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RESEARCH ARTICLE

Effects of Dentifrices With Antierosive Potential on the Surface of Bovine Enamel Submitted to Acidic Beverage

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ABSTRACT

Objectives: To evaluate the effects of dentifrices containing sodium fluoride (NaF) combined with NovaMin (Sensodyne Repair & Protect—SRP), NaF combined with stannous fluoride (SnF_2, Oral-B Pro-Gengiva—OBP), and amine fluoride (AmF, Colgate Elmex—ELM) on enamel subjected to simulated erosive cycling.

Materials and Methods: Bovine enamel-dentin discs ($n = 10$/group) were subjected to erosive cycling with orange juice (pH = 3.29, 5 min, 3x/day), artificial saliva (SA–2 h, 3x/day and overnight) and treated with dentifrice (2 min, 2x/day) or without treatment (CONT). Surface microhardness (SMH) was evaluated at baseline (T_0), on the first (T_1) and fifth (T_5) days. SMH loss (%SHL) was calculated. Surface roughness (Ra, μm) was determined at T_0 and T_5. Morphology and mineral content were evaluated under scanning electron microscopy and energy-dispersive x-ray spectroscopy. Data were analyzed using ANOVA/Tukey or Bonferroni ($\alpha = 5\%$).

Results: No differences in %SHL were detected among groups at T_1. At T_5, OBP promoted %SHL, Ra, and ΔRa significantly lower than all the other groups ($p < 0.05$). All groups exhibited morphological changes in topography and similar Ca/P means before and after treatments.

Conclusions: Dentifrice containing SnF_2 minimized the negative effects on the SMH and Ra caused by exposure to orange juice after 5 days of simulated cycling.

Clinical Relevance: Patients who are more exposed to risk factors for dental erosion could benefit from the use of dentifrice containing SnF_2.

1 | Introduction

Dental erosion is a chemical process characterized by the progressive and irreversible loss of the dental hard tissue, not involving acids of bacterial origin [1]. Erosive demineralization depends on an acidic condition that may be caused by intrinsic and extrinsic factors [1]. Intrinsic erosion originates from metabolism, habits, and conditions that promote dental wear due to the acidification of the environment [2]. These clinical oral challenges are a result of the action of endogenous acids, such as gastric acid in patients suffering from bulimia and/or gastroesophageal reflux disease (GERD) [2], and exogenous acids, which are related to the consumption of acidic foods and beverages such as soft drinks, sports drinks, and citrus fruit juice with low pH values [1]. Saliva plays an important role in neutralizing oral acidity due to its buffer system composed of bicarbonate and ions. However, the frequency of acidic challenges surpasses the regulatory and reparative capacity of saliva, resulting in a mineral loss on the dental surface [3].

The gradual softening of dental tissue by nonbacterial acid byproducts weakens the enamel and increases the loss of tooth structure [4], negatively influencing enamel physical properties such as microhardness, roughness, surface morphology, and mineral content [5]. To minimize these effects, dentifrices with antierosive potential containing sodium fluoride (NaF) or amine fluoride (AmF) may produce a barrier composed of calcium fluoride (CaF_2) on the dental surface that may lead to the formation of fluorapatite (FA) on the enamel surface and promote higher resistance during acid attacks compared with hydroxyapatite [6]. This barrier protects the dental structure from acid exposure and assists the buffering capacity during the acid challenge.

However, a recent study demonstrated that stannous fluoride (SnF_2) plays a highly effective role in protecting enamel against acid challenges compared with conventional fluorides [7]. The mechanism of action of dentifrices containing SnF_2 involves the deposition of a compound similar to CaF_2, and it has been demonstrated that Sn can interact with dental surfaces to form other acid-resistant precipitates [8]. Moreover, tin could be incorporated into the enamel, modifying its top layer and increasing the surface resistance [9].

Other components have been proposed to suppress mineral loss, such as NovaMin, which is a commercial biocompatible bioactive glass that consists of calcium sodium phosphosilicate [10]. Its action mechanism relies on the formation of a calcium (Ca) and phosphate (PO_4^{3-}) ion layer similar to hydroxyapatite on the dental surface. This layer provides a reservoir capable of releasing Ca and PO_4^{3-} ions when in contact with an aqueous medium or saliva. Calcium sodium phosphosilicate has demonstrated its ability to reduce dental surface loss due to its high concentration of calcium and phosphate, however, the evidence regarding NovaMin's action against an erosive challenge is scarce [10].

