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Research Article

Preventing Enamel Demineralization Using Propolis Fluoride and Sodium Fluoride Varnishes: A Comparison

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KEYWORDS

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ABSTRACT

Introduction: Soft drink are one of the most frequently consumed beverages that can cause enamel demineralization, with a pH ranging from 1 to 4. The use of fluoride varnishes in clinical dentistry is the most common and favored method of countering this process, and the newest innovation made from natural substances is propolis fluoride. **Objective:** To compare the enamel structures of samples treated with propolis fluoride and sodium fluoride after being demineralized by carbonated liquid. **Methods:** Twenty-seven permanent premolar teeth were equally divided into three groups for treatment. One group received propolis fluoride, one received sodium fluoride, and the control group was not treated. The samples were exposed to 5% acetic acid for 20 minutes and subsequently varnished with fluoride. The samples were then exposed to Fusayama's artificial saliva for 30 minutes, followed by a carbonated liquid for 1 hour. The samples were analyzed with an EDX or the quantitative analysis and a SEM was used to photograph the surfaces of all the samples to compare them qualitatively. **Results:** The percentage of the fluoride element inside the enamel surfaces from the EDX showed no significant differences between any of the groups ($P \geq 0.05$), although the control group showed differences in the enamel surface structure compared to the other groups. **Conclusion:** Propolis fluoride and sodium fluoride have the same effectivity in inhibiting enamel demineralization by carbonated drinks.

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INTRODUCTION

The continuous process of demineralization in teeth beyond their ability to remineralize is one of the causes of caries.¹ Enamel demineralization is the process of dissolving the minerals in enamel.¹⁻⁴ Enamel demineralization occurs when the pH in plaque or the oral environment drops below 5.5.^{1,3} The cause of decreased pH in plaque is the acid produced when carbohydrates are metabolized by bacteria present in the plaque.^{3,5}

Streptococcus mutans, *Streptococcus sobrinus*, and *Lactobacillus sp.* are the main bacteria classified as cariogenic pathogens due to their ability to produce strong lactic acid after sugar fermentation. Moreover, they can withstand a low pH environment.^{1,5} Unchecked enamel demineralization will form cavities that expose the dentin layer underneath. The pH of the oral environment is influenced by the food and drink an individual consumes:^{2,5} Foods and drinks that contain simple carbohydrates or that have high levels of acidity, such as carbonated drinks, can exacerbate the demineralization caused by bacteria.

Remineralization is the reintegration of dissolved calcium (Ca^{2+}) and phosphorus ions (PO_4^{3-}) ions after demineralization.^{1,3,5} Under normal circumstances, both ions dissolve and are contained in saliva. However, remineralization must take place in partially demineralized hydroxyapatite crystals so that they can grow back to their original size,^{1,5} and the oral environment must support remineralization, such as having a neutral pH and reduced plaque. The process of demineralization and remineralization occurs continuously on the surface of the tooth, but cavities will still form if the demineralization process is dominant.^{1,3,5,6}

One mean of preventing demineralization and supporting the remineralization process is the use of fluoride varnish containing fluoride ions in concentrations higher than that used in toothpaste.⁷ The most common main component of fluoride varnish is sodium fluoride.⁸ The latest innovation developed as the main component of fluoride varnish is propolis fluoride, which utilizes the antibacterial properties and fluoride composition of propolis.

No studies have tested the effect of propolis fluoride on tooth enamel surfaces in the demineralization process due to carbonated drinks. Therefore, in this in vitro study, the effects of propolis fluoride and sodium fluoride varnishes on the surface structure of enamel by demineralized carbonated beverages will be compared.

MATERIALS AND METHODS

This research was exempt from the requirement for ethical approval (No.09/Ethical Exempted/FKG UI/VIII/2019, Protocol Number: 010920819) in its implementation. Criteria for dental samples inclusion: premolar teeth; teeth with intact crowns and roots; teeth that have not been restored in any way; teeth with enamel surfaces that have not been demineralized and do not have visible caries; teeth that are not discolored; and teeth that have only been cleaned and soaked with water during storage.

