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ORIGINAL ARTICLE

Effect of Xylitol Varnishes on the Inhibition of Demineralization *in Vitro*

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ABSTRACT

Objective: To evaluate the efficacy of xylitol varnishes in the inhibition of enamel demineralization *in vitro*. **Material and Methods:** Bovine enamel blocks (n=120) were randomly allocated to four groups (n = 30), and the surface hardness (SH) was measured at baseline. The blocks were treated with the following varnishes: 20% xylitol, 20% xylitol plus F (5% NaF), Duraphat™ (5% NaF, positive control), and placebo (no-F/xylitol, negative control). The varnishes were applied and removed after 6 h of immersion in artificial saliva. The blocks were subjected to pH cycles (demineralization and remineralization for 2 and 22h/day, respectively, for 8 days). Surface and cross-sectional hardnesses were measured to calculate the percentage of SH loss (%SHL) and the integrated loss of the subsurface hardness (ΔKHN). Data were statistically analyzed using Kruskal-Wallis and Tukey's tests ($p<0.05$). **Results:** %SHL was significantly decreased by 20% xylitol plus F, Duraphat™, and 20% xylitol varnishes compared to placebo. The use of 20% xylitol plus F varnish led to a significantly lower percentage of SH loss compared to the use of 20% xylitol varnish without F. However, the experimental and commercial varnishes led to significantly lower subsurface demineralization compared to placebo and did not differ from each other. **Conclusion:** Xylitol varnishes, especially when combined with F, effectively prevent enamel demineralization.

Keywords: Dental Caries; Tooth Demineralization; Xylitol.

Introduction

Xylitol is a natural sweetener used as a substitute for table sugar (sucrose) due to their identical sweetness. In addition, it has other properties, such as increasing the production of saliva and reducing the growth of bacteria associated with acid production, both of which help prevent tooth decay [1,2]. The importance of xylitol in the prevention of dental caries has been recognized, and it is now used in chewing gums, syrups, lozenges, sprays, mouthwashes, gels, toothpastes, and candies [3]. A recent systematic review reported that over 2.5 to 3 years of use, a F toothpaste containing 10% xylitol may reduce caries by 13%, compared to that by using a F toothpaste. Furthermore, the authors found low-quality evidence suggesting that F-containing xylitol toothpaste may be more effective than F toothpaste for preventing caries in the permanent teeth of children, with no associated adverse effects [4]. Janakiram et al. [5] favored the use of xylitol in comparison with other caries prevention strategies. Xylitol was found to be effective as a self-applied caries prevention agent or as an over-the-counter (OTC) sweetener; however, the studies included herein were found to have an unclear risk of bias. A significant salivary level reduction of *Streptococcus mutans* can be achieved with longstanding and frequent exposure to chewing gums with xylitol [6-8]. Although it has been reported that the additional use of xylitol in existing F regimes could help in preventing caries [5,9], there is still uncertainty on whether the reduction of microorganisms at the intra-oral levels is clinically relevant [10].

Dental varnish is a promising alternative to vehicles such as chewing gums containing xylitol. While chewing gums require a high frequency of use to reach a satisfactory salivary concentration of xylitol, which also depends on the patient's discipline, varnishes maintain prolonged contact with the enamel surface. Two recent studies conducted by our group showed that a 20% xylitol varnish combined with or without F was able to promote enamel surface remineralization just as well as commercial F varnishes. Considering subsurface remineralization, a blend of xylitol and F led to worse results, with one possible explanation being that F may have blocked superficial enamel pores, preventing access to the deeper lesion areas [11,12]. This remineralizing effect of xylitol, even in the absence of bacteria, has been demonstrated in an *in vitro* study [12], and a probable mechanism is that xylitol can induce remineralization of deeper demineralized enamel layers by facilitating Ca^{+2} movement and accessibility [13].

Since the remineralizing potential of xylitol is well described, its use in preventing demineralization also deserves attention. It has been suggested that xylitol can alter the mechanism of polysaccharide production, which facilitates bacterial adherence to enamel [14,15]. Considering that *streptococci* cannot process xylitol for polysaccharide synthesis, they can potentially mitigate biofilm formation and acid production [7,16,17].

Since there are very few studies testing xylitol varnishes for caries prevention [18], the present study aimed to analyze the efficacy of varnishes containing 20% xylitol combined with or without F in inhibiting the demineralization of a bovine enamel *in vitro*.

