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The Effect of Almond Milk on Tooth Enamel Surface Hardness

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Abstract

Introduction: Enamel is the hardest substance in the human body and serves as the wear-resistant outer layer of the dental crown. Demineralization releases mineral ions which cause a decrease in the enamel surface hardness. Demineralization could be neutralized to recover minerals, and this process is called remineralization. Almond milk was an alternative to cow's milk, supporting tooth enamel surface remineralization. **Purpose:** To determine the effect of almond milk on enamel hardness. **Methods:** Twenty maxillary premolars were divided into group A (control) and group B (almond milk). Before treatment, samples were soaked in 1% citric acid. Enamel hardness was tested with Vickers Hardness Tester. The statistical tests used in this study were Paired t-test and Independent Sample t-Test. **Results:** The results of the paired sample test got significant results in groups A and B in the comparison of pretest - 1% citric acid, and 1% citric acid - posttest, which means that there was a significant difference in enamel hardness values. However, the results obtained were not substantial in groups A and B in the posttest- pretest comparison. There was no significant difference between Group A and Group B in the independent sample test results. There was an increase before and after, but it was not much different when compared to the control. **Conclusion:** Almond milk can increase enamel surface hardness but cannot reverse the initial enamel surface hardness value.

Keywords: almond milk; demineralization; enamel; enamel hardness; remineralization

Introduction

Enamel is the hardest substance in the human body and serves as the wear-resistant outer layer of the dental crown.[1] Chemically, enamel consists of about 90%-92% hydroxyapatite crystals, 4%-12% water, and 1%-2% enamel organic matrix proteins.[2] Hydroxyapatite crystals are composed of calcium and mostly phosphate.[1] The loss of mineral ions from the hydroxyapatite crystals in the teeth is called the demineralization process. Demineralization could be affected due to the presence of bacteria in plaque as well as carbohydrates.[3] A few bacteria could ferment carbohydrates from food according to the substrate (e.g., sucrose and glucose), to produce an acid, which can cause plaque pH to drop below 5 within 1-3 minutes. Continuous decreases in pH could lead to demineralization of the tooth surface.[4] Demineralization could be neutralized so that the pH increases and minerals could be recovered, this process is called remineralization.[4] Factors that favor remineralization are an increase in Ca^{2+}, PO_4^{2-}, an increase in pH, and the presence of F^-.[5] There were several ideal requirements for remineralizing materials, namely having a low viscosity to diffuse to the

subsurface or providing calcium and phosphate to the subsurface.[1] Demineralization and remineralization are dynamic physicochemical processes that affect the hardness and strength of enamel.[1]

Several studies have shown the potential of dairy foods and beverages to protect the enamel from tooth erosion.[6] Milk was an important drink for daily consumption because it contains very good nutrition for the health of the body.[7] The lactose content of milk could cause some people to not be able to consume milk because they had a history of allergy or intolerance to lactose, known as lactose intolerance. Plant-based milk has characteristics close to animal milk and it was safe for consumption by people who have lactose intolerance.[8] Recently almond milk has been used as an alternative to cow's milk, especially for those who had a milk allergy or lactose intolerance and/or are vegetarians.[6] Almond milk has become the most popular plant-based milk in the United States and sales had surpassed soy milk.[9] In the United States, it has been proven that sales of almond milk had increased by up to 250% in the last 5 years and occupied the top position when compared to milk from nuts and other cereals.[10]

Research conducted by Abd-elmonsif et al (2017) on the comparison of the effects of cow's milk and plant-based milk on enamel erosion, showed that after immersion in almond milk, teeth experienced a significant increase in the ratio of calcium (Ca) and phosphate (P) and a decrease in carbon content (C). The high remineralizing effect of almond milk might be because almonds contain Ca, which is one of the best alternatives, which is 45% of the daily value per serving. Almond milk showed better results than other types of milk (cow's milk, soy milk, oat milk) regarding Ca and P levels and changed surface morphology.[6] Based on this description, research on the effect of almond milk on tooth enamel surface hardness has never been done, so the researchers will conduct a test that aims to determine the effect of almond milk on enamel hardness.

Methods

This type of research was quasi-experimental research with a pretest and posttest design with a control group design. A total of ten maxillary premolar crowns were cut mesiodistally using a separating disk that produced a total of 20 samples. The inclusion criteria were as follows: first and second maxillary premolars free of caries and/or restorations, fractured or cracked. The following exclusion criteria were adopted: teeth with abrasion, attrition, and incomplete tooth anatomy.

