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(Research Article)

The effect of milk types on enamel density in primary teeth: Research article

Luthfiyah Nadhira [1,*], Soegeng Wahluyo [2] and Prawati Nuraini [2]

[1] Bachelor of Dental Science, Faculty of Dental Medicine, Univesitas Airlangga, Surabaya 60132, Indonesia.
[2] Département of Pediatric Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia.

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Abstract

Background: Early Childhood Caries (ECC) is a condition affecting primary teeth, caused by enamel demineralization due to bacterial acids from sugar metabolism. Remineralization, supported by calcium and phosphate, is crucial for repairing damage. Milk, rich in these minerals, may aid enamel remineralization and improve dental health.

Purpose: To analyze the effects of various bovine milk types on the enamel density of primary teeth.

Methods: Twelve extracted primary incisors were divided into four groups: distilled water (control), pure bovine milk, UHT bovine milk, and low-fat bovine milk. Teeth were immersed for 21 days, and enamel density was evaluated using a scanning electron microscope (SEM-EDX) and ImageJ software. The data were analyzed using One-Way ANOVA and Tukey HSD tests.

Results: Significant differences were observed between treatment groups ($p = 0.006$). The highest enamel density was found in the low-fat bovine milk group (0.83±0.64 µm), followed by UHT milk (0.95±0.16 µm), and the lowest in pure bovine milk (2.09±0.19 µm). Tukey HSD results showed significant differences between the control and UHT milk groups ($p = 0.11$) and the control and low-fat milk groups ($p = 0.09$).

Conclusion: Milk consumption positively affects enamel density in primary teeth, with low-fat and UHT bovine milk showing the most significant impact.

Keywords: Caries; Bovine milk; Enamel density; Microporosity; Primary teeth

1. Introduction

Globally, approximately 2.4 billion people suffer from dental caries in permanent teeth, while 486 million children are affected by early childhood caries (ECC), a condition specific to primary teeth [1,2]. ECC arises due to enamel demineralization, driven by acids produced during sugar metabolism by oral bacteria, primarily *Streptococcus mutans*. Prolonged demineralization leads to significant enamel loss, compromising its structure [3]. Enamel, the hardest tissue in the human body, consists predominantly of hydroxyapatite, making up about 95% of its weight. Despite its strength, enamel is vulnerable to acid exposure, particularly in conditions where the availability of calcium and phosphate ions in saliva is insufficient for natural remineralization [4,5].

Remineralization plays a critical role in restoring lost minerals, reducing enamel porosity, and preventing further damage. Calcium and phosphate are essential for the formation of hydroxyapatite, the mineral responsible for enamel's

* Corresponding author: Luthfiyah Nadhira

resilience [3]. Milk, rich in these minerals, has been widely recognized for its protective and reparative effects on enamel. Its casein proteins also form a protective barrier against acid erosion and reduce the risk of caries [6,7].

Conversely, formula milk, though beneficial for child growth due to its essential nutrients, poses a higher risk of ECC. This risk stems from the added sugars like sucrose and glucose, which serve as substrates for acidogenic bacteria in the oral cavity. These sugars promote acid production, leading to enamel demineralization. Research suggests that children consuming formula milk without proper oral hygiene are at a significantly higher risk of developing caries compared to those consuming unsweetened milk or breast milk [8].

While existing studies emphasize the general benefits of milk, limited research compares the effects of different bovine milk types—pure, UHT, and low-fat—on enamel density. This study aims to fill this gap, providing specific evidence to guide milk-based interventions for enhancing dental health in children.

2. Material and methods

This study investigates the effect of different types of milk on the enamel density of primary incisors. An experimental, in vitro laboratory design was employed, utilizing a true experimental post-test-only control group design. The study used extracted primary incisors, which were prepared and submerged in various milk solutions for 21 days.

2.1. Sample Preparation

The sample consisted of 12 primary incisors, which were free from caries, abrasion, and fractures. These teeth were disinfected by immersion in a 2.5% NaOCl solution for 15 minutes and then cleaned using a fine abrasive. The teeth were then embedded in self-cured acrylic resin, leaving only the crown exposed for treatment.

2.2. Treatment Groups

The samples were divided into four groups:

1. Control Group: Teeth immersed in sterile aquadest (distilled water).
2. Treatment Group 1: Teeth immersed in Hometown Dairy Fresh Milk®.
3. Treatment Group 2: Teeth immersed in Ultra Milk Susu UHT®.
4. Treatment Group 3: Teeth immersed in Ultra Milk Susu Low-fat®.

Each milk solution was replaced daily, and all samples were incubated at 37°C to simulate oral conditions.

