0233 ± 0.0527	0.8264	0.3870	0.7726	0.4469 ± 0.1612	0.6665 ± 0.1196	0.7060 ± 0.3293	0.0018	0.0111	0.2738
C	Colgate Optic White High Impact White Advanced	0.0043 ± 0.0088	0.0059 ± 0.0103	0.0067 ± 0.0109	0.5836	0.5530	1.0000	0.5857 ± 0.9150	0.0295 ± 0.1185	0.1112 ± 0.4162	0.1587	0.2880	0.9717	0.0481 ± 0.1188	0.0281 ± 0.0406	0.0436 ± 0.1062	0.7410	0.8994	0.6915
D	Colgate Peroxyl	0.0195 ± 0.0081	0.0177 ± 0.0110	0.0136 ± 0.0169	0.2768	0.0267	0.0486	0.2125 ± 0.6411	0.1771 ± 0.6193	0.4279 ± 0.7861	0.7441	0.3474	0.1977	0.0002 ± 0.0037	0.0058 ± 0.0054	0.0053 ± 0.0149	0.0215	0.7102	0.4179
E	Oral B Gum Detoxify	0.0000 ± 0.0000	0.0793 ± 0.0158	0.0502 ± 0.0184	0.0001	0.0001	0.0121	0.0876 ± 0.1954	0.2208 ± 0.4545	0.1441 ± 0.5094	0.5154	0.8201	0.4849	0.0000 ± 0.0000	0.0272 ± 0.0097	0.0149 ± 0.0076	0.0002	0.0008	0.0140
F	Crest Pro-Health Bacteria Guard	0.0041 ± 0.0067	0.0409 ± 0.0161	0.0350 ± 0.0152	0.0001	0.0001	0.0528	0.4562 ± 0.8266	0.4303 ± 0.8749	0.1913 ± 0.3527	0.7904	0.4792	0.6236	0.0000 ± 0.0000	0.0193 ± 0.0079	0.0139 ± 0.0089	0.0002	0.0002	0.1879
G	Crest Pro-Health Clinical	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0762 ± 0.0279	1.0000	0.0001	0.0001	0.2198 ± 0.4959	0.0313 ± 0.0999	0.0265 ± 0.1058	0.5828	0.9027	0.7726	0.0003 ± 0.0012	0.0685 ± 0.0234	0.0278 ± 0.0139	0.0002	0.0002	0.0008
H	Ultimate Essential MouthCare. Eco-Dent	0.0067 ± 0.0076	0.2616 ± 0.2163	0.3873 ± 0.0821	0.0001	0.0001	0.2918	-0.0070 ± 0.0151	0.0719 ± 0.0514	0.0111 ± 0.0058	0.0013	0.0081	0.0097	0.0198 ± 0.0302	0.3832 ± 0.5224	0.8929 ± 0.6409	0.0111	0.0004	0.1415
I	Listerine Freshburst	0.0320 ± 0.0080	0.2980 ± 0.0377	0.3693 ± 0.0292	0.0001	0.0001	0.0016	0.1357 ± 0.4105	0.1602 ± 0.3397	0.4318 ± 0.7020	0.0099	0.0167	0.9991	0.1292 ± 0.3945	0.2614 ± 0.1892	0.4700 ± 0.3554	0.0549	0.1564	0.2076
J	Pre Brush Dental Rinse	0.0458 ± 0.0048	0.0263 ± 0.0061	0.0208 ± 0.0078	0.0001	0.0001	0.0475	0.5856 ± 0.7629	0.5849 ± 0.8801	0.2005 ± 0.5941	0.5004	0.0293	0.1503	0.0033 ± 0.0045	0.0039 ± 0.0043	0.0675 ± 0.2198	0.7744	0.4462	0.3843
K	Listerine Limited Edition Coconut and Lime Blend	0.0125 ± 0.0143	0.2589 ± 0.0578	0.3582 ± 0.0803	0.0001	0.0001	0.0014	0.2914 ± 0.5600	0.5566 ± 0.8060	0.0245 ± 0.0060	0.8264	0.4531	0.1977	0.0093 ± 0.0062	0.3858 ± 0.0683	0.5283 ± 0.1648	0.0002	0.0002	0.0493
L	Oral B Breath Purify	0.1578 ± 0.0357	0.3366 ± 0.0718	0.4073 ± 0.0631	0.0006	0.0001	0.0181	0.0060 ± 0.0072	0.0185 ± 0.0214	0.0158 ± 0.0220	0.1400	0.1712	0.8354	0.3938 ± 0.1111	0.4814 ± 0.2217	0.5881 ± 0.1390	0.3456	0.0061	0.0701
M	Thera Breath Dentist Formulated Withening Fresh Breath	0.0249 ± 0.0127	0.0736 ± 0.0298	0.2650 ± 0.0656	0.0011	0.0001	0.0002	0.5100 ± 0.6388	0.7393 ± 0.9389	0.4211 ± 0.7996	0.7464	0.9518	0.7670	0.0406 ± 0.0388	0.1193 ± 0.0632	0.6408 ± 0.2142	0.0066	0.0009	0.0014
N	Colgate Total Whole Mouth Health	0.0618 ± 0.0200	0.0443 ± 0.0186	0.0318 ± 0.0129	0.0445	0.0031	0.0528	0.5683 ± 0.8752	0.1094 ± 0.2025	0.2739 ± 0.4012	0.1412	0.8901	0.3667	0.0213 ± 0.0052	0.0246 ± 0.0249	0.0176 ± 0.0067	0.3456	0.1414	0.7410
O	Listerine Ultra Clean Zero Alcohol	0.0279 ± 0.0179	0.0679 ± 0.0457	0.4144 ± 0.0309	0.0113	0.0001	0.0001	0.0743 ± 0.1770	0.0208 ± 0.0456	0.1229 ± 0.3891	0.8354	0.5154	0.5004	0.0085 ± 0.0181	0.1657 ± 0.0833	0.7723 ± 0.0998	0.0002	0.0002	0.0002
P	Cepacol Antibacterial Multi-Protection Mouthwash	0.0452 ± 0.0190	0.0513 ± 0.0225	0.0526 ± 0.0179	0.4653	0.3025	0.8956	0.1480 ± 0.1814	0.0694 ± 0.1143	0.0068 ± 0.0128	0.4531	0.0428	0.3223	0.0273 ± 0.0177	0.0306 ± 0.0216	0.0383 ± 0.0361	0.8326	0.4476	0.8326
Q	Perio Brite Complete Oral Care Natural Mouthwash	0.0408 ± 0.0248	0.1519 ± 0.0578	0.2961 ± 0.0335	0.0002	0.0001	0.0002	0.0031 ± 0.0101	0.0133 ± 0.0097	0.0111 ± 0.0143	0.0870	0.1712	0.3140	0.0059 ± 0.0205	0.3043 ± 0.1638	0.7464 ± 0.0993	0.0002	0.0002	0.0002
R	Crest Pro-Health Intense	0.0000 ± 0.0000	0.0603 ± 0.0200	0.0528 ± 0.0121	0.0001	0.0001	0.2786	0.3662 ± 0.4933	0.4103 ± 0.7296	0.3117 ± 0.7017	0.3474	0.2880	0.4849	0.0367 ± 0.0174	0.0233 ± 0.0169	0.0347 ± 0.0537	0.0892	0.1805	0.7410
S	Hello Activated Charcoal	0.2532 ± 0.0510	0.4134 ± 0.0492	0.3984 ± 0.0332	0.0001	0.0001	0.6224	0.3400 ± 0.7389	0.0401 ± 0.1076	0.1713 ± 0.5456	0.0844	0.0694	0.5828	0.5332 ± 0.0694	0.7093 ± 0.0899	0.7182 ± 0.0565	0.0005	0.0003	0.7744
T	Jason Healthy Mouth Tartar Control Cinnamon Clove	0.1149 ± 0.1911	0.1226 ± 0.0573	0.2088 ± 0.0764	0.0761	0.0014	0.0274	0.0003 ± 0.0012	0.0093 ± 0.0197	0.1658 ± 0.3592	0.0362	0.0501	0.8186	0.1354 ± 0.1011	0.2185 ± 0.1174	0.3449 ± 0.0754	0.1564	0.0005	0.0174
U	Thera Breath Dentist Formulated Fresh Breath	0.0166 ± 0.0142	0.0187 ± 0.0187	0.0793 ± 0.0353	0.8796	0.0002	0.0003	-0.0052 ± 0.0073	0.0208 ± 0.0660	0.0044 ± 0.0120	0.8040	0.1092	0.6236	0.0161 ± 0.0143	0.0196 ± 0.0256	0.1340 ± 0.0901	0.9699	0.0002	0.0004
V	Parodontax Active Gum Health Mint	0.0000 ± 0.0000	0.0850 ± 0.0105	0.0717 ± 0.0091	0.0001	0.0001	0.0142	0.3059 ± 0.6002	0.4843 ± 0.6468	0.2454 ± 0.5705	0.9518	0.0657	0.1587	0.0000 ± 0.0000	0.0485 ± 0.0234	0.0650 ± 0.0292	0.0002	0.0002	0.1002
W	Biotene Dry Mouth Oral Rinse	0.2088 ± 0.0642	0.3591 ± 0.0343	0.4080 ± 0.0433	0.0001	0.0001	0.0192	0.0181 ± 0.0161	0.1420 ± 0.2634	0.0969 ± 0.2675	0.2061	0.7441	0.5683	0.2517 ± 0.1068	0.5922 ± 0.1591	0.7134 ± 0.0696	0.0002	0.0002	0.0111
X	ARC Turn Up The Bright	0.0180 ± 0.0077	0.0236 ± 0.0094	0.0230 ± 0.0075	0.2034	0.1934	1.0000	0.3101 ± 0.6215	0.3758 ± 0.6631	0.2823 ± 0.5576	0.4631	0.9791	0.5683	0.0437 ± 0.0672	0.0204 ± 0.0101	0.0233 ± 0.0118	0.7928	0.6220	0.4915
Y	Crest Pro-Health Clean Mint Multiprotection	0.0033 ± 0.0106	0.0838 ± 0.0078	0.0698 ± 0.0133	0.0001	0.0001	0.0191	0.3713 ± 0.4373	0.2611 ± 0.4365	0.2684 ± 0.5389	0.1800	0.1503	0.7441	0.0120 ± 0.0265	0.0496 ± 0.0193	0.0319 ± 0.0066	0.0029	0.0174	0.0657
Z	Mouthwash Up and Up	0.0447 ± 0.0064	0.0492 ± 0.0047	0.0541 ± 0.0102	0.0596	0.0236	0.3025	0.4224 ± 0.5922	0.3081 ± 0.6137	0.2791 ± 0.5654	0.1800	0.6702	0.7464	0.0325 ± 0.0090	0.0437 ± 0.0144	0.0404 ± 0.0147	0.0735	0.1962	0.5656
AA	Dr. Tichenor's All Natural Peppermint Mouthwash Concentrate	0.0838 ± 0.0407	0.1548 ± 0.0362	0.1733 ± 0.0681	0.0015	0.0016	0.3162	0.0000 ± 0.0000	0.0013 ± 0.0021	0.0035 ± 0.0040	0.0715	0.0116	0.3190	0.1765 ± 0.0819	0.4488 ± 0.1072	0.4218 ± 0.1378	0.0002	0.0017	0.5292
AB	Antiseptic Mouthwash Antigingivits Antiplaque	0.0693 ± 0.0031	0.3385 ± 0.0286	0.4252 ± 0.0336	0.0001	0.0001	0.0002	0.0340 ± 0.0051	0.1136 ± 0.2681	0.0777 ± 0.1922	0.7441	0.0815	0.0870	0.0166 ± 0.0187	0.5812 ± 0.0901	0.8477 ± 0.0612	0.0002	0.0002	0.0002
AC	Hello Peace Out, Plaque	0.0203 ± 0.0063	0.0258 ± 0.0086	0.0325 ± 0.0115	0.1739	0.0113	0.2361	0.3053 ± 0.5749	0.5941 ± 0.8561	0.2721 ± 0.5023	0.5468	0.9438	0.7410	0.0153 ± 0.0095	0.0124 ± 0.0044	0.0131 ± 0.0057	0.7605	0.9153	0.9851
AD	Listerine Original	0.1320 ± 0.1817	0.2493 ± 0.0724	0.4313 ± 0.0429	0.0599	0.0041	0.0001	0.0209 ± 0.0118	0.1054 ± 0.2811	0.1043 ± 0.2565	0.3667	0.3014	0.8354	0.2738 ± 0.2739	0.5321 ± 0.2277	0.8036 ± 0.2799	0.0428	0.0042	0.0549
AE	Lavoris Freash Breath Mouthwash	0.3138 ± 0.0579	0.3543 ± 0.0593	0.3560 ± 0.0414	0.1524	0.0856	0.8273	0.0682 ± 0.0994	0.1754 ± 0.3027	0.1998 ± 0.5469	0.7726	0.6802	0.8264	0.6733 ± 0.1795	0.7300 ± 0.1632	0.6980 ± 0.1681	0.4621	0.7744	0.6553
AF	Oral B Dry mouth	0.2740 ± 0.0566	0.3913 ± 0.0248	0.3686 ± 0.0319	0.0002	0.0016	0.0856	0.3093 ± 0.6471	0.6713 ± 0.7098	0.0480 ± 0.0722	0.1800	0.6802	0.0355	0.3913 ± 0.2389	0.7025 ± 0.1896	0.8222 ± 0.3233	0.0153	0.0131	0.6036
AG	Plax Softmint Flavor Mouthwash	0.0580 ± 0.0039	0.0394 ± 0.0100	0.0353 ± 0.0078	0.0005	0.0001	0.5841	0.1863 ± 0.3454	0.3033 ± 0.6192	0.1688 ± 0.3285	0.7441	0.2165	0.1800	0.0004 ± 0.0022	-0.0006 ± 0.0020	-0.0006 ± 0.0028	0.4915	0.4179	0.8326
AH	ARC Fresh Breath Mouth Rinse	0.1317 ± 0.0580	0.1470 ± 0.0257	0.1128 ± 0.0282	0.1582	0.7475	0.0142	0.1529 ± 0.4226	0.1480 ± 0.2622	0.0660 ± 0.1096	0.2061	0.7019	0.3870	0.1533 ± 0.1618	0.1035 ± 0.0641	0.0761 ± 0.0333	0.3127	0.0735	0.4915
AI	Antiseptic Mouthwash Up and Up	0.0761 ± 0.0381	0.3122 ± 0.0652	0.3794 ± 0.0548	0.0001	0.0001	0.0341	0.0773 ± 0.1762	0.5054 ± 0.6047	0.3770 ± 0.5393	0.0116	0.0844	0.7441	0.1011 ± 0.1015	0.6718 ± 0.2366	0.8971 ± 0.2777	0.0002	0.0002	0.1002
AJ	Oral B Mouth Sore	0.0228 ± 0.0284	0.0218 ± 0.0110	0.0193 ± 0.0099	0.5011	0.4148	0.8273	0.4518 ± 0.5712	0.6930 ± 0.6238	0.5463 ± 0.7732	0.3140	0.8264	0.3740	0.0084 ± 0.0071	0.0193 ± 0.0125	0.0328 ± 0.0202	0.0231	0.0067	0.1632
AK	Tom`s Natural Fluoride-Free Wicked Fresh	0.0800 ± 0.0256	0.2603 ± 0.0413	0.3693 ± 0.0409	0.0001	0.0001	0.0003	0.0573 ± 0.1555	0.0129 ± 0.0233	0.0062 ± 0.0110	0.7441	0.7237	0.8901	0.3253 ± 0.1799	0.7179 ± 0.1924	0.9050 ± 0.1807	0.0017	0.0002	0.0403
AL	Listerine Cool Mint	0.0809 ± 0.0102	0.3182 ± 0.0319	0.3831 ± 0.0392	0.0001	0.0001	0.0015	0.2385 ± 0.5521	0.3577 ± 0.4520	0.3598 ± 0.7112	0.0870	0.7441	0.4849	0.0103 ± 0.0066	0.5799 ± 0.1189	0.9329 ± 0.1551	0.0002	0.0002	0.0005
AM	The Natural Dentist Healthy Gums	0.0528 ± 0.0112	0.1591 ± 0.0340	0.2834 ± 0.0320	0.0001	0.0001	0.0001	0.1407 ± 0.2973	0.4956 ± 0.6180	0.1113 ± 0.3465	0.1800	0.2880	0.0559	0.0600 ± 0.0308	0.3318 ± 0.1422	0.5067 ± 0.1802	0.0002	0.0002	0.0428
AN	Thera Breath Periodontist Formulated Healthy Gums Oral Rinse	0.0466 ± 0.0193	0.0488 ± 0.0169	0.0480 ± 0.0167	0.8670	0.8796	1.0000	0.2043 ± 0.3297	0.4126 ± 0.7690	0.5707 ± 0.6350	0.5828	0.3740	0.3667	0.0273 ± 0.0071	0.0353 ± 0.0165	0.0353 ± 0.0117	0.4621	0.1632	0.8198