Material and Methods

Study Design

Three experimental varnishes, 20% xylitol combined with F (5% NaF), 20% xylitol without F and control varnish (no-F/xylitol), were tested (FGM Dental Group Joinville, SC, Brazil). The varnishes contained colophonium, synthetic resin, thickening polymer, essence, and ethanol, as informed by the manufacturer. Xylitol was procured from Danisco (Xylitab™ 300, Danisco Brasil Ltd., Cotia, SP, Brazil).

Material Characterization

To elucidate the mechanism of action of the xylitol varnish, characterization was performed. The theoretical density of the xylitol particles was determined using a helium pycnometer (Ultrapyc 1200e, Quantachrome Instruments, Boynton Beach, FL, USA), and the agglomerate size distribution was estimated using dynamic light scattering (DLS, Nanotrac 252, Microtrac, Montgomeryville, PA, USA). Xylitol was dispersed in ethyl alcohol and sonicated for 1 min. After 20 min, the supernatant was collected for further analysis. The morphology of the particles was evaluated using scanning electron microscopy (SEM, LEO, Germany), at 10–15 kV and 1.2× magnification.

Preparation of Bovine Enamel Specimens

Enamel specimens (4 × 4 × 2.5 mm) were prepared following the method described by Cardoso et al. [12]. One hundred and twenty enamel specimens were selected based on the baseline SH (mean Knoop hardness (KHN), 354 ± 25), and one-third of their surfaces was protected by a nail varnish (control area).

Treatment and pH Cycling

The enamel specimens were randomly allocated to four groups (n = 30/group), according to the type of varnish applied: (1) 20% xylitol (pH 5); (2) 20% xylitol + 5% NaF (pH 5); (3) Duraphat™ (5% NaF, 2.26% F, and pH 5; Colgate, São Bernardo, SP, Brazil); and (4) placebo, no xylitol or F (pH 5, control; FGM-Dentscare). The varnish was applied as a thin layer onto the enamel using a microbrush, and the samples were immediately immersed in artificial saliva (0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2.2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4, and traces of ascorbic acid; pH 6.8; 30 mL per sample) [19] for 6 h at 25 °C [20]. Thereafter, the varnishes were removed using a surgical blade and cotton swabs were soaked in a 50% acetone solution [21].

The specimens were then subjected to pH cycling for 8 d, following the method reported by Queiroz et al. [22]. The bovine enamel blocks were immersed in the demineralizing solution (0.05 mol/L acetate buffer, pH 5; containing 1.28 mmol/L Ca, 0.74 mmol/L P, and 0.03 µg F/mL) for 2 h and in the remineralizing solution (0.1 mol/L Tris buffer, pH 7; containing 1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, and 0.05 µg F/mL) for 22 h, at 37 °C. The proportions of the demineralizing and remineralizing solutions per unit area of the enamel were 6.25 and 3.12 mL/mm², respectively. On the fourth day, the two solutions were replaced with fresh ones. After another four days, the enamel remineralization was evaluated.

Hardness Determination

The baseline SH was determined by measuring three indentations at distances of 100 µm from one another (Knoop diamond, 25 g, 10 s, HMV- 2; Shimadzu Corporation, Tokyo, Japan). After treatment and pH cycling, the hardness was re-assessed, and the percentage of SH loss (%SHL) was calculated as follows:

$$\%SHL = 100 \times (SH\ final - SH\ baseline)/SH\ baseline.$$

For the cross-sectional hardness (CSH) tests, the blocks were longitudinally sectioned through the center, embedded, and polished. Two rows of eight indentations each were made—one in the central region of the exposed dental enamel and the other in the control area (one-third of the surface was protected with the nail varnish)—and placed under a load of 25 g for 10 s. The indentations were made at 10, 30, 50, 70, 90, 110, 220, and 330 mm from the outer enamel surface in two sequences. The mean values of the two measuring points at a distance of 100 µm from the surface were then averaged. The integrated area under the curve (CSH

profiles into the enamel), using the hardness values (KHN), was calculated by the trapezoidal rule for each depth (μm) from the lesion to the sound enamel. This value was subtracted from the integrated area of the sound enamel to obtain the integrated area of the subsurface regions in the enamel. This was denoted as the integrated loss of subsurface hardness (ΔKHN) [23].

The specimens treated with varnishes were added to stubs with carbon adhesive tapes and gold-sputtered (Balzers SDC 050, Oerlikon Balzers, Liechtenstein), and the surface morphology was evaluated using SEM (LEO 430i, Germany) at 15 kV and 7.5× magnification.

Statistical Analysis

The software SigmaPlot 11.0 (SigmaPlot Inc., La Jolla, CA) was used for statistical analysis. The assumptions of equality of variances and normal distribution of errors were checked for all data. Kruskal-Wallis and Tukey's tests were performed for the SH and ΔKHN. Statistical comparisons of the CSH at each distance were performed using one-way ANOVA ($p<0.05$), with a significance level of 5%.

