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Effect of cow and soy milk on enamel hardness of immersed teeth

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Abstract. Cow milk and soy milk have different mineral contents and this can affect the tooth remineralization process. The aim of this study was to determine the effect of cow and soy milk on immersed teeth after demineralization. Twenty-one specimens, of human maxillary premolars, were measured for enamel hardness before immersion and demineralization in orange juice. The teeth were divided into three groups (n = 7) with each group immersed in either distilled water, cow milk, or soy milk. There was a significant increase in enamel hardness in all groups (p < 0.05). Cow milk provided the highest increase in enamel hardness, of all the three groups, but was not able to restore the initial enamel hardness.

1. Introduction

Dental caries is the most common oral health problem caused by several factors, including the host, food leftovers, bacteria, and time span. When all these factors interact, the caries process is progressed [1]. Food leftovers, that have carbohydrate content, will be metabolized by oral bacteria creating acid as a byproduct that will later decrease saliva pH. Decreasing saliva pH will cause demineralization or the dissolution of enamel minerals into the saliva. Ongoing demineralization will cause cavities in teeth, which is called caries [2,3].

The types of food and drink consumed can also affect the demineralization process of tooth enamel, such as apple juice and orange juice. The acid content in these products can accelerate the demineralization process of tooth enamel [3]. Milk is one of the essential nutrient sources for the human body. Milk contains several nutritious substances such as fat, lactose, protein, energy, vitamins, minerals, and calcium, which are for the teeth. Cow milk contains around 69 mg of calcium per 100 ml [4].

Milk has high potential in supporting dental enamel remineralization [5]. Casein protein in milk prevents caries through its ability to produce high concentrations of phosphate calcium in the plaque structure. This can prevent demineralization and initiate remineralization [6]. Milk can be obtained from many sources. The most common milk consumed is milk from cows and its relatives, such as sheep and goats. Unfortunately, not all individuals can consume animal milk. For example, individuals can be allergic to cow milk and have other digestion problems. Cow milk allergy is an unwanted reaction from an immune response to cow milk protein. Cow milk contains more than 20 proteins (allergens) that can cause allergic reactions. [7].

As well as people allergic to cow milk, vegetarians are also unable to consume animal milk. However, there are now milks from plant sources, such as soy milk. Soy milk is believed to have lower cholesterol content than cow milk and to also have several advantages compared to animal milk

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[8]. In previous research of soy milk, it has been shown that soy milk can have a remineralization effect on human teeth [9]. Other benefits of soy milk encompass anti-carcinogenic effects, lowering of cholesterol serum, and preventing the cardiovascular disease [10]. As the effects of soy milk are being understood, the difference in the remineralization process of cow milk and soy milk in permanent teeth needs to be investigated, considering the consumers of these two products vary in age.

2. Materials and Methods

Before beginning this research, an ethics assessment was completed with the Research Ethic Commission, Dentistry Faculty, Universitas Indonesia. For this research, maxillary premolars were used as specimens. The inclusion criteria for premolar teeth were free from caries, abrasion, and erosion. The exclusion criteria for premolar teeth were discoloration and cracks. The total number of specimens was counted using a G*Power application, Version 3.1.9.2, for Windows, and the result obtained was 21 specimens. The premolar teeth were collected and stored in a saline solution. Specimen preparation involved rinsing the tooth with aquades and then drying the specimen. The tooth was cut along the cervical margin, to separate the crown and root, using a carborundum disk and a micro-motor. The tooth crown was cleaned and dried before mounting.

The specimens were mounted using sticky paper, PVC pipe, silicon oil, and decorative resin. The PVC pipe was first covered with silicon oil to ease the demounting process. The tooth crown was stuck with sticky paper, with the buccal side face upward. Then, decorative resin was poured into the PVC pipe and left for the setting time. After setting the decorative resin, the specimen was taken out of the PVC pipe and prepared by smoothing and polishing. First, the smoothing was done to the lower part of the specimen (side without tooth) in order to produce equal dimensions. Then, the specimen thickness was measured by a digital micrometer. The specimen smoothing was done by a grinding/polishing machine (Struers LaboPol-21 with SiC sandpaper number 2000).

