

Original article

## Effect of formula milk on the roughness and hardness of tooth enamel

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### ABSTRACT

**Background:** Demineralisation and remineralisation is a natural process in tooth enamel. It is influenced by the content of calcium and phosphorus in saliva, which concentrations are affected by the consumption of food, including formula milk. Demineralisation and remineralisation determine the roughness and hardness of the enamel surface. **Purpose:** This study compared the effect of formula milk on the roughness and hardness of tooth enamel. **Methods:** Maxillary premolar extracted teeth were demineralised with 37% phosphoric acid for 90 seconds and then divided into four treatment groups. For four days, the teeth were immersed twice a day in cow formula for five and ten minutes (Group I and II) and soy formula for five and ten minutes (Group III and IV). Before and after the immersion in milk, the teeth were submerged in artificial saliva. The enamel surface roughness and hardness were measured three times using a surface roughness tester and a Vickers microhardness tester, before and after demineralisation and after immersion in milk. Data were analysed using Kruskal–Wallis and post hoc Mann–Whitney tests. **Results:** There was no significant difference ( $p=0.88$ ) observed in the roughness reduction among the treatment groups. The highest increase in hardness was noted for the ten-minute cow formula milk group ( $93.27 \pm 16.00$ ). The increase of hardness was higher after immersion for ten minutes. A substantial difference ( $p=0.03$ ) was seen in the increase of hardness between the treatment groups. **Conclusion:** Immersion in cow and soy formula milk for five and ten minutes does not reduce the enamel roughness, but it increases the enamel hardness.

**Keywords:** demineralisation; enamel hardness; enamel roughness; milk formula; remineralisation

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### INTRODUCTION

Enamel is the outermost tooth layer and the hardest tissue in the human body. The components in enamel are inorganic materials (95%), organic materials (1–2%) and water (2–4%). Most of the inorganic materials are hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) crystals containing mineral ions, especially calcium and phosphate.<sup>1</sup> Saliva is a source of calcium and phosphate, which maintains the mineral saturation of teeth. Its role is to inhibit demineralisation at low pH and to initiate remineralisation when the pH returns to normal.<sup>2</sup> Demineralisation is the process of removing mineral ions, especially calcium and phosphate, from the hydroxyapatite (HA) crystals of the tooth enamel. Remineralisation is the

process of restoring the mineral ions in the HA crystals.<sup>3</sup> The required calcium and phosphate can be obtained from the consumption of food and drink, including formula milk.

Formula milk is frequently consumed by children. It contains protein, fat, calcium, phosphorus, zinc and other minerals. The calcium ions that are present in saliva have a buffering effect on dental plaque, preventing demineralisation and promoting remineralisation.<sup>4</sup> Formula milk is made from animal milk or plants such as soybean. Some children are allergic to animal milk lactose, therefore they consume milk derived from plants, such as soy milk.<sup>5</sup> Soy milk contains more protein, but the amino acids are not as complete as in cow protein. The calcium content is also lower than in cow milk.<sup>6</sup>

Milk can decrease the pH of plaque ten minutes after being consumed because of the fermentation of carbohydrates. The pH will increase 20 minutes later because hydrolysis of milk protein occurs.<sup>7</sup> There are various ways for infants and children to consume formula milk, such as bottle feeding, spoon feeding, drinking using a straw or cup feeding. In this way, a contact is established between milk and teeth. The longer and more frequently milk is consumed, the longer the enamel is exposed to calcium and phosphorus, which results in higher remineralisation.<sup>8</sup>

Surface roughness and hardness indirectly indicate the occurrence of demineralisation and remineralisation of hard tooth tissue.<sup>9</sup> Demineralisation in enamel starts with the release of minerals, especially calcium and phosphate, which causes the surface to become softer and rougher.<sup>10</sup> Hardness is defined as the resistance of a solid material to penetration.<sup>11</sup> Calcium and phosphate ions form HA crystals in enamel and the number of HA crystals formed affects the thickness of the enamel.<sup>12</sup> The purpose of this study was to compare the different effects of immersion in cow milk formula and soy formula on the roughness and hardness of the enamel surface.

## MATERIALS AND METHODS

This is a quasi-experimental laboratory (in vitro) study using 12 caries-free maxillary premolar extracted teeth. After separating from the root, the crown was cut mesiodistally to obtain the labial and lingual surfaces and then cultivated in resin. In this way, 24 specimens were prepared. The enamel surface was coated with nail polish, except for a circular work area with a diameter of 5 mm.<sup>5</sup> The first measurement of roughness and hardness of the enamel surface was done prior to the demineralisation with 37% phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) for 90 seconds.<sup>13</sup> Then, specimens were immersed for three hours in artificial saliva with a pH of 7.0. Thereafter, the second measurement was done. The specimens were grouped into four treatment groups. Group I was immersed in cow formula milk for five minutes and group II for ten minutes. Group III was immersed in soy formula milk for five minutes and group IV for ten minutes. For four days, the immersion was done twice a day (07:30 and 16:30) at a temperature

of 37 °C.<sup>8</sup> The milk was produced by Morinaga™ (PT. Kalbe Morinaga, Indonesia). The composition of both milk formulas was similar, namely, calcium, phosphorus, magnesium, iron, zinc and docosahexaenoic acid (DHA) in the same proportions, but the soy formula contained also arachidonic acid (AA), phospholipids, nucleotides, and other ingredients. The third measurement was done after the immersion was completed.