The prevalence and severity of dental erosion in the adult population are significant and have become an increasingly frequent problem in adolescents and young adults [11]. In this regard, it is paramount to analyze the effectiveness of noninvasive therapies to uphold the enamel mineral content. Previous studies assessed the efficacy of the aforementioned dentifrices [6, 12, 13], but the results are still controversial, and the potential antierosive effects of such dentifrices have so far not been compared simultaneously. This study aimed to evaluate enamel resistance against erosive challenge with an acidic beverage using an adapted erosive cycling model [14, 15], and compared the protective ability of dentifrices containing NovaMin bioactive glass (5% calcium and sodium phosphosilicate), AmF (1250 ppm F), and SnF_2 (1100 ppm F). The null hypotheses postulated were that the dentifrices would not impact enamel surface (i) microhardness and (ii) roughness during erosive challenges.

2 | Methodology

2.1 | Experimental Design

Forty bovine enamel-dentin discs were randomized into groups ($n = 10$/group), according to the dentifrice treatment:

- SRP: a bioactive glass of calcium sodium phosphosilicate combined with NaF (1426 ppm F) (Sensodyne Repair & Protect, GSK);
- ELM: AmF (1250 ppm F, Colgate Elmex);
- OBP: SnF_2 combined with NaF (SnF_2 1100 ppm F + NaF 350 ppm F, Oral-B Pro-Gengiva);
- CONT: Control (without treatment).

Enamel surface microhardness (SMH), percentage of microhardness loss (%SHL), surface roughness (Ra and ΔRa), morphology, and calcium-to-phosphorus ratio were evaluated (Ca/P). SMH data were obtained before cycling (baseline, T_0), on the first day of cycling (T_1), and after erosive cycling (fifth day of cycling, T_5). Ra data were obtained at T_0 and T_5, while morphology and Ca/P ratio were determined at T_5.

2.2 | Preparation of Specimens

Forty bovine incisors were collected and cleaned using periodontal scalers and scalpel blades. Enamel-dentin discs (diameter = 5.6 mm, thickness = 3.0 mm) were obtained from the central third of the buccal surface of the incisors using a bench drill (FSB 16—Pratika Schultz; SP, Brazil). The dentin was flattened, and the enamel surface was polished in a polishing machine (Arotec; Cotia, São Paulo, Brazil) with #600- and #1200-grit sandpapers (Norton Saint-Gobain; Guarulhos, SP, Brazil). The discs were ultrasonically cleaned in distilled water for 10 min. The discs were fixed into acrylic plates using sticky wax to cover all the exposed dentin surfaces, and only the enamel remained exposed. One-half of the exposed enamel surface of the specimen was covered with adhesive tape to create a reference area unaffected by erosion.

2.3 | SMH

At the baseline (T_0), three indentations were performed in the central area of each specimen, 100 μm-distant from each other, with a Knoop-type indenter (Future Tech-FM-1e; Tokyo, Japan) under a static load of 50 g/5 s. The mean SMH values of all specimens were obtained (320.0 kg/cm^2), and a 10% (+/−) variation of the mean values was used for the specimens' selection. The specimens were randomly distributed into four experimental groups and submitted to one-way ANOVA, and no statistical differences were found among groups ($p > 0.05$). During the simulated cycling on the first and fifth days (T_1 and T_5), three indentations were performed in the exposed area. At T_1 and T_5, the percentage of the SMH loss (%SHL = Initial SMH–Final SMH/Initial SMH × 100) was calculated.

2.4 | pH Analysis and Solution Preparation

The pH of the acidic beverage (orange juice, Del Valle) was measured (pH = 3.29) at 0 min, 5 min, and 24 h using a pH meter (Equilam; Diadema, SP, Brazil) coupled with a potentiometer (Orion Research Incorporated; Boston, MA), previously calibrated with pH 4.0 and 7.0 standards.

The artificial saliva (SA) was prepared containing 1.5 mM Ca, 0.9 mM PO_4, 150 mM KCl, and 20 mM Tris, pH 7.0 [16]. The slurries for each dentifrice were diluted in distilled water at a 1:3 ratio (dentifrice, g/distilled water, mL), according to the groups previously described. Table 1 displays the complete composition of the dentifrices used.