The smoothing result was checked using a microscope until the specimen surface result obtained was focused and even. The enamel thickness, after smoothing, was limited to >0.1 mm. After the even specimen surface was obtained, the polishing process was done to remove scratch lines that had formed during the smoothing process. The polishing process was done using the same equipment with an alumina water based suspension (1 µm size) [10]. Twenty-one specimens were tested for initial hardness using a Knoop Zwick Roell Micro hardness Tester. The enamel surface hardness was tested five times by indentation on five different spots with a 25 g weight for 15 seconds. The test result was the average score of the indentations [11,12].

Before the specimen was immersed in several solutions, a pH measurement was done. There were four solutions: Buavita® orange juice; Indomilk® Fresh Milk cow milk; Soylicious® Unsweetened soy milk; and aquades. The pH measurement was done three times using a Thermo Orion pH meter (290A⁺⁾. The result was the average of the three measurements. All of the specimens were demineralized by immersion in the orange juice. The immersing was done for 5 minutes and the temperature was kept at 37 °C by being inserted into an incubator. After that, the specimens were rinsed with aquades and dried. Then, the specimens were tested for hardness after the demineralization process, with the same method as the initial hardness test.

The specimens were divided into three groups for the handling stage. The first group was the control group, having been immersed in the aquades. The second group was immersed in cow milk and the third group was immersed in soy milk. All of the three groups were immersed for 150 minutes (on the assumption of a milk drinking duration of 30 days, 1 time for every 10 minutes of drinking) and kept at 37 °C by being inserted into an incubator. The hardness test, after remineralization, for each group, was later measured by the same method as the initial hardness test. The data analysis was done with quantitative comparison of the mean score of the enamel hardness between each group with a one-way analysis of variance (ANOVA) test to find the significance. The criteria for the comparison were the difference between enamel hardness after demineralization and after remineralization. The ANOVA test was repeated to find the significance of the difference in the hardness score for each group.

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3. Results and Discussion

3.1 Results

In this research, the enamel hardness test was carried out for the initial stage, after demineralization, with Buavita® processed orange juice, and after the handling stage in the form of immersion in the remineralization solutions of aquades, processed cow milk, or processed soy milk. The hardness score for the three groups had a normal distribution (p > 0.05) and were homogenous. The mean scores of the hardness tests are shown in Table 1.

Variahla	Enamel Surface Hardness ± SD (Knoop Hardness Number)		
variable	Initial	Remineralization	
Aquades	438.87 ± 22.44	333.11 ± 3.42	
Cow milk	438.87 ± 22.44	415.17 ± 2.90	
Soy milk	438.87 ± 22.44	345.40 ± 3.13	

Table 1. Results of enamel surface hardness

The results in Table 1 show that the mean score of enamel hardness was 438.87 ± 22.44 KHN. After the demineralization occurred, the enamel hardness score decreased to 318.04 ± 11.24 KHN. The ANOVA test, shown in Table 2, shows the decrease is significant (p < 0.05).

After remineralization with aquades, cow milk, and soy milk there was significant increase in enamel hardness (p < 0.05). The increase in enamel hardness from aquades or soy sauce immersion showed a significant result (p < 0.05).

However, the enamel hardness after immersion in aquades or soy milk has insignificantly different results (p > 0.05). Differing from the enamel hardness after immersion in cow milk, the test showed the significantly different results compared to the immersion in aquades and soy milk (p < 0.05). These results are shown in Table 3.

Remineralization, with the three solutions, onto the enamel demineralized by the processed orange juice could not restore the initial hardness. This is shown in Table 2, where the comparison of the initial hardness and the hardness after remineralization is significantly different (p < 0.05).

Variable	Comparison	Significant Score	
Aquades	Initial-Demineralization	<0.05	
	Demineralization-Remineralization	<0.05	
	Remineralization-Initial	< 0.05	
Cow milk	Initial-Demineralization	< 0.05	
	Demineralization-Remineralization	<0.05	
	Remineralization-Initial	< 0.05	
Soy milk	Initial-Demineralization	< 0.05	
	Demineralization-Remineralization	<0.05	
	Remineralization-Initial	< 0.05	

Table 2. Repeated ANOVA test results

T	able	3. Significat	ncy results	of <i>Post I</i>	Hoc ANOVA	test reminera	lization score
		0	2				

Groups	Significancy score		
Aquades-Cow milk	<0.05		
Aquades-Soy milk	>0.05		
Cow milk-Soy milk	< 0.05		

