



## Research article

Protective effect of calcium silicate toothpaste on enamel erosion and abrasion *in vitro*

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## ARTICLE INFO

## Keywords:

Enamel  
Erosion  
Abrasion  
Toothbrushing  
Toothpaste

## ABSTRACT

**Objectives:** To compare *in vitro* the effect of a toothpaste containing fluoride (F), calcium silicate (CaSi) and sodium phosphate salts to conventional toothpaste (NaF) on human enamel specimens submitted to erosive and abrasive challenges.

**Methods:** 48 sound and 48 enamel samples pre-treated with 1% citric acid were divided into 4 groups (n = 12): Group 1- Non-fluoride toothpaste; Group 2- NaF toothpaste (1450 ppmF); Group 3- CaSi toothpaste (1450 ppmF; MFP); Group 4- Erosion only. The samples were subjected to pH cycling (3 cycles/day; 90s; 1% citric acid, pH 3.6) and to abrasion for 7 days. After the 1<sup>st</sup> and the last cycle, they were submitted to abrasion (15s, 1.5N load), using a brushing machine, soft toothbrush and toothpaste slurry (1:3; 15ml/sample) and then immersed in the slurry for 45s. Samples were immersed in artificial saliva between the challenges. Enamel loss was evaluated using profilometry on days 3 and 7. Data were analysed by ANOVA and Tukey's test (p < 0.05).

**Results:** For sound enamel at baseline, mean (±SD) enamel loss (µm) for groups 1–4 on day 3 was 2.15 ± 0.35<sup>a</sup>, 1.20 ± 0.22<sup>b</sup>, 0.95 ± 0.19<sup>b</sup> and 1.98 ± 0.32<sup>a</sup>; on day 7 was 3.05 ± 0.40<sup>a</sup>, 2.07 ± 0.32<sup>b</sup>, 1.36 ± 0.33<sup>c</sup> and 3.69 ± 0.27<sup>d</sup> respectively. For acid-softened enamel at baseline, enamel loss on day 3 was 3.16 ± 0.19<sup>a</sup>, 2.17 ± 0.14<sup>b</sup>, 1.70 ± 0.11<sup>c</sup> and 3.04 ± 0.19<sup>a</sup>; on day 7 was 3.92 ± 0.25<sup>a</sup>, 3.07 ± 0.13<sup>b</sup>, 2.09 ± 0.15<sup>c</sup> and 3.87 ± 0.25<sup>a</sup> respectively.

**Conclusions:** Both F toothpastes led to significantly higher enamel protection from short-term erosion and abrasion in comparison to the non-F toothpaste and erosion only. In the longer term, CaSi toothpaste conferred significantly higher protection than NaF toothpaste.

**Clinical significance:** The results showed that for the longer term the CaSi toothpaste provided significantly higher protection than the NaF toothpaste, which indicates a good potential of the former to help prevent erosive tooth wear.

## 1. Introduction

Tooth wear refers to the loss of mineralized tissue caused by chemical and mechanical challenges, excluding caries. It involves distinct processes, termed as erosion, abrasion and attrition. Erosion typically involves chemical loss of dental tissue provoked by dietary or gastric acids; abrasion involves physical removal of dental tissue provoked by insertion of foreign bodies into the oral cavity, and attrition is the physical removal of dental hard tissue provoked by the direct contact between teeth [1]. Tooth wear is considered a multi-factorial process in which interactions between distinct mechanisms act in concert to lead to the clinically

observed pattern of wear. The interactions between the processes of chemical (erosion) and mechanical wear are the most common ones and this is termed as erosive tooth wear [2,3].