2.5 | Erosive Cycling Model

This study used a modified version of an in situ erosion-remineralization cycling model [14, 15]. The specimens were immersed in dentifrice slurries (10 mL, 2 min, 2x/day) under constant agitation, in orange juice (20 mL, 5 min, 3x/day), and the %SHL was measured on the first and fifth days of cycling. The specimens were stored in SA (20 mL, 120 min) at intervals among treatments and overnight, after the last slurry treatment. Figure 1 shows a schematic representation of the erosive cycling model, which was repeated for five consecutive days at 37°C, always initiated with slurry immersion. The control group (CONT) was maintained in SA during the slurry agitation.

2.6 | Surface Roughness (Ra)

The surface roughness (Ra, µm) was determined at the baseline (T_0) and after the treatments (T_5) using a roughness meter (Surfcorder SE 1700, Kosalab), calibrated at a 0.8 mm cutoff. The measuring tip of the equipment remained perpendicular to the surface and three measurements were performed on each side of the sample (untreated at T_0 and treated at T_5), rotating the specimen 45°, and the mean Ra value was calculated. The average variation of surface roughness (ΔRa) was determined (T_5–T_0).

TABLE 1 | Composition of the antierosive dentifrices used.

Dentifrices	Ingredients
Sensodyne Repair & Protect (GlaxoSmithKline, GSK; Brazil)	Calcium sodium phosphosilicate 5% (NovaMin), sodium fluoride (1426 ppm F), Glycerin, PEG 8, hydrated silica, cocamidopropyl betaine, sodium methyl cocoyl taurate, aroma, titanium dioxide, carbomer, sodium sacchari, limonene, flavor.
Colgate Elmex (Colgate Palmolive Company; São Paulo, SP, Brazil)	Amine fluoride (1250 ppm F), water, hydrated silica, sorbitol, hydroxyethylcellulose, olaflur, aroma, saccharin, CI 77891, Limonene, 3-(N-hexdecyl-N-2-hydroxyethylammonium) propyl bis (2-hydroxyethyl) ammonium dihydrofluoride.
Oral-B Pro-Gengiva (Procter & Gamble, P&G; Brazil)	Stannous fluoride (1100 ppm F) + sodium fluoride (350 ppm F), glycerin, silica, sodium hexametaphosphate, PEG-6, propylene glycol, water, zinc lactate, aroma, sodium gluconate, titanium dioxide (CI 77891), sodium lauryl sulfate, sodium saccharin, stannous chloride, carrageenan, trisodium phosphate, xanthan gum, cinnamal.

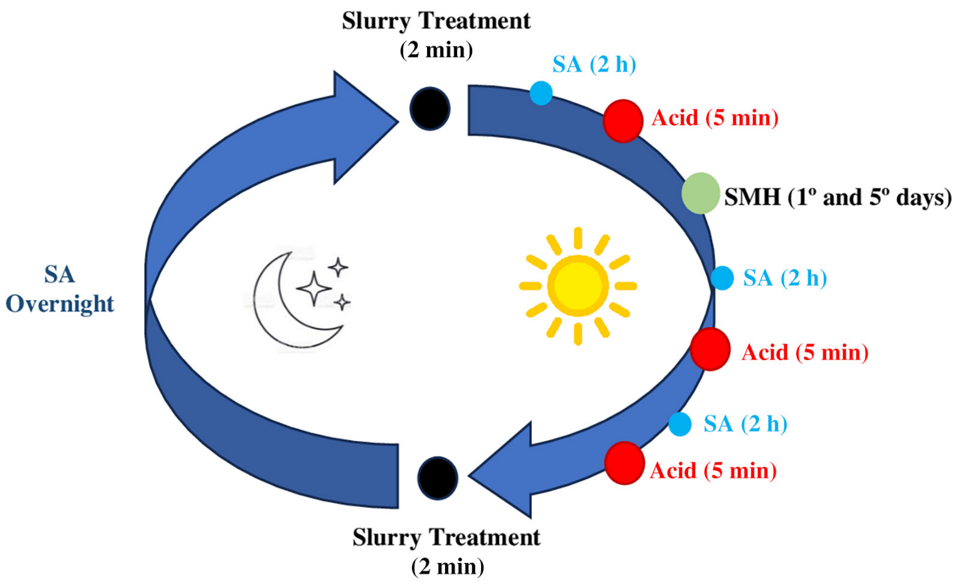

FIGURE 1 | Schematic representation of the erosive cycling for 5 days.