Ethical Aspects

The ethical approval for this study involving bovine teeth was granted by the local ethics committee (Protocol no. 033-2016; Ethics Committee of Cruzeiro do Sul University, São Paulo, SP, Brazil).

Results

Through characterization analysis, the theoretical density of xylitol particles was obtained (average: 1.57 g/mL). Figure 1 shows the monomodal distribution of xylitol particles, with a size range of 64.7–306.5 μm and an average size (D_{50}) of 145.8 μm. Figure 2 shows that the xylitol particles have rounded shapes.

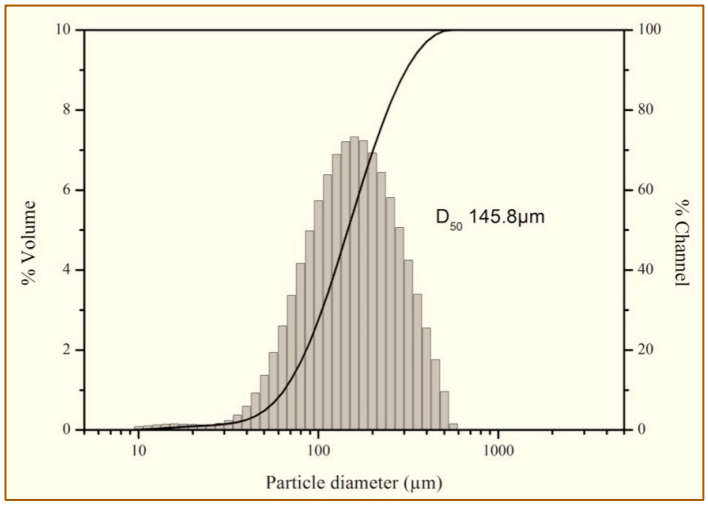

Figure 1. Distribution of xylitol particles obtained by DLS.

The varnishes 20% xylitol, 20% xylitol plus F, and Duraphat™ were able to significantly inhibit %SHL compared to the placebo (Table 1; $p<0.05$). There were no significant differences between the experimental varnishes and Duraphat™. However, the use of 20% xylitol plus F led to a significantly lower %SHL than that of 20% xylitol without F, indicating its greater capability to inhibit lesion formation when used in combination with F than that when used without.

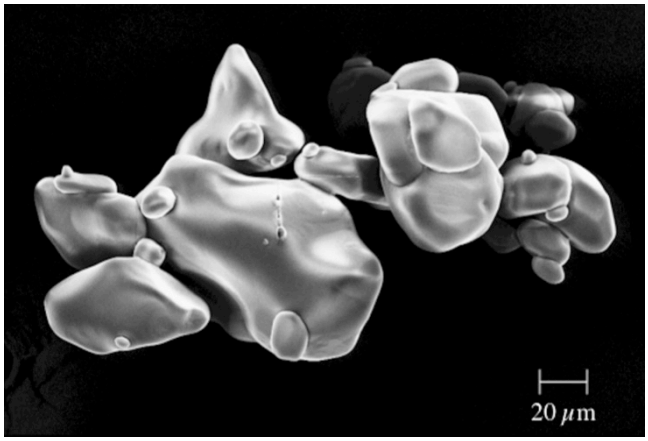

Figure 2. Morphology of xylitol particles obtained by SEM.

Table 1. SH measurements at the baseline (SH baseline), after treatment and pH cycling (SH final), %SHL, and ΔKHN of the enamel specimens treated with different varnishes (n = 30).

Varnishes	SH Baseline (KHN)	SH Final (KHN)	% SHL	ΔKHN
20% Xylitol	355±24.8	262±26.3	26.5(CI 21.7/30.4)[a]	2102 (CI, 1127/3450)[a]
20% Xylitol + 5% NaF	356±24.8	288±34.7	17.7(CI 13.5/27.0)[b]	1522 (CI, 910/2615)[a]
Duraphat™	352±26.2	269±36.2	21.7(CI 16.9/30.6)[ab]	2569 (CI, 1282/3125)[a]
Placebo	354±25.3	227±32.4	35.5(CI 30.5/39.6)[c]	5015 (CI, 4675/5040)[b]

The results of SHL and ΔKHN are provided as medians (minimum/maximum). Values in the same column with different superscript letters differ significantly from each other. The significance was determined using the Kruskal-Wallis test, followed by Tukey's test ($p<0.05$).