The enamel wear during tooth brushing is complex, being affected by many variables [4]. Some degree of abrasivity is acceptable in toothpastes provided that proper teeth cleaning is achieved [5,6] since low or non-abrasive toothpastes do not prevent extrinsic tooth stain formation [7]. However, the toothpaste abrasivity needs to be moderated and is controlled by national and international standards [8]. The wear of sound enamel due to the toothbrush alone is minimal but in combination with toothpaste can lead to some enamel wear *in vitro* [8,9]. However,

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<https://doi.org/10.1016/j.heliyon.2021.e06741>

Received 30 October 2020; Received in revised form 19 January 2021; Accepted 1 April 2021

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evidence from clinical (*in situ* and *in vivo*) studies demonstrates that brushing with a toothbrush and toothpaste does not reach clinically significant levels in a lifetime [8,10,11,12,13]. However, following exposure of enamel to acid softening, this makes the enamel surface more vulnerable to abrasion, not only to the effects of toothbrush and toothpaste, but also toothbrush alone or even friction from the human tongue [2,8,14].

The prevalence of erosive tooth wear is generally increasing [15] and thus the need for protective agents that are easily applicable such as via toothpaste are required. This has led to the development of a number of toothpaste formulations with potential anti-erosive properties [16], for example, toothpaste containing materials such as polyvalent metal fluorides [17], hydroxyapatite (HAP) [18], Zinc-carbonate HAP [19] and calcium silicate (CaSi) [20].

The particular interest in CaSi arises from the fact that it can be deposited on sound and eroded enamel surfaces and transformed into HAP [21]. Therefore, it is a material with potential to protect and repair early stages of enamel erosion. Indeed, it has been demonstrated that CaSi incorporated into a toothpaste can form HAP on enamel surfaces *in vitro* and *in situ* [22] and to prevent enamel demineralisation and to promote enamel remineralisation compared to a NaF toothpaste *in vitro* [23,24]. There is a need to further evaluate the potential erosive tooth wear benefits of CaSi containing toothpaste versus control toothpastes under protocols that incorporate pH cycling to mimic erosion challenges during the day and twice/day toothbrushing challenges.

The objective of this *in vitro* study is to compare the effect of a toothpaste containing CaSi, sodium phosphate salts and fluoride to control toothpastes containing fluoride or no fluoride on sound and pre-acid softened human enamel specimens, subjected to both erosive and abrasive challenges. The null hypothesis tested was that there is no difference in the protective effect between CaSi and either NaF or non-fluoride control toothpastes on enamel wear, after short-term (3 days) or long-term (7 days) erosive-abrasive challenges.

## 2. Material and methods

The Ethics Committee of Bauru School of Dentistry, University of São Paulo approved the protocol of the study (CAAE: 56407715.2.00005417). The teeth were obtained from the dental clinics of Bauru School of Dentistry, University of São Paulo, after the donors signed an informed consent document.

### 2.1. Sample preparation

Human enamel specimens were prepared from recently extracted unerupted 3<sup>rd</sup> molars that had been stored in 0.1% thymol solution (pH 7.0). Roots and crowns were separated using a cutting machine (Maruto, Tokyo, Japan) and a diamond disc (Maruto, Tokyo, Japan). The crowns were placed in a silicone mold (Biopdi, São Carlos, Brazil) and embedded in autopolymerising acrylic resin with the labial surface exposed. After polymerisation, silicon carbide sandpapers were employed to polish the samples (320, 600 and 1200 grades of Al<sub>2</sub>O<sub>3</sub> paper; Buehler, Lake Bluff, USA). Thereafter, the baseline profile was taken with a contact profilometry and 2/3 of the surface of the samples were sealed with purple nail varnish (Risqué, Sao Paulo, Brazil), providing 2 control areas.

The samples were randomly assigned to treatment groups, with 48 enamel samples allocated to sound enamel at baseline and a further 48 samples allocated to an initial pre-treatment of 1% citric acid (pH 3.6) for 10 min. The samples were then allocated to 4 groups (n = 12/group) according to the treatment: Group 1 – brushing with non-fluoride toothpaste; Group 2 – brushing with 1450 ppm F (as NaF) silica toothpaste (positive control); Group 3 – brushing with CaSi toothpaste (CaSi, sodium phosphate salts and 1450 ppm F as MFP); Group 4 – erosion only with no brushing. All the test toothpastes were prepared by Unilever Oral Care (UK) to have closely matched relative dentine abrasivity (RDA) and

relative enamel abrasivity (REA) values (Table 1). The RDA and REA values were provided by the manufacturer.