Dental crowns are placed in self-curing acrylic that has been imprinted with the buccal, lingual, or palatal side of the tooth upward (Figure 1). The buccal/lingual/palatal surfaces of the teeth were smoothed using sandpaper sizes 400, 1000, and 1500 respectively to obtain a smooth surface. The initial hardness test of the enamel surface used a Vickers Hardness Tester with a load of 5 kg for 15 seconds and carried out at two indentation points to avo=id discrepancies (Figure 2). The diameter of the rhombus-shaped indentation impression was measured using a Zoom Stereo Microscope (Figure 3). Email hardness was calculated using the formula VHN = $\frac{1.854\ x\ F}{d^2}$. The enamel surface hardness of each sample was taken from the average.

Figure 1. Samples placed in self-curing acrylic mould

Figure 2. Surface hardness of the enamel test using the Vickers Hardness Tester

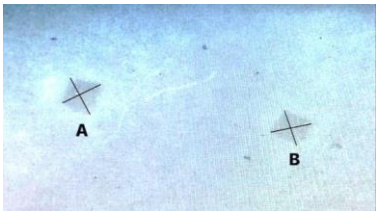

Figure 3. Impression results of Vickers Hardness Test; (A) first impression, (B) second impression

Research subjects were grouped into 2 groups (A and B) with each consisting of 10 samples. Each sample was assigned a serial number according to the group (A1-A10, B1-B10) on each side in the surface of the acrylic cylinder. Acrylic cylinders were marked with strokes using a small diamond bur on the top surface to distinguish the points to be indented by the penetrator needle by dividing the sample into three parts, namely a (for measurements before demineralization), b (for measurements after demineralization), and c (for measurements after immersion in the remineralization solution). All dental samples were immersed in 100 ml of 1% citric acid for 90 minutes at 37◦C in an incubator. Group A as a control group was immersed in distilled water solution and group B was immersed in 100 ml of almond milk (Almond Breeze®, Blue Diamond) for 90 minutes at 37◦C in an incubator (assuming the consumption of almond milk was carried out per 1-minute one-time consumption/days for 3 months) (Figure 4). The data were analyzed by Independent Sample t-test to compare the enamel surface hardness between one group and another after the remineralization treatment.

Figure 4. Sample storage in an incubator at 37◦C for 90 minutes

Results

Figure 4 below shows a graph of enamel hardness provided an overview of the average decrease in enamel hardness in all groups after demineralization treatment, namely group A (264.49 VHN) and group B (271.36 VHN). The average enamel hardness in all groups then showed an increase after being given remineralization treatment, namely group A (343.34 VHN) and group B (350.72 VHN).

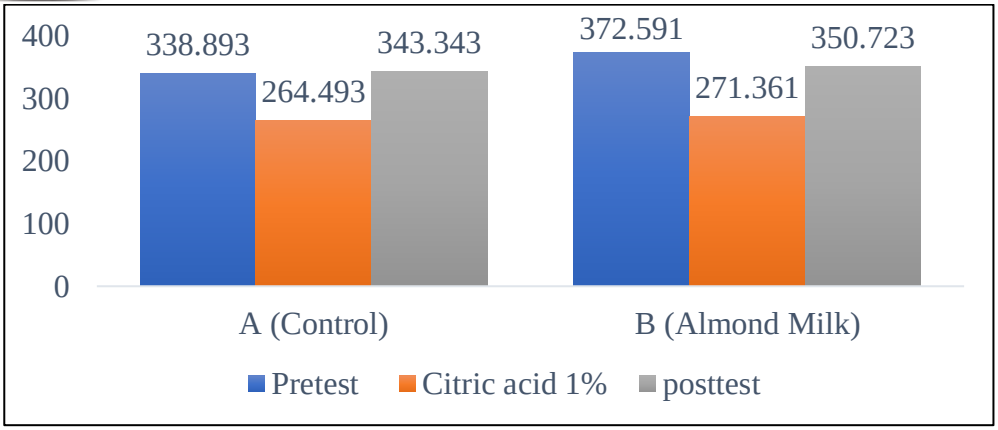

Figure 4. Graph of average enamel hardness pretest, 1% citric acid, posttest (VHN)

Based on Table 1 and 2, there were significant results in groups A and B in the comparison of pretest - 1% citric acid and 1% citric acid - posttest. However, the results were not significant in groups A and B in the posttest-pretest. The results of the independent samples test (Table 3) showed a probability of 0,735 (p>0,05), which means that there was no significant difference between the control and almond milk.