AO	Oral B Dry Mouth	0.0470 ± 0.0191	0.0565 ± 0.0256	0.0552 ± 0.0261	0.4490	0.2439	1.0000	0.6339 ± 0.5682	0.0860 ± 0.1731	0.0509 ± 0.1103	0.0116	0.0116	0.3740	0.0150 ± 0.0052	0.0226 ± 0.0079	0.0321 ± 0.0171	0.0316	0.0069	0.1717
AP	Swan Mouthwash Fresh Mint	0.0716 ± 0.0242	0.0996 ± 0.1301	0.0598 ± 0.0173	0.7867	0.3554	0.5540	0.2035 ± 0.4993	0.1161 ± 0.2446	0.6363 ± 0.8861	0.9518	0.0524	0.0509	0.0423 ± 0.0165	0.0353 ± 0.0160	0.0503 ± 0.0328	0.3456	0.8038	0.4476
AQ	Crest Scope Mouthwash Rince-Bouche	0.0232 ± 0.0082	0.0319 ± 0.0089	0.0415 ± 0.0194	0.0468	0.0060	0.3841	0.2434 ± 0.3825	0.0948 ± 0.1310	0.1682 ± 0.4618	0.4792	0.4373	0.9027	0.0688 ± 0.2244	0.0494 ± 0.1098	0.0276 ± 0.0215	0.0549	0.0108	0.4179
AR	Family Wellness Blue Mint Mouthwash	0.0185 ± 0.0049	0.0270 ± 0.0079	0.0271 ± 0.0072	0.0179	0.0031	0.8796	0.3598 ± 0.6025	0.1673 ± 0.2880	0.0942 ± 0.1877	0.1800	0.1587	0.7410	0.0269 ± 0.0510	0.0158 ± 0.0062	0.1078 ± 0.1903	0.3456	0.0549	0.1252
AS	Crest All Fresh No Stress Scope All Day	0.1053 ± 0.0239	0.0992 ± 0.0182	0.0909 ± 0.0208	0.6036	0.2439	0.2439	0.0612 ± 0.1919	0.3545 ± 0.4625	0.0609 ± 0.1229	0.0220	0.0312	0.6802	0.1723 ± 0.1523	0.0828 ± 0.0298	0.0363 ± 0.0087	0.0029	0.0002	0.0002
AT	Desert Essence Tea Tree Oil Spearmint	0.2227 ± 0.0447	0.2794 ± 0.0525	0.2965 ± 0.0599	0.0445	0.0097	0.4000	0.0038 ± 0.0040	0.0145 ± 0.0109	0.1220 ± 0.2199	0.0167	0.0220	0.8264	0.4458 ± 0.0807	0.5611 ± 0.0962	0.5653 ± 0.0546	0.0174	0.0056	0.9153
AU	Jason Healthy Powersmile Brightening	0.0222 ± 0.0154	0.0461 ± 0.0178	0.0973 ± 0.0911	0.0121	0.0004	0.0104	0.0000 ± 0.0000	0.0010 ± 0.0016	0.0035 ± 0.0040	0.0715	0.0116	0.2284	0.0543 ± 0.0427	0.0785 ± 0.0680	0.1659 ± 0.1473	0.4915	0.0657	0.1879
AV	Lumineux Oral Essentials	0.2233 ± 0.0496	0.2710 ± 0.0524	0.2905 ± 0.0690	0.0528	0.0237	0.6825	0.0892 ± 0.3079	0.0028 ± 0.0035	0.0044 ± 0.0036	0.2865	0.0627	0.3740	0.4725 ± 0.0897	0.5611 ± 0.0557	0.4273 ± 0.3701	0.0255	0.6749	0.8038
AW	Jason Total Protection Sea Salt Mouthrinse	0.0153 ± 0.0188	0.1400 ± 0.0566	0.2090 ± 0.0601	0.0002	0.0001	0.0192	0.3231 ± 0.5333	0.1817 ± 0.2987	0.0204 ± 0.0364	0.7441	0.7721	0.9438	0.0641 ± 0.0309	0.4110 ± 0.1108	0.5998 ± 0.0955	0.0002	0.0002	0.0025
AX	Tea Tree Therapy Mouth Wash	0.1573 ± 0.0461	0.2258 ± 0.0363	0.2846 ± 0.0432	0.0034	0.0002	0.0048	0.2002 ± 0.4655	0.2868 ± 0.3157	0.2178 ± 0.4318	0.1529	0.0428	0.9518	0.4051 ± 0.1242	0.4999 ± 0.0809	0.5992 ± 0.0807	0.0549	0.0025	0.0174
AY	HPM Hydrogen Peroxide Mouthwash	0.0011 ± 0.0032	0.0044 ± 0.0065	0.0072 ± 0.0060	0.1165	0.0204	0.3376	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	1.0000	1.0000	1.0000	0.0409 ± 0.0302	0.0343 ± 0.0155	0.0607 ± 0.0758	0.7410	0.8038	0.6411
AZ	Bubble Gum Kids Spry Mouthwash	0.0169 ± 0.0097	0.0482 ± 0.0280	0.2922 ± 0.0249	0.0048	0.0001	0.0001	0.0532 ± 0.1227	0.1196 ± 0.2992	0.0571 ± 0.1439	0.1006	0.0293	0.3667	0.0317 ± 0.0322	0.0942 ± 0.1263	0.5637 ± 0.0714	0.0107	0.0002	0.0002
BA	Jason Healthy Powersmile Brightening	0.1628 ± 0.0332	0.2466 ± 0.0794	0.2848 ± 0.0831	0.0907	0.0029	0.0599	0.0044 ± 0.0153	0.0119 ± 0.0285	0.0039 ± 0.0089	0.3667	0.3908	0.8594	0.3010 ± 0.1060	0.4898 ± 0.1031	0.6259 ± 0.1235	0.0012	0.0002	0.0174
BB	Swan Alcohol Free Mouthwash	0.2479 ± 0.0204	0.3217 ± 0.0223	0.3247 ± 0.0574	0.0001	0.0057	0.5699	0.0226 ± 0.0492	0.1384 ± 0.2969	0.1873 ± 0.3523	0.5468	0.1503	0.4849	0.5050 ± 0.0348	0.7013 ± 0.1043	0.7525 ± 0.2794	0.0002	0.0003	1.0000
BC	Orajel 2x Mouth Sores Rinse Medicated	0.0165 ± 0.0105	0.0098 ± 0.0083	0.0151 ± 0.0094	0.2108	0.9821	0.2506	0.3112 ± 0.5068	0.0003 ± 0.0009	0.2158 ± 0.3472	0.0428	0.7441	0.1529	0.0003 ± 0.0008	0.0032 ± 0.0056	0.0064 ± 0.0063	0.2335	0.0111	0.2609
BD	Crest Bacteria Blast	0.0000 ± 0.0000	0.0018 ± 0.0034	0.0055 ± 0.0058	0.1088	0.0056	0.0922	0.3382 ± 0.3992	0.0263 ± 0.0912	0.0393 ± 0.1098	0.0817	0.1457	0.7464	0.0007 ± 0.0013	0.0015 ± 0.0021	0.0027 ± 0.0054	0.4127	0.8114	0.7928
BE	Clean Mint Withening Alcohol Free Rinse	0.0001 ± 0.0003	0.0024 ± 0.0049	0.0013 ± 0.0023	0.1524	0.1615	0.9208	0.0000 ± 0.0000	0.0617 ± 0.2136	0.0953 ± 0.1893	0.5633	0.0715	0.2644	0.0247 ± 0.0513	0.0091 ± 0.0181	0.0183 ± 0.0084	0.5967	0.0543	0.0210
BF	Thera Breath Dentist Formulated Fresh Breath Oral Rinse	0.0047 ± 0.0061	0.0165 ± 0.0074	0.0731 ± 0.0299	0.0018	0.0001	0.0002	0.0001 ± 0.0003	0.0008 ± 0.0012	0.0096 ± 0.0131	0.1751	0.0011	0.0013	0.0256 ± 0.0204	0.0377 ± 0.0196	0.1383 ± 0.0708	0.1492	0.0002	0.0004
BG	Listerine Sensitivity Zero Alcohol	0.0248 ± 0.0065	0.0312 ± 0.0056	0.0339 ± 0.0057	0.0199	0.0028	0.3838	0.0872 ± 0.1838	0.1287 ± 0.2647	0.1656 ± 0.2311	0.7726	0.2375	0.5683	0.0000 ± 0.0000	0.0017 ± 0.0025	0.0105 ± 0.0049	0.0647	0.0002	0.0008
BH	Splendid White Whitening Mouth Rinse Alcohol Free Up and Up	0.0003 ± 0.0009	0.0001 ± 0.0003	0.0058 ± 0.0086	1.0000	0.0705	0.0705	0.3113 ± 0.5131	0.0607 ± 0.1435	0.1398 ± 0.4187	0.3605	0.5217	0.8515	0.0027 ± 0.0052	0.0188 ± 0.0344	0.0193 ± 0.0157	0.2280	0.0108	0.3842
BI	Crest Scope Classic	0.0323 ± 0.0072	0.0400 ± 0.0128	0.0459 ± 0.0155	0.2439	0.0528	0.3554	0.2273 ± 0.3308	0.0153 ± 0.0045	0.0815 ± 0.2419	0.0307	0.0627	0.3474	0.0138 ± 0.0245	0.0113 ± 0.0067	0.0164 ± 0.0106	0.3372	0.1182	0.1868
BJ	BR Rinse Organic Mouthwash	0.0000 ± 0.0000	0.0065 ± 0.0067	0.0070 ± 0.0067	0.0056	0.0023	0.7826	0.0031 ± 0.0107	0.0000 ± 0.0000	0.0002 ± 0.0004	0.5633	0.7950	0.3474	0.0286 ± 0.0129	0.0388 ± 0.0129	0.0806 ± 0.0892	0.1189	0.0069	0.3127
BK	Tom's Sea Salt	0.0213 ± 0.0117	0.2081 ± 0.0518	0.2639 ± 0.0849	0.0001	0.0006	0.0045	0.0007 ± 0.0010	0.0161 ± 0.0071	0.0169 ± 0.0084	0.0013	0.0013	0.6802	0.1266 ± 0.0433	0.5575 ± 0.1905	0.5887 ± 0.1493	0.0002	0.0002	0.1717
BL	CloSYS	0.0092 ± 0.0102	0.0433 ± 0.0177	0.1436 ± 0.0319	0.0006	0.0001	0.0001	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0070 ± 0.0020	1.0000	0.0011	0.0011	0.0543 ± 0.0108	0.0793 ± 0.0149	0.4003 ± 0.1001	0.0020	0.0002	0.0002
BM	Antiseptic Mouthwash Antigingivits Antiplaque	0.0138 ± 0.0109	0.2630 ± 0.0258	0.4370 ± 0.0318	0.0001	0.0001	0.0001	0.0042 ± 0.0057	0.0166 ± 0.0051	0.0191 ± 0.0126	0.0091	0.0081	0.8201	0.0018 ± 0.0034	0.2778 ± 0.1280	0.4259 ± 0.1011	0.0002	0.0002	0.0069
BN	Crest Gum and Breath Purify	0.0990 ± 0.0509	0.1945 ± 0.0341	0.1546 ± 0.0181	0.0012	0.0041	0.0020	0.2068 ± 0.5178	0.0471 ± 0.1178	0.1638 ± 0.2554	0.7441	0.0657	0.0249	0.0142 ± 0.0264	0.1291 ± 0.0189	0.0907 ± 0.0195	0.0002	0.0002	0.0018
BO	Swan Antiseptic Mouth Rinse Original	0.1229 ± 0.1514	0.2334 ± 0.0333	0.3453 ± 0.0624	0.0636	0.0068	0.0001	0.0094 ± 0.0063	0.0193 ± 0.0037	0.0118 ± 0.0032	0.0119	0.4849	0.0078	0.0619 ± 0.1251	0.2994 ± 0.1266	0.5092 ± 0.1487	0.0029	0.0002	0.0012
BP	Swan Antiseptic Mouth Rinse Spring Mint	0.0295 ± 0.0105	0.2628 ± 0.0318	0.3558 ± 0.0456	0.0001	0.0001	0.0005	0.0129 ± 0.0042	0.1803 ± 0.4167	0.0149 ± 0.0028	0.0092	0.4598	0.0140	0.0338 ± 0.0518	0.3733 ± 0.0807	0.4962 ± 0.0808	0.0002	0.0002	0.0069
BQ	Advanced Mouth Rinse	0.0531 ± 0.0107	0.1867 ± 0.0316	0.3637 ± 0.0386	0.0001	0.0001	0.0001	0.1234 ± 0.3353	0.0253 ± 0.0087	0.0248 ± 0.0083	0.1213	0.1712	0.7904	0.0013 ± 0.0021	0.3102 ± 0.0843	0.6015 ± 0.1600	0.0002	0.0002	0.0002
BR	Crest 3D White Brilliance	0.0095 ± 0.0068	0.0188 ± 0.0110	0.0281 ± 0.0111	0.0445	0.0015	0.0761	0.0003 ± 0.0006	0.1348 ± 0.3502	0.1416 ± 0.3431	0.4598	0.1800	0.7464	0.0037 ± 0.0059	0.0008 ± 0.0012	0.0027 ± 0.0053	0.3127	0.7102	0.6412
BS	Multi-Action Alcohol Free Antiseptic Mouth Rinse	0.0128 ± 0.0281	0.1434 ± 0.0174	0.1089 ± 0.0166	0.0001	0.0001	0.0005	0.0735 ± 0.0680	0.1471 ± 0.2430	0.0183 ± 0.0278	0.7464	0.0116	0.0116	0.0000 ± 0.0000	0.0527 ± 0.0240	0.0332 ± 0.0084	0.0002	0.0002	0.0579
BT	BR Rinse Organic Mouthwash	0.0143 ± 0.0158	0.0145 ± 0.0151	0.0128 ± 0.0176	0.8956	0.8580	0.5544	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	1.0000	1.0000	1.0000	0.0250 ± 0.0120	0.1058 ± 0.2472	0.1456 ± 0.2747	0.0735	0.2738	0.5656
BU	Guru Nanda Oxyburst Whitening Technology	0.0065 ± 0.0087	0.0083 ± 0.0118	0.0158 ± 0.0152	1.0000	0.1557	0.1582	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	1.0000	1.0000	1.0000	0.0312 ± 0.0512	0.0138 ± 0.0087	0.0195 ± 0.0114	0.3127	0.9699	0.3456
BV	PerCara Mouthwash and Gargle Refresh Mint	0.0319 ± 0.0162	0.0427 ± 0.0164	0.0458 ± 0.0126	0.1138	0.0237	0.5540	0.0353 ± 0.0643	0.1578 ± 0.2609	0.1478 ± 0.4424	0.5631	0.1457	0.1712	0.0337 ± 0.0140	0.0311 ± 0.0307	0.0302 ± 0.0083	0.2629	0.9699	0.2390
BW	Spry Oral Rinse (Dental Defense System)	0.2678 ± 0.0357	0.3589 ± 0.0164	0.3434 ± 0.0201	0.0001	0.0003	0.0808	0.0253 ± 0.0330	0.0112 ± 0.0134	0.0118 ± 0.0117	0.0524	0.1587	0.8354	0.2572 ± 0.1076	0.4943 ± 0.1176	0.5002 ± 0.1082	0.0009	0.0008	0.9851
BX	Desert Essence Tea Tree Oil Whitening Plus Mouthwash	0.2611 ± 0.0233	0.3649 ± 0.0274	0.4193 ± 0.0239	0.0001	0.0001	0.0012	0.1318 ± 0.4523	0.0408 ± 0.0880	0.1211 ± 0.2032	0.0211	0.0293	0.9791	0.2937 ± 0.1216	0.5083 ± 0.1251	0.4281 ± 0.0688	0.0029	0.0111	0.1002
BY	Desert Essence Prebiotic Plant Based Brushing Rinse Mint	0.2364 ± 0.0128	0.3671 ± 0.0160	0.4167 ± 0.0251	0.0001	0.0001	0.0003	0.1438 ± 0.4766	0.0144 ± 0.0021	0.0111 ± 0.0081	0.0965	0.5468	0.0524	0.3218 ± 0.0858	0.4506 ± 0.0676	0.5852 ± 0.0598	0.0029	0.0002	0.0007
BZ	Smart Mouth Sore Zinc Activatted Oral Rinse	0.0061 ± 0.0066	0.0132 ± 0.0086	0.0484 ± 0.0140	0.0991	0.0001	0.0002	0.0005 ± 0.0012	0.0152 ± 0.0354	0.0045 ± 0.0131	0.5468	0.4373	0.9791	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0846 ± 0.0185	1.0000	0.0002	0.0002
CA	Smart Mouth Original	0.0021 ± 0.0033	0.0158 ± 0.0128	0.0629 ± 0.0138	0.0006	0.0001	0.0002	0.0005 ± 0.0017	0.0101 ± 0.0340	0.3124 ± 0.7273	0.7464	0.0116	0.0293	0.0027 ± 0.0092	0.0175 ± 0.0267	0.1188 ± 0.0317	0.0106	0.0002	0.0003
CB	Smart Mouth Clinical Zinc Activated Oral Rinse	0.0078 ± 0.0088	0.0303 ± 0.0157	0.0240 ± 0.0103	0.0014	0.0015	0.3291	0.6565 ± 0.8365	0.0554 ± 0.1600	0.4536 ± 0.7321	0.9791	0.8869	0.7441	0.0002 ± 0.0006	0.1144 ± 0.2429	0.0222 ± 0.0128	0.0003	0.0002	0.3752
CC	Parodontax Active Gum Health Clear Mint	0.0109 ± 0.0210	0.1335 ± 0.0166	0.1028 ± 0.0168	0.0001	0.0001	0.0015	0.2783 ± 0.3606	0.1373 ± 0.4276	0.2369 ± 0.4129	0.0116	0.1977	0.9027	0.0000 ± 0.0000	0.0701 ± 0.0254	0.0558 ± 0.0129	0.0002	0.0002	0.3372

Appendix 3: MBC and percentage of reduction of values compared with the negative control, and the statistical difference in the first dilution (1:3) of each mouthwash with other for the Total absorbance and biofilm analysis.

Code	MBC	Total absorbance analys 1:3 dilution		Biofilm analysis 1:3 dilution	
		% of reduction comparative with negative control	Statistical diference p value < 0.05; (code of the other mouthwash)	% of reduction comparative with negative control	Statistical diference p value < 0.05; (code of the other mouthwash)
A	>1:12	90.20	B, C, D, E, F, G, H, I, K, L, M, N, O, R, S, T, U, V, W, X, Y, AA, AB, AC, AE, AF, AH, AI, AJ, AK, AL, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	98.42	B, D, E, F G, J, L, M, N, Q, R, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AK, AM, AN, AP, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BG, BH, BJ, BK, BL, BM, BQ, BR, BS, BT, BV, BW, BX, BY, BZ, CA, CB, CC
B	<1:3	40.56	A, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BY, BZ, CA, CB, CC	42.34	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, T, U, V, W, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW AY AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
C	>1:12	99.06	A, B, D, I, J, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AX, AZ, BA, BB, BC, BG, BI, BK, BM, BN, BO, BP, BQ, BV, BW, BX, BY	93.80	B, D, E, F, G, L, Q, R, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AK, AM, AP, AS, AT AU, AV, AW, AX, AY, BA, BB, BC, BD, BG, BH, BK, BL, BM, BQ, BS, BW, BX, BY, BZ, CA, CB, CC
D	>1:12	95.77	A, B, C, E, F, G, H, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BI, BJ, BL, BN, BP, BQ, BR, BS, BU, BV, BW, BX, BY, BZ, CA, CB	99.98	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AR, AS, AT AU, AV, AW, AX, AY AZ, BA, BB, BF, BI, BJ, BK, BL, BM, BP, BT, BU, BV, BW, BX, BY
E	>1:12	100.00	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BF, BG, BI, BK, BL, BM, BN, BO, BP, BQ, BR, BT, BU, BV, BW, BX, BY, BZ, CB, CC	100.00	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT AU, AV, AW, AX, AY, AZ, BA, BB, BE, BF, BI, BJ, BK, BL, BM, BO, BP, BR, BT, BU, BV, BW, BX, BY
F	>1:12	99.11	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AX, AZ, BA, BB, BC, BG, BI, BK, BM, BN, BO, BP, BQ, BR, BV, BW, BX, BY	100.00	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BE, BF, BI, BJ, BK, BL, BO, BP, BR, BT, BU, BV, BW, BX, BY
G	>1:12	100.00	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BF, BG, BI, BK, BL, BM, BN, BO, BP, BQ, BR, BT, BU, BV, BW, BX, BY, BZ, CB, CC	100.00	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT AU, AV, AW, AX, AY AZ, BA, BB, BE, BF, BI, BJ, BK, BL, BO, BP, BR, BT, BU, BV, BW, BX, BY
H	<1:3	98.54	A, B, D, E, G, I, J, L, M, N, O, P, Q, R, S, T, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AX, AY, AZ, BA, BB, BC, BD, BE, BG, BH, BI, BJ, BK, BN, BO, BP, BQ, BV, BW, BX	97.45	B, D, E, F, G, L, M, N, R, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AK, AM, AN,AP, AS, AT AU, AV, AW, AX, AY, BA, BB, BC, BD,BG, BJ, BK, BL, BQ, BS, BV, BW, BX, BY, BZ, CB, CC
I	<1:3	93.07	A, B, C, D, E, F, G, H, J, K, L, N, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AP, AQ, AR, AS, AT, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BJ, BK, BL, BM, BN, BQ, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	83.33	B, D, E, F, G, I, J, L, M, Q, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AK, AM, AN, AP, AS, AT AU, AV, AW, AX, AY, BA, BB, BC, BD, BG, BH, BK, BL, BM, BQ, BR, BS, BV, BW, BX, BY, BZ, CA, CB, CC
J	<1:6	90.08	B, C, D, E, F, G, H, I, K, L, M, N, O, R, S, T, U, V, W, X, Y, AA, AB, AC, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	99.58	A, B, I, K, L, M, N, P, R, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AH, AI, AK, AL, AM, AN, AO, AP, AR, AS, AT AU, AV, AW, AX, AY AZ, BA, BB, BF, BJ, BK, BL, BP, BT, BU, BV, BW, BX, BY	
K	>1:12	97.29	A, B, E, F, G, I, J, L, M, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AQ, AS, AT, AU, AV, AX, AY, BA, BB, BD, BE, BG, BH, BI, BJ, BN, BO, BP, BQ, BS, BV, BW, BX, BY, CA	98.81	B, D, E, F, G, J, L, M, N, P, Q, R, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, ,AK, AL, AM, AN, AO, AP, AS, AT AU, AV, AW, AX, AY AZ, BA, BB, BC, BD, BF, BG, BH, BJ, BK, BL, BM, BQ, BR, BS, BT, BV, BW, BX, BY, BZ, CA, CB, CC	
L	<1:6	65.78	A, B, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	49.19	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BZ, CA, CB, CC	
M	<1:6	94.60	A, B, C, E, F, G, H, J, K, L, N, P, R, S, T, V, W, Y, Z, AA, AB, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BJ, BL, BN, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC	94.76	A, B, C, D, E, F, G, H, I, J, K, L, N, O, Q, S, U, V, W, Y, AA, AB, AC, AD, AE, AF, AG, AH, AJ ,AK, AL, AO, AP, AQ, AR, AS, AT, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BO, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC	
N	>1:12	86.59	A, B, C, D, E, F, G, H, I, J, K, L, M, O, Q, R, S, U, V, W, X, Y, Z, AC, AE, AF, AH, AJ, AL, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	97.26	A, B, D, E, F, G, J, K, L, M, N, Q, S, T, V, W, Z, AA, AB, AD, AE, AF, AG, AH, AI, AJ ,AK, AL, AM, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BQ, BR, BS, BV, BW, BX, BY, BZ, CA, CB, CC	
O	>1:3	93.95	A, B, C, E, F, G, H, J, L, N, P, R, S, T, V, W, Y, Z, AA, AB, AE, AF, AG, AH, AI, AK, AL, AM, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BJ, BL, BN, BQ, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB	98.90	B, L, M, P, R, S, T, W, Z, AA, AD, AE, AF,AH, AI, AK, AM, AN, AP, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BJ, BK, BL, BT, BV, BW, BX, BY	
P	>1:12	90.20	B, C, D, E, F, G, H, K, L, M, O, R, S, U, V, W, X, Y, AA, AB, AC, AE, AF, AH, AI, AJ, AK, AL, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BJ, BK, BL, BM, BN, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	96.48	B, D, E, F, G, J, K, L, O, Q, S, T, V, W, AA, AD, AE, AF, AG, AH, AI, AJ ,AK, AM, AQ, AS, AT, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BK, BL, BM, BN, BQ, BR, BS, BW, BX, BY, BZ, CA, CB, CC	
Q	<1:3	91.16	B, C, D, E, F, G, H, K, L, N, R, S, T, U, V, W, X, Y, AA, AB, AC, AE, AF, AG, AH, AI, AJ, AK, AL, AP, AR, AS, AT, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BH, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	99.23	A, B, C, I, L, M, N, P, R, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BI, BJ, BK, BL, BO, BP, BR, BT, BU, BV, BW, BX, BY	
R	>1:12	100.00	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BF, BG, BI, BK, BL, BM, BN, BO, BP, BQ, BR, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	95.27	A, B, C, D, E, F, G, H, J, K, L, O, Q, S, T, U, V, W, Y, AA, AB, AC, AD, AE, AF, AG, AH, AJ ,AK, AL, AO, AQ, AR, AS, AT, AV, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BQ, BR, BS, BW, BX, BY, BZ, CA, CB, CC	
S	<1:3	45.12	A, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, X, Y, Z, AA, AB, AC, AD, AE, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC	31.23	A, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AG, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BX, BW, BY, BZ, CA, CB, CC	
T	<1:3	75.07	A, B, C, D, E, F, G, H, I, J, K, L, M, O, Q, R, S, U, V, W, X, Y, Z, AC, AE, AF, AH, AJ, AL, AN, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	82.53	A, B, C, D, E, F, G, H, I, J, K, L, N, O, P, Q, R, S, T, V, W, X, Y, AB, AC, AE, AF, AG, AJ ,AK, AL, AN, AO, AQ, AR, AT, AV, AX, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	
U	>1:12	96.40	A, B, C, E, F, G, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BH, BI, BJ, BN, BP, BQ, BV, BW, BX, BY, CA	97.92	B, D, E, F, G, J, L, M, Q, R, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, ,AK, AM, AP, AS, AT, AU, AV, AW, AX, AY, BA, BB, BC, BD, BG, BH, BK, BL, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	
V	>1:12	100.00	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BF, BG, BI, BK, BL, BM, BN, BO, BP, BQ, BR, BT, BU, BV, BW, BX, BY, BZ, CB, CC	100.00	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BE, BF, BI, BJ, BK, BL, BM, BO, BP, BR, BT, BU, BV, BW, BX, BY	
W	<1:3	54.70	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, X, Y, Z, AA, AB, AC, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BZ, CA, CB, CC	67.53	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, X, Y, Z, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC	
X	>1:12	96.10	A, B, C, E, F, G, H, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BI, BJ, BL, BN, BP, BQ, BR, BS, BU, BV, BW, BX, BY, BZ, CA	94.37	B, D, E, F, G, J, L, Q, S, T, V, W, AA, AD, AE, AF, AG, AH, AI, AK, AP, AQ, AS, AT, AU, AV, AW, AX, BA, BB, BC, BD, BG, BK, BL, BS, BV, BW, BX, BY, BZ, CB, CC	
Y	>1:12	99.35	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BG, BI, BK, BL, BM, BN, BO, BP, BQ, BR, BV, BW, BX, BY, BZ, CB	98.45	B, D, E, F, G, L, M, R, S, T, V, W, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	
Z	>1:12	90.31	B, C, D, E, F, G, H, I, K, L, M, N, O, R, S, T, U, V, W, X, Y, AA, AB, AC, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	95.81	A, B, C, D, E, F, G, H, I, J, K, L, N, O, Q, S, U, V, W, Y, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ ,AK, AL, AO, AQ, AR, AS, AT, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC	
AA	<1:3	81.81	A, B, C, D, E, F, G, H, I, J, K, L, M, O, P, Q, R, S, U, V, W, X, Y, Z, AC, AE, AF, AG, AI, AM, AN, AO, AQ, AR, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	77.23	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, U, V, X, Y, Z, AB, AC, AE, AF, AG, AJ ,AK, AL, AM, AN, AO, AP, AQ, AR, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BX, BY, BZ, CA, CB, CC	
AB	<1:3	84.96	A, B, C, D, E, F, G, H, I, J, K, L, M, O, P, Q, R, S, U, V, W, X, Y, Z, AC, AD, AE, AF, AG, AH, AI, AL, AM, AN, AO, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	97.86	B, D, E, F, G, J, L, M, N, Q, R, S, U, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AK, AM, AN, AP, AQ, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BG, BH, BJ, BK, BL, BM, BQ, BR, BS, BV, BW, BX, BY, BZ, CA, CB, CC	
AC	>1:12	95.59	A, B, C, E, F, G, H, I, J, K, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BI, BJ, BL, BN, BP, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC	98.02	B, D, E, F, G, J, L, M, Q, R, S, U, V, W, Z, AA, AB, AD, AE, AF, AG, AH, AI ,AK, AM, AN, AP, AQ, AS, AT, AU, AV, AW, AX, AY, BA, BB, BC, BD, BE, BG, BH, BJ, BK, BL, BM, BQ, BR, BS, BV, BW, BX, BY, BZ, CA, CB, CC	
AD	<1:3	71.36	B, C, D, E, F, G, H, K, R, S, U, V, X, Y, AB, AC, AE, AF, AH, AI, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BH, BJ, BK, BL, BM, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	64.68	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, U, V, X, Y, Z, AB, AC, AE, AG, AI, AL, AM, AN, AO, AP, AQ, AR, AU, AW, AY, AZ, BC, BD, BE, BF, BG, BH, BI, BJ, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC	
AE	<1:3	31.94	A, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	13.12	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AF, AG, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	
AF	<1:3	40.56	A, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC	49.51	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, T, U, V, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AY, AZ, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC	
AG	>1:12	87.42	B, C, D, E, F, G, H, I, J, K, L, M, O, Q, R, S, U, V, W, X, Y, Z, AA, AB, AC, AE, AF, AH, AI, AK, AL, AN, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	99.95	A, B, C, H, I,K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ ,AK, AL, AM, AN, AO, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BI, BJ, BK, BL, BP, BT, BU, BV, BW, BX, BY	
AH	>1:12	71.44	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AB, AC, AD, AE, AF, AG, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AT, AU, AV, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	80.22	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S,U, V, W, X, Y, Z, AB, AC, AE, AF, AG, AJ, AL, AM, AN, AO, AP, AQ, AR, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ,BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	
AI	<1:3	83.50	A, B, C, D, E, F, G, H, I, J, K, L, M, O, P, Q, R, S, U, V, W, X, Y, Z, AC, AE, AF, AH, AJ, AN, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	86.96	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, S, U, V, W, X, Y, Z, AB, AC, AE, AF, AG, AJ ,AK, AL, AN, AO, AQ, AR, AS, AT, AV, AX, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	
AJ	>1:12	95.07	A, B, C, E, F, G, H, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BG, BH, BI, BN, BP, BQ, BS, BU, BV, BW, BX, BY, BZ, CA, CB	98.91	B, D, E, F, G, L, M, N, P, Q, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AK, AM, AN, AO, AP, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BF, BG, BJ, BK, BL, BQ, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	
AK	<1:3	82.65	A, B, C, D, E, F, G, H, I, J, K, L, M, O, P, Q, R, S, U, V, W, X, Y, Z, AC, AE, AF, AG, AH, AI, AM, AN, AO, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	58.02	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AU, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC	
AL	<1:3	82.45	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AB, AC, AE, AF, AG, AH, AJ, AM, AN, AO, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	98.67	B, D, E, F, G, J, L, M, N, Q, R, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AK, AM, AN, AP, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BF, BG, BH, BJ, BK, BL, BM, BQ, BR, BS, BT, BV, BW, BX, BY, BZ, CA, CB, CC	