### 2.2. Erosive-abrasive cycling and treatments

A day before the beginning of the study (day 0), the samples were immersed in artificial saliva overnight. From day 1 until day 7, the samples were subjected to erosive and abrasive challenges daily. Three times per day, the erosive challenges were performed by immersion of the samples in 1% citric acid solution (pH 3.6, 30 mL/sample) unstirred for 90 s at 25 °C. They were then washed with deionised water (5 s) and immersed in unstirred artificial saliva (pH 6.8, 30 mL/sample) [25], between the erosive and abrasive challenges, at 25 °C.

After the first erosive challenge and 30 min of immersion in artificial saliva, the samples were submitted to abrasion for 15 s. For this, a tooth brushing machine (Biopdi®, São Carlos, Brazil), toothbrushes (5460 ultrasoft Curaprox®, Kriens, Switzerland, 1 toothbrush/sample) and slurry of the toothpastes (1:3 water, 15 mL/sample) under standardised force (1.5 N), at 37 °C were employed. After brushing, the samples were kept in contact with the slurries for further 45 s, then washed with deionised water (5 s) and immersed in artificial saliva. After the last erosive challenge and 30 min of immersion in artificial saliva, the samples were again brushed as described above.

The specimens were kept in artificial saliva along the night to complete 24 h of pH-cycling each day, as schematically shown in Figure 1. The composition of the artificial saliva was: 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM CaCl<sub>2</sub>·2H<sub>2</sub>O, 3 mM NH<sub>4</sub>Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K<sub>2</sub>HPO<sub>4</sub>, 3.3 mM urea, 2.4 mM NaH<sub>2</sub>PO<sub>4</sub> and traces of ascorbic acid (pH 6.8) [25]. The volume of artificial saliva to be used in the whole experiment was prepared once and stored at 8 °C. The artificial saliva was changed on a daily basis.

On days 3 and 7, the nail polish was removed and the tooth wear was assessed using contact profilometry. After the profile measurement on day 3, the nail polish was reapplied.

### 2.3. Contact profilometry

Tooth wear was determined with a contact profilometer (Mahr Perthometer, Göttingen, Germany). Five equidistant surface scans of each specimen were taken (reading of 5 mm, 250 µm apart from each other with an area of 5 mm<sup>2</sup>) at the baseline, and on days 3 and 7. To achieve the repeatability, the specimens presented an identification mark (small drill holes made with drill 1/4) and two scratches at the area exposed to the erosive challenge. The samples were inserted into a metal apparatus (x and y axes determination, SD 0.08) that allowed the stylus was accurately repositioned at each measurement. The baseline profile was compared to the final profiles to calculate tooth wear, by using the software Marh Surf XCR20. The scans were superimposed (Figure 2) and this allowed the calculation of the average depth of the area-under the curve in µm.

### 2.4. Statistical analysis

The data were analysed using Graph Pad InStat Software version 6.0 for Windows (San Diego, USA). Data were checked for homogeneity (Bartlett

**Table 1.** Mean and standard deviations for relative dentine abrasivity (RDA) and relative enamel abrasivity (REA) values of test toothpastes.

	RDA	REA
Non-fluoride Toothpaste	104.94 ± 4.51 <sup>a</sup>	5.45 ± 0.76 <sup>a</sup>
NaF Toothpaste	113.61 ± 10.12 <sup>ab</sup>	4.70 ± 0.92 <sup>a</sup>
CaSi Toothpaste	117.35 ± 10.36 <sup>b</sup>	1.96 ± 1.70 <sup>b</sup>

Distinct letters in each column show significant differences among the groups (ANOVA and Tukey's test, p < 0.05). These values were provided by the manufacturer (Unilever Oral Care, UK).