2.7 | Scanning Electron Microscopy (SEM) and Energy-Dispersive X-Ray Spectroscopy (EDS)

One representative specimen from each group was selected and analyzed for morphology (SEM) and mineral content (EDS) at T_5. After treatment, the specimens were washed in an ultrasonic bath (Ultra Cleaner, Unique; Indaiatuba, SP, Brazil) for 10 min and allowed to dry for 24 h in an oven at 37°C. After drying, the specimens were sputter-coated with a thin carbon layer and observed under SEM (JEOL–JSM, 6460 LV; Tokyo, Japan), operating at 15 kV in vacuum mode (45 Pa) [17]. Images were obtained at 1000× magnification. Concurrent to obtaining the SEM images, the software of the EDS (Vantage System—Easymicro Noran Instruments; Middleton, Wisconsin, USA) provided semiquantitative data on the percentage of chemical elements (atomic percentage) in the selected area of the sample surface.

2.8 | Statistical Analyses

The collected data, except for the EDS analysis, were submitted to an exploratory analysis to verify the normal distribution (Shapiro–Wilk and Levene, $p > 0.05$). The values obtained from the analyzed variables met the assumptions of normality and homoscedasticity. The results of %SHL and ΔRa were submitted to one-way ANOVA and Tukey post hoc tests when significance was observed. The values of SMH and Ra assessed over time were submitted to two-way ANOVA (treatment × time) and Bonferroni post hoc tests. A 5% significance level was set for all the analyses.

3 | Results

3.1 | SMH

Figure 2A shows that no significant differences in the %SHL were detected among groups ($p > 0.05$) on the first day of cycling (T_1). Figure 2B shows that, on the last day of cycling (T_5), OBP exhibited significantly lower %SHL than the CONT, SRP, and ELM ($p < 0.05$), and no differences were found among them ($p > 0.05$). Figure 2C depicts a significant decrease in SMH for all groups over time, and no significant differences were found among the groups at each time point (T_0, T_1, and T_5).

3.2 | Surface Roughness

Figure 3A displays the mean surface roughness results. No significant differences were observed among the groups at T_0, indicating that the initial condition was homogeneous. After 5 days of cycling (T_5), although the mean values increased for all groups, OBP significantly reduced Ra values compared with the others. SRP showed significantly lower mean Ra compared with ELM ($p = 0.044$), but both dentifrices exhibited no differences in comparison with CONT at T_5. Figure 3B shows ΔRa values, in which the SnF_2 (OBP) treatment showed a Ra variation significantly lower than all the other groups. No differences in ΔRa were noted among SRP, ELM, and CONT groups ($p > 0.05$).