AM	<1:3	88.54	B, C, D, E, F, G, H, I, J, K, L, M, O, R, S, U, V, W, X, Y, Z, AA, AB, AC, AE, AF, AH, AJ, AK, AL, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	92.26	A, B, C, D, E, F, G, H, I, J, K, L, N, O, P, Q, S, U, V, W, Y, AA, AB, AC, AD, AE, AF, AG, AH, AJ, AK, AL, AN, AO, AQ, AR, AS, AT, AV, AX, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC
AN	>1:12	89.90	B, C, D, E, F, G, H, I, K, L, M, R, S, T, U, V, W, X, Y, AA, AB, AC, AE, AF, AG, AH, AI, AJ, AK, AL, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	96.47	A, B, D, E, F, G, H, I, J, K, L, O, Q, S, T, V, W, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AO, AP, AQ, AR, AS, AT, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
AO	>1:12	89.80	B, C, D, E, F, G, H, K, L, M, O, R, S, U, V, W, X, Y, AA, AB, AC, AE, AF, AH, AJ, AK, AL, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	98.06	B, D, E, F, G, J, K, L, M, N, Q, R, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AJ, AK, AM, AN, AP, AQ, AS, AT, AU, AV, AW, AX, AY, BA, BB, BC, BD, BG, BH, BI, BJ, BK, BL, BM, BQ, BR, BS, BT, BV, BW, BX, BY, BZ, CA, CB, CC
AP	>1:12	84.47	A, B, C, D, E, F, G, H, I, J, K, L, M, O, P, Q, R, S, U, V, W, X, Y, Z, AC, AE, AF, AH, AJ, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	94.54	A, B, C, D, E, F, G, H, I, J, K, L, N, O, Q, S, U, V, W, X, Y, AA, AB, AC, AD, AE, AF, AG, AH, AJ, AK, AL, AN, AO, AQ, AR, AS, AT, AV, AX, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BM, BN, BQ, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC
AQ	>1:12	94.97	A, B, C, E, F, G, H, I, J, K, L, N, P, R, S, T, V, W, Y, Z, AA, AB, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BI, BJ, BL, BM, BN, BP, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC	91.13	B, E, F, G, H, I, J, K, L, M, N, P, R, S, T, V, W, X, Z, AA, AB, AC, AD, AE, AF, AH, AI, AK, AM, AN, AO, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BG, BJ, BK, BL, BS, BT, BU, BV, BW, BX, BY, BZ, CB, CC
AR	>1:12	95.99	A, B, C, E, F, G, H, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AC, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BG, BH, BI, BJ, BL, BN, BP, BQ, BR, BS, BU, BV, BW, BX, BY, BZ, CA, CB	96.53	B, D, E, F, G, J, L, M, N, Q, R, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AK, AM, AN, AP, AQ, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BG, BH, BJ, BK, BL, BM, BQ, BR, BS, BV, BW, BX, BY, BZ, CA, CB, CC
AS	>1:12	77.15	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AB, AC, AD, AE, AF, AG, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	77.76	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AB, AC, AE, AF, AG, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
AT	<1:3	51.70	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BZ, CA, CB, CC	42.47	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
AU	<1:6	95.19	A, B, C, E, F, G, H, J, K, L, N, P, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BJ, BL, BN, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB	92.99	A, B, C, D, E, F, G, H, I, J, K, L, N, O, Q, S, U, V, W, X, Y, AA, AB, AC, AD, AE, AF, AG, AH, AJ, AK, AL, AO, AQ, AR, AS, AT, AV, AX, BA, BB, BC, BD, BE, BF, BG, BH, BI, BK, BM, BN, BO, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC
AV	<1:3	51.57	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BZ, CA, CB, CC	39.03	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, T, U, V, W, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
AW	<1:3	98.07	A, B, E, G, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BG, BH, BI, BJ, BN, BO, BP, BQ, BV, BW, BX, BY, CA	91.73	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, S, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AJ, AK, AL, AN, AO, AQ, AR, AS, AT, AV, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
AX	<1:3	65.87	A, B, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	47.73	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BZ, CA, CB, CC
AY	>1:12	99.77	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AG, AH, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BG, BI, BK, BL, BM, BN, BO, BP, BQ, BR, BT, BV, BW, BX, BY, BZ, CB	94.72	A, B, C, D, E, F, G, H, I, J, K, L, N, O, Q, S, U, V, W, Y, AA, AB, AC, AD, AE, AF, AG, AH, AJ, AK, AL, AO, AQ, AR, AS, AT, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BO, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC
AZ	<1:3	96.33	A, B, C, E, F, G, H, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BI, BJ, BN, BP, BQ, BS, BU, BV, BW, BX, BY, BZ, CA, CB	95.91	A, B, D, E, F, G, J, K, L, O, Q, S, T, V, W, AA, AB, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AP, AQ, AR, AS, AT, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BQ, BR, BS, BW, BX, BY, BZ, CA, CB, CC
BA	<1:3	64.68	A, B, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	61.16	A, B, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC
BB	<1:3	46.22	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, X, Y, Z, AA, AB, AC, AD, AE, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC	34.84	A, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, T, U, V, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AU, AW, AY, AZ, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
BC	>1:12	96.42	A, B, C, E, F, G, H, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BG, BH, BI, BJ, BN, BP, BQ, BS, BU, BV, BW, BX, BY, BZ, CA, CB	99.96	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BI, BJ, BK, BL, BO, BP, BR, BT, BU, BV, BW, BX, BY
BD	>1:12	100.00	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BF, BG, BI, BK, BL, BM, BN, BO, BP, BQ, BR, BT, BU, BV, BW, BX, BY, BZ, CB, CC	99.91	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BI, BJ, BK, BL, BP, BT, BU, BV, BW, BX, BY
BE	>1:12	99.98	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BF, BG, BI, BK, BL, BM, BN, BO, BP, BQ, BR, BT, BU, BV, BW, BX, BY, BZ, CB, CC	96.82	B, E, F, G, L, M, N, P, R, S, T, V, W, X, Z, AA, AB, AC, AD, AE, AF, AH, AI, AK, AM, AN, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BG, BJ, BK, BL, BS, BT, BU, BV, BW, BX, BY, BZ, CB, CC
BF	>1:12	98.99	A, B, D, E, G, I, J, L, M, N, O, P, Q, R, S, T, V, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AX, AZ, BA, BB, BC, BD, BE, BG, BH, BI, BJ, BK, BM, BN, BO, BP, BQ, BV, BW, BX, BY	96.70	B, D, E, F, G, J, K, L,Q, S, T, V, W, AA, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AP, AQ, AS, AT, AU, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BQ, BR, BS, BW, BX, BY, BZ, CA, CB, CC
BG	>1:12	94.63	A, B, C, E, F, G, H, I, J, K, L, N, P, R, S, T, V, W, Y, Z, AA, AB, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AR, AS, AT, AV, AW, AX, AY, BA, BB, BC, BD, BE, BF, BH, BI, BJ, BL, BM, BN, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC	100.00	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BE, BF, BI, BJ, BK, BL, BM, BO, BP, BR, BT, BU, BV, BW, BX, BY
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BJ	>1:12	100.00	A, B, D, H, I, J, K, L, M, N, O, P, Q, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AZ, BA, BB, BC, BF, BG, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BT, BU, BV, BW, BX, BY, BZ, CB, CC	96.31	A, B, D, E, F, G, H, J, K, L, O, Q, S, T, V, W, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AO, AP, AQ, AR, AS, AT, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BQ, BR, BS, BW, BX, BY, BZ, CA, CB, CC
BK	<1:3	95.39	A, B, C, E, F, G, H, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AJ, AK, AL, AM, AN, AO, AP, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BH, BI, BJ, BL, BN, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB	83.67	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, U, V, W, X, Y, Z, AB, AC, AE, AF, AG, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ,BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
BL	>1:12	98.01	A, B, D, E, G, I, J, L, M, N, O, P, Q, R, S, T, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AX, AY, BA, BB, BD, BE, BG, BH, BI, BJ, BK, BM, BN, BO, BP, BQ, BV, BW, BX, BY	92.99	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AJ, AK, AL AN, AO, AQ, AR, AS, AT, AV, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BM, BN, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
BM	<1:3	97.02	A, B, C, E, F, G, I, J, L, N, P, Q, R, S, T, V, W, Y, Z, AA, AB, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AQ, AS, AT, AV, AX, AY, BA, BB, BD, BE, BF, BG, BH, BI, BJ, BN, BP, BQ, BS, BV, BW, BX, BY, BZ, CA	99.77	A, B, C, E, F, I, K, L, M, N, P, R, S, T, V, W, X, Z, AA, AB, AC, AD, AE, AF, AH, AI, AK, AL, AM, AN, AO, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BG, BI, BJ, BK, BL, BP, BS, BT, BU, BV, BW, BX, BY, BZ, CB, CC
BN	>1:12	78.52	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, U, V, W, X, Y, Z, AC, AE, AF, AG, AJ, AM, AN, AO, AQ, AR, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BP, BQ, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	98.19	B, L, M, N, P, R, S, T, W, X, Z, AA, AD, AE, AF, AH, AI, AK, AM, AN, AP, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BJ, BK, BL, BT, BV, BW, BX, BY
BO	<1:3	73.34	B, C, E, F, G, H, K, R, S, V, Y, AE, AF, AT, AV, AW, AY, BB, BD, BE, BF, BH, BJ, BL, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	92.01	B, E, F, G, L, M, Q, S, T, V, W, AA, AD, AE, AF, AH, AI, AK, AM,AS, AT, AU, AV, AW, AX, AY, BA, BB, BC, BG, BK, BS, BW, BX, BY, BZ, CA, CB, CC
BP	<1:3	93.60	A, B, C, D, E, F, G, H, J, K, L, N, Q, R, S, T, U, V, W, X, Y, Z, AA, AB, AC, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AP, AQ, AR, AS, AT, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BH, BJ, BL, BM, BN, BQ, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	95.63	B, D, E, F, G, J, L, Q, S, T, V, W, AA, AD, AE, AF, AG, AH, AI, AK, AM, AS, AT, AV, AW, AX, BA, BB, BC, BD, BG, BH, BK, BL, BM, BQ, BR, BS, BW, BX, BY, BZ, CA, CB, CC
BQ	<1:6	88.49	B, C, D, E, F, G, H, I, K, L, M, O, R, S, U, V, W, X, Y, AA, AB, AC, AE, AF, AH, AJ, AK, AL, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BP, BR, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC	99.84	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BF, BI, BJ, BK, BL, BP, BT, BU, BV, BW, BX, BY
BR	>1:12	97.94	A, B, D, E, F, G, I, J, L, M, N, O, P, Q, R, S, T, V, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AX, AY, BA, BB, BD, BE, BG, BH, BI, BJ, BK, BN, BO, BP, BQ,BV, BW, BX, BY, CA	99.53	A, B, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, X, Z, AA, AB, AC, AD, AE, AF, AH, AI, AK, AL, AM, AN, AO, AP, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BC, BF, BG, BJ, BK, BL, BP, BS, BT, BU, BV, BW, BX, BY, BZ, CA, CB, CC
BS	>1:12	97.23	A, B, D, E, J, K, L, M, N, O, P, Q, S, T, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AX, AZ, BA, BB, BC, BG, BI, BK, BM, BN, BO, BP, BQ, BV, BW, BX, BY	100.00	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BE, BF, BI, BJ, BK, BL, BM, BO, BP, BR, BT, BU, BV, BW, BX, BY
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BU	>1:12	98.59	A, B, D, E, G, I, J, L, M, N, O, P, Q, R, S, T, V, W, X, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AX, AZ, BA, BB, BC, BD, BE, BG, BH, BI, BJ, BK, BN, BO, BP, BQ, BV, BW, BX, BY	95.98	B, D, E, F, G, J, L, M, Q, S, T, V, W, Z, AA, AD, AE, AF, AG, AH, AI, AJ, AK, AM, AP, AQ, AS, AT, AU, AV, AW, AX, AY, BA, BB, BC, BD, BE, BG, BH, BK, BL, BM, BQ, BR, BS, BV, BW, BX, BY, BZ, CA, CB, CC
BV	>1:12	93.08	A, B, C, D, E, F, G, H, J, K, L, N, R, S, T, U, V, W, X, Y, Z, AA, AB, AE, AF, AG, AH, AI, AJ, AK, AL, AM, AN, AP, AR, AS, AT, AV, AW, AX, AY, AZ, BA, BB, BC, BD, BE, BF, BH, BJ, BL, BM, BN, BQ, BR, BS, BT, BU, BW, BX, BY, BZ, CA, CB, CC	95.66	A, B, D, E, F, G, H, I, J, K, L, N, O, Q, S, U, V, W, Y, AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL, AO, AQ, AR, AS, AT, AV, AW, AX, BA, BB, BC, BD, BE, BG, BH, BI, BK, BL, BM, BN, BQ, BR, BS, BU, BW, BX, BY, BZ, CA, CB, CC
BW	<1:3	41.90	A, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BY, BZ, CA, CB, CC	66.82	A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, X, Y, Z, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC
BX	<1:3	43.37	A, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, T, U, V, W, X, Y, Z, AA, AB, AC, AD, AE, AG, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AW, AX, AY, AZ, BA, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BY, BZ, CA, CB, CC	62.11	A, B, C, D, E, F, G, H, I, J, K, M, N, O, P, Q, R, S, T, U, V, X, Y, Z, AA, AB, AC, AE, AG, AH, AI, AJ, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AY, AZ, BB, BC, BD, BE, BF, BG, BH, BI, BJ, BK, BL, BM, BN, BO, BP, BQ, BR, BS, BT, BU, BV, BZ, CA, CB, CC
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CC	>1:12	97.63	A, B, E, G, I, J, L, M, N, P, Q, R, S, T, V, W, Z, AA, AB, AC, AD, AE, AF, AG, AH, AI, AK, AL, AM, AN, AO, AP, AQ, AS, AT, AV, AX, BA, BB, BD, BE, BG, BH, BI, BJ, BN, BO, BP, BQ, BV, BW, BX, BY	100.00	A, B, C, H, I, K, L, M, N, P, R, S, T, U, W, X, Y, Z, AA, AB, AC, AD, AE, AF, AH, AI, AJ, AK, AL, AM, AN, AO, AP, AQ, AR, AS, AT, AU, AV, AW, AX, AY, AZ, BA, BB, BE, BF, BI, BJ, BK, BL, BM, BO, BP, BR, BT, BU, BV, BW, BX, BY

2.3. Erosion potential of commercial fluoride-free mouthwashes on dental hard tissues

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Short Title: Erosion potential of fluoride-free mouthwashes

ABSTRACT:

Purpose: Mouthwashes often present acidic pH, which raises concerns about their potential contribution to erosive tooth wear. This laboratory study evaluated the erosive potential of commercial fluoride-free mouthwashes using a screening method. **Methods:** A convenience sample of 81 different mouthwashes were evaluated for their erosion potential using a standard method (International Organization for Standardization, ISO/FDIS 28888:2013). This method measured pH changes (ΔpH) of a 25 mL of calcium phosphate solution (baseline pH 5.05±0.05; mean±standard deviation) once the test mouthwash was added (0.25 mL). Three reference citric acid/citrate buffers were included. The maximum allowable ΔpH corresponds to that of the strongest buffer. Each mouthwash was tested four times, and the means and standard deviations were calculated. **Results:** Mouthwashes presented a baseline pH range of 3.00-9.47. The reference solutions resulted in ΔpH of 0.58±0.00, 0.89±0.00, and 1.20±0.01, respectively. None of the mouthwashes resulted in ΔpH higher than the strongest standard. Several unexpected results were observed: I) nine mouthwashes with an initial pH>5.00 caused a pH drop when added to the screening solution; II) one mouthwash with an initial pH of 4.27 caused a pH drop to 4.17 once added to the screening solution; and III) one mouthwash with an initial pH of 4.93 caused a pH increase to 6.18 when added to the screening solution. In conclusion, all tested mouthwashes do not appear to present any erosive potential. Due to the wide range of ingredients used in mouthwash formulations, the test method resulted in unexpected pH changes for more than 10% of the test products. Further development of suitable test methods to evaluate the potential contribution of fluoride-free mouthwashes to erosive tooth wear may be needed.