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3.2 Discussion

The research results showed a decrease in the initial hardness score of 27.5% after being immersed in processed orange juice with pH4. The decrease of enamel hardness occurred because of the acid content of the orange juice. The dominant acid of orange juice is citric acid. Jamil *et al.* state that orange contains 2.13 ± 0.026 g/ml of citric acid. This is more than the acid content of a lemon or a sweet lime [13]. The acid content of orange triggers tooth demineralization by decreasing the oral pH below the critical hydroxyapatite pH of 5.5. The dissolved hydroxyapatite crystal in tooth enamel creates a micro gap in enamel structure so the hardness decreases [14,15]. Research by Margolis and Moreno showed that there is a linear increase of dissolving mineral content in the immersion of tooth enamel in lactic acid [16].

The remineralization process was done in three kinds of solution: aquades, processed cow milk, and processed soy milk. Seven specimens were immersed in aquades for 150 minutes and the increase in enamel hardness was 4%. Aquades has near-neutral pH 7.3 and contain minerals, even in low quantities [17]. Increase in enamel hardness can occur because of the increasing of pH and the mineral content in aquades. Increasing pH facilitate the occurance of remineralization. In the remineralization process, the minerals in oral cavity, especially calcium and phosphate ions were binded back, so that the carbonated-hydroxyapatite crystals formed covering the demineralized area resulting in increased enamel hardness [18].

The immersion in soy sauce (pH 7.1) resulted in an increased enamel hardness score of 8.6%. This increase was insignificantly different compared to immersion in aquades. The enamel hardness increase occurred because of the increasing pH of the solution that triggered remineralization. It was also supported by the calcium and the phosphate, in the soy milk, that is required for remineralization. However, the content of calcium in soy milk is 9.8 mg with 120 mg of phosphate per glass (245 g). This amount is less than the content of cow milk. Widyaningtyas *et al.* showed that immersion in soy milk would decrease demineralized enamel microporocity by acid etching. This is caused by calcium and phosphate ions from the soy milk diffusing inside the pores of the enamel and increasing the hydroxyapatite saturation [9,10].

Immersion in aquades and soy milk was significant enough to increase enamel hardness, but immersion in cow milk showed the greatest increase compared to the immersion in aquades and soy milk. This showed that cow milk is more effective for remineralization of demineralized tooth enamel. The enamel hardness score, with pH 7.1, increased significantly to 30.5% above the demineralized hardness score. This was caused by the cow milk that provided higher remineralization during the same immersion time compared to the others solutions. Remineralization with cow milk can happened to a greater degree because cow milk has a higher mineral quantity for remineralization, compared to aquades and soy milk [8].

Pereira *et al.* broke down the contents of cow milk. It contained 1200 mg of calcium per liter. One glass of cow milk (245 g) contained 119–124 mg of calcium that fulfills 37–40% of the body's calcium requirement [4]. Cow milk is also a good phosphate source. One liter of milk can contain 950 mg of phosphate that can also play a role in the remineralization process. This research showed that an increase in enamel hardness on immersion in cow milk is more effective than immersion in aquades and soy milk. Immersion in soy milk gave the same result as immersion in aquades.

Further research should be done on the topic of the effect of immersion in cow milk and soy milk for a longer time period, and the effect of immersion in processed cow milk and processed soy milk, with a control specimen for all cases being immersion in artificial saliva, to adjust for the condition in the oral cavity. Additionally, the effect of tooth immersion in a remineralization agent to analyze the decrease of enamel hardness after remineralization has ended should be investigated.

4. Conclusion

Demineralization occurred in tooth enamel after being immersed in processed orange juice for 5 minutes, which is equal to one drink cycle, as evidenced by decreasing enamel hardness. Remineralization occurred on the demineralized tooth enamel after being immersed for 150 minutes

(equivalent to one month's consumption) in cow milk, as shown from the statistically significant increase of enamel hardness. However, remineralization cannot restore initial hardness. There was insignificant remineralization on the tooth that had been immersed in soy milk, as shown by the increasing in enamel hardness. However, remineralization cannot restore the enamel to its initial hardness. The hardness scores for the teeth that were immersed in cow milk were higher than for immersion in soya milk. The addition of essential minerals is needed for the body, especially for minerals that are not contained in soy milk.

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