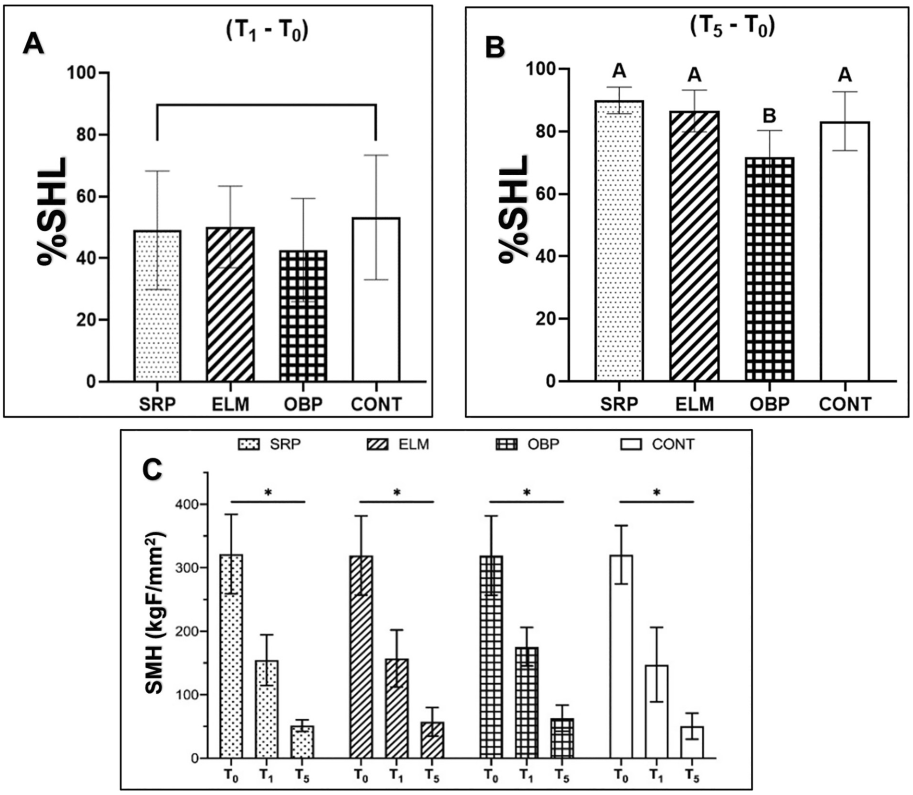

FIGURE 2 | (A) Graphical representation of the %SHL results on the 1st day of cycling (T_1) compared with the baseline (T_0). Bars connected by brackets indicate that there were no differences between the groups according to the one-way ANOVA. (B) Graphical representation of the %SHL results on the fifth day of cycling (T5). Different letters indicate differences between the groups according to one-way ANOVA and Tukey post hoc test. (C) Graphical representation of the mean values and standard deviation of SMH over time (T_0, T_1, and T_5). Horizontal lines with asterisks (*) indicate statistical differences within each group at all evaluation times.

3.3 | Morphology of Enamel Surface

Representative images of SEM Figure 4 show that all groups exhibited noticeable enamel surface alterations in the treated areas (T) compared with the nontreated areas (NT). The interface between the areas (IF) demonstrates the transition from a polished enamel surface (NT) to another with different degrees of grooves, valleys, depressions, and surface porosity that represent the enamel submitted to acid cycling (T). The eroded area of the CONT and SRP exhibited more irregularities and porosities than OBP and ELM, which showed areas compatible with mineral precipitate formation indicated by the white arrows. The mean percentages (%) of atomic weight for calcium (Ca) and phosphorus (P), as well as the Ca/P, are displayed under each SEM image. The only group showing a mean reduction after treatment was ELM, whereas all the others showed closer Ca/P means before and after treatments.