CLINICAL SIGNIFICANCE Fluoride-free mouthwashes vary greatly in pH, although, based on an ISO screening method, none of the presently tested 81 products were found to have detrimental effects on the dental hard tissues.

Introduction

Erosive tooth wear is a condition that has dental erosion as the primary etiological factor (Schlueter et al., 2019). Dental erosion is process that can lead to dental structure loss, due to the exposure to acids of extrinsic or intrinsic nature. For instance, mouthwashes are extrinsic agents that may present an erosive potential, depending on chemical factors, such as pH, buffering capacity, and the type of acid (Zero, 1996; Lussi et al., 2011; Kanzow et al., 2016). Several types of mouthwash are available worldwide (Van der Weijden et al., 2015; Valdivia-Tapia et al., 2021; Radzki et al., 2022). These are individual/home use oral products that can be sold with or without a prescription. Depending on their formulation and purpose, they may have a therapeutic or cosmetic purpose. Ideally, mouthwashes should present benefits for oral health and, while avoiding adverse effects on the oral soft and hard tissues, when used according to the manufacturer's recommendations.

The American Dental Association (ADA) states that mouthwashes can be formulated with acidic, neutral, or alkaline pH, with permissible pH values ranging from 3.0 and 10.5. For mouthwashes with a pH below 5.5, the American National Standards Institute (ANSI)/ADA Standard 116 calls for further demonstration of product safety, either through a demineralization test, erosion test, or other appropriate methods (ADA, 2010, 2019). Considering the large number of commercially available products and that previous studies only tested a small proportion of these products; it is necessary to evaluate their risk of causing dental erosion. Therefore, this study aimed to evaluate the erosive potential of commercial non-fluoridated mouthwashes on dental hard tissues, using an International Organization for Standardization (ISO) Standard (ISO, 2013).

Material and Methods

Experimental design

A convenience sample of 81 different fluoride-free-mouthwashes (FFM) were purchased in grocery, pharmacy, and variety stores in Indianapolis, IN, USA. The International Standard ISO/FDIS 28888:2013; Dentistry - Screening method for erosion potential of mouthwashes on dental hard tissues as a methodology was used (ISO, 2013). This method is based on measuring pH changes of a calcium phosphate solution (pH = 5.05 ± 0.05; mean ± standard deviation) once the test mouthwash has been added. Three reference solutions, citric acid/citrate buffers, varying in expected pH decreases (0.5; 0.7; 1.0) once added to the calcium phosphate solution, were included. The maximum allowable decrease in pH is 1.0, corresponding to the expected pH change of the strongest buffer. Initial pH measurements of

the undiluted mouthwashes were conducted, followed by measuring said pH changes. Each mouthwash was tested in quadruplicates.

Samples

Eighty-one different FFM were purchased in August 2022 in Indianapolis, IN, USA. Only FFM available in 'brick-and-mortar' stores but not those available online were purchased. One of each grocery and variety store chain, as well as one of each pharmacy chain were visited. No repeat purchases were made, i.e., the same product available in a grocery store was not purchased again in a pharmacy. The rinses were coded with capital letters (A-CC) (Annex 1).

Preparation of reference buffer solutions

Three citric acid/citrate buffers were prepared from sodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) and citric acid ($H_3C_6H_5O_7$): 1) 3.67 mM citric acid and 1.84 mM sodium citrate dihydrate (expected pH 3.60, expected pH decrease once added to screening solution 1.0); 2) 0.93 mM citric acid and 0.39 mM sodium citrate dihydrate (pH 3.68/ΔpH -0.7); and 3) 0.25 mM citric acid and 0.11 sodium citrate dihydrate (pH 3.77/ΔpH -0.5).

Screening solutions

Two stock solutions were prepared as described in the ISO/FDIS 28888:2013 manual (ISO, 2013): Stock solution A. 1 M $CaCl_2$ and 0.03 mM NaN_3; Stock solution B1 M KH_2PO_4 and 0.03 mM NaN_3. The screening solution was prepared by adding 1.266 mL of stock solution A and 0.760 mL of stock solution B to 500 mL of deionized water. The pH was adjusted to 5.05 ± 0.05 with HCl. The solution was then made up to 1 L with deionized water. This solution was prepared fresh daily from the stock solutions.

Test procedure

The test was performed at an ambient temperature (approx. 20 °C). For the screening method, 25 mL of the screening solution were added to a 50 mL reaction vessel. The solution was stirred at a moderately fast rate (100 r/min) and held at this rate throughout the experiment. A pH-electrode (Fisher, accumet® cat. #13-620-631), previously calibrated with standard buffer solutions of pH 4.0 and 7.0 (Fisher Chemical, Fisher Scientific ™), was used. The pH of the screening solution was determined. Then, 250 µl (0.25 ml) of the test material (reference buffer or mouthwash) were added to the screening solution. The reaction was terminated after a steady pH value was recorded. The test was repeated four times for each test material.

Data recording and evaluation

For each test, the pH of the test material (buffer or mouthwash), starting pH of the screening solution, the pH of the screening solution after adding the test material, and the pH change (starting pH minus final pH) were recorded. Furthermore, the pH of each mouthwash was also determined (without dilution). Only descriptive statistics were performed (means, standard deviations).

Results

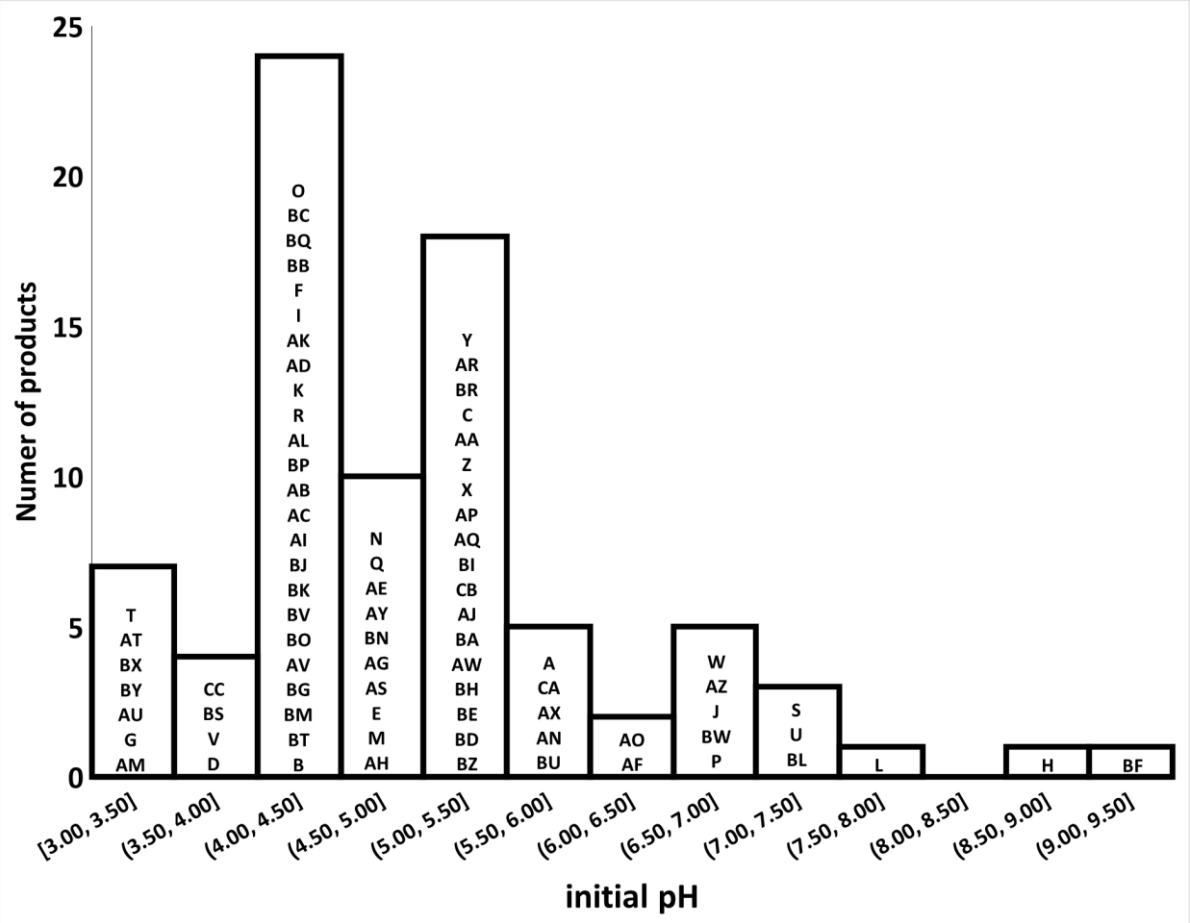

Fig 1: Initial pH distribution of the commercial fluoride-free mouthwashes.

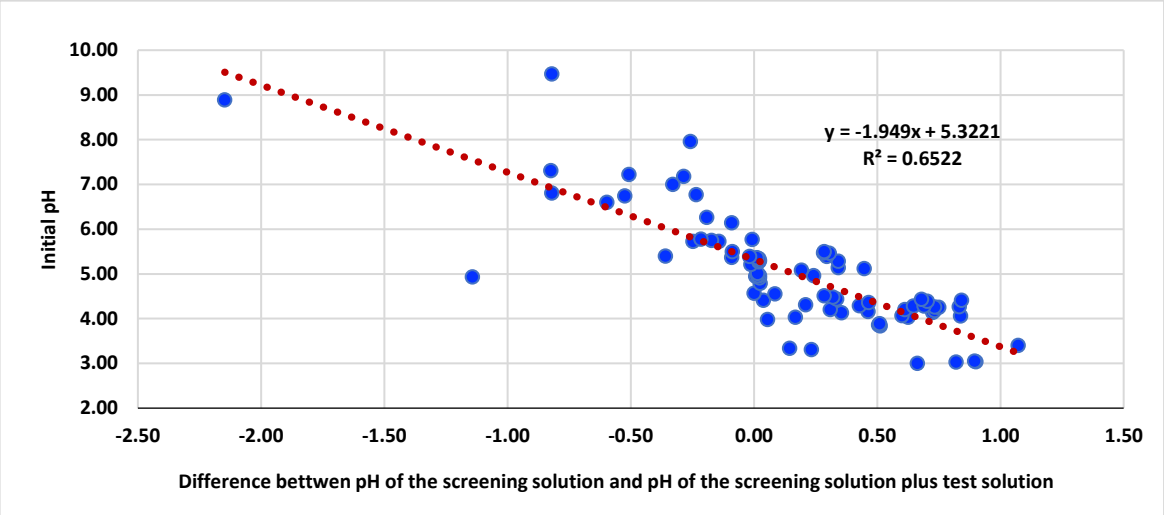

Fig 2: Correlation between the initial pH of the mouthwash with the result value after the ISO-method test.

Table 1 shows the different mouthwashes with their respective codes, initial pH, pH of the used screening solution, pH of the screening solution + mouthwashes, mean and standard deviation of the differences. The initial pH of the mouthwashes ranged from 3.00-9.47, with 63 mouthwashes exhibiting a pH < 5.5. Eleven mouthwashes presented a pH of 3.00 to 4.00, twenty-eight had a pH of 4.01 to 5.00, six had a pH of 4.95 to 5.05, twenty-three had a pH of 5.01 to 6.00, seven had a pH of 6.01 to 7.00, four had a pH of 7.01 – 8.00, one had a pH of 8.01 to 9.00, and one had a pH > 9.01 (Fig. 1). Thirty-two mouthwashes contained benzoic acid, 19 citric acid, six ascorbic acid, and four contained phosphoric acid.

The reference solutions resulted in pH decreases of 0.58 ± 0.00, 0.89 ± 0.00, and 1.20 ± 0.01, respectively (all mean ± standard deviation). There was a moderate correlation between the initial pH of each product and that of the screening solution once the mouthwash was added (Fig. 2). Only one mouthwash (AM) resulted in a pH decrease of more than 1.0 (1.07±0.00).

Several unexpected pH changes were observed: I) nine mouthwashes (C: hydrogen peroxide; M: plant extract; X, BD, BE, BH: hydrogen peroxide and sodium hexametaphosphate; AJ: hydrogen peroxide and sodium hexametaphosphate; AR: cetylpyridinium chloride; BR: sodium hexametaphosphate) with an initial pH > 5.00 caused a pH drop when added to the screening solution; II) one mouthwash (AB: essential oils) with an initial pH of 4.27 caused a pH drop to 4.17 once added to the screening solution; and III) one mouthwash (AG: tetrasodium pyrophosphate) with an initial pH of 4.93 caused a pH increase to 6.18 when added to the screening solution. Their ingredients can be found in table 2.

Table 1. ISO method results. Values found after adding the test solution to the screening solution.

Name	ISO method					
	Code	Initial pH control/mouthwash	A pH screening solution	B pH Screening solution + test solution	Difference A-B	SD
Citric Acid 1 %	1	3.60	5.00	3.80	1.20	0.01
Citric Acid 0.25 %	2	3.68	5.00	4.11	0.89	0.00
Citric Acid 0.07 %	3	3.77	5.00	4.43	0.58	0.00
Close UP Mouthwash with Calcium cinnamon	A	5.73	5.00	5.14	-0.14	0.00
Sea salt oral rinse	B	4.48	5.05	4.73	0.32	0.01
Colgate Optic White High Impact White Advanced	C	5.14	5.00	4.66	0.34	0.01
Colgate Peroxyl	D	3.98	5.06	5.01	0.05	0.00
Oral B Gum Detoxify	E	4.96	5.06	5.04	0.02	0.00
Crest Pro-Health Bacteria Guard	F	4.13	5.06	4.71	0.36	0.00
Crest Pro-Health Clinical	G	3.34	5.06	4.92	0.15	0.00
Ultimate Essential MouthCare. Eco dent	H	8.89	5.06	7.21	-2.15	0.00
Listerine Freshburst	I	4.14	5.06	4.34	0.73	0.01
Pre Brush Dental Rinse	J	6.77	5.04	5.28	-0.24	0.01
Listerine edition coconut & lime blend	K	4.20	5.03	4.42	0.61	0.00
Oral B Breath Purify	L	7.96	5.01	5.27	-0.26	0.00
Thera Breath Dentist formulated whitening fresh breath	M	4.96	5.06	4.82	0.24	0.01
Colgate Total Whole Mouth Health	N	4.51	5.04	4.76	0.29	0.00
Listerine Ultra Clean Zero alcohol	O	4.03	5.05	4.43	0.63	0.00
Cepacol Antibacterial multi -protection mouthwash	P	7.00	5.05	5.38	-0.33	0.00
Perio Brite Complete Oral Care Natural Mouthwash	Q	4.55	5.05	4.97	0.09	0.00
Crest Pro-Health Intense	R	4.20	5.05	4.74	0.31	0.01
Hello Activated Charcoal	S	7.18	5.04	5.33	-0.29	0.00
Jason Halthy mouth Tartar Control cinnamon clove	T	3.00	5.04	4.38	0.66	0.01
Thera Breath Dentist formulated fresh breath	U	7.22	5.04	5.55	-0.51	0.00
Parodontax active gum health Mint	V	3.89	5.02	4.51	0.51	0.01
Biotène dry mouth oral rinse	W	6.60	5.02	5.62	-0.60	0.01
ARC turn up the bright	X	5.28	5.03	4.69	0.34	0.00
Crest Pro-Health Clean Mint multiprotection	Y	5.01	5.01	5.00	0.01	0.00
Mouthwash up & up	Z	5.27	5.02	5.00	0.02	0.00
Dr. Tichenor's All Natural Peppermint Mouthwash Concentrate	AA	5.21	5.00	5.01	-0.01	0.00
Antiseptic Mouthwash antigingivits antiplaque	AB	4.27	5.00	4.17	0.83	0.00
Hello Peace, out, plaque	AC	4.28	5.00	4.35	0.65	0.01
Listerine Original	AD	4.18	5.00	4.27	0.73	0.00
Lavoris Fresh Breath Mouthwash	AE	4.57	5.00	5.00	0.00	0.00
Oral B Dry mouth	AF	6.26	5.00	5.19	-0.19	0.00
Plax soft mint flavor mouthwash	AG	4.93	5.04	6.18	-1.14	0.00
ARC fresh breath mouth rinse	AH	4.96	5.03	5.02	0.02	0.00
Antiseptic Mouthwash up &up	AI	4.28	5.00	4.31	0.69	0.01
Oral B Mouth Sore	AJ	5.39	5.01	4.72	0.30	0.00
Tom`s Natural Fluoride-free Wicked Fresh	AK	4.16	5.01	4.55	0.46	0.03
Listerine Cool Mint	AL	4.25	5.07	4.32	0.75	0.00
The Natural Dentist Healthy Gums	AM	3.40	5.05	3.98	1.07	0.00
Thera breath periodontist formulated Healthy gums oral rinse	AN	5.77	5.03	5.04	-0.01	0.00
Oral B Dry mouth	AO	6.14	5.01	5.10	-0.09	0.00
Swan Mouthwash fresh mint	AP	5.30	5.01	4.99	0.02	0.00
Crest Scope mouthwash rince-bouche	AQ	5.34	5.04	5.02	0.02	0.00
Family Wellness Blue Mint Mouthwash	AR	5.08	5.01	4.82	0.19	0.00
Crest all fresh, no stress Scope All day	AS	4.95	5.01	5.00	0.01	0.00
Desert Essence Tea Tree Oil Spearmint	AT	3.03	5.04	4.22	0.82	0.01
Jason Healthy power smile brightening	AU	3.31	5.05	4.82	0.23	0.01
Lumineux Oral essentials	AV	4.41	5.00	4.96	0.04	0.00
Jason Total Protection Sea salt mouthrinse	AW	5.40	5.05	5.41	-0.36	0.01
Tea Tree Therapy Mouth Wash	AX	5.75	5.01	5.18	-0.17	0.00
HPM Hydrogen Peroxide Mouthwash	AY	4.79	5.00	4.98	0.02	0.01
Bubble Gum Kid's Spry Mouth wash	AZ	6.74	5.06	5.59	-0.53	0.00
Jason Healthy powersmile brightening	BA	5.39	5.04	5.06	-0.02	0.00
Swan Alcohol-free Mouthwash	BB	4.07	5.01	4.41	0.60	0.01
Orajel 2x Mouth Sores Rinse Medicated	BC	4.03	5.00	4.83	0.17	0.00
Crest Bacteria Blast	BD	5.50	5.00	4.72	0.29	0.00
Clean mint Whitening alcohol free rinse	BE	5.47	5.00	4.72	0.28	0.01
Thera Breath dentist formulated fresh breath oral rinse	BF	9.47	5.00	5.82	-0.82	0.01
Listerine Sensitivity Zero alcohol	BG	4.41	5.05	4.21	0.84	0.01
Splendid white whitening mouth rinse alcohol free Up & Up	BH	5.46	5.00	4.70	0.31	0.00
Crest Scope Classic	BI	5.36	5.03	5.02	0.01	0.00
BR rinse. Organic Mouthwash	BJ	4.29	5.03	4.60	0.43	0.00
Tom´s Sea Salt	BK	4.31	5.00	4.79	0.21	0.00
CloSYS	BL	7.31	5.02	5.85	-0.83	0.00
Antiseptic Mouthwash antigingivits antiplaque	BM	4.43	5.04	4.36	0.68	0.01
Crest GUM and Breath Purify	BN	4.91	5.04	5.02	0.02	0.00
Swan Antiseptic mouth rinse Original	BO	4.39	5.00	4.30	0.70	0.00
Swan Antiseptic Mouth Rinse Spring Mint	BP	4.26	5.03	4.30	0.73	0.00
Advanced Mouth Rinse	BQ	4.06	5.00	4.16	0.84	0.00
Crest 3D white brilliance	BR	5.12	5.00	4.55	0.45	0.01
Multi-action alcohol free. Antiseptic mouth rinse	BS	3.86	5.01	4.51	0.51	0.00
BR rinse. Organic Mouthwash	BT	4.44	5.04	4.71	0.34	0.00
Guru Nanda Oxyburst Whitening Technology	BU	5.78	5.05	5.27	-0.22	0.00
PerCara Mouthwash & Gargle Refresh mint	BV	4.36	5.05	4.59	0.47	0.00
Spry Oral Rinse (Dental defense system)	BW	6.81	5.06	5.88	-0.82	0.02
Desert Essence Tea Tree Oil Whitening Plus Mouthwash	BX	3.04	5.03	4.13	0.90	0.01
Desert Essence Prebiotic Plant-based brushing rinse Mint	BY	3.05	5.03	4.14	0.90	0.00
Smart mouth Mouth Sore zinc activated oral rinse	BZ	5.50	5.06	5.15	-0.09	0.01
Smart mouth Original	CA	5.73	5.06	5.31	-0.25	0.01
Smart mouth Clinical zinc activated oral rinse	CB	5.37	5.06	5.15	-0.09	0.01
Parodontax active gum health Clear mint	CC	3.84	5.01	4.50	0.51	0.01

Table 2. Information about the ingredients of FFM with specific phenomena after the test.