2.3. Enamel Density Measurement

After 21 days of immersion, the enamel density of the samples was analyzed using Scanning Electron Microscope with Energy Dispersive X-ray Spectroscopy (SEM-EDX). The microporosity of the enamel, indicating the degree of mineralization, was measured using the ImageJ software, focusing on the depth of enamel micro-pores. Smaller pore depths indicate better remineralization due to milk's calcium and phosphate content.

2.4. Statistical Analysis

The data obtained from the SEM-EDX analysis were evaluated for normality using the Shapiro-Wilk test and homogeneity using Levene's test. Differences between the groups were analyzed using One-Way ANOVA. If significant differences were found, post-hoc analysis with Tukey's Honest Significant Difference (HSD) test was applied to compare the groups. The significance level was set at $p<0.05$.

3. Results and discussion

The analysis of enamel density using SEM-EDX showed varying degrees of microporosity and mineralization across the treatment groups. Images in Figure 1 provide a detailed overview of the enamel surface from samples in different groups after 21 days of immersion. Figure 2 shows the electron micrograph of the enamel surface of primary teeth from each group as analyzed using ImageJ.

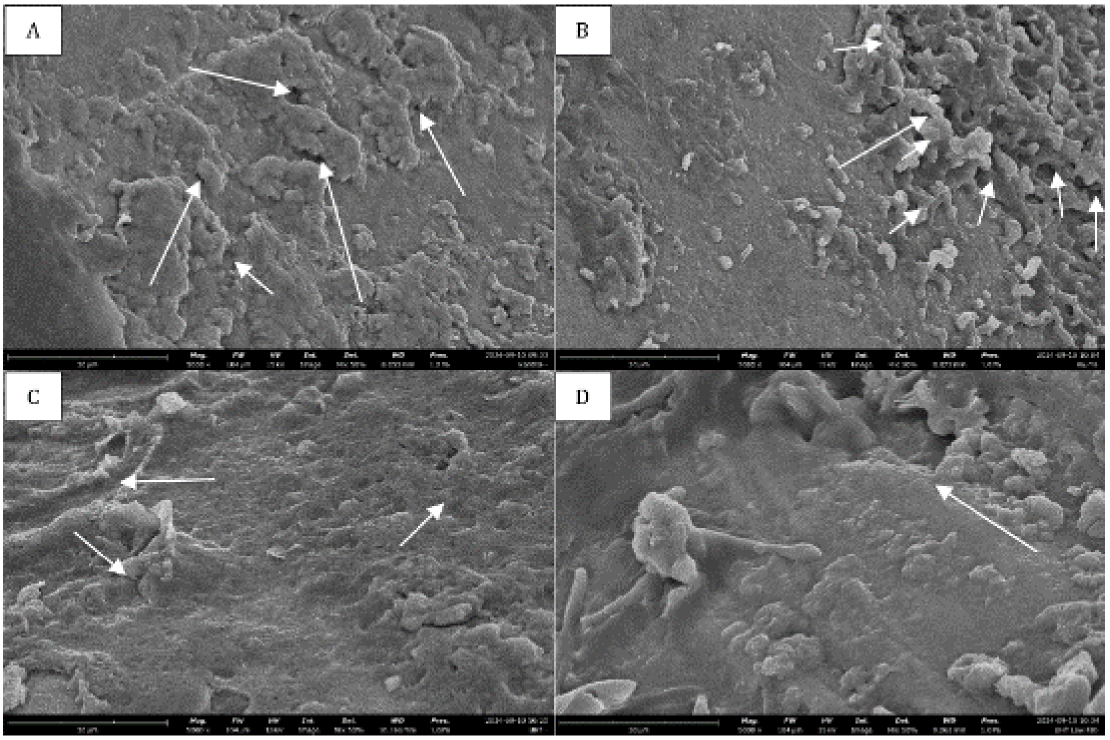

Figure 1 The electron micrograph of the enamel surface of primary teeth from the control group (A), the group immersed in pure bovine milk (B), UHT milk (C), and low-fat milk (D) reveals the presence of microporosity and deposits on the enamel surface that fill the enamel irregularities, leading to the loss of interprismatic substance (arrows indicate enamel microporosity). The observations were randomly made on the labial enamel surface using SEM-EDX with a magnification of 5,000x.