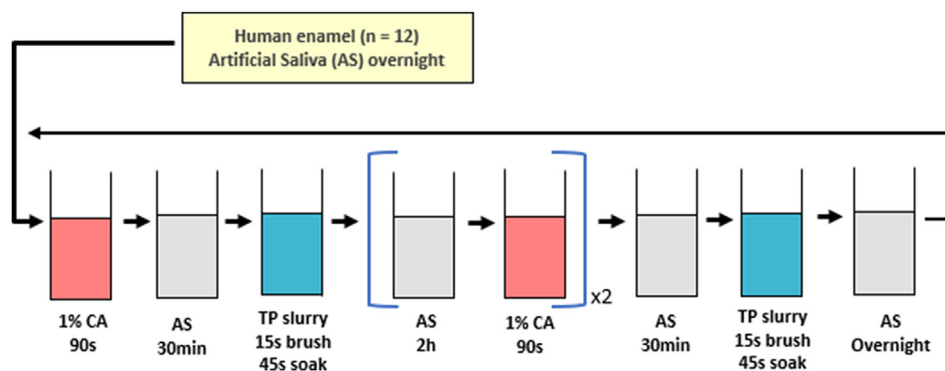


Figure 1. Outline of erosion-abrasion protocol. CA = Citric Acid; AS = Artificial Saliva; TP = Toothpaste; s = seconds; h = hours; X2 = twice.

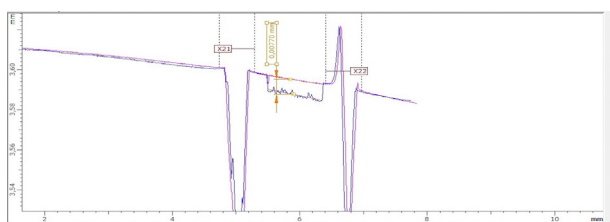


Figure 2. Superimposition of the initial (pink line) and final (blue line) profile scans.

test) and normal distribution (Kolmogorov-Smirnov test). ANOVA and Tukey's test were applied. The level of significance was set at 5%.

### 3. Results

The results of erosion/abrasion study after 3 and 7 days are shown in Table 2. For initially sound enamel specimens, after 3 days, NaF and CaSi toothpaste treatments were similar and both able to reduce enamel wear in comparison to the non-fluoride toothpaste. After 7 days, NaF and CaSi toothpaste treatments were still able to reduce enamel wear progression compared to placebo toothpaste. However, the CaSi toothpaste presented the greatest protective effect, differing significantly from the NaF toothpaste ( $p < 0.05$ ). The percentage enamel protection for sound enamel versus placebo toothpaste was 32 and 55% for NaF toothpaste and CaSi toothpaste, respectively, after 7 days.

For initially acid-softened enamel specimens, after 3 and 7 days, NaF and CaSi toothpaste treatments were both able to reduce enamel wear in comparison to the placebo toothpaste. However, the CaSi toothpaste presented the greatest protective effect, differing significantly from the NaF toothpaste ( $p < 0.05$ ) at both time points. The percentage enamel protection for sound enamel versus non-fluoride toothpaste was 22 and 47% for NaF toothpaste and CaSi toothpaste, respectively, after 7 days.

### 4. Discussion

The present study has assessed the effect of a toothpaste formulation containing CaSi/phosphate and fluoride on human enamel surfaces using

an *in vitro* model which followed the recommendations provided during the Workshop on Methodology in Erosion Research in Zürich, 2010 [26]. This includes standardization of important experimental factors, for example, specimen preparation; acid challenge (type/duration), and toothbrushing parameters that mimic normal brushing behaviors [27, 28].

Based on the results, for the short-term (3 days) and long-term (7 days) erosive-abrasive challenge on pre-acid softened specimens, the null hypothesis is rejected since CaSi toothpaste significantly reduced enamel wear compared to NaF and non-fluoride toothpaste at both time points. The null hypothesis is also rejected for initial sound enamel and long-term results. The null hypothesis is partially rejected for sound enamel and short-term results where the CaSi toothpaste, although gave the lowest enamel wear, was not significantly different to the NaF toothpaste but was significantly different from the non-fluoride toothpaste.