Code	Commercial name	Active ingredients	Other ingredients	Alcohol
C	Colgate Optic White High Impact White Advanced	Hydrogen peroxide	Water, Glycerin, Propylene Glycol, Sorbitol, Hydrogen Peroxide, Polysorbate 20, Sodium Acrylates/Methacryloylethyl Phosphate Copolymer, Phosphoric Acid, Citric Acid, Flavor, PVM/MA Copolymer, Sodium Saccharin.	–
M	Thera Breath Dentist formulated whitening fresh breath	–	Water, glycerin, polysorbate 20, sodium benzoate, PVP, Natural mint flavor, papain, D-limonene, menthol, glucose oxidase	
X	ARC turn up the bright	Hydrogen peroxide and SH	Water, glycerin, propylene glycol, hydrogen peroxide, sodium hexametaphosphate, poloxamer 407, flavor, sodium citrate, sodium saccharin, citric acid, sucralose	–
AB	Antiseptic Mouthwash antigingivits antiplaque	Eucalyptol (0.092%), Menthol (0.042%), Methyl Salicylate (0.060%), Thymol (0.064%)	Water, Alcohol (26.9 % v/v), poloxamer 407, benzoic acid sodium benzoate, Caramel	Alcohol 26.9 % v/v
AG	Plax soft mint flavor mouthwash	Sodium lauryl sulfate, %), tetrasodium pyrophosphate	Water, sorbitol, alcohol (8.6%), tetrasodium pyrophosphate, sodium benzoate, benzoic acid, sodium lauryl sulfate, poloxamer 407, flavor, xanthan gum, sodium saccharin, blue 1, yellow 5	Alcohol 8.6 % v/v
AJ	Oral B Mouth Sore	Hydrogen peroxide 1.5% /W/v) and sodium hexametaphosphate	Citric acid, flavor, glycerin, poloxamer 407, propylene glycol, sodium citrate, sodium hexametaphosphate, sodium saccharin, sucralose, water	–
AR	Family Wellness Blue Mint Mouthwash	Cetylpyridinium Chloride	Water, alcohol (12.25 wt%), glycerin and/or sorbitol, polysorbate 80 and/or polysorbate 20, flavor, sodium saccharin, sodium benzoate, cetylpiridinium chloride, benzoic acid, blue 1	12.25%
BD	Crest Bacteria Blast	Hydrogen peroxide and sodium hexametaphosphate	Water, glycerin, alcohol (5wt%), hydrogen peroxide, sodium hexametaphosphate, poloxamer 407, flavor, sodium citrate, sodium saccharin, citric acid, sucralose	Alcohol 5% wt%
BE	Clean mint Whitening alcohol-free rinse	Hydrogen peroxide and sodium hexametaphosphate	Water, glycerin, hydrogen peroxide, propylene glycol, sodium hexametaphosphate, poloxamer 407, sodium citrate, flavor, PEG-40, Hydrogenated castor oil, sodium saccharin, citric acid.	–
BH	Splendid white whitening mouth rinse alcohol-free Up & Up	Hydrogen peroxide and sodium hexametaphosphate	Water, glycerin, hydrogen peroxide, propylene glycol, sodium hexametaphosphate, poloxamer 407, sodium citrate, flavor, PEG-40, Hydrogenated castor oil, sodium saccharin, citric acid.	–
BR	Crest 3D white brilliance	Hydrogen peroxide and sodium hexametaphosphate	Water, glycerin, sodium hexametaphosphate, poloxamer 407, sodium benzoate, sodium lauryl sulfate, flavor, phosphoric acid, sodium saccharin, sucralose, red 33, green 3	–

Discussion

Dental erosion is caused by the chemical dissolution of the hard tooth surfaces by frequent exposure to acids not produced by oral bacteria. The buffer systems present in human saliva can help neutralizing the pH of potentially erosive acids (Reddy et al., 2016); however, it is known that the dilution of mouthwashes in the oral cavity is minimal, and the initial pH of each product would not be significantly affected when used (Delgado et al., 2016). In fact, oral hygiene products of acidic nature have been implicated as a cause of dental erosion (Rytomaa et al., 1989; Bhatti et al., 1994).

The present study highlighted that fluoride-free mouthwashes vary greatly in their pH (3.00 - 9.47), with the majority displaying a pH between pH 4.0 and 5.0 (Figure 1). Acids in

mouthwashes serve different purposes. They can act as preservatives (benzoic acid/sodium benzoate buffer), aid in the stabilization of ingredients (e.g., citric acid/sodium citrate buffer for hydrogen peroxide, citric acid acting as an antioxidant), and enhance flavor perception (some flavors require low pH values) (Radzki et al., 2022). However, mouthwashes with a pH less than 5.5 are recommended to demonstrate safety using a demineralization test, erosion test, or other appropriate methods (ADA, 2010).

Since the pH alone is not indicative of the erosive potential of a mouthwash, a screening method was developed by the ISO (ISO/FDIS 28888:2013; Dentistry — Screening method for erosion potential of mouthwashes on dental hard tissues). The motivation for the present study was therefore to not only evaluate FFM for their erosive potential but also the screening method as proposed by the ISO.

A moderate, linear correlation (r^2=0.6522) was found between the initial pH of the mouthwash and the final pH of the screening solution (Fig. 1 e 2). According to the ISO guidelines, only one mouthwash (AM) did not pass the test, recording a pH decrease of 1.07 (Table 1). Its main ingredients are Aloe Vera 20% (*Aloe brabadensis*) and citric acid, among others (Table 2). However, it should be noted that the citric acid/sodium citrate buffers did not register the expected pH changes. The strongest buffer was expected to lead to a pH decrease of 1.0, as expected. Instead, it led to a decrease of 1.20 which is greater than what was registered for mouthwash AM. Therefore, it could be argued that this mouthwash did not fail the test. Furthermore, several unexpected pH changes were observed as outlined in the results. There is no plausible explanation for these occurrences as mouthwash formulations are complex and contain a wide range of ingredients. Taken together, these observations highlight that further refinements of this test method are needed.

The rationale for using the ISO method is to detect those that are potentially erosive, and in those that are outside the limits (Delta pH >1) or there is some type of question, a more elaborate study should be carried out. A recent study proposed a different method to determine “the safety of low pH oral care rinse products to dental enamel” (Moore et al., 2020). their method is more sophisticated and clinically relevant as it involves enamel specimens that undergo a cycling procedure with changes in surface microhardness being used as the outcome measure. However, the ideal would be to carry out a previous study such as the ISO method.

Another point to assess is that this method alone is not suitable as a screening method as testing the presently evaluated 81 mouthwashes would likely take several months. Another methodology used to evaluate the erosive potential of different substances is the pH-stat method. It uses an automatic titrator that adds an acidic titrant to maintain a constant pH of a

solution containing hydroxyapatite and the test agent. The necessary volume of titrator to maintain the pH is then converted to the amount of hydroxyapatite dissolved (Scaramucci et al., 2011). This test can also be conducted manually (Tenuta et al., 2015). The pH-stat method may be more clinically relevant because it is a technique that studies dental erosion and incorporates hydroxyapatite crystals that provide information on its interactions with acid in erosive challenges. A balance needs to be found for a screening method that is both clinically relevant yet can be accomplished in a short time frame.

In conclusion according to our results using the ISO method, none of the fluoride-free mouthwashes evaluated have an erosive potential. However, different unusual behaviors were reported in some products, which, although they passed the test, could be indicating a non-stabilization of some of its components.

Clinical significance

Fluoride-free mouthwashes vary greatly in pH (3.0-9.47), with 63 out of 81 mouthwashes displaying pH values below 5.5. However, none of the presently tested mouthwashes were found to have detrimental effects on the dental hard tissues, based on an ISO screening method. Nonetheless, further method development is needed.

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References:

1. Schlueter N, Amaechi BT, Bartlett D, Buzalaf MAR, Carvalho TS, Ganss C, Hara AT, Huysmans MDNJM, Lussi A, Moazzez R, Vieira AR, West NX, Wiegand A, Young A, Lippert F. Terminology of Erosive Tooth Wear: Consensus Report of a Workshop Organized by the ORCA and the Cariology Research Group of the IADR. Caries Res. 2020;54(1):2-6. doi: 10.1159/000503308.

2. Zero DT. Etiology of dental erosion--extrinsic factors. Eur J Oral Sci. 1996 Apr;104(2 (Pt 2)):162-77. Doi 10.1111/j.1600-0722.1996.tb00065.x.
3. Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental erosion--an overview with emphasis on chemical and histopathological aspects. Caries Res. 2011;45 Suppl 1:2-12. Doi 10.1159/000325915. Epub 2011 May 31. PMID: 21625128.
4. Kanzow P, Wegehaupt FJ, Attin T, Wiegand A. Etiology and pathogenesis of dental erosion. Quintessence Int. 2016 Apr;47(4):275-8. doi: 10.3290/j.qi.a35625. PMID: 27022647
5. Van der Weijden FA, Van der Sluijs E, Ciancio SG, Slot DE. Can Chemical Mouthwash Agents Achieve Plaque/Gingivitis Control? Dent Clin North Am. 2015 Oct;59(4):799-829. doi: 10.1016/j.cden.2015.06.002.
6. Valdivia-Tapia AC, Botelho JN, Giacaman RA, Tabchoury CPM, Cury JA. Fluoride concentration in mouth rinses marketed in Chile and Brazil, and a discussion regarding their legislations. Braz Oral Res. 2021 Aug 18;35:e083. Doi 10.1590/1807-3107bor-2021.vol35.0083.
7. Radzki D, Wilhelm-Węglarz M, Pruska K, Kusiak A, Ordyniec-Kwaśnica I. A Fresh Look at Mouthwashes-What Is Inside and What Is It For? Int J Environ Res Public Health. 2022 Mar 25;19(7):3926. doi: 10.3390/ijerph19073926.
8. 2019 American Dental Association. Reproduction or republication is strictly prohibited without prior written permission https://www.ada.org/resources/research/science-and-research-institute/oral-health-topics/mouthrinse-mouthwash
9. International Standard (ISO). Dentistry — Screening method for erosion potential of oral rinses on dental hard tissues. ISO/FDIS 28888:2013(E)
10. Reddy A, Norris DF, Momeni SS, Waldo B, Ruby JD. The pH of beverages in the United States. J Am Dent Assoc 2016;147(4):255-63
11. Delgado AJ, Olafsson VG, Donovan TE. pH and Erosive Potential of Commonly Used Oral Moisturizers. J Prosthodont 2016;25(1):39-43
12. Rytömaa I, Meurman JH, Franssila S, Torkko H. Oral hygiene products may cause dental erosion. Proc Finn Dent Soc. 1989;85(3):161-6.
13. Bhatti SA, Walsh TF, Douglas CW. Ethanol and pH levels of proprietary mouthrinses. Community Dent Health. 1994 Jun;11(2):71-4.
14. American National Standards Institute ADA. ANSI/ADA Standard No. 116 for Oral Rinses. American National Standards Institute, American Dental Association. Chicago: American Dental Association. 2010Moore J, White DJ, Schneiderman E, Chen H, Strand

R, Grender JM. Laboratory screening evaluation of the safety of low pH oral care rinse products to dental enamel. Am J Dent. 2020 Jun;33(3):161-164.

15. Moore J, White DJ, Schneiderman E, Chen H, Strand R, Grender JM. Laboratory screening evaluation of the safety of low pH oral care rinse products to dental enamel. Am J Dent. 2020 Jun;33(3):161-164. PMID: 32470243.
16. Scaramucci T, Hara AT, Zero DT, Ferreira SS, Aoki IV, Sobral MA. In vitro evaluation of the erosive potential of orange juice modified by food additives in enamel and dentine. J Dent. 2011 Dec;39(12):841-8. doi: 10.1016/j.jdent.2011.09.004.
17. Tenuta LM, Fernández CE, Brandão AC, Cury JA. Titratable acidity of beverages influences salivary pH recovery. Braz Oral Res. 2015;29:S1806-83242015000100234. doi: 10.1590/1807-3107BOR-2015.vol29.0032.

2.4. The in vitro erosive potential of fluoride-free mouthwashes

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Abstract

Objectives: To investigate the enamel and dentin surface loss caused by acidic fluoride-free mouthwashes using erosion pH-cycling. **Methods:** Enamel and dentin incisor bovine slabs (n = 8 per group) were subjected to 5 days of erosion cycling (four 1-min mouthwash treatments/day). Six fluoride-free mouthwashes (MW1-pH 3.98; MW2-pH 3.03; MW3-pH 4.51; MW4-pH 4.41; MW5-pH 5.12; MW6-pH 4.14) were investigated. Controls were three 0.3% citric acid solutions (PC3-pH 3.0; PC4-pH 4.0; PC5-pH 5.0) and deionized water (NC). Non-contact profilometry was used to determine surface loss (SL). Data were analyzed using one-way ANOVA. **Results:** SL: For both enamel and dentin, the model was able to differentiate between PC3 (enamel/dentin; mean±standard deviation [µm]; -3.32±0.27/-7.08±0.60), PC4 (-0.76±0.26/-4.25±0.61), and PC5 (-0.23±0.19/-3.04±0.67; all comparisons $p \leq 0.012$). Differences between PC5 and NC (0.06±0.11/-0.13±0.28) were only directional for enamel ($p=0.528$) but not in dentin ($p<0.001$). SL ranged considerably between MW1-MW6; however, all were less erosive than PC3 in both enamel and dentin (all $p<0.001$). In enamel, MW1 (0.20±0.12), MW3 (-0.26±0.29), and MW6 (0.01±0.12) were not different from NC ($p \geq 0.399$). MW4 (-0.48±0.29) was not different from PC4 and PC5 ($p \geq 0.590$), whereas MW5 (-1.19±0.59) was more erosive than PC5 ($p<0.001$) but only directionally more erosive than PC4 ($p=0.081$). However, MW2 (-1.37±0.21) was more erosive than PC4 ($p=0.002$). The SL data for dentin were mostly comparable to enamel. In dentin, MW1 (-0.34±0.30), MW3 (-0.68±0.45), and MW6 (-0.79±0.17) were also not different from NC ($p \geq 0.566$). However, MW4 (-1.38±0.52) was less erosive than PC5 ($p<0.001$) but more than NC ($p=0.008$). As observed in enamel, MW5 (-4.51±0.86) was not different from PC4 ($p=0.999$) but more erosive than PC5 ($p=0.001$). Unlike in enamel, MW2 (-3.51±1.28) was indistinguishable from both PC4 ($p=0.400$) and PC5 ($p=0.902$).

Conclusion: Some acidic fluoride-free mouthwashes can cause erosive mineral loss in the dental hard tissues, with the effects more pronounced in dentin.

Keywords: Dental Erosion, Mouthwash, Enamel, Dentin

Introduction

Erosive tooth wear is a condition of the oral cavity that has become more prevalent over time, especially in younger populations (Jaeggi and Lussi, 2014). It is one of the most common conditions that produce progressive and irreversible loss of hard dental tissues (Gandara and Truelove, 1999; Jaeggi and Lussi, 2006). There is insufficient evidence that remineralization of erosive lesions occurs; however, surface deposition of minerals may be possible. A significant contribution to erosive tooth wear is from the process of dental erosion which is "the chemical loss of mineralized tooth substance caused by the exposure to acids not derived from oral bacteria" (Schlueter et al., 2019).

Extrinsic and intrinsic factors modulate the erosive process based on their inherent characteristics, such as pH and buffering capacity, calcium and phosphate contents (degree of saturation), fluoride content, and temperature (Zero, 1996; Lussi et al., 2011). Some dental products for individual use, such as mouthwashes, can be potentially erosive. When they contain fluoride, this can mitigate the effect of a low pH. However, a recent study identified 81 different fluoride-free mouthwashes that are commercially available in retail stores and pharmacies (Valdivia-Tapia et al., 2023 manuscript in preparation). Most of these products use a buffer such as citric acid/citrate or phosphoric acid/phosphate, with 63 out of 81 mouthwashes displaying a pH of less than 5.5. Given their often low pH, the presence of buffering agents, and their frequency of use (2×/day every day), these products may be potentially erosive, especially in dentin. Therefore, this laboratory study aimed to investigate the enamel and dentin surface loss caused by acidic fluoride-free mouthwashes using a validated erosion pH- cycling model.

Methods

Experimental design

Enamel and dentin incisor bovine slabs were subjected to 5 days of erosion cycling which included four 1-min mouthwash treatments/day, with exposure to artificial saliva at all other times). Six fluoride-free mouthwashes (pH 3.03 - 5.12), chosen from a previous study (Valdivia-Tapia et al., 2023 manuscript in preparation), were investigated. Controls were three 0.3% citric acid solutions (pH 3.0/4.0/5.0) and deionized water (negative control). Non-contact profilometry was used to determine surface loss (SL). Knoop microhardness was utilized to calculate the percentage of surface hardness loss (%SHL) in enamel.

Preparation and selection of the specimens

Enamel and dentin slabs of 4×4×2 mm were prepared from bovine incisors stored in 0.1% thymol solution pH (7.0) at 4°C. The slabs were prepared and flattened with silicon carbide grinding papers (Struers RotoPol 31/RotoForce 4 polishing unit: Struers, Cleveland, PA, USA). The slabs were embedded in acrylic resin (Varidur acrylic system, Buehler, Lake Bluff, IL, USA) blocks utilizing a custom-made silicon mold, exposing the enamel and dentin surfaces with a space between each other. The embedded blocks were serially ground and polished up to 4000-grit silicon carbide grinding paper, followed by 1-μm diamond polishing suspension. The first selection of the specimens was based on the visual quality of enamel and dentin. Those with cracks or other defects were rejected. The second selection was based on their surface hardness (+/- 10% of variability inter and intra blocks was considered acceptable). Five indentations, 100 μm apart from one another, were placed on both specimens (Knoop, 50 g and 10g for enamel and dentin, respectively, Tukon ®2100B, INSTRON model; Clemex CMT.HD software (Fushida and Cury, 1999)). The slabs were randomly assigned to the selected experimental groups with eight specimens per group (n=8) (Albeshir et al., 2022 in press). Adhesive unplasticized polyvinyl chloride (uPVC) tapes were placed on the specimens' surface, leaving an exposed area of 1×4 mm in the center of each enamel and dentin block.

Initial pH and titratable acidity

The pH of each sample (50 mL) was determined by using a pH-electrode (Fisher, accumet® cat. #13-620-631), previously calibrated with standard buffer solutions of pH 4.0 and 7.0 (Fisher Chemical, Fisher Scientific ™) coupled to a potentiometer, previously calibrated with pH 4.0 and 7.0 buffers. The titratable acidity was then measured by adding aliquots of 0.1 N NaOH to each beverage until the pH 7.0. The base (mmol) required to reach pH 7.0 in the tested solution was calculated (Table 1). pH record reached after each addition of NaOH. Titratable acidity was expressed as mmol OH^- needed to reach neutral pH (Tenuta et al., 2015)

Delta pH

The International Standard ISO/FDIS 28888:2013; Dentistry — Screening method for erosion potential of mouthwashes on dental hard tissues as a methodology was used (ISO, 2013). The test was performed at an ambient temperature (approx. 20 °C). For the screening method, 25 mL of the screening solution were added to a 50 mL reaction vessel. The solution was stirred at a moderately fast rate (100 r/min) and held at this rate throughout the experiment.

A pH-electrode (Fisher, accumet® cat. #13-620-631), previously calibrated with standard buffer solutions of pH 4.0 and 7.0 (Fisher Chemical, Fisher Scientific ™), was used. The pH of the screening solution was determined. Then, 250 µl (0.25 ml) of the test material (reference buffer or mouthwash) were added to the screening solution. The reaction was terminated after a steady pH value was recorded. The test was repeated four times for each test material. (Valdivia-Tapia et al., 2023 in preparation)

Artificial saliva preparation

Artificial saliva [1.45 mM Ca^{2+}, 5.4 mM PO_4^{3-}, 0.1 M Tris buffer, 2.2 g/l of porcine gastric mucin, pH 7.0] was used as the remineralization medium (Hara et al., 2009).

Control solutions and Treatments

Table 1 displays the positive and negative controls as well as the test mouthwashes and their compositions. For the positive control, three different solutions of 0.3% (w/v) anhydrous citric acid (Sigma C1857, St. Louis, MO, USA) were prepared in deionized water at pH 3.0, 4.0, and 5.0. The pH was adjusted with NaOH when necessary (Hara et al., 2009). Deionized water (diH_2O) served as a negative control. The fluoride-free mouthwashes were selected from a recently concluded study (Valdivia-Tapia et al., 2023 manuscript in preparation), with the aim to include products with different pH values (3.03 - 5.12) and compositions.

Table 1. Controls and commercial mouthwashes information.

Purpose	Name	Code	Ingredients	Initial pH	Titratable acidity (to pH 7.0; mM OH^-)	Delta pH (ISO-method)
Positive Controls	0.3% citric acid pH 3.0	PC3	0.3% citric acid anhydrous in diH_2O	3.00	2.13	1.20
	0.3% citric acid pH 4.0	PC4	0.3% citric acid anhydrous in diH_2O	4.00	1.37	0.89
	0.3% citric acid pH 5.0	PC5	0.3% citric acid anhydrous in diH_2O	5.00	0.77	0.58
Negative Control	Deionized water	NC	diH_2O	5.70	0.001	0.05
Test Mouthwashes	Colgate Peroxyl	MW1	Hydrogen peroxide 1.5%, water, sorbitol, propylene glycol, poloxamer 338, polysorbate 20, flavor, sodium saccharin, FD&C blue no.1	3.98	0.28	0.05
	Desert Essence Tea Tree OilSpearmint	MW2	Water, glycerin, polysorbate 80, Eco-Harvest *Melaleuca alternifolia* leaf oil, *Aloe barbadensis* Leaf, juice, *Mentha Viridis* leaf oil, *Hamamelis virginiana* extract, ascorbic acid, calcium ascorbate, citric acid	3.03	0.63	0.66
	Colgate Total Whole Mouth Health	MW3	Cetylpyridinium chloride 0.075%, water, glycerin, propylene glycol, sorbitol, poloxamer 407, flavor, potassium sorbate, citric acid, sodium saccharin, blue 1	4.51	0.19	0.29
	Listerine Sensitivity Zero Alcohol	MW4	Water, sorbitol, propylene glycol, dipotassium oxalate monohydrate, flavor, phosphoric acid, poloxamer, sodium benzoate, sodium methyl cocoyl taurate, sodium lauryl sulfate, sodium saccharin, sucralose	4.41	2.53	0.84
	Crest 3D white brilliance	MW5	Water, glycerin, sodium hexametaphosphate, poloxamer 407, sodium benzoate, sodium lauryl sulfate, flavor, phosphoric acid, sodium saccharin, sucralose, red 33, green 3	5.12	1.35	0.45
	Listerine Freshburst	MW6	Eucalyptol (0.092%), menthol (0.042%), methyl salicylate (0.060%), thymol (0.064%), alcohol. 21.6% (v/v), water, sorbitol, poloxamer 407, benzoic acid, sodium saccharin, flavor, sodium benzoate, yellow 10, green	4.14	0.57	0.73

Daily treatment regimen

The daily treatment regimen (table 2) comprised four mouthwash/solution treatments/day for 1 min under gentle agitation (50 rpm; orbital shaker) over five days. Between the treatments, the blocks remained in artificial saliva and after the last cycle each day (Romão et al., 2023, manuscript in preparation).