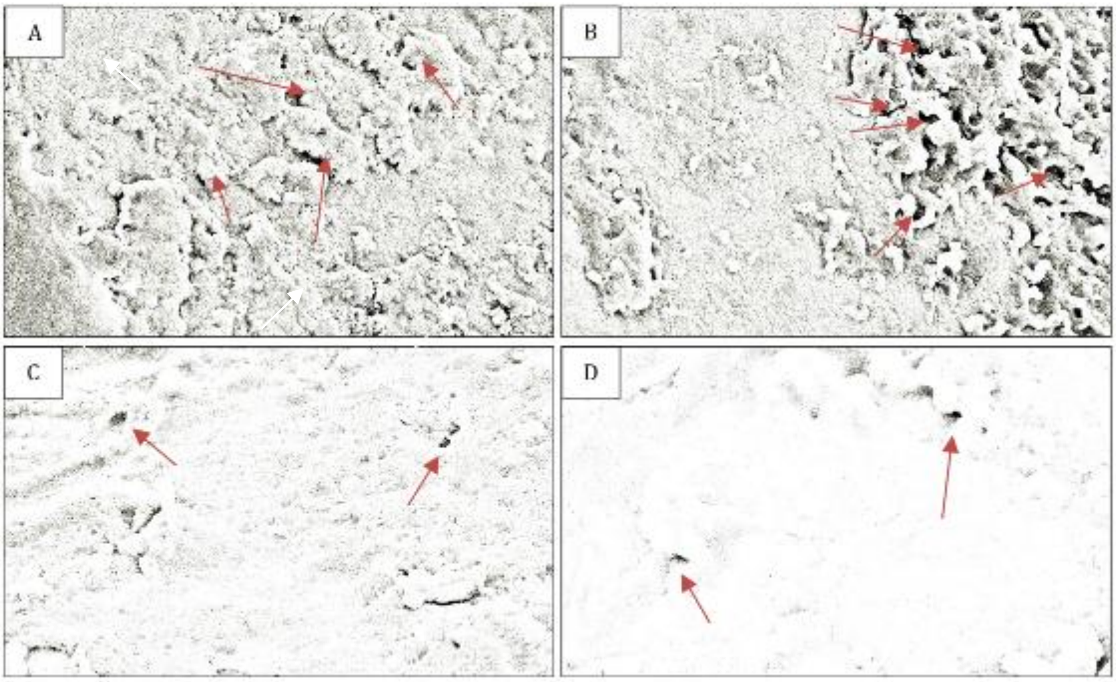

Figure 2 The electron micrograph of the enamel surface of primary teeth from the control group (A), the group immersed in pure bovine milk (B), UHT milk (C), and low-fat bovine milk (D) when analyzed using ImageJ shows black-colored areas (indicated by red arrows), which represent the extent of microporosity on the enamel.

Table 1 The average, standard deviation, as well as the minimum and maximum values of microporosity of the enamel surface after being immersed in various types of milk, measured in μm.

No	Treatment Groups	Average	Standard Deviation	Minimum	Maximum
1	Control	3,25	±1,28	1,96	4,53
2	Pure	2,09	±0,19	1,90	2,28
3	UHT	0,95	±0,16	0,78	1,11
4	Low-fat	0,83	±0,64	0,77	0,89

The control group, immersed in distilled water, exhibited significant microporosity with exposed prisms and surface cracks. The microporosities observed ranged from 1.08 μm to 5.93 μm. In contrast, the group immersed in pure milk showed fewer micro-pores, indicating some remineralization. The micro-pores observed were smaller compared to the control group, further suggesting the presence of minerals that reduced enamel damage. The samples treated with UHT milk exhibited some unevenness on the enamel surface, with microporosities ranging from 0.05 μm to 3.30 μm. Lastly, the group treated with low-fat milk demonstrated the smallest micro-porosities, with measurements ranging from 1.70 μm to 2.23 μm. The average depth of microporosity was lowest in the low-fat milk group (0.83±0.64 μm), followed by the UHT milk group (0.95±0.16 μm). The pure milk group had a higher average micro-porosity depth (2.09±0.19 μm), and the control group exhibited the largest micro-porosity depth (3.25±1.28 μm).

Table 2 outlines the atomic and weight concentrations of calcium and phosphorus on the enamel surfaces across all groups. The results revealed that the enamel samples immersed in low-fat milk had the highest concentrations of calcium (91.78 mol; 88.51%wt), followed by UHT milk (58.80 mol; 69.70%wt) and pure milk (27.43 mol; 49.34%wt). The control group had the lowest calcium concentration (6.21 mol; 12.50%wt). For phosphorus, UHT milk showed the highest concentration (19.53 mol; 17.90%wt), while the control group had none (0.00 mol; 0.00%wt).