Sample Preparation

Twenty-seven premolar teeth that met the criteria stated above and were to be used in the research were previously cleaned using water and a toothbrush. All teeth had been previously coated with nail polish (Revlon, USA) over the entire root surface. The application was refined with nail polish remover using a microbrush (TPC, United States). Afterwards, the teeth were divided into 3 groups, each consisting of 9 teeth, labeled A, B, and C. The teeth in Group A were treated with propolis fluoride (Flolis, Indonesia), those in Group B were treated with sodium fluoride (Clinpro White Varnish, 3M, United States), and those in Group C, the control group, were not treated with fluoride varnish. Each tooth was placed in a plastic pot labeled A, B, or C, with the crown at the bottom.

Demineralization Treatment and Assessing the Results

All specimens were immersed in 5% acetic acid solution for 20 minutes to demineralize teeth so that the fluoride varnish could penetrate the substitute hydroxyl groups in hydroxyapatite crystals. Prior to soaking, the pH of the acetic acid was measured (pH 3,2). After soaking, each tooth was once again cleaned with water. Propolis fluoride was applied with a microbrush on the surfaces of the teeth in Group A, while sodium fluoride was applied to the surfaces of the teeth in Group B teeth that had not been sealed with nail polish. Afterwards, the teeth were left for 30–60 seconds before being returned to their plastic pots. All specimens in Groups A–C were coated in artificial saliva using a syringe to cover the entire surface of the crown and were left for 30 minutes.

The carbonated liquid (Sprite, The Coca-Cola Company, United States) used to instigate the enamel demineralization process had been previously measured using a pH meter. The measurements were taken in a plastic container. All Group A specimens were transferred into containers (labeled “Group A”) that

contained a carbonated liquid with the crown completely immersed and were left for 1 hour to replicate the demineralization process. All specimens in Groups B and C were subjected to the same treatment. All specimens from Groups A–C were cleaned with running water to remove any residue from the carbonated liquid and were returned to their respective pots.

Specimen Analysis with an Energy-Dispersive X-ray

Representative specimens from Groups A, B, and C were analyzed using an energy-dispersive x-ray (EDX) to calculate the percentages of the elements Ca (Calcium), P (Phosphorus), and F (Fluorine). The results were averaged by each group. Topographic photographs of the specimens closest to the group average were then taken using a scanning electron microscope (SEM). These results were quantitatively analyzed to calculate the value of the significance between groups.

Photograph Specimen with Scanning Electron Microscope

Representative specimens from Groups A–C were photographed using SEM so that they could be compared and qualitatively analyzed.

Data Analysis

The data obtained from the EDX were quantitatively analyzed using the Shapiro–Wilk normality test to obtain the data distribution. To obtain the P value, a one-way ANOVA test was performed when the data distribution was normal; the Kruskal–Wallis test was used when the data distribution was not normal. Statistical analysis was performed with SPSS v.23 for Windows. The data obtained from the SEM were qualitatively analyzed.

RESULTS

Prior to starting the research, the pH of the carbonated beverage to be used (Sprite) was measured with a pH meter and found to be 2.6. After Groups A–C were treated according to the research method, 2 locations on the representative samples from the three groups were viewed using the EDX in order to determine the percentages of Ca, P, and F present. The one-way ANOVA test results are presented in Table 1.

The *P* value produced by the one-way ANOVA test indicated that there was no significant difference in the percentage of elemental fluorine between propolis fluoride and sodium fluoride, propolis fluoride with control, and sodium fluoride with statistical control ($P \geq 0.05$). Next, images of the enamel surface samples demineralized using 5% acetic acid for 20 minutes were

compared to determine whether fluoride varnish affects the remineralization of the enamel surface (Figure 1).

Figure 1 shows that, at a glance, demineralizing an enamel surface with 5% acetic acid for 20 minutes at 3000x magnification revealed that large amounts of pores were formed by the demineralization. Afterwards, images of enamel surface samples that had been soaked in carbonated liquid for 1 hour were taken using the SEM. Determination of samples tested by SEM using magnifications of 100x, 1000x, and 3000x. Therefore, the samples viewed with the SEM were A4, B3, and C1. The SEM images showed different surface modifications. The A4 sample images taken with the SEM are presented in Figure 2.