The non-fluoride toothpaste gave, in general, similar levels of enamel loss as the erosion only group. This is in contrast to a number of previous studies that have shown that enamel undergoing erosion and abrasion from toothpaste gave greater tissue loss than erosion effects only [29,30, 31]. For example, Ganss et al. [31] showed in an *in vitro* erosion/abrasion study that a non-fluoride control toothpaste significantly increased enamel tissue loss by about 30% compared to erosion only. However, in the same study, a non-fluoride toothpaste containing chitosan gave a 7% increase in tissue loss compared to erosion only, and the group differences were not of statistical significance. This latter result suggests that certain polymeric materials may have the potential to reduce the effects of abrasion on eroded enamel. Indeed, Bezerra et al. [32] showed that solutions containing the materials poly[methylvinylether-maleic anhydride], poly[vinylpyrrolidone] or carboxy-methyl-cellulose had an anti-erosive effect on enamel. The non-fluoride toothpaste used in the current study contained carboxy-methylcellulose, primarily as a rheology-modifying ingredient, but this inclusion may in part explain the unexpected reduced erosion/abrasion effects of the non-F toothpaste versus erosion only.

The NaF toothpaste gave a significantly lower level of enamel wear than the non-fluoride toothpaste and erosion only groups. The level of abrasive in these products is similar and gives further evidence that fluoride can protect enamel undergoing erosion/abrasion protocols. The level of protection in the current study of the NaF product versus non-

Table 2. Mean and standard deviation of wear of sound and acid-softened enamel after 3 and 7 days of erosive-abrasive challenges ( $\mu\text{m}$ ).

Treatment	Sound Enamel		Acid-softened Enamel	
	3 days	7 days	3 days	7 days
Non-fluoride Toothpaste	2.15 $\pm$ 0.35 <sup>a</sup>	3.05 $\pm$ 0.40 <sup>a</sup>	3.16 $\pm$ 0.19 <sup>a</sup>	3.92 $\pm$ 0.25 <sup>a</sup>
NaF Toothpaste	1.20 $\pm$ 0.22 <sup>b</sup>	2.07 $\pm$ 0.32 <sup>b</sup>	2.17 $\pm$ 0.14 <sup>b</sup>	3.07 $\pm$ 0.13 <sup>b</sup>
CaSi Toothpaste	0.95 $\pm$ 0.19 <sup>b</sup>	1.36 $\pm$ 0.33 <sup>c</sup>	1.70 $\pm$ 0.11 <sup>c</sup>	2.09 $\pm$ 0.15 <sup>c</sup>
Erosion only	1.98 $\pm$ 0.32 <sup>a</sup>	3.69 $\pm$ 0.27 <sup>d</sup>	3.04 $\pm$ 0.19 <sup>a</sup>	3.87 $\pm$ 0.25 <sup>a</sup>

Distinct letters in each column show significant differences among the groups (ANOVA and Tukey's test,  $p < 0.05$ ).

fluoride product was 32 and 22% after 7 days for initially sound and pre-acid softened enamel, respectively. This is largely in agreement with a review of *in vitro* erosion/abrasion studies which reported the overall effects of conventional NaF toothpaste compared to non-fluoride controls that ranged from no effect to 37% enamel protection [33].

Other materials have been studied for their potential anti-erosion effects. For example, stannous and fluoride containing toothpastes when applied as slurries gave a reduction between 55–95% of tissue loss compared to a control. However, when applied with brushing their protecting efficacy was reduced and were either not or only slightly better in comparison to toothpastes with monovalent fluorides [29].