Table 1. Paired samples test results in group A

Group A	**Pretest**	**Citric acid 1%**	**posttest**
Pretest		0,017*	0,855
Citric acid 1%			0,000*
postest			

* Shows a significant difference at the 0.05 level

Table 2. Paired samples test results in group B

Group B	**Pretest**	**Citric acid 1%**	**posttest**
Pretest		0,000*	0,342
Citric acid 1%			0,006*
Posttest			

* Shows a significant difference at the 0.05 level

Table 3. Independent Samples test results

Between groups	P Value
Pretest	0,250
Citric acid 1%	0,663
Posttest	0,735
	Significant at level p≤0,05

Discussion

The results of the paired sample test showed that all groups (A and B) in the 1% citric acid treatment experienced a significant decrease in enamel hardness. Demineralization occurs at low pH when the oral environment was below saturated with mineral ions, relative to the mineral content of the teeth.[6] The decrease in enamel hardness indicated that 1% citric acid with a pH of 2.27 (measured by a pH meter) could dissolve the minerals contained in the enamel. The citrate ion contained in citric acid as a chelating agent could form complex compounds along with calcium ions which result in the dissolution of tooth minerals.[3] Demineralization caused calcium and phosphate in hydroxyapatite crystals became unstable, so that it could reduce enamel hardness.[1]

The demineralized samples were soaked according to the treatment of each group. Group A was a control group that was immersed in distilled water. Group B soaked in almond milk. Immersion was carried out for 90 minutes in an incubator at 37◦C. This study was using Almond Breeze Almond Milk which contains vitamin E 85% of the RDA (Recommended Dietary Allowances) and calcium 25% of the RDA per serving, which means that by consuming according to the serving size, nutritional needs have met 85% of the need for Vitamin E and 25% for Calcium per day.

The results of the Paired samples test also showed that group A (control) and group B (almond milk) could significantly increase the enamel hardness value in each group in the posttest. The process of moving a substance from high concentration to low concentration is called diffusion. Almond milk and distilled water solution could diffuse into the microporosity of the tooth surface. Ion diffusion could be affected by the viscosity of the solution. Low viscosity was a good solution condition for remineralization which caused the solution to experience rapid penetration into the enamel microporosity. Distilled water has a low viscosity when compared to almond milk, this made the diffusion process to take place quickly so that the remineralization process in distilled water occurred faster and the level of enamel hardness

increased. Almond milk has a higher viscosity (thicker) so that the diffusion process became slower and the remineralization process was inhibited.[7]

High value in enamel hardness that occurred in group B (almond milk) was due to the remineralization process involving the diffusion of calcium and phosphate ions in the defect to neutralize the tooth surface.[11] The remineralization process was an important process and has a significant influence on the strength and hardness of the tooth.[7] The process of formation of the mineral apatite occurred as soon as calcium, phosphate, and fluorine ions had contact with the enamel surface. Factors that favor remineralization were an increase in Ca^{2+}, PO_4^{2-}, an increase in pH, and the presence of F^-.[5] This is in accordance with research conducted by Abd-elmonsif et al (2017) which showed that after soaking in almond milk, there was an almost regular smooth enamel surface with scattered mineral deposits without obvious cracks which meant a significant increase in the Ca/P ratio and a decrease in significant levels of C. Almond milk contained good Ca so it has a high remineralizing effect.[6]

The Independent Samples test showed a probability of 0.735 ($p>0.05$) in the post test, meaning that there was no significant or significant difference in enamel hardness after the administration of remineralization materials between group A (distilled water) and group B (almond milk), which means that both groups both could increase the hardness of tooth enamel. From the test results in this study, it was found that distilled water and almond milk were significant in increasing the surface hardness of tooth enamel. Consumption of almond milk was significant in increasing the surface hardness of tooth enamel, but has not been able to restore the initial surface hardness of the enamel.

The limitations of this study were that there was no examination of the content of distilled water used for immersion in the control group, so it was recommended that further research be investigated first. In addition, the limitations of this study were that there was no control over the age of tooth extraction (distance between tooth extraction) in each sample and the lack of maintenance of the integrity of the condition of the teeth during storage so that this was thought to be the cause of the large difference in tooth enamel surface hardness between each sample.

Conclusion

This current research has shown that distilled water could increase the hardness of tooth enamel and could restore the initial enamel surface hardness value.

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