The 100x magnified image of the surface of sample A4 showed demineralization that had formed a striped pattern on the enamel surface. The 1000x and 3000x magnified images showed that these lines were areas of craters with small pores—these can be seen more clearly on the bottom of the crater in the 3000x magnified image. The surface of sample B3 in Figure 3 showed that the enamel surface looked more structured than that of A4 at 100x magnification, but the 1000x magnified image revealed that the more opaque areas were more structured and deeper than the darker regions. Basically, this indicates that the brighter areas are the result of demineralization due to the carbonated liquid, but a magnification of 3000x indicated that globules are spread

Table 1. Characteristics of research subjects by gender, age level, education level, and stroke level.

Group	Mean ± SD	Significance Value
Propolis Fluoride	1.418 (0.50450)	0.067
Sodium Fluoride	1.840 (0.81765)	
Control	0.888 (0.27490)	

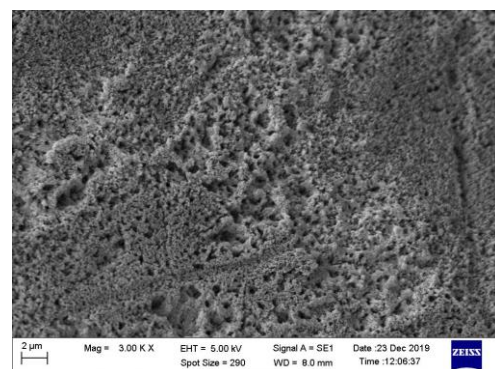


Figure 1. Electron microscope scans of tooth enamel after demineralization with acetic acid at 3000x magnification.

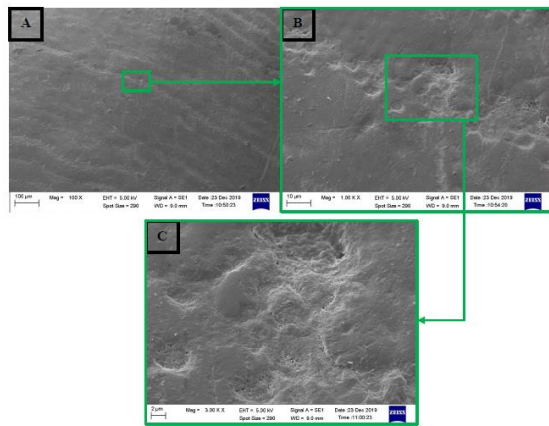


Figure 2. Electron microscope scans of samples A4 (propolis fluoride), after being soaked in carbonated liquid for 1 hour at magnifications of (A) 100x, (B) 1000x, and (C) 3000x.

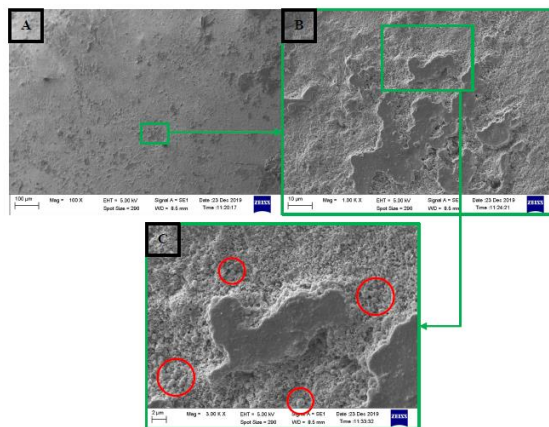


Figure 3. Electron microscope scans of samples B3 (sodium fluoride) after being soaked in carbonated liquid for 1 hour at magnifications of (A) 100x, (B) 1000x, and (C) 3000x.