The CaSi toothpaste gave a greater enamel protective effect than the NaF toothpaste and Non-F toothpaste. The enamel protection effects of CaSi containing toothpaste versus control toothpaste formulations from acid challenges has been confirmed in a series of *in vitro* studies [23,24]. This protective effect is probably due to the deposition and retention of CaSi particles onto the enamel surface [21,22] with a greater delivery of CaSi to eroded than sound enamel surfaces [21]. When CaSi particles are deposited onto enamel surfaces, the rate constant of calcium loss from the enamel surface is reduced by 39%, which decreases the impact of future acidic challenges. Several mechanisms may be involved in the mode of action of CaSi to protect enamel. CaSi may function as a physical barrier or as a chemical barrier due to its buffering action that reduces pH fall and further enamel demineralisation; CaSi increases the degree of saturation with respect to hydroxyapatite by releasing calcium into the surrounding oral fluids, thereby inhibiting dissolution [21,34]. Indeed, in an enamel erosion/abrasion *in vitro* study, higher concentrations of calcium and phosphate in the test toothpastes were associated with less surface loss [35]. Furthermore, CaSi is able to nucleate hydroxyapatite, which shifts the equilibrium towards remineralisation and reduces enamel demineralisation [21,22]. In addition, the nucleated hydroxyapatite itself may act as a sacrificial material to the enamel during subsequent acid exposure. One point that deserves attention is that the CaSi toothpaste contains 1450 ppm F as MFP. The protocol that we used did not allow the hydrolysis of MFP to orthophosphate and free fluoride. Thus, it is expected that in *in situ* or in clinical studies the performance of the CaSi toothpaste might be better, since besides the effect of CaSi, there will also be the effect of free F released from MFP through the action salivary phosphatases.

The effect of the CaSi containing formulation in the current study giving protection of enamel versus the fluoride control is in contrast to a study by João-Souza et al. [35]. This erosion/abrasion study reported that a toothpaste containing CaSi, sodium phosphate and 1450 ppm F (as MFP) gave greater enamel surface loss than a 1450 ppm F (as NaF) toothpaste but similar enamel surface loss to a stannous containing toothpaste (1450 ppm F – 1100 ppm as SnF<sub>2</sub> and 350 ppm as NaF). João-Souza et al. [35] used commercially available toothpastes so it was not possible to control for toothpaste abrasivity across treatment groups which may explain some of the observed differences and the daily protocol used consisted of the following steps for 5 days: artificial saliva (60 min); 1% citric acid, pH 3.6 (3 min); toothpaste slurry (2 min, brushed 25 s); measure enamel wear. This protocol is quite different to the current protocol and confirms the impact that the protocol can have on study outcome [33]. However, the João-Souza et al. [35] protocol does not represent typical consumer/patient behavior, for example, where toothbrushing happens immediately after the acid erosion challenge and there is no exposure to artificial saliva between acid erosion challenge and toothpaste treatment. The importance of this has been shown in a number of studies where following acid treatment, the subsequent exposure to artificial saliva or saliva for 30–60 min before brushing, reduced the overall wear to enamel compared to no exposure [36,37]. In addition, the enamel specimens in the João-Souza et al. [35] protocol do not have any artificial saliva exposure immediately after the toothpaste treatment. This will provide little opportunity for either CaSi to show its potential enamel remineralisation and protection benefits post brushing or for the stannous toothpaste to form a significant tin-containing protective layer [38].

The role of the toothpaste abrasive and the abrasivity level of a toothpaste formulation on enamel tissue loss undergoing abrasion or erosion/abrasion cycles is complex and generally poorly understood. An obvious assumption is that the higher the toothpaste abrasivity values (RDA and REA), the greater will be the tissue loss. For sound dentine, it has been shown that as the concentration of abrasive particles is increased there is a concomitant increase in RDA value [39] and it has also been shown that there is a positive relationship between toothpaste RDA value and wear to sound dentine specimens *in vitro* [40]. However, RDA values do not reflect the complex multifactorial characteristic of the toothbrushing process clinically and thus are not intended and should not be used as a prediction tool of dental abrasion *in vivo* [39]. For sound enamel, RDA level has no significant impact on tissue loss as confirmed by *in vitro* and *in situ* studies [11,12,13]. Similarly, toothpastes with REA values in the range 1.1–4.1 have been shown to have no significant effect on sound enamel wear using *in vitro* and *in situ* models whereas a prophylaxis paste with an REA value of 70.9 gave significant levels of wear to sound enamel [11,40]. In addition, an *in situ* enamel wear study with test toothpastes with REA values of 3.86 and 7.68 showed no significant difference in tissue loss after 12 weeks with twice/day brushing [13].