Table 2 The concentration of atomic (mol) and weight (%wt) content of calcium and phosphorus elements on the enamel surface of primary teeth from various treatment groups. Observations were made randomly on the enamel surface using SEM-EDX at 5,000x magnification.

Element	Concentration	Control	Pure	UHT	Low-fat
Calcium	Atom	6.21	27.43	58.80	91.78
	Weight	12.50	49.34	69.70	88.51
Phosphorus	Atom	0.00	8.35	19.53	0.93
	Weight	0.00	11.61	17.90	0.69

Previous research has shown that bovine milk is non-cariogenic and even offers protective effects against dental caries, largely due to its ability to prevent enamel demineralization. Milk proteins adhere to the enamel surface, inhibiting demineralization, while the enzymes in milk reduce the acidogenic activity of plaque bacteria. Furthermore, the high protein content in milk binds calcium and phosphate ions, initiating the remineralization process. This process begins as milk proteins bond to the enamel, allowing calcium and phosphate ions to diffuse into the enamel subsurface, filling voids caused by demineralization, and thereby reforming hydroxyapatite crystals. This reduces microporosity and increases enamel density or hardness [9].

Statistical analysis through Shapiro-Wilk and Levene's tests confirmed the normal distribution and homogeneity of the data. One-Way ANOVA showed significant differences in micro-porosity depths among the groups ($p=0.006$), with Tukey's post-hoc test indicating that the control group significantly differed from the milk-treated groups ($p<0.05$). No significant difference was found between the pure milk and UHT milk groups. These results suggest that milk, especially low-fat milk, contributes to enamel remineralization and reduces micro-porosity compared to untreated enamel.

The findings of this study are supported by previous research, which showed that milk contains sufficient minerals to enhance enamel hardness following demineralization, though this increase is still lower than pre-demineralization hardness. Similarly, found that soaking enamel in cow's milk significantly improved enamel hardness post-remineralization, with cow's milk outperforming distilled water and soy milk in enhancing enamel hardness [10,11]. The current study confirmed that microporosity and enamel density are inversely related—larger microporosity correlates with a more porous enamel surface, while smaller values result in denser enamel surfaces. Among the groups, the low-fat milk group showed the smallest microporosity and the highest enamel density, indicating the positive effect of higher mineral content on remineralization. The control group, with no milk soaking, showed the highest microporosity and the lowest enamel density.

The mineral content of milk is a key factor in enhancing enamel density during remineralization. Calcium and phosphate ions are crucial in the formation of hydroxyapatite crystals, which repair enamel damage caused by demineralization. The concentration of these ions must be controlled to avoid excessive accumulation on the enamel surface, which can block tubules and hinder ion penetration into white spot lesions [12]. The viscosity of the milk solution also influences the diffusion rate of these ions. UHT milk, which has a lower viscosity than fresh milk, promotes faster ion diffusion and enhances remineralization, whereas fresh milk, with higher viscosity, slows ion diffusion, hindering the remineralization process [13]. Fructose and sucrose in milk also play a role in dental caries. Although fructose has lower cariogenic potential compared to sucrose, it can still contribute to enamel damage. Bacteria in the plaque ferment fructose, generating organic acids like lactic acid, which lower the oral pH, triggering demineralization and increasing the risk of tooth decay. Despite this, the overall effect of milk on enamel density is beneficial due to the high concentrations of calcium and phosphate [14,15].

During remineralization, calcium and phosphate ions accumulate on the enamel surface before diffusing into the enamel's microporosity. These ions help repair damage caused by demineralization by reforming hydroxyapatite crystals, which reduces the gaps in the enamel. The higher the concentration of calcium and phosphate, the faster the mineral precipitation, leading to the closure of microporosity and the remineralization of enamel [16].

However, the study has some limitations. It focused only on primary incisors, so the findings may not be generalizable to other tooth types, such as premolars or permanent teeth. Additionally, while the study examined three types of bovine milk (fresh, UHT, and low-fat), it did not explore other milk varieties or brands that may have different effects on enamel density. Lastly, the SEM-EDX method used for analysis may have limitations in resolution and data interpretation, which could affect the results.

4. Conclusion

Bovine milk, particularly low-fat bovine milk, positively impacts the enamel density of primary teeth. A reduction in microporosity observed in samples soaked in low-fat bovine milk correlates with an increase in enamel density, highlighting the important role of milk in the remineralization process. The higher calcium and phosphate content, along with the lower viscosity of UHT and low-fat milk, facilitates the diffusion of mineral ions into the enamel. Therefore, bovine milk can serve as a protective agent against dental demineralization, especially in children.

Compliance with ethical standards

Disclosure Conflict of interest

The authors have no conflict of interest to declare.

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