The ΔKHN data also showed that the use of the experimental and commercial varnishes led to significantly lower subsurface demineralization when compared to the use of the placebo, which did not differ from each other (Table 1; $p<0.05$). Regarding the CSH (Kg/mm²) data at every 100 μm from the outer surface, all varnishes were able to significantly reduce the hardness loss (up to 30 μm) when compared to the placebo (one-way ANOVA, $p<0.001$). In the deeper layers, there were no significant differences between the varnishes and placebo groups. Figure 3 shows the representative profiles of the CSH (kg/mm²) of enamel specimens subjected to pH cycling after treatment with different varnishes.

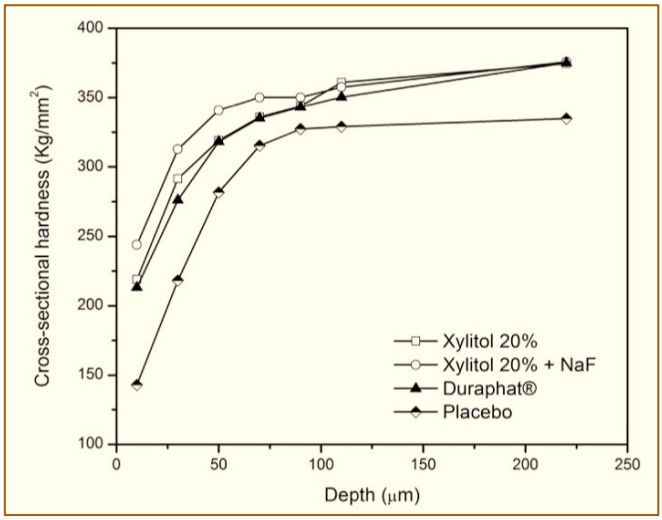

Figure 3. Mean of cross-sectional hardness (Kg/mm2) vs. distance of enamel specimens subjected to pH cycling after treatment with different varnishes. All varnishes could significantly reduce the hardness loss (up to 30 μm) when compared to that of the placebo (one-way ANOVA, $p<0.001$). In the deeper layers of the enamel, there were no significant differences between the varnishes and placebo.

Using SEM, the dental surfaces of the different groups were observed after the demineralizing and remineralizing treatments. The dental blocks treated with varnishes 20% xylitol, 20% xylitol plus F, and Duraphat™ showed a sound enamel surface. Although the surface was not completely smooth, uniformity of the surface layer was observed. The control dental block (placebo varnish without additive agents) showed a dental surface that was exposed to enamel prisms (Figure 4).

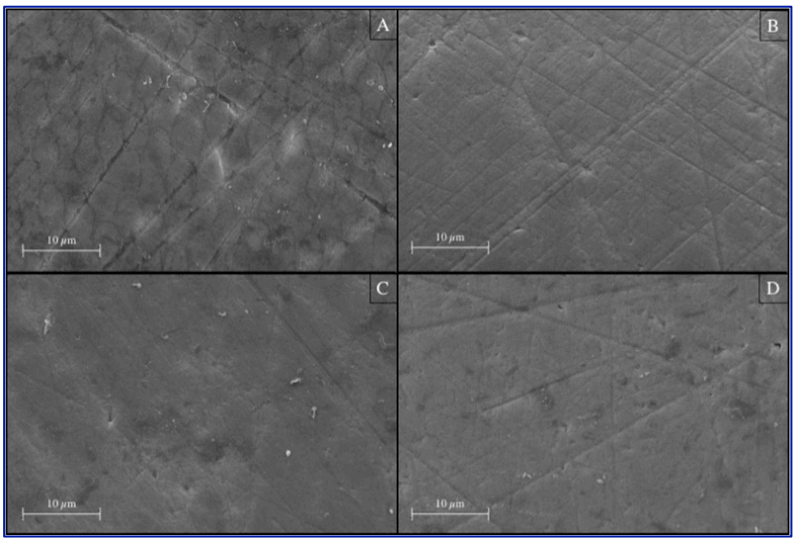

Figure 4. SEM images of the dental surfaces of the different groups after demineralizing and remineralizing treatments. A) Placebo, B) 20% xylitol, C) 20% xylitol plus 5% NaF, and D) 5% NaF (Duraphat™) varnishes.