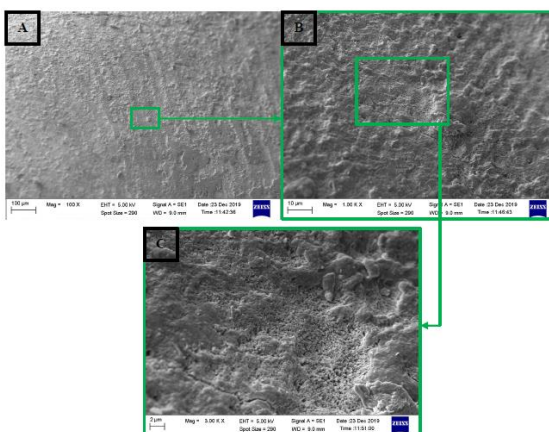


Figure 4. Electron microscope scans of samples C1 (control) after being soaked in carbonated liquid for 1 hour at magnifications of (A) 100x, (B) 1000x, and (C) 3000x.

evenly across the surface (marked with a red circle). These globules comprise CaF_2 , which is formed by applying sodium fluoride to a partially demineralized enamel surface in order to protect the enamel structure from further demineralization.⁹

The control group's sample surface, which was not treated with fluoride varnish, showed that the enamel surface, viewed at magnifications of 100x and 1000x, was rougher and more structured than the previous two groups. Magnifications of 1000x and 3000x showed large amounts of pores on all visible surfaces (Figure 4).

DISCUSSION

Fluoride in its ion form is the core component of all caries preventive material in dentistry. At concentrations of 1000–1500 mg/L in toothpaste and more than 5000 mg/L in varnish, fluoride has been proven to be effective in preventing the demineralization of tooth enamel.¹⁰ The demineralization process is slowed by the substitution of hydroxyl ions with fluoride ions in hydroxyapatite crystals; this prompts the formation of fluorapatites, which are more resistant to low pH environments.^{5,9,10} Fluoride is involved in three interrelated objectives in the dynamics of demineralization and remineralization.

First, fluoride inhibits the demineralization process. This can occur because of the integration of fluoride ions (F^-), which will replace hydroxyl groups (OH^-) in the structure of hydroxyapatite crystals in enamel so that they become fluorapatite crystals ($\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$).^{4,7,10,11} Fluorapatite crystals are more resistant to acid solubility than hydroxyapatite, as evidenced by its ability to maintain its structure in environments with a $\text{pH} \geq 4.5$.¹² Second, fluoride ions in a saturated state in the oral environment will trigger an increase in the speed of the remineralization process due to previously formed fluorapatites.¹⁰ The highest concentration of fluoride in teeth is on the enamel surface. Fluoride levels on the enamel surface can reach 1000–2000 ppm on non-fluoridated surfaces and 3000 ppm in fluoridated areas. In the enamel subsurface, fluoride levels are very low, around 20–100 ppm depending on fluoride absorption during the tooth development stage, which is not sufficient to inhibit demineralization. Previous studies have shown that dissolved fluoride at low concentrations (close to 1 ppm) can inhibit enamel demineralization, indicating that the fluoride dissolved around the crystal (liquid enamel) is strongly absorbed by the crystal surface because of apatite and acts as a potent defense mechanism against acids that dissolve the surface of the crystal. Both of the above processes occur during long-term exposure to low concentrations of fluoride.¹⁰

High levels of fluoride in sodium fluoride varnish can lead to the formation of CaF_2 globules across the entire surface of the teeth coated with the different varnishes. These globules later function as fluoride ion reservoirs in a neutral pH environment. In a low pH environment these globules will dissolve, causing the integration of fluoride ions into hydroxyapatite crystals and neutralizing the plaque and saliva pH with calcium ions.^{4,9}

Saliva plays an important role in the tooth remineralization process.³ It contains various kinds of electrolytes, namely bicarbonates and phosphates of sodium, potassium, calcium, and magnesium. Immunoglobulin, proteins, enzymes, mucin (a lubricant), urea, and ammonia can also be found. If adequate amounts are present in the mouth, saliva will reduce the accumulation of plaque on the tooth surface.¹