Table 2. Daily treatment schedule

	Steps for each day	Duration
0	Artificial saliva	1 h (only first day)
Step 1	Exposure to treatment rinse (1 of 4)	1 min
Step 2	Remineralization in artificial saliva (1 of 4)	1 h
Step 3	Exposure to treatment rinse (2 of 4)	1 min
Step 4	Remineralization in artificial saliva (2 of 4)	1 h
Step 5	Exposure to treatment rinse (3 of 4)	1 min
Step 6	Remineralization in artificial saliva (3 of 4)	1 h
Step 7	Exposure to treatment rinse (4 of 4)	1 min
Step 8	Remineralization in artificial saliva (4 of 4)	Overnight
Step 9	Surface hardness in enamel	After the last day
Step 10	Profilometry enamel and dentin	After the last day

Data Analysis

Surface Hardness: The surface hardness of the enamel specimens was measured again after completion of the 5-day cycling procedure. Five indentations were placed to the right of the baseline indentations as described above. The %SHL was then calculated as: %SHL = ((Knoop Hardness pre-cycling – Knoop Hardness post-cycling) x 100)/Knoop Hardness pre-cycling. Higher values indicate greater hardness loss (Romão et al., 2023, manuscript in preparation). Hardness measurements were not performed on dentin specimens after the cycling procedure.

Profilometry: The surface loss of all specimens was measured after completion of the hardness measurements. The UPVC tapes were removed from the specimens, and the specimens placed onto the stage of the optical profilometer (Proscan 2000, Scantron) with the experimental surface parallel to the horizontal plane. An area of 2×1 mm^2 covering both reference areas (non-exposed to treatment - previously protected with UPVC tapes) and treated (exposed to treatment) surfaces was scanned using horizontal resolutions of 0.01 and 0.05 mm in the x and y directions, respectively. The images were analyzed using dedicated software (Proscan, 2000; Scantron application software v. 2.0.17), which calculates the average height of the two reference areas and subtracts it from the experimental area. The difference in depth (surface loss) was expressed in micrometers (μm). Dentin specimens were scanned under moistened condition to prevent collagen shrinkage [Attin et al., 2009].

Statistical Analysis

The assumptions of equality of variances and normal distribution of errors were checked for the response variable tested. Then, an analysis of variance (ANOVA) was performed, followed by Tukey's test for comparisons between experimental groups. Pearson correlations were used to evaluate associations between outcomes. The significance level was α=0.05. The analyses were

performed using the SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).

Results

The surface loss and %SHL data for all groups and the results of the statistical analysis can be found in Table 3. Surface loss in enamel ranged between -3.32±0.27 to 0.23±0.19 (mean±standard deviation [µm] µm for the controls and between -1.37±0.21 to 0.20±0.12 for the mouthwashes. For dentin, the surface loss for the controls ranged between -7.08±0.60 to -3.04±0.67 and for the mouthwashes between -4.51±0.86 to -0.34±0.30. The present model was able to differentiate between the positive control citric acid solutions (PC3, PC4, PC5) in both enamel ($p \leq 0.05$) and dentin ($p \leq 0.05$), with solutions of lower pH resulting in greater surface loss (SL). Similar observations were made for the %SHL data, although differences between PC3 and PC4 as well as PC4 and PC5 were only directional ($p=0.055$ and $p=0.606$, respectively). Likewise, the enamel profilometry data were not able to differentiate between PC5 and deionized water ($p=0.528$); however, there were differences in dentin ($p<0.001$). Somewhat contrasting the enamel profilometry data, the %SHL data revealed differences between these two groups, with more softening observed in PC5 than in NC ($p<0.001$). Irrespective of the investigated variable, diH_20 did not cause any surface loss or surface softening.

SL ranged considerably between MW1-MW6; all less erosive than PC3 in both enamel and dentin (all $p<0.001$). In enamel, MW1 (0.20±0.12), MW3 (-0.26±0.29), and MW6 (0.01±0.12) were not different from NC ($p \geq 0.399$). MW4 (-0.48±0.29) was not different from PC4 and PC5 ($p \geq 0.590$), whereas MW5 (-1.19±0.59) was more erosive than PC5 ($p<0.001$) but only directionally more erosive than PC4 ($p=0.081$). However, MW2 (-1.37±0.21) was more erosive than PC4 ($p=0.002$). The SL data for dentin were mostly comparable to the enamel. In dentin, MW1 (-0.34±0.30), MW3 (-0.68±0.45), and MW6 (-0.79±0.17) were also not different from NC ($p \geq 0.566$). However, MW4 (-1.38±0.52) was less erosive than PC5 ($p<0.001$) but more than NC ($p=0.008$). As observed in enamel, MW5 (-4.51±0.86) was not different from PC4 ($p=0.999$) but more erosive than PC5 ($p=0.001$). Unlike in enamel, MW2 (-3.51±1.28) was indistinguishable from both PC4 ($p=0.400$) and PC5 ($p=0.902$).

The %SHL data were less discerning than the SL data for the more erosive test solutions and mouthwashes but showed similar trends overall. Unlike in the SL data, there were no differences between PC3 and PC4 ($p=0.055$) as well as between PC4 and PC5 ($p=0.606$). Comparable to the SL data, however, PC3 caused more softening than PC5. The %SHL data indicated less

softening for NC vs. PC5 ($p<0.001$) which contrasted with the SL data. Similarly, to the SL data, there were no differences between NC, MW1, MW3, and MW6 ($p\geq0.320$). Contrasting the SL data, MW2 ($p=1.000$), MW4 ($p<0.001$), and MW5 ($p=0.304$) all caused at least numerically less surface softening than PC5, although the order of softening caused by these mouthwashes followed the same rank order as in the SL data.

There were nonsignificant, weak correlations between the initial pH and SL for enamel ($r=0.125$/ $p=0.814$) and dentin ($r=0.074$/$p=0.890$) as well as the initial pH and %SHL ($r=0.149$/$p=0.778$). Likewise, there were nonsignificant, weak correlations between titratable acidity and SL for enamel ($r=0.287$/$p=0.581$), dentin ($r=0.275$/$p=0.598$), and for %SHL ($r=0.450$/$p=0.370$). For Delta pH and SL for enamel ($r=0.364$/$p=0.479$), dentin ($r=0.255$/$p=0.626$), and for %SHL ($r=0.526$/$p=0.284$).

Table 3. Surface loss and percent surface hardness loss (%SHL) data and results of the statistical analysis for both enamel and dentin and all treatment groups. Data are mean (standard deviation).

Code	Surface loss (µm)		%SHL
	Enamel	Dentin	Enamel
PC3	-3.32 (0.27) ^{A}	-7.08 (0.60) ^{A}	61.09 (6.86) ^{a}
PC4	-0.76 (0.26) $^{C, D}$	-4.25 (0.61) ^{B}	49.89 (7.45) ^{a,b}
PC5	-0.23 (0.19) $^{E, F}$	-3.04 (0.67) ^{C}	42.98 (9.13) ^{b,c}
NC	0.06 (0.11) ^{F}	-0.13 (0.28) ^{F}	1.05 (3.23) ^{e}
MW1	0.20 (0.12) ^{F}	-0.34 (0.30) $^{E, F}$	-2.81 (6.45) ^{e}
MW2	-1.37 (0.21) ^{B}	-3.51 (1.28) $^{B, C}$	42.31 (7.84) ^{b,c}
MW3	-0.26 (0.29) $^{E, F}$	-0.68 (0.45) $^{E, F}$	6.07 (5.61) ^{e}
MW4	-0.48 (0.29) $^{D, E}$	-1.38 (0.52) ^{E}	25.10 (2.57) ^{d}
MW5	-1.19 (0.59) $^{B, C}$	-4.51 (0.86) ^{B}	34.43 (5.42) ^{c,d}
MW6	0.01 (0.12) ^{F}	-0.79 (0.17) $^{E, F}$	5.88 (4.90) ^{e}

Different letters indicate significant differences between treatments within each variable ($\alpha=0.05$)

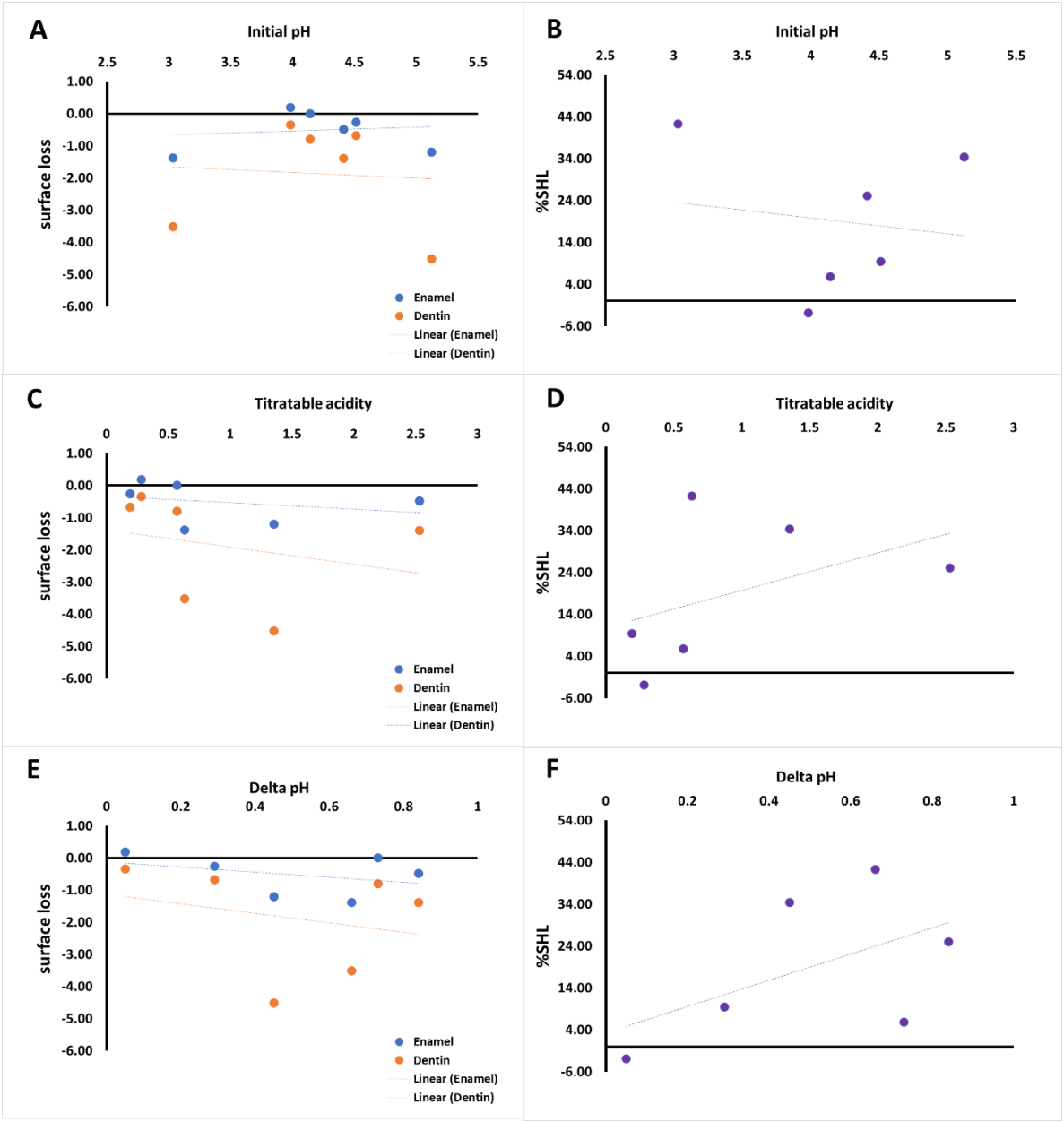

Figure. Correlations between initial pH of the test solutions and mouthwashes and SL for enamel and dentin (A), initial pH and %SHL (B), titratable acidity and SL (C), and titratable acidity and %SHL (D), Delta pH and SL (E), and Delta pH and %SHL (F).

Discussion

The cycling model (Romão et al., 2023, manuscript in preparation) was chosen to investigate the inherent erosive potential of the mouthwashes under realistic conditions (i.e., 1-min exposures instead of continuous exposure for prolonged periods) rather than to mimic their everyday use as the impact of toothpastes and dietary variables would not allow for meaningful conclusions to be drawn. Three citric acid solutions varying in pH were included as positive controls to not only demonstrate model sensitivity but also to allow to determine the relative

erosion potential of the studied mouthwashes. The six mouthwashes were chosen from a previous study (Valdivia-Tapia et al., 2023, manuscript submitted for publication) and represent a range of different formulations with low pH (table 1). All products presented pH values (3.03 to 5.12) below the critical for enamel and dentin dissolution (5.5 and 6.5, respectively). Furthermore, these products present other ingredients that can interfere with the buffering capacity and titratable acidity of the buffer present in the product (Zero, 1996).

The European Organization for Caries Research (ORCA) and the Cariology Research Group of the International Association for Dental Research (CRG-IADR) held a consensus workshop and defined dental erosion as a *"process where the chemical loss of mineralized tooth substance is caused by exposure to acids which are not derived from oral bacteria*", and that this process can be influenced by internal or external factors (Schlueter et al., 2019). As pointed out earlier, extrinsic factors, such as mouthwashes, can potentially contribute to erosion of dental substrates, although our understanding of their relative contribution to the overall prevalence of dental erosion is still poor. The erosive impact of mouthwashes depends on their pH, buffer capacity, calcium, phosphate, and fluoride contents (Zero, 1996; Lussi et al., 2011; Kanzow et al., 2016).

None of the test products contained fluoride; therefore, they did not contain any known protective factors that could prevent mineral loss. High-concentrated fluoride applications can increase abrasion resistance and decrease the development of erosion in enamel and dentine (Wiegand and Attin, 2003). Fluoride mouthwashes that are considered remineralizing agents in dental caries prevention (226 ppm F for daily use and 900 ppm F for weekly use; Marinho et al., 2016), can potentially be effective in preventing dental erosion (Sorvari et al., 2004; Wiegand and Attin, 2003; Amechi and Higham et al., 2004; Venasakulchai et al., 2010). However, in the market, it is possible found different products without fluoride. Representing more than 90% of the products used in US (Zero et al., 2006)

The results for the six mouthwashes were not necessarily as expected. For example, MW2 presented a similar pH and titratable acidity as PC5 (Table 1) yet was found to be considerably higher erosive in enamel, but similar in dentin for surface loss (Table 3).

A potential explanation of this is due to the product's composition, since in addition to presenting citric acid in its composition, it presents ascorbic acid and aloe vera in composition, all of which can affect the erosive potential of this product.

MW1 had the second lowest pH of all mouthwashes yet was not found to present an erosive potential as it was comparable to NC. MW1 contains hydrogen peroxide, which is a weak acid. Over-the-counter tooth whitening/bleaching products typically contain higher concentrations of

hydrogen or carbamide peroxide than MW1 and have not been associated with detrimental effects on the mechanical properties of dental hard tissues (Zanolla et al., 2017). Therefore, the present results were not surprising.

MW5 had the highest pH of the tested mouthwashes yet caused the numerically second-highest SL in enamel and the highest SL in dentin. Regarding this product, it was expected to have one of the lowest surface losses. However, it was the opposite; this could be explained due to His composition. A previous study (Valdivia-Tapia et al., 2023 in preparation) evaluated the pH variation (the mouthwash was added to a screening solution containing calcium and phosphate). The pH was expected to increase since the initial pH was 5.05, and that of the product was 5.12. However, the opposite happened; the resulting pH decreased (4.55). This can reveal a possible instability of the formulation, which is reflected in a more significant loss with mouthwashes with a lower pH.

MW4 ranks third in terms of surface loss in enamel and dentin and in %SHL. Although it presents a pH of 4.41, it presents a more significant loss of surface in enamel than PC5. However, in dentin, it presents a lower SL than this same control. Already for %SHL, it presents a lower percentage than PC5; it presents phosphoric acid in its composition, which may explain our results. Well, we use citric acid as a control.

MW3 and MW6 are the products with the least SL and %SHL. These presented a pH of 4.51 and 4.14, respectively. The first presents CPC in its composition with citric acid and others, and the second presents essential oils and benzoic acid.

Different limitations need to be considered in the interpretation of the present data. A previous study highlighted the commercial availability of a vast number of acidic fluoride-free mouthwashes with a variety of ingredients and purposes. This made it challenging to choose a representative sample as not all mouthwashes can be evaluated using the present 5-day model. The present model only included mouthwash treatments with artificial saliva exposure at all other times. While this allows for the determination of the inherent erosive potential of mouthwashes, it does not mimic their clinical use and protection afforded by previously applied fluoride toothpaste, for example. There is considerable further research needed to understand how toothpastes affect mouthwash and vice versa in both caries and erosion prevention. There are many oral hygiene adjuvants frequently used after tooth brushing. Hence, further studies are necessary to assess whether other ingredients modulate the presently found erosive potential, evaluate the interaction between these products, and determine the abrasion-erosion potential from the combined use of these products.

Conclusion

Some commercial products analyzed present an erosive potential on hard dental tissues, especially dentin. However, this effect was not dependent on the pH or titratable capacity. Therefore, these products must be cautiously recommended to individuals at risk of erosive tooth wear. These products should be used with care since they do not contain fluoride, which is a protective factor.

Author contributions

ACVT, FL, and JAC designed the study. ACVT and FL were responsible for data collection and analysis. All the authors interpreted the data. ACVT and FL prepared the article. Finally, all the authors revised and contributed to the final version of the manuscript.

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Conflict of interest

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References

1. Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monogr Oral Sci. 2014;25:55-73. doi: 10.1159/000360973. Epub 2014 Jun 26. PMID: 24993258.
2. Gandara BK, Truelove EL. Diagnosis and management of dental erosion. J Contemp Dent Pract. 1999 Nov 15;1(1):16-23. PMID: 12167897.
3. Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. In: Lussi A, editor. Dental erosion: from diagnosis to therapy. Basel: Karger; 2006. p.44−65.
4. Schlueter N, Amaechi BT, Bartlett D, Buzalaf MAR, Carvalho TS, Ganss C, Hara AT, Huysmans MDNJM, Lussi A, Moazzez R, Vieira AR, West NX, Wiegand A, Young A,

Lippert F. Terminology of Erosive Tooth Wear: Consensus Report of a Workshop Organized by the ORCA and the Cariology Research Group of the IADR. Caries Res. 2020;54(1):2-6. doi: 10.1159/000503308. Epub 2019 Oct 14. PMID: 31610535.