The surface roughness of the tooth enamel was measured using a surface roughness tester (Starrett SR300, Taylor Hobson, Berwyn, PA, USA).<sup>13</sup> The value of the roughness was obtained from the motion signal of a diamond-shaped stylus moving along a straight line on the enamel surface. The roughness was expressed as roughness average (Ra) and stated in µm. The roughness was measured at three different points on each research object. The magnitude of indentation was determined using a microhardness tester (HMV-2, Shimadzu, Japan) by looking at the area of the imprint on the indenter. A load of 100 grams was applied for ten seconds onto the enamel surface.<sup>8,14</sup> The enamel surface hardness was obtained from the tooth resistance to indentation. The units in the enamel surface hardness test were expressed in Vickers hardness (HV). The enamel surface hardness was measured at three different points on each research object and obtained as an average of three measurements. The data were analysed using the Kruskal–Wallis test ( $p < 0.05$ ) and the post hoc Mann–Whitney test ( $p < 0.05$ ).

## RESULTS

The Kruskal–Wallis test on mean and standard deviation of surface roughness reduction based on milk type and immersion time (Table 1) showed no significant difference ( $p > 0.05$ ) between the treatment groups. The largest average reduction in enamel surface roughness was observed after immersion in soy formula for ten minutes, followed by immersion in cow and soy formula for five minutes. The reduction in surface roughness was lowest after immersion in cow formula for ten minutes, namely  $0.21 \pm 0.06$ .

The Kruskal–Wallis test on mean and standard deviation of increased surface hardness based on milk type and immersion time (Table 2) exhibited a significant difference ( $p < 0.05$ ) between the treatment groups.

**Table 1.** The averages and standard deviations of the enamel surface roughness based on formula milk and immersion time and the Kruskal–Wallis test results in µm

Treatment group	N	Before immersion ( $x \pm SD$ )	After immersion ( $x \pm SD$ )	Reduction of enamel surface roughness ( $x \pm SD$ )	Kruskal–Wallis test		
					X <sup>2</sup>	Df	p
Group I	6	0.98 ± 0.29	0.73 ± 0.22	0.25 ± 0.18	0.66	3	0.82
Group II	6	0.91 ± 0.19	0.70 ± 0.15	0.21 ± 0.06			
Group III	6	0.82 ± 0.20	0.57 ± 0.14	0.25 ± 0.13			
Group IV	6	0.74 ± 0.12	0.48 ± 0.05	0.26 ± 0.12			

Description: N: Number of research objects; Df: Degrees of freedom; X<sup>2</sup>: Chi square; p: Probability

**Table 2.** The averages and standard deviations of the enamel surface hardness based on formula milk and immersion time and the Kruskal–Wallis test results (as HV)

Treatment group	N	Before immersion ( $x \pm SD$ )	After immersion ( $x \pm SD$ )	Increase in enamel surface hardness ( $x \pm SD$ )	Kruskal–Wallis test		
					X <sup>2</sup>	Df	p
Group I	6	170.43 ± 12.05	248.41 ± 23.31	77.98 ± 13.29	9.34	3	0.025
Group II	6	170.88 ± 24.86	264.15 ± 34.60	93.27 ± 16.00			
Group III	6	124.23 ± 23.66	190.45 ± 20.68	66.22 ± 9.32			
Group IV	6	130.30 ± 37.28	211.53 ± 39.33	81.23 ± 6.31			

Description: N: Number of research objects; Df: Degrees of freedom; X<sup>2</sup>: Chi square; p: Probability

**Table 3.** Post hoc Mann–Whitney test comparing the increasing average of the treatment groups

Comparison between groups	Post hoc Mann–Whitney test		
	Z	p	
Group I	Group II	-1.92	0.06
	Group III	-1.60	0.11
	Group IV	-0.48	0.63
Group II	Group III	-2.40	0.02*
	Group IV	-1.12	0.26
Group III	Group IV	-2.25	0.03*

Description: Z: Statistical value of Z table, p: Probability; \*: Significant

The highest average increase in hardness was observed after immersion in cow formula for ten minutes, namely  $93.27 \pm 16.00$ . Using the post hoc Mann–Whitney test, the mean comparison of the increase in enamel surface hardness between the treatment groups (Table 3) displayed only significant differences ( $p < 0.05$ ) between the groups of teeth immersed in soy formula for five minutes and those immersed in cow formula for ten minutes and between the groups of teeth immersed in soy formula for five minutes and those immersed in soy milk formula for ten minutes.

## DISCUSSION

The study showed that there was no significant difference in enamel surface roughness between