For enamel undergoing erosion/abrasion the level of tissue loss compared to RDA and REA is more complex and shown to be non-linear [41]. In terms of RDA, Ganss et al. [41] found that the association with enamel tissue loss fitted a power function and in the reported range of RDA values between 25–125 there was an increase in tissue loss with RDA with the slope of the fitted curve decreasing with increasing RDA. In contrast, Lippert et al. [42] found that the enamel wear by toothpastes with RDA of 69 and 208 gave the same level of tissue loss in an erosion/abrasion study. In terms of REA, Ganss et al. [41] found the association with enamel tissue loss fitted a cubic function (S-shaped curve) which meant that only very low or very high REA values had an impact but REA values in the range 2–8 had very similar levels of enamel tissue loss undergoing erosion/abrasion, as confirmed by Lippert et al. [42] who showed no significant differences between toothpastes with REA values of 4.0 and 7.1 on enamel surface loss. Under acid erosion conditions *in vitro*, the enamel surface will become significantly softer with a surface hardness approaching that of dentine and therefore the impact of RDA value becomes more dominant than REA under such conditions. Thus, the RDA values of toothpaste formulations evaluated in *in vitro* erosion/abrasion studies ideally needs to be controlled and of similar magnitude in order to elucidate any significant differences between anti-erosion technologies. However, the level of toothpaste abrasivity is rarely controlled in erosion/abrasion studies. This makes the direct comparison of the enamel protective effects of different technologies within the same experiment more challenging. In one reported study the experimental amine fluoride, NaF and stannous chloride formulations tested contained different levels of two types of silica abrasives in order to maintain their RDA values in the range 77–82 [29]. In another *in vitro* erosion/abrasion study, Pini et al. [43] controlled the abrasive level in order to demonstrate the impact of chitosan viscosity on the efficacy of Sn/F toothpaste formulations. In the current study, the level of abrasion was controlled so that REA values of test formulations were in the range of REA values which have no significant impact on enamel loss and described as of minor relevance for enamel wear undergoing erosion/abrasion [41]. The RDA values of the test formulations were also of similar values in the range to 104–118. Thus, it is possible in the current study to be able to compare the impact of different enamel protection and remineralising technologies during an erosion/abrasion protocol with the results demonstrating additional enamel protection from CaSi/-phosphate than fluoride alone.

In conclusion, both CaSi and NaF containing toothpastes were able to reduce enamel wear after short-term erosion and abrasion. However, CaSi toothpaste was superior in respect to conventional toothpaste in reducing the progression of enamel wear, after long-term challenges. Therefore, according to the current protocol, the CaSi toothpaste has good potential to help protect enamel against erosive and abrasive

challenges. It should be highlighted that our conclusions are based on a single characterization (surface profilometry). In future studies, other analytical tools should be employed to reinforce the findings.

## Declarations

### Author contribution statement

Marília Afonso Rabelo Buzalaf, Ana Carolina Magalhães: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Flávia Mauad Levy, Beatriz Gomes, Aline Dionizio Valle, Juliana Sanches Trevizol: Performed the experiments.

Andrew Joiner: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

### Funding statement

This work was supported by Unilever Oral Care.

### Data availability statement

Data will be made available on request.

### Declaration of interests statement

The authors declare the following conflict of interests: Dr Andrew Joiner works at Unilever. Unilever holds a patent on NR5 technology.

### Additional information

No additional information is available for this paper.

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