Saliva has a bicarbonate buffer system that prevents the pH of the oral environment from reaching a critical pH, thus allowing the demineralization process to occur. This reaction neutralizes acids in the oral environment, but the pH and buffering capacity are influenced by the level of secretion. Increased salivary secretion increases the buffering capacity of saliva due to the increased number of sodium and bicarbonate ions. According to the Stephan curve, saliva has the ability to neutralize the pH of the oral environment for an average of 20 minutes.¹

Table 1 shown the percentage of fluorine in the samples that had been treated with sodium fluoride was greater than that of the samples treated with propolis fluoride, and both had a greater percentage than the control samples, but a one-way ANOVA revealed that statistically there were no significant differences between the groups. However, the data indicates that there is a substantial difference between group A, group B and group C. The element fluorine is considered to be able to represent the fluorapatites formed after the application of fluoride varnish, so it was used as a reference in determining the sample to be examined using the SEM at greater magnifications.

An SEM image of an enamel surface after demineralization using 5% acetic acid (Figure 1) shows a rougher structure with pores over the entire surface compared to the enamel surface of the whole group after the final treatment of the research. This proves that sodium fluoride, propolis fluoride, and saliva can inhibit demineralization and enhance remineralization of enamel surfaces using the fluoride content of varnish as well as the calcium, phosphate, and alkaline pH from saliva. According to Demarco et al. (2011), darker areas of enamel indicate demineralization and the pocked enamel surface indicates erosion.^{13,14}

The enamel surface of the tooth treated with propolis fluoride (Figure 2) shows a flat area with craters formed with pores at the bottom of the crater. This indicates that the enamel surface of propolis fluoride group was demineralized to an extent but that the process can be inhibited by the formed fluorapatite. At the same time, the fluorine in the propolis fluoride group was lower than the fluorine in the sodium fluoride group, which explains why the SEM image of the CaF_2 globules in the propolis fluoride group were not found on the surface of the enamel. The enamel surface that appears darker compared to that of the sodium fluoride group also shows that the level of mineral density on the enamel surface of the propolis fluoride group is lower than that the sodium fluoride group. However, if implemented in a normal oral environment, propolis fluoride has advantages due to its propolis content, which has antibacterial properties.^{15,16,17}

The combination of propolis, which inhibits bacterial reproduction and plaque formation, and fluoride, which can inhibit demineralization and accelerate remineralization, is superior to sodium fluoride.^{9,10,15} The enamel surface of the sample treated with sodium fluoride (Figure 2) shows an area with CaF_2 globules scattered on its surface at a magnification of 3000x, marked by a red circle on the image. This picture is consistent with an image published by Brar et al. in 2017, which shows a picture of SEM on an enamel surface treated with sodium fluoride varnish that has formed CaF_2 globules.¹⁸ This also explains why Group B had the highest percentage of fluorine. According to previous research, the surface structure of demineralized enamel will be darker than the surrounding surface. Figure 2 shows that the enamel surface treated with sodium fluoride is brighter than that of the other groups, indicating greater mineral density.¹⁷ The enamel surface of the control sample (Figure 2) is rougher and more structured than those treated with propolis fluoride and sodium fluoride. These images indicate that Group C (the control group) had the lowest fluorine composition and did not inhibit demineralization at all. In addition, the higher concentration of fluorine compared to the propolis fluoride group may be caused by the absence of shellac and mastic compositions present in propolis fluoride. Shellac and mastic are resins contained in sodium fluoride varnishes that prevent it from dissolving quickly in saliva or water.¹⁹ Conversely, the presence of fluorine in the enamel surface is thought to be due to the people who donated their teeth using fluoridated toothpaste, which left residual fluorine from the previous fluorapatite structure.

The effect of propolis fluoride in preventing demineralization of enamel might be more visible if the number of samples used were larger. In addition, it would

be even better if the samples used were paired with the EDX and SEM tests before and after the test treatment.

CONCLUSION

Based on quantitative analysis with EDX and qualitative analysis with SEM, propolis fluoride and sodium fluoride were found to have the same effectivity in inhibiting enamel demineralization by carbonated drinks.

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CONFLICT OF INTEREST

Declared none.

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