5. ten Cate JM, Duijsters PP. Influence of fluoride in solution on tooth demineralization. I. Chemical data. Caries Res. 1983;17(3):193-9. doi: 10.1159/000260667. PMID: 6573960.
6. Dawes C. What is the critical pH and why does a tooth dissolve in acid? J Can Dent Assoc. 2003 Dec;69(11):722-4. PMID: 14653937.
7. Radzki D, Wilhelm-Węglarz M, Pruska K, Kusiak A, Ordyniec-Kwaśnica I. A Fresh Look at Mouthwashes-What Is Inside and What Is It For? Int J Environ Res Public Health. 2022 Mar 25;19(7):3926. doi: 10.3390/ijerph19073926. PMID: 35409608; PMCID: PMC8997378.
8. Valdivia-Tapia AC, Kelly AB, Hara AT, Cury JA, Ricomini-Filho AP, Lippert F. Erosion potential of commercial fluoride-free mouthwashes on dental hard tissues. Am Dent J. 2003. (manuscript in preparation)
9. Fushida CE, Cury JA: In situ evaluation of enameldentin erosion by beverage and recovery by saliva (in Portuguese). Rev Odontol Univ Sao Paulo 1999;13:127–134
10. Albeshir EG, Cook NB, Eckert GJ, Hara AT,Lippert F. Impact of mouthrinses on fluoride dentifricesefficacy preventing enamel and dentin erosion/abrasion. 2022 (in press)
11. Hara AT, González-Cabezas C, Creeth J, Parmar M, Eckert GJ, Zero DT. Interplay between fluoride and abrasivity of dentifrices on dental erosion-abrasion. J Dent. 2009 Oct;37(10):781-5. doi: 10.1016/j.jdent.2009.06.006. Epub 2009 Jun 18. PMID: 19577835.
12. Romão DA, Amoedo MM, Noronha MS, Hara AT, Cury JA, Tabchoury CPM. Validation of an erosion cycling model to estimate the pH dose-response effect on initial erosion of dental enamel. (manuscript in preparation).
13. Zero DT. Etiology of dental erosion--extrinsic factors. Eur J Oral Sci. 1996 Apr;104(2 (Pt 2)):162-77. Doi 10.1111/j.1600-0722.1996.tb00065.x. PMID: 8804884.
14. Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental erosion--an overview with emphasis on chemical and histopathological aspects. Caries Res. 2011;45 Suppl 1:2-12. Doi 10.1159/000325915. Epub 2011 May 31. PMID: 21625128.
15. Kanzow P, Wegehaupt FJ, Attin T, Wiegand A. Etiology and pathogenesis of dental erosion. Quintessence Int. 2016 Apr;47(4):275-8. doi: 10.3290/j.qi.a35625. PMID: 27022647
16. Wiegand A, Attin T. Influence of fluoride on the prevention of erosive lesions—a review. Oral Health and Preventive Dentistry 2003;4:245–53

17. Marinho VC, Chong LY, Worthington HV, Walsh T. Fluoride mouthrinses for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2016 Jul 29;7(7): CD002284. doi: 10.1002/14651858.CD002284.pub2. PMID: 27472005; PMCID: PMC6457869.
18. Sorvari R, Meurman JH, Alakuijala P, Frank RM. Effect of fluoride varnish and solution on enamel erosion in vitro. Caries Research 1994;28:227–32
19. Amaechi BT, Higham SM. Dental erosion: possible approaches to prevention and control. J Dent. 2005 Mar;33(3):243-52. doi: 10.1016/j.jdent.2004.10.014. Epub 2004 Nov 26. PMID: 15725524.
20. Venasakulchai A, Williams NA, Gracia LH, Rees GD. A comparative evaluation of fluoridated and non-fluoridated mouthrinses using a 5-day cycling enamel erosion model. J Dent. 2010 Nov;38 Suppl 3:S21-9. doi: 10.1016/S0300-5712(11)70005-7. PMID: 21256401
21. Joshi M, Joshi N, Kathariya R, Angadi P, Raikar S. Techniques to Evaluate Dental Erosion: A Systematic Review of Literature. J Clin Diagn Res. 2016 Oct;10(10):ZE01-ZE07. doi: 10.7860/JCDR/2016/17996.8634. Epub 2016 Oct 1. PMID: 27891489; PMCID: PMC5121827.
22. Azzopardi A, Bartlett DW, Watson TF, Sherriff M. The measurement and prevention of erosion and abrasion. J Dent. 2001;29:395-400
23. Lynch MC, Perfekt R, McGuire JA, Milleman J, Gallob J, Amini P, Milleman K. Potassium oxalate mouthrinse reduces dentinal hypersensitivity: A randomized controlled clinical study. J Am Dent Assoc. 2018 Jul;149(7):608-618. doi: 10.1016/j.adaj.2018.02.027. Epub 2018 May 2. PMID: 29728195.
24. Eisenburger M, Addy M, Hughes JA, Shellis RP. Effect of time on the remineralisation of enamel by synthetic saliva after citric acid erosion. Caries Res. 2001 May-Jun;35(3):211-5. doi: 10.1159/000047458. PMID: 11385202.
25. Zanolla J, Marques A, da Costa DC, de Souza AS, Coutinho M. Influence of tooth bleaching on dental enamel microhardness: a systematic review and meta-analysis. Aust Dent J. 2017 Sep;62(3):276-282. doi: 10.1111/adj.12494. Epub 2017 May 22. PMID: 27997982.
26. Tenuta LM, Fernández CE, Brandão AC, Cury JA. Titratable acidity of beverages influences salivary pH recovery. Braz Oral Res. 2015;29:S1806-83242015000100234. doi: 10.1590/1807-3107BOR-2015.vol29.0032. Epub 2015 Feb 11. PMID: 25715032.

3 DISCUSSÃO

Muitos enxaguatórios bucais não fluoretados (ENF) são comercializados com diferentes finalidades. Neste estudo, os ENF foram diferenciados de acordo ao declarado pela *Food and Drug Administration* (FDA). As informações coletadas para ENF de livre comercialização foram analisadas para fornecer uma visão geral da finalidade terapêutica e cosmética. De modo geral, apenas dois tipos de ENF com finalidade terapêutica podem ser identificados: I) antiplaca/antigengivite, e II) desbridamento oral/antisséptico/limpeza de feridas/enxaguatórios bucais para alívio da dor. Dos que apresentam objetivo terapêutico como controle de placa ou antigengivite, possuem como constituintes cloreto de cetilpiridínio (0,05-0,1%), óleos essenciais (0,092% eucaliptol/ 0,042% mentol/ 0,060% salicilato de metila/ 0,064% timol) e *Aloe vera* (20%).

Evidências substanciais apóiam a eficácia do cloreto de cetilpiridínio (CPC) e dos óleos essenciais como agentes antiplaca/antigengivite [Gusolley, 2010; Figuero et al., 2020]. O CPC é um agente tensoativo catiônico com amplo espectro antimicrobiano, com rápida eliminação de patógenos gram-positivos, e como um composto quaternário, inibe os primeiros estágios da formação do biofilme [Schroeder et al., 1962; Pitten e Kramer, 2001]. Sobre os óleos essenciais de acordo a FDA eles devem apresentar um veículo alcoólico para terem o efeito esperado [Fine et al., 1985]. Em altas concentrações, os óleos essenciais quebram as paredes celulares e causam precipitação de proteínas celulares, enquanto em concentrações mais baixas, provocam inativação de enzimas essenciais [Ross et al., 1989]. Sobre a *Aloe vera*, a FDA observou em sua proposta de regulamentação que "não há dados suficientes para permitir a classificação final da segurança e eficácia de *Aloe vera* como ingrediente antiplaca/antigengivite de livre comercialização" [FDA, 2003]. Vários estudos foram realizados desde então. Esses estudos eram geralmente a favor da ação de *Aloe vera*, porém considerados de baixa qualidade [Al-Maweri et al., 2020; Tidke et al., 2022].

Alguns produtos sem uma concentração declarada de peróxido de hidrogênio destinavam-se ao desbridamento oral, limpeza de feridas ou, às vezes, como antisséptico. Sabe-se que este agente em enxaguatórios bucais usados por si só não previne o acúmulo de placa, no entanto, quando utilizado como adjuvante na higiene bucal promove redução da inflamação gengival [Hossainian et al., 2011]. Um dos produtos possuía bicarbonato de sódio a 2,5% para limpeza de feridas orais e desbridamento oral. O mentol também estava presente nos produtos, tendo sido usado mais como agente aromatizante do que como ingrediente ativo, no entanto, foi comprovado que possui considerável atividade antimicrobiana, sendo considerado GRAS (*Generally Regarded as Safe*) pela FDA [Van Leeuwen et al., 2014; Freires et al., 2015]. Um

dos produtos cosméticos continha oxalato dipotássico monoidratado. Este pertence à família dos oxalatos, que são utilizados no tratamento da hipersensibilidade dentinária porque podem reduzir a permeabilidade dos túbulos dentinários. No entanto, não há fortes evidências de estudos clínicos que demonstrem um benefício consistente [Lynch et al., 2018; Cunha-Cruz et al., 2011].

O clareamento foi o objetivo mais comum dentre ENF cosmético, o que pode ser atribuído à presença de peróxido de hidrogênio e/ou hexametafosfato de sódio. Uma ampla gama de produtos contendo extratos vegetais foi observada. Ao todo 15 produtos possuíam extratos de plantas, contendo de um a mais de 10 extratos. Há um aumento constante no uso e na variedade de extratos de plantas em produtos de higiene bucal, com a intenção de tratar naturalmente doenças e condições bucais. Os extratos de plantas contêm diferentes classes de compostos, incluindo polifenóis, óleos essenciais e alcalóides, afetando potencialmente o controle de patologias associadas ao biofilme oral [Cardoso et al., 2021]. No entanto, atualmente existem poucas evidências clínicas para apoiar seu uso como agentes terapêuticos em enxaguatórios bucais.

Um número considerável de ENF (n=25) contém álcool (etanol), com até 26,9%. O álcool é usado como solubilizante, estabilizador, conservante, para aumentar a eficácia dos agentes antiplaca e para obter um sabor distinto. No entanto, existe controvérsia, pois estudos comparativos entre enxaguatórios bucais contendo álcool e não-alcoólicos mostraram que o álcool adiciona pouco à eficácia ao produto [Werner e Seymour, 2009].

Verificou-se que vários ENF cosméticos continham CPC ou óleos essenciais, mas não indicavam suas concentrações. Em relação ao CPC, isso provavelmente se deve ao uso de concentrações de CPC clinicamente ineficazes ou à sua baixa disponibilidade química na formulação. Com relação aos óleos essenciais, isso se deve ao fato de a formulação não conter álcool.

Dos produtos comerciais analisados no efeito em *S. mutans*, os produtos contendo peróxido de hidrogênio e hexametafosfato apresentaram os melhores resultados tanto para CIM quanto para biofilme. Isso acontece já que o peróxido de hidrogênio como foi mencionado anteriormente tem um efeito antimicrobiano em bactérias Gram-positivas e Gram-negativas [Brown et al., 1947]. Vários fatores são necessários para que ocorra o efeito antimicrobiano do peróxido de hidrogênio como concentração e duração da exposição são os fatores mais críticos. Para maximizar o efeito e reduzir os efeitos colaterais, é apropriado usar concentrações $\leq 1,5\%$ [Hossainian et al., 2011]. Os produtos que contêm CPC foram os segundos com melhor resultado no controle bacteriano. Este composto apresenta benefícios na redução do acúmulo

de biofilme dental. Formulações com CPC estão associadas a maior atividade biológica e, portanto, sugerem maior probabilidade de eficácia clínica [Versteeg et al., 2010]. *S mutans*, sendo uma bactéria gram-positiva, é reduzida na presença de produtos contendo CPC. Embora haja controvérsia de que estes tendem a ter um efeito clínico aumentado quando usados como adjuvantes em regimes mecânicos de higiene bucal [Barnett, 2003]. Em acréscimo, sugere-se que os enxaguatórios bucais com CPC promovem alterações na estrutura microbiana oral e/ou reduções na diversidade da comunidade que favorecem a resolução da disbiose e o restabelecimento de uma comunidade microbiana compatível com a saúde [do Amaral et al., 2022], sendo reconhecido e recomendado pela ADA com ação antiplaca/antigengivite [ADA, 2021]. Os produtos com óleos essenciais apresentaram um menor efeito que os mencionados anteriormente, porém com efeito maior que o grupo de extratos de plantas.

No primeiro estudo de erosão foi observado a comercialização de produtos com uma ampla faixa de pH, variando de 3,03 a 9,47. Apesar de não ser obrigatório descrever o valor de pH do produto no rótulo da embalagem, esta informação é relevante ao considerar que produtos de higiene bucal têm sido apontados como uma possível causa extrínseca da erosão dentária. No estudo, foi encontrado um elevado número de produtos (n=63) com pH menor que 5,5, valor baixo de pH que possibilitaria dissolução de esmalte e dentina, sendo relevante avaliar o potencial erosivo. De acordo a ADA, produtos com pH 5,5 são recomendados para demonstrar a segurança usando um teste de desmineralização, teste de erosão ou outros métodos apropriados [ADA, 2010].

Devido ao elevado número de amostras de ENF e o interesse de avaliar o efeito erosivo destes enxaguatórios, foi utilizado método ISO, um método padronizado para enxaguatórios sem fluoreto e que seria viável frente ao número de amostras. Segundo este estudo, os enxaguatórios bucais não apresentariam potencial erosivo, pois todos teriam resultados dentro da faixa considarada como não erosiva. No entanto, existem produtos com pH baixo e diferentes comportamentos foram encontrados, como aumentar o pH quando era esperado que diminuísse, ou vice-versa, o que pode indicar alguma desestabilização na fórmula do produto.

Embora este estudo consiga dar uma perspectiva sobre o potencial erosivo, era importante verificar o que aconteceria quando os enxaguatórios fossem avaliados utilizando substratos dentários. Sendo assim, para o segundo estudo de erosão, 6 enxaguatórios de 81 foram selecionados com base em seu pH inicial e comportamento no primeiro estudo de erosão. O segundo trabalho mostrou que alguns dos produtos avaliados possuem potencial erosivo e que nem o pH nem a acidez titulável são bons indicadores. Não foi encontrada correlação entre o pH inicial do produto e a perda de superfície dos dois substratos, nem entre a acidez titulável

e a perda de superfície. Indicar se o nível de erosão exibido é clinicamente relevante depende de uma variedade de fatores que não podem ser estudados satisfatoriamente em condições de laboratório.

Algumas limitações dos estudos consitiam em que foram considerados apenas produtos disponíveis pessoalmente em lojas convencionais. Sabe-se que atualmente através de diversos sites *web* é possível adquirir produtos que são vendidos em qualquer lugar do mundo. A diferenciação entre ENF terapêutico e cosmético foi impulsionada pela presença ou ausência de um rótulo de medicamento, em vez de haver evidências clínicas irrefutáveis para apoiar qualquer uma das reivindicações feitas. Finalmente, novos produtos estão sendo comercializados, outros são retirados ou reformulados, o que sugere que a repetição periódica de tal estudo pode ser justificada.

Em estudos de erosão, nenhum dos dois permite um entendimento completo, pois aspectos comportamentais também precisam ser considerados. No entanto, o presente estudo estabeleceu que alguns enxaguatórios de baixo pH têm potencial erosivo e que nem o pH nem a acidez titulável são bons indicadores. Se o nível de erosão exibido é clinicamente relevante depende de uma variedade de fatores que não podem ser estudados satisfatoriamente em condições de laboratório.

4 CONCLUSÃO

Considerando os resultados dos estudos realizados, pode-se concluir:

Um grande número de enxaguatórios bucais não fluoretados (ENF) está disponível comercialmente. Os ENF variam consideravelmente em sua composição e finalidade, muitos fornecendo apenas benefícios cosméticos. Entre os ENF terapêuticos, a maioria fornece benefícios antiplaca/antigengivite.

Os enxaguatórios bucais encontrados apresentam algum efeito sobre biofilme de *S. mutans*, porém e os produtos contendo agentes clareadores (peróxido de hidrogênio/ hexametafosfato de sódio) e CPC têm efeito mais significativo sobre o biofilme de *S. mutans*.

De acordo com nossos resultados usando o método ISO, todos os enxaguatórios seriam potencialmente seguros em erosão. Porém com a ciclagem de pH podemos observar que alguns produtos produzem perda de superfície em especial em dentina.

REFERÊNCIAS

Abdelwahed AG, Temirek MM, Hassan FM. Antierosive Effect of Topical Fluorides: A Systematic Review and Meta-Analysis of *In Situ* Studies. Open Access Maced J Med Sci. 2019 May 12;7(9):1523-1530. doi: 10.3889/oamjms.2019.291. PMID: 31198467; PMCID: PMC6542392.

Albeshir EG, Cook NB, Eckert GJ, Hara AT, Lippert F. Impact of mouthrinses on fluoride dentifrices efficacy preventing enamel and dentin erosion/abrasion. 2022 (in press)

Al-Maweri SA, Nassani MZ, Alaizari N, Kalakonda B, Al-Shamiri HM, Alhajj MN,et al.,. Efficacy of aloe vera mouthwash versus chlorhexidine on plaque and gingivitis: A systematic review. Int J Dent Hyg. 2020 Feb;18(1):44-51. doi: 10.1111/idh.12393. Epub 2019 Mar 25. PMID: 30829440.

Almoudi MM, Hussein AS, Abu Hassan MI, Mohamad Zain N. A systematic review on antibacterial activity of zinc against *Streptococcus mutans*. Saudi Dent J. 2018 Oct;30(4):283-291. doi: 10.1016/j.sdentj.2018.06.003. Epub 2018 Jun 18. PMID: 30202164; PMCID: PMC6128804.

Amaechi BT, Higham SM. Dental erosion: possible approaches to prevention and control. J Dent. 2005 Mar;33(3):243-52. doi: 10.1016/j.jdent.2004.10.014. Epub 2004 Nov 26. PMID: 15725524.

American Dental Association *(ADA). 2021. Department of Scientific Information, Evidence Synthesis & Translation Research, ADA Science & Research Institute, LLC.* https://www.ada.org/resources/research/science-and-research-institute/oral-health-topics/mouthrinse-mouthwash

American Dental Association (ADA). Mouthwash. MouthHealthy™. 2023. https://www.mouthhealthy.org/all-topics-a-z/mouthwash [accessed April 6, 2023]

American National Standards Institute ADA. ANSI/ADA Standard No. 116 for Oral Rinses. American National Standards Institute, American Dental Association. Chicago: American Dental Association. 2010.

American Dental Association *(ADA). 2019.* Reproduction or republication is strictly prohibited without prior written permission https://www.ada.org/resources/research/science-and-research-institute/oral-health-topics/mouthrinse-mouthwash

Moore J, White DJ, Schneiderman E, Chen H, Strand R, Grender JM. Laboratory screening evaluation of the safety of low pH oral care rinse products to dental enamel. Am J Dent. 2020 Jun;33(3):161-164.

Angelillo IF, Nobile CG, Pavia M. Evaluation of the effectiveness of a pre-brushing rinse in plaque removal: a meta-analysis. J Clin Periodontol. 2002 Apr;29(4):301-9. doi: 10.1034/j.1600-051x.2002.290405.x.

Azzopardi A, Bartlett DW, Watson TF, Sherriff M. The measurement and prevention of erosion and abrasion. J Dent. 2001;29:395-400

Barnett ML. The role of therapeutic antimicrobial mouthrinses in clinical practice. Control of supragingival plaque and gingivitis. J Am Dent Assoc 2003; 134: 699–701.

Batra C, Alalshaikh M, Gregory RL, Windsor LJ, Blanchard SB, Hamada Y. An in vitro comparison of four antibacterial agents with and without nicotine and their effects on human gingival fibroblasts. J Periodontol. 2022 Feb;93(2):e24-e33. doi: 10.1002/JPER.21-0262. Epub 2022 Jan 7.

Bhatti SA. Walsh TF Douglas WL. Ethanol and pH levels of proprietary mouthrinses. Comm Dent Health1994; 11:71-74

Brown EA, Krabek W, Skiffington R. Glycerite of hydrogen peroxide: I. Comparison of its bacteriotoxic with that of mercurial solutions. J Bacteriol 1947; 53: 793–799.

Cabrera-Jaime S, Martínez C, Ferro-García T, Giner-Boya P, Icart-Isern T, Estrada-Masllorens JM, Fernández-Ortega P. Efficacy of Plantago major, chlorhexidine 0.12% and sodium bicarbonate 5% solution in the treatment of oral mucositis in cancer patients with solid tumour: A feasibility randomised triple-blind phase III clinical trial. Eur J Oncol Nurs. 2018 Feb;32:40-47. doi: 10.1016/j.ejon.2017.11.006.

Cardoso VFDS, Amaral Roppa RH, Antunes C, Silva Moraes AN, Santi L, Konrath EL. Efficacy of medicinal plant extracts as dental and periodontal antibiofilm agents: A systematic review of randomized clinical trials. J Ethnopharmacol. 2021 Dec 5;281:114541. doi: 10.1016/j.jep.2021.114541.

Chaudhary P, Melkonyan A, Meethil A, et al. Estimating salivary carriage of severe acute respiratory syndrome coronavirus 2 in nonsymptomatic people and efficacy of mouthrinse in reducing viral load: A randomized controlled trial. J Am Dent Assoc 2021;152(11):903-08.

Cortelli SC, Cortelli JR, Wu MM, Simmons K, Charles CA. Comparative antiplaque and antigingivitis efficacy of a multipurpose essential oil-containing mouthrinse and a cetylpyridinium chloride-containing mouthrinse: A 6-month randomized clinical trial. Quintessence Int. 2012 Jul-Aug;43(7):e82-94.

Cunha-Cruz J, Stout JR, Heaton LJ, Wataha JC; Northwest PRECEDENT. Dentin hypersensitivity and oxalates: a systematic review. J Dent Res. 2011 Mar;90(3):304-10. doi: 10.1177/0022034510389179.

Cvjetinovic A, Ramseier CA, Salvi GE, Laugisch O. Chemische Zusätze in Zahnpasten zur Hemmung der Zahnsteinbildung. Eine Literaturübersicht [Chemical additives in toothpastes to inhibit calculus formation]. Swiss Dent J. 2020 Jun 15;130(6):503-513. German.

David, MPR, and Shree, MKT. Effectiveness of turmeric mouthwash and sodium bicarbonate mouthwash to reduce oral mucositis among patient undergoing radiation therapy. Scholars International Journal of Traditional and Complementary Medicine. (2019). 2(7), 124-127.

Mohammadi F, Oshvandi K, Kamallan SR, Khazaei S, Ranjbar H, Ahmadi-Motamayel F, Gillespie M, Jenabi E, Vafaei SY. Effectiveness of sodium bicarbonate and zinc chloride

mouthwashes in the treatment of oral mucositis and quality of life in patients with cancer under chemotherapy. Nurs Open. 2022 May;9(3):1602-1611. doi: 10.1002/nop2.1168.

Dawes C. What is the critical pH and why does a tooth dissolve in acid? J Can Dent Assoc. 2003 Dec;69(11):722-4.

Delgado AJ, Olafsson VG, Donovan TE. pH and Erosive Potential of Commonly Used Oral Moisturizers. J Prosthodont 2016;25(1):39-43

Denton GW. Chlorhexidine. In: Block SS, ed. Disinfection, Sterilization and preservation. 4th edn. Philadelphia: Lea and Febiger, 1991: 274-289.

do Amaral GCLS, Hassan MA, Sloniak MC, Pannuti CM, Romito GA, Villar CC. Effects of antimicrobial mouthwashes on the human oral microbiome: Systematic review of controlled clinical trials. Int J Dent Hyg. 2023 Feb;21(1):128-140. doi: 10.1111/idh.12617.

Dong L, Tong Z, Linghu D, Lin Y, Tao R, Liu J, Tian Y, Ni L. Effects of sub-minimum inhibitory concentrations of antimicrobial agents on *Streptococcus mutans* biofilm formation. Int J Antimicrob Agents. 2012 May;39(5):390-5. doi: 10.1016/j.ijantimicag.2012.01.009.

Duckworth RM, Maguire A, Omid N, Steen IN, McCracken GI, Zohoori FV. Effect of rinsing with mouthwashes after brushing with a fluoridated toothpaste on salivary fluoride concentration. Caries Res. 2009;43(5):391-6. doi: 10.1159/000239753.