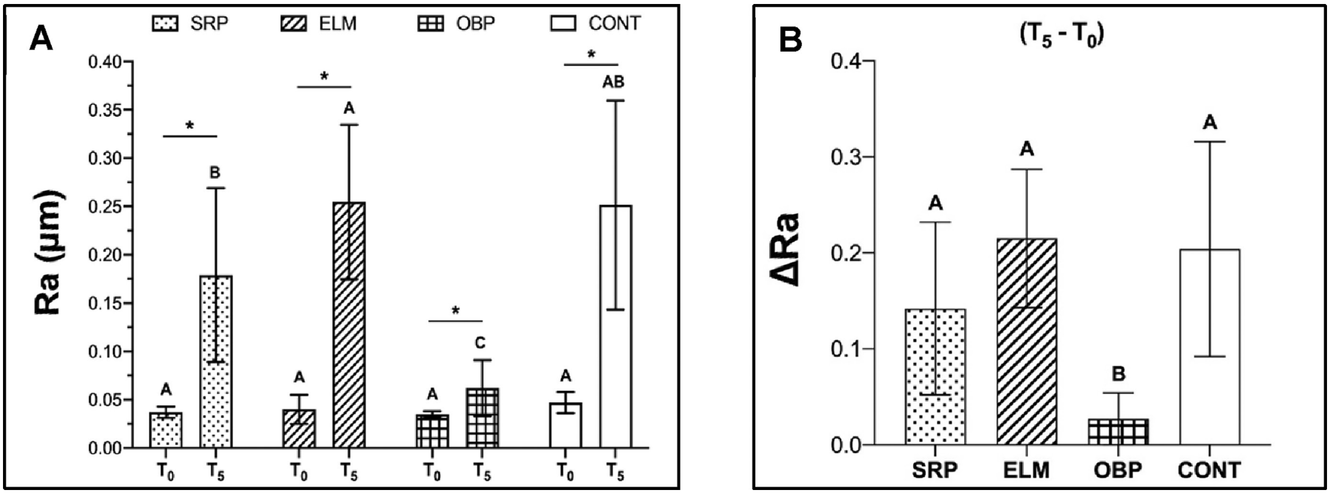

FIGURE 3 | (A) Mean values and standard deviation of Ra at the baseline (T_0) and after the treatments (T_5). Horizontal lines with asterisks (*) indicate statistical differences within each group between the evaluation times, and different letters represent statistical differences among groups within each time point according to one-way ANOVA and Tukey ($\alpha = 5\%$). (B) Graphical representation of the ΔRa results (T_5–T_0), different letters represent statistical differences according to one-way ANOVA and Tukey ($\alpha = 5\%$).

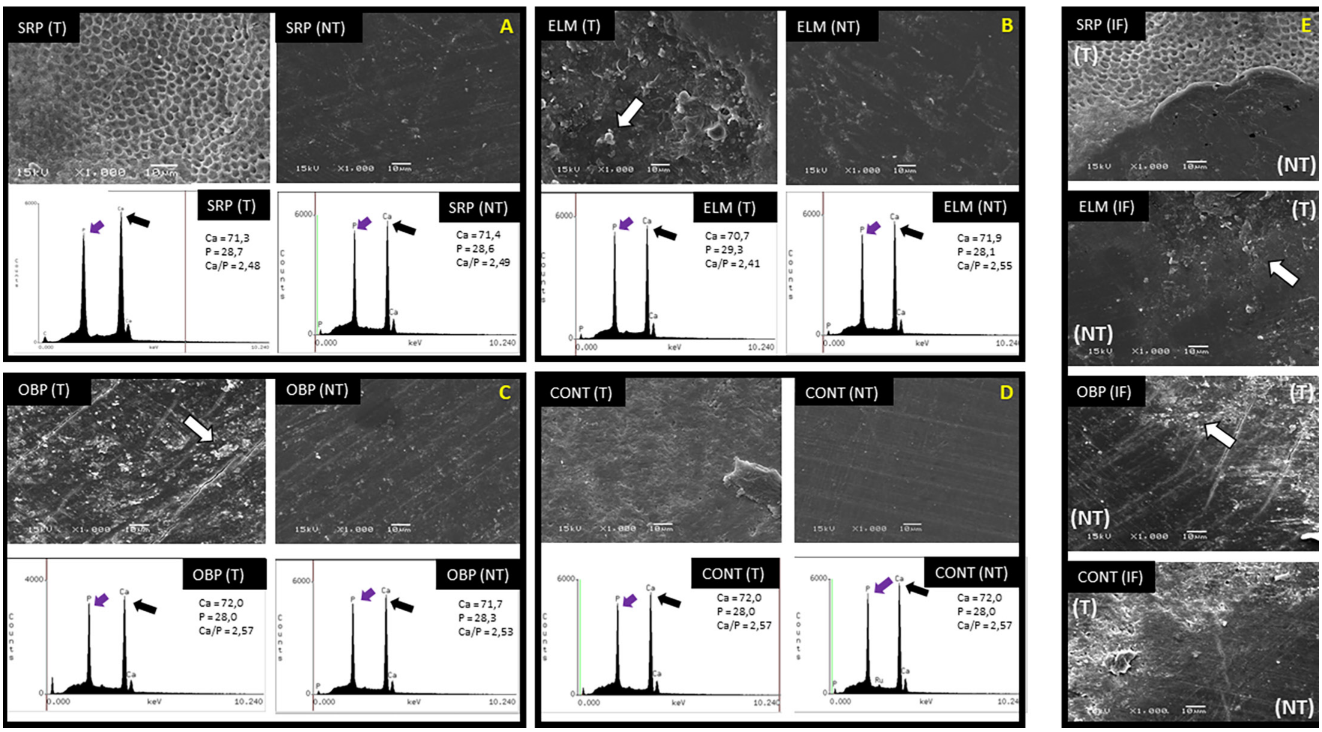

FIGURE 4 | Representative images collected from SEM and EDS for SRP (A), ELM (B), OBP (C), and CONT (D) groups, showed that the nontreated area (NT) presented a polished, flat, and regular surface of enamel in comparison with the treated area one (T), in which enamel exhibited valleys, pits, and depressions. ELM and OBP presented depositions that could be the result of precipitates from the slurries used specifically in these groups' treatments. The interface (IF) for each group is shown (E), indicating a clear transition between the sound and eroded enamel. White arrows represent mineral precipitate formation. In the EDS plots, black arrows represent calcium (Ca) peaks, and purple arrows represent phosphorus (P) peaks.

4 | Discussion

The results of this study showed that orange juice can cause dental enamel erosion after successive immersion cycles. This erosive effect was confirmed by the significant decrease in SMH, regardless of the dentifrice treatment [18]. Even though all groups presented microhardness loss levels above 70%, it is noteworthy that the SnF_2-containing dentifrice combined with a small amount of NaF (OBP) promoted a lower decrease in microhardness. Therefore, the first null hypothesis that no differences in terms of SMH would be detected among treatments was rejected. Authors of previous studies reported that tin-containing salts can deposit a more resistant layer on the enamel surface compared with CaF_2 alone, through the interaction with other ions, such as Ca and PO_4, present in the resulting pellicle [19]. These salts can be incorporated into the demineralized enamel surface and react to hydroxyapatite, which results in a reduced solubility of the dental element [20]. A recent meta-analysis demonstrated that, indeed, none of the potential antierosive dentifrices included in that systematic review were capable of avoiding dental erosion. However, SnF_2 showed a higher effectiveness against erosion when compared with dentifrices containing monovalent fluoride (NaF) [21]. In this context, the present study supports the idea that SnF_2 would be an effective agent to control the microhardness loss promoted by acidic beverages, suggesting a lower softening of this eroded surface [15].

On the other hand, the NovaMin-containing dentifrice (SRP), which was combined with a large amount of NaF (1426 ppm F), was not capable of reducing the microhardness loss detected in the control group. Although it was expected that the formation of a calcium (Ca) phosphate (PO_4^{3-}) ion layer similar to hydroxyapatite would make the enamel surface more resistant to the erosive cycling, the short slurry application time may have hindered the formation of this layer, or it may have prevented the formation of a layer that was sufficient to combat the acidic attack [15]. It is important to note that since this toothpaste occludes dentinal tubules effectively, it would be more strategically efficient to use it as a treatment for patients who already experience dentin hypersensitivity due to exposed dentin [22]. Therefore, the use of dentifrices with NovaMin technology for preventing or minimizing the enamel microhardness loss due to erosion should be discouraged.

The AmF-containing dentifrice was herein evaluated because there is evidence that it is more effective in preventing carious demineralization among the monovalent fluoride dentifrices (containing solely either NaF, AmF, or sodium monofluorophosphate) [23]. Nevertheless, the present findings demonstrated that such monovalent fluoride compound in the dentifrice was not effective in preserving the enamel SMH when facing an erosive challenge. On the contrary, previous studies have shown that a combination with SnF_2 or chloride could be more effective in preventing dental erosion [7, 19]. Further studies could investigate whether AmF could synergistically decrease the mineral loss observed in enamel treated with $SnF_2 + NaF$.

Even though SA, containing calcium, potassium, and phosphate, was used to mimic the remineralizing effect played by human saliva as much as possible, the acidic challenge likely overcame the buffering capacity provided by saliva in this in vitro model. It is important to bear in mind that factors, such as pH, abrasivity [24], and mechanical force, may modulate the effect of a dentifrice on dental erosion and abrasion. Due to the contact with acid, it is expected that toothbrushing leads to greater tissue loss as the eroded enamel becomes softened and, consequently, more vulnerable when submitted to abrasive forces [19]. Therefore, aiming to evaluate the dentifrice's effect on SMH, the slurry agitation was performed, based on previous studies [15, 19], to analyze more effectively the dentifrice's ability to protect the enamel surface without the influence of other factors, such as dental abrasion.

It is important to emphasize that previous studies [15, 25] assessed surface loss through microhardness and optical profilometry methods. Optical profilometry analysis is essential in erosion studies and its absence may be a limitation to the current study. However, microhardness evaluation is considered an indirect method for assessing surface loss [26]. As mentioned before, the findings from this research might indicate that the softening of the eroded enamel treated with SnF_2 was lower than it was with other dentifrices, possibly inferring that the progression of enamel loss for this group was slower. Further studies could indicate if there is a correlation between the enamel SMH and volume loss under the present treatments and the orange juice challenge.

The enamel surface roughness increased over time following the exposure to orange juice. This event is in accordance with previous research [27], which reported that the immersion in acidic beverages itself increases the surface roughness. Under clinical examination, at the end of the erosive cycling, the enamel showed an opaque surface similar to a previous study [28], wherein the enamel surfaces placed in orange juice were visibly roughened and exhibited a loss of shine. However, enamel treated with a dentifrice containing SnF_2 showed more brightness than the others, and such a group had a significantly lower Ra increase compared with all the other groups. Therefore, the second null hypothesis that no differences in terms of surface roughness would be detected among treatments was rejected. A recent network meta-analysis [29] demonstrated that the enamel wear is lower following the use of the polyvalent fluoride group (SnF_2) compared with monovalent fluoride. The tin ion, which has a strong affinity to hydroxyapatite, can embed into the dental structure, thereby reducing the solubility of the dental hard tissue [30]. Furthermore, the enamel volume loss due to erosion may be associated with surface roughness, according to a study reported in the literature [31], in which polished enamel exhibited higher surface roughness and loss after a 45-min immersion in orange juice compared with 15- and 30-min immersion times. Considering the ability of the SnF_2 to minimize the increase of enamel surface roughness, as suggested by the current study, it can be inferred that there is a relationship between this fluoride and the maintenance of the surface volume.

Although quantitative analyses of hard dental tissues altered by erosion offer more objective results, SEM can be used for the qualitative assessment of changes in the tissue surface morphology [32, 33]. Images obtained by SEM depicted that the treated area (T) of the OBP group, containing SnF_2, presented a uniform surface with precipitate formation. Similar results were obtained with the formation of mineral precipitates on the enamel surface, such as calcium SnF_2, stannous hydroxyphosphate, and stannous fluorophosphate, which are acid-resistant layers [8]. This complementary analysis confirms the observed roughness results with OBP, which showed lower Ra compared with the other groups. Furthermore, the ELM group also showed a uniform surface with possible precipitate formation, which could be explained through the mechanisms of action of CaF_2 precipitation on the enamel surface [23]. However, according to the authors, these surface precipitates were not sufficiently acid-resistant to effectively prevent demineralization, confirming the findings of the current study. On the other hand, the SRP and CONT images showed changes in the interprismatic space on the enamel surface, which are characteristic of enamel submitted to acid attack [5].

Even though the specimens treated with SnF_2 in our study demonstrated a positive effect in minimizing damage caused by acid exposure, the EDS (Figure 4) did not detect the presence of stannous ions in the mineral composition of specimens treated with a dentifrice containing tin fluoride, which supports the findings from a previous study [34]. According to these authors, this absence can be attributed to the complex compositions of excipients in dentifrice such as detergents and abrasives like silica, which may adsorb the stannous ion, reducing its availability [34]. However, these findings should be carefully evaluated, since EDS analysis is relative (in %) and performed on a random section of the enamel.

The present outcomes should be interpreted with caution because surface dissolution behavior can be influenced by the presence of the resulting pellicle and saliva under in situ/in vivo conditions, which are essential factors in the protection against erosion [35]. Clinical investigations are necessary to improve these results. The clinical relevance of this study is associated with the increasingly excessive consumption of citrus beverages and food directly influencing the dental erosion process, which has become a global issue in terms of hard dental tissue wear. Thus, patients who are more exposed to these risk factors for dental erosion development could benefit from the use of dentifrice containing SnF_2. Nevertheless, while this approach may not offer a comprehensive solution to completely forestall erosion, evaluating the inherent efficacy of these dentifrices in preventing reduction in microhardness and increase in surface roughness remains essential.

5 | Conclusions

None of the commercial dentifrices tested were able to reverse or prevent microhardness loss and the increase in surface roughness promoted by the erosive challenge. However, the OBP dentifrice, which contains SnF_2, minimized the average loss of SMH and the roughness increase caused by exposure to orange juice after 5 days of simulated cycling.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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