Discussion

Other studies using transverse microradiography (TMR) analysis (*in vitro* and *in situ*) have demonstrated the remineralization capacity of 20% xylitol varnish; this capacity was influenced by the enamel region that was analyzed in combination with fluoride [11,12]. In the *in situ* study, the use of the experimental 20% xylitol varnish led to a significant decrease in lesion depth (ΔLD) compared to that of the positive control varnish (Duraphat™). Thus, the mineral gain observed by the integrated mineral loss (ΔΔZ) for Duraphat™ was mostly situated on the outer surface and intermediate enamel layers, as F may have blocked superficial enamel pores, preventing access to and the remineralization of deeper areas of the lesion. In contrast, the experimental varnish may have favored remineralization in deeper layers, either by decreasing the acidogenic potential of plaque or by facilitating the movement of ions from the saliva toward the enamel [11].

The concentration of xylitol (20%) employed in the present experimental varnishes was chosen because of its better performance in comparison to other concentrations, as confirmed by previous studies [11,12,24].

Regarding prevention of lesion formation, the present study demonstrates that all experimental and gold standard varnishes are able to inhibit %SHL and ΔKHN, respectively, compared to the placebo. However, the use of the 20% xylitol plus F varnish led to a significantly lower %SHL than that of the 20% xylitol varnish, showing the better capacity of the varnish combined with F to inhibit the formation of a lesion. The cross-sectional hardness data at every 100 µm from the outer surface indicated a significant reduction in enamel loss (up to 30 µm) from all experimental varnishes and Duraphat™, when compared to the placebo. As reported recently [25], demineralization due to early carious lesions can be sufficiently studied by the SH;

however, its limitations in assessing the mineral status of lesions that have undergone further demineralization must be considered. Due to the shallowness of the formed lesions in this study, the SH was chosen as an outcome instead of TMR. Furthermore, the cross-sectional hardness and integrated area under the curve (calculated by the trapezoidal rule) were calculated to show ΔKHN [23]. Ideally, both methods should be combined as the hardness is not necessarily a measure of the mineral content, and some studies regarding the conversion of the hardness to the mineral volume are controversial [26]; however, they provide important information regarding the mechanical properties (physical strength) of the lesions, which is not provided by TMR. Complementary techniques should also be employed to assess the changes in the physical and chemical characteristics of the lesion [25]. The SEM analyses was aimed at complementing and illustrating the results obtained in the analysis of the surface and longitudinal hardness. Considering Figure 4, the dental substrate treated with the placebo varnish is exposed to the enamel prisms, denoting the beginning of a demineralization process, which did not occur with the experimental and gold standard varnishes. The experimental varnishes showed a homogeneous surface and no signs of demineralization, which was very similar to the commercial control material (Duraphat) [27].

One of the challenges for implementing this material in its commercial form is the decantation of xylitol particles. In this study, the characterization analyses of xylitol particles aimed to contribute to the advancement of the clinical application of the material. It was observed that these particles have dimensions of more than 100 μm (D50 = 145.8 μm) and density of 1.57 g/mL (varnish density = 0.84 g/mL). The dimensions obtained in the DLS analysis are shown in Figure 2, using the xylitol particles from SEM. Because of these characteristics, with the addition of 20% by mass of xylitol particles, a large part of these particles decants at the bottom of the bottle, necessitating the exploration of some alternatives to promote improvements in the material.

Based on these findings, it may be inferred that the 20% xylitol varnish without fluoride is the product of choice for the remineralization of pre-existing white spot lesions. Moreover, the 20% xylitol varnish combined with fluoride performed better in terms of prevention of lesion formation, acting on the inhibition of demineralization. According to two systematic reviews [28,29], the application of fluoride varnishes for 2 to 4 times a year was associated with a significant decrease in tooth decay in populations with different levels of caries risk. Thus, as there is only one randomized controlled trial testing varnishes containing xylitol with promising results for the use of 10 and 20% xylitol compared to fluoride varnish in caries prevention, and the authors of this trial applied the varnishes with a 3 month interval [18] the protocol used for conventional fluoride varnishes should be followed for this varnish as well.

The present results should be confirmed *in vivo* using more properly designed randomized controlled clinical trials. The beneficial effect of xylitol on the inhibition of bacterial metabolism and growth should also be investigated, as it could improve its remineralizing and preventive effects.

Conclusion

Despite the fact that all the experimental varnishes were able to inhibit demineralization when compared to placebo in the present study, it can be concluded that 20% xylitol varnish without fluoride performs better for the remineralization of pre-existing white spot lesions, while the 20% xylitol varnish combined with fluoride is the best product when the goal is the inhibition of demineralization.

Authors' Contributions

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VEAC	https://orcid.org/0000-0002-6767-7812	Resources and Writing - Review and Editing.
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CABC	https://orcid.org/0000-0003-3645-5368	Formal Analysis, Writing - Review and Editing, Supervision and Funding Acquisition

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

University of São Paulo has a patent request in Brazil (INPI) for "Xylitol-containing dental varnish".

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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