Eisenburger M, Addy M, Hughes JA, Shellis RP. Effect of time on the remineralisation of enamel by synthetic saliva after citric acid erosion. Caries Res. 2001 May-Jun;35(3):211-5. doi: 10.1159/000047458.

Figuero E, Herrera D, Tobías A, Serrano J, Roldán S, Escribano M, Martín C. Efficacy of adjunctive anti-plaque chemical agents in managing gingivitis: A systematic review and network meta-analyses. J Clin Periodontol. 2019 Jul;46(7):723-739. doi: 10.1111/jcpe.13127.

Figuero E, Roldán S, Serrano J, Escribano M, Martín C, Preshaw PM. Efficacy of adjunctive therapies in patients with gingival inflammation: A systematic review and meta-analysis. J Clin Periodontol. 2020 Jul;47 Suppl 22:125-143. doi: 10.1111/jcpe.13244.

Fine DH, Letizia J, Mandel ID. The effect of rinsing with Listerine antiseptic on the properties of developing dental plaque. J Clin Periodontol 1985;12:660-666.

Fine DH. Mouthrinses as adjuncts for plaque and gingivitis management. A status report for the American Journal of Dentistry. Am J Dent 1988;1:259-263.

Finney, M.; Walker, J.T.; Marsh, P.D.; Brading, M.G. Antimicrobial effects of a novel Triclosan/zinc citrate dentifrice against mixed culture oral biofilms. *Int. Dent. J.* **2003**, *53* (Suppl. S1), 371–378.

Food and Drug Administration (FDA). Department of Health and Human Services. 21 CFR Part 356 Oral Health Care Drug ProducIOverthe-Counter Human Use; Antigingivitis/ Antiplaque Drug Products; Establishment of a Monograph; Proposed Rules. Federal Register / Vol. 68, No. 103 / Thursday, May 29, 2003 / Proposed Rules. https://www.govinfo.gov/content/pkg/FR-2003-05-29/pdf/03-12783.pdf. [accessed April 1, 2023]

Food and Drug Administration (FDA). Oral Healthcare Drug Products: Anesthetic, Astringent, Antimicrobial, Debriding, Demulcent, Expectorant, Decongestant. 2017. https://www.fda.gov/drugs/historical-status-otc-rulemakings/rulemaking-history-otc-oral-healthcare-drug-products#oral [accessed April 1, 2023]

Freires IA, Denny C, Benso B, de Alencar SM, Rosalen PL. Antibacterial Activity of Essential Oils and Their Isolated Constituents against Cariogenic Bacteria: A Systematic Review. Molecules. 2015 Apr 22;20(4):7329-58. doi: 10.3390/molecules20047329.

Fushida CE, Cury JA: In situ evaluation of enameldentin erosion by beverage and recovery by saliva (in Portuguese). Rev Odontol Univ Sao Paulo 1999;13:127–134

Gandara BK, Truelove EL. Diagnosis and management of dental erosion. J Contemp Dent Pract. 1999 Nov 15;1(1):16-23.

Gunsolley JC. Clinical efficacy of antimicrobial mouthrinses. J Dent. 2010 Jun;38 Suppl 1:S6-10. doi: 10.1016/S0300-5712(10)70004-X.

Gunsolley, J.C. A Meta-Analysis of Six-Month Studies of Antiplaque and Antigingivitis Agents. J. Am. Dent. Assoc. 2006, 137, 1649–1657.

Hall PJ, Green AK, Horay CP, de Brabander S, Beasley TJ, Cromwell VJ, Holt JS, Savage DJ. Plaque antibacterial levels following controlled food intake and use of a toothpaste containing 2% zinc citrate and 0.3% Triclosan. Int Dent J. 2003 Dec;53(6 Suppl 1):379-84. doi: 10.1111/j.1875-595x.2003.tb00913.x.

Haps S, Slot DE, Berchier CE, van der Weijden GA. The effect of cetylpyridinium chloride-containing mouth rinses as adjuncts to toothbrushing on plaque and parameters of gingival inflammation: a systematic review. Int J Dent Hyg 2008;6(4):290–303. 5.

Hara AT, González-Cabezas C, Creeth J, Parmar M, Eckert GJ, Zero DT. Interplay between fluoride and abrasivity of dentifrices on dental erosion-abrasion. J Dent. 2009 Oct;37(10):781-5. doi: 10.1016/j.jdent.2009.06.006. Epub 2009 Jun 18.

Hasturk H, Nunn M, Warbington M, Van Dyke TE. Efficacy of a fluoridated hydrogen peroxide-based mouthrinse for the treatment of gingivitis: a randomized clinical trial. J Periodontol. 2004 Jan;75(1):57-65. doi: 10.1902/jop.2004.75.1.57.

Hosida TY, Pessan JP, Cavazana TP, Sampaio C, Monteiro DR, Botazzo Delbem AC. Effect of sodium hexametaphosphate and fluoride on dual-species biofilms of *Candida albicans* and *Streptococcus mutans*. Biofouling. 2021 Oct-Nov;37(9-10):939-948. doi: 10.1080/08927014.2021.1916816.

Hossainian N, Slot DE, Afennich F, Van der Weijden GA. The effects of hydrogen peroxide mouthwashes on the prevention of plaque and gingival inflammation: a systematic review. Int J Dent Hyg. 2011 Aug;9(3):171-81. doi: 10.1111/j.1601-5037.2010.00492.x.

Huang R, Li M, Gregory RL. Effect of nicotine on growth and metabolism *of Streptococcus mutans*. Eur J Oral Sci. 2012;120(4):319–25.

International Standard (ISO). Dentistry — Screening method for erosion potential of oral rinses on dental hard tissues. ISO/FDIS 28888:2013(E)

Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monogr Oral Sci. 2014;25:55-73. doi: 10.1159/000360973.

Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. In: Lussi A, editor. Dental erosion: from diagnosis to therapy. Basel: Karger; 2006. p.44−65.

Jones CG. Chlorhexidine: is it still the gold standard? Periodontol 2000. 1997 Oct;15:55-62. doi: 10.1111/j.1600-0757.1997.tb00105.x.

Jose A, Siddiqi M, Cronin M, DiLauro TS, Bosma ML. A randomized clinical trial in subjects with dry mouth evaluating subjective perceptions of an experimental oral gel, an oral rinse and a mouth spray compared to water. Am J Dent. 2016 Feb;29(1):58-64. PMID: 27093778.

Joshi M, Joshi N, Kathariya R, Angadi P, Raikar S. Techniques to Evaluate Dental Erosion: A Systematic Review of Literature. J Clin Diagn Res. 2016 Oct;10(10):ZE01-ZE07. doi: 10.7860/JCDR/2016/17996.8634. Epub 2016 Oct 1.

Kanzow P, Wegehaupt FJ, Attin T, Wiegand A. Etiology and pathogenesis of dental erosion. Quintessence Int. 2016 Apr;47(4):275-8. doi: 10.3290/j.qi.a35625.

Khoramian Tusi S, Jafari A, Marashi SMA, Faramarzi Niknam S, Farid M, Ansari M. The effect of antimicrobial activity of Teucrium Polium on Oral *Streptococcus mutans*: a randomized cross-over clinical trial study. BMC Oral Health. 2020 May 1;20(1):130. doi: 10.1186/s12903-020-01116-4.

Langa GPJ, Muniz FWMG, Costa RDSA, da Silveira TM, Rösing CK. The effect of cetylpyridinium chloride mouthrinse as adjunct to toothbrushing compared to placebo on

interproximal plaque and gingival inflammation-a systematic review with meta-analyses. Clin Oral Investig. 2021 Feb;25(2):745-757. doi: 10.1007/s00784-020-03661-2.

Lee SS, Suprono MS, Stephens J, Withers SA, Oyoyo U, Li Y. A randomized, blinded, clinical investigation of breath odor reduction efficacy of a stabilized chlorine-dioxide containing flavored mouthwash. Am J Dent. 2021 Aug;34(4):195-200.

Lussi A, Schlueter N, Rakhmatullina E, Ganss C. Dental erosion--an overview with emphasis on chemical and histopathological aspects. Caries Res. 2011;45 Suppl 1:2-12. Doi 10.1159/000325915.

Lynch MC, Cortelli SC, McGuire JA, Zhang J, Ricci-Nittel D, Mordas CJ, Aquino DR, Cortelli JR. The effects of essential oil mouthrinses with or without alcohol on plaque and gingivitis: a randomized controlled clinical study. BMC Oral Health. 2018 Jan 10;18(1):6. doi: 10.1186/s12903-017-0454-6.

Lynch MC, Perfekt R, McGuire JA, Milleman J, Gallob J, Amini P, Milleman K. Potassium oxalate mouthrinse reduces dentinal hypersensitivity: A randomized controlled clinical study. J Am Dent Assoc. 2018 Jul;149(7):608-618. doi: 10.1016/j.adaj.2018.02.027.

Lynch, R.J.M., 2011. Zinc in the mouth, its interactions with dental enamel and possible effects on caries; a review of the literature. Int. Dent. J. 61, 46–54.

Marinho VC, Chong LY, Worthington HV, Walsh T. Fluoride mouthrinses for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2016 Jul 29;7(7):CD002284. doi: 10.1002/14651858.CD002284.pub2.

Marui VC, Souto MLS, Rovai ES, et al. Efficacy of preprocedural mouthrinses in the reduction of microorganisms in aerosol: A systematic review. J Am Dent Assoc 2019;150(12):1015-26.e1.

Mohd-Said S, Mohd-Dom TN, Suhaimi N, Rani H, McGra th C. Effectiveness of Pre-procedural Mouth Rinses in Reducing Aerosol Contamination During Periodontal Prophylaxis: A Systematic Review. Front Med (Lausanne) 2021;8:600769.

Moore J, White DJ, Schneiderman E, Chen H, Strand R, Grender JM. Laboratory screening evaluation of the safety of low pH oral care rinse products to dental enamel. Am J Dent. 2020 Jun;33(3):161-164.

National archives and records administration. Code of Federal Regulatio–s. Subpart C - Labeling Requirements for Over-the-Counter Drugs. Nomenclature changes to part 201 appear at 69 FR 13717, Mar. 24, 2004. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-201/subpart-C [accessed April 6, 2023]

Ouhayoun JP. Penetrating the plaque biofilm: Impact of essential oil mouthwash. J Clin Periodontol 2003; 30(Suppl. 5):10-12

Paraskevas, S.; Rosema, N.A.M.; Versteeg, P.; Van der Velden, U.; Van der Weijden, G.A. Chlorine Dioxide and Chlorhexidine Mouthrinses Compared in a 3-Day Plaque Accumulation Model. J. Periodontol. 2008, 79, 1395–1400.

Phan, T.-N., Buckner, T., Sheng, J., Baldeck, J.D., Marquis, R.E., 2004. Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. Oral Microbiol. Immunol. 19, 31–38.

Pitten FA, Kramer A. Efficacy of cetylpyridinium chloride used as oropharyngeal antiseptic. Arzneim Forsch/Drug Res 2001; 51: 588–595.

Radzki D, Wilhelm-Węglarz M, Pruska K, Kusiak A, Ordyniec-Kwaśnica I. A Fresh Look at Mouthwashes-What Is Inside and What Is It For? Int J Environ Res Public Health. 2022 Mar 25;19(7):3926. doi: 10.3390/ijerph19073926.

Rajendiran M, Trivedi HM, Chen D, Gajendrareddy P, Chen L. Recent Development of Active Ingredients in Mouthwashes and Toothpastes for Periodontal Diseases. Molecules. 2021 Apr 1;26(7):2001. doi: 10.3390/molecules26072001.

Reddy A, Norris DF, Momeni SS, Waldo B, Ruby JD. The pH of beverages in the United States. J Am Dent Assoc 2016;147(4):255-63

Romão DA, Amoedo MM, Noronha MS, Hara AT, Cury JA, Tabchoury CPM. Validation of an erosion cycling model to estimate the pH dose-response effect on initial erosion of dental enamel. (manuscript in preparation).

Ross NM, Charles CH, Dills SS. Long-term effects of Listerine antiseptic on dental plaque and gingivitis. J Clin Dent 1989;1:92-95.

Rytomaa L. Meurman JH, Franssila S, Torkko H. Oral hygiene products may cause dental erosion. Proc FinnDent Soc 1989; 85: 161-166.93.

Scaramucci T, Hara AT, Zero DT, Ferreira SS, Aoki IV, Sobral MA. In vitro evaluation of the erosive potential of orange juice modified by food additives in enamel and dentine. J Dent. 2011 Dec;39(12):841-8. doi: 10.1016/j.jdent.2011.09.004.

Tenuta LM, Fernández CE, Brandão AC, Cury JA. Titratable acidity of beverages influences salivary pH recovery. Braz Oral Res. 2015;29:S1806-83242015000100234. doi: 10.1590/1807-3107BOR-2015.vol29.0032.

Schlueter N, Amaechi BT, Bartlett D, Buzalaf MAR, Carvalho TS, Ganss C, Hara AT, Huysmans MDNJM, Lussi A, Moazzez R, Vieira AR, West NX, Wiegand A, Young A, Lippert F. Terminology of Erosive Tooth Wear: Consensus Report of a Workshop Organized by the ORCA and the Cariology Research Group of the IADR. Caries Res. 2020;54(1):2-6. doi: 10.1159/000503308.

Schroeder HE, Marthaler TM, Mühlemann HR. Effects of some potential inhibitors on early calculus formation. Helv Odont Acta 1962; 6: 6–9.

Shaw E, Wuest WM. Virulence attenuating combination therapy: a potential multi-target synergy approach to treat Pseudomonas aeruginosa infections in cystic fibrosis patients. RSC Med Chem. 2020 Feb 19;11(3):358-369. doi: 10.1039/c9md00566h.

Shibata H, Morioka T. Antibacterial action of condensed phosphates on the bacterium *Streptococcus mutans* and experimental caries in the hamster. Arch Oral Biol. 1982;27(10):809-16. doi: 10.1016/0003-9969(82)90034-6.

Sorvari R, Meurman JH, Alakuijala P, Frank RM. Effect of fluoride varnish and solution on enamel erosion in vitro. Caries Research 1994;28:227–32

Stoeken JE, Paraskevas S, van der Weijden GA. The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: a systematic review. Journal of Periodontology 2007;78(7):1218−28.

Subramaniam P, Nandan N. Effect of xylitol, sodium fluoride and triclosan containing mouth rinse on *Streptococcus mutans*. Contemp Clin Dent. 2011;2(4):287.

Takenaka S, Sotozono M, Ohkura N, Noiri Y. Evidence on the Use of Mouthwash for the Control of Supragingival Biofilm and Its Potential Adverse Effects. Antibiotics (Basel). 2022 May 28;11(6):727. doi: 10.3390/antibiotics11060727.

ten Cate JM, Duijsters PP. Influence of fluoride in solution on tooth demineralization. I. Chemical data. Caries Res. 1983;17(3):193-9. doi: 10.1159/000260667. PMID: 6573960.

Tidke S, Chhabra GK, Madhu PP, Reche A, Wazurkar S, Singi SR. The Effectiveness of Herbal Versus Non-Herbal Mouthwash for Periodontal Health: A Literature Review. Cureus. 2022 Aug 13;14(8):e27956. doi: 10.7759/cureus.27956. PMID: 36120261; PMCID: PMC9465625.

Valdivia-Tapia AC, Botelho JN, Giacaman RA, Tabchoury CPM, Cury JA. Fluoride concentration in mouth rinses marketed in Chile and Brazil, and a discussion regarding their legislations. Braz Oral Res. 2021 Aug 18;35:e083. Doi 10.1590/1807-3107bor-2021.vol35.0083. PMID: 34431848.

Valdivia-Tapia AC, Kelly AB, Hara AT, Cury JA, Ricomini-Filho AP, Lippert F. Erosion potential of commercial fluoride-free mouthwashes on dental hard tissues. Am Dent J. 2023 (manuscript in preparation)

Van der Weijden FA, Van der Sluijs E, Ciancio SG, Slot DE. Can Chemical Mouthwash Agents Achieve Plaque/Gingivitis Control? Dent Clin North Am. 2015 Oct;59(4):799-829. doi: 10.1016/j.cden.2015.06.002.

Van Leeuwen MP, Slot DE, Van der Weijden GA. The effect of an essential-oils mouthrinse as compared to a vehicle solution on plaque and gingival inflammation: a systematic review and meta-analysis. Int J Dent Hyg. 2014 Aug;12(3):160-7. doi: 10.1111/idh.12069.

Van Leeuwen, M.P.; Slot, D.E.; van der Weijden, G.A. Essential Oils Compared to Chlorhexidine with Respect to Plaque and Parameters of Gingival Inflammation: A Systematic Review. J. Periodontol. 2011, 82, 174–194

Venasakulchai A, Williams NA, Gracia LH, Rees GD. A comparative evaluation of fluoridated and non-fluoridated mouthrinses using a 5-day cycling enamel erosion model. J Dent. 2010 Nov;38 Suppl 3:S21-9. doi: 10.1016/S0300-5712(11)70005-7.

Versteeg PA, Rosema NA, Hoenderdos NL, et al. The plaque inhibitory effect of a CPC mouthrinse in a 3-day plaque accumulation model – a cross-over study. Int J Dent Hyg 2010;8:269–75.

Werner CW, Seymour RA. Are alcohol containing mouthwashes safe? Br Dent J. 2009 Nov 28;207(10):E19; discussion 488-9. doi: 10.1038/sj.bdj.2009.1014.

Wiegand A, Attin T. Influence of fluoride on the prevention of erosive lesions—a review. Oral Health and Preventive Dentistry 2003;4:245–53

Zero DT. Dentifrices, mouthwashes, and remineralization/caries arrestment strategies. BMC Oral Health. 2006 Jun 15;6 Suppl 1(Suppl 1):S9. doi: 10.1186/1472-6831-6-S1-S9.

Zero DT. Etiology of dental erosion--extrinsic factors. Eur J Oral Sci. 1996 Apr;104(2 (Pt 2)):162-77. doi 10.1111/j.1600-0722.1996.tb00065.x.

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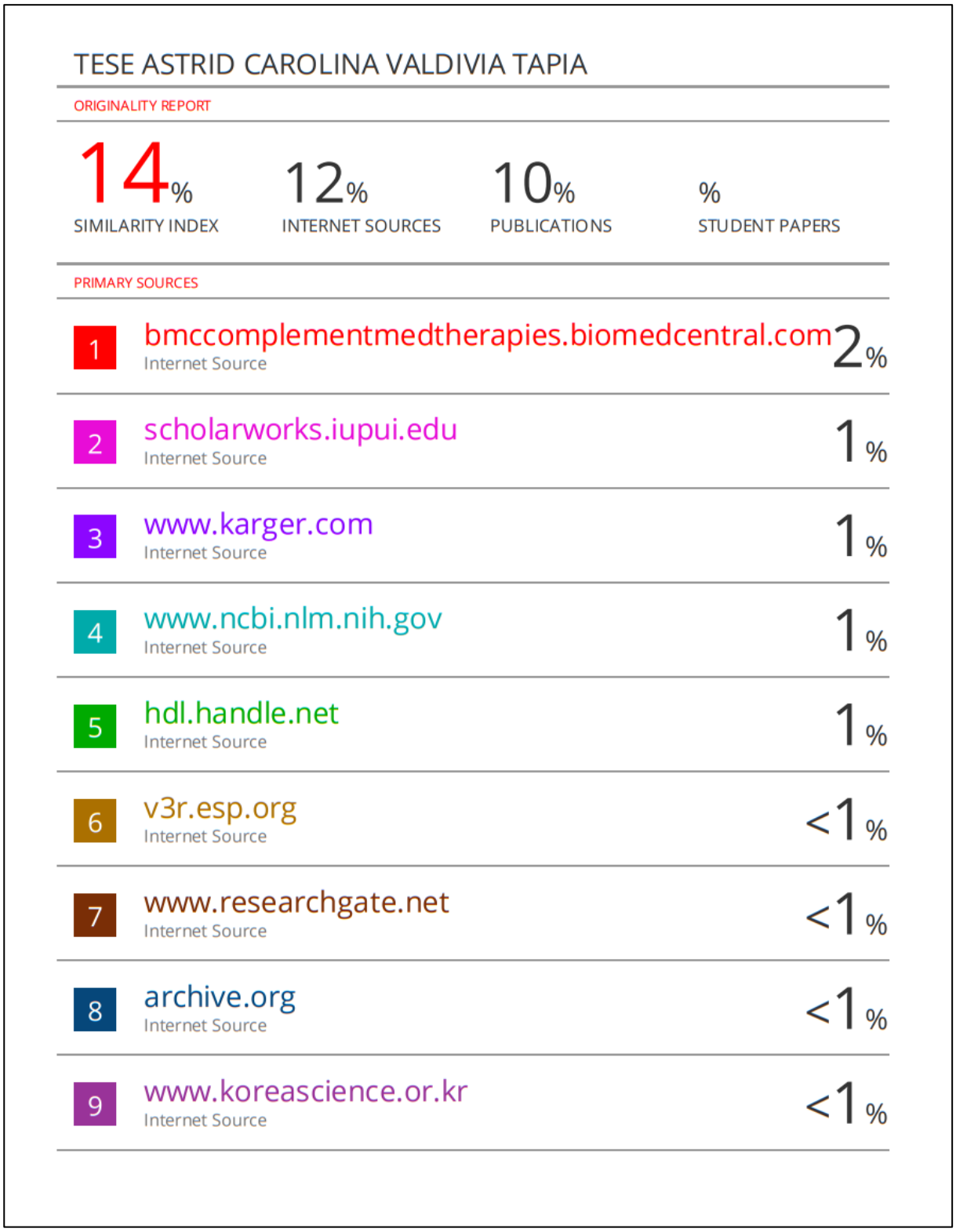

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Fluoride-free mouthwashes available over-the-counter in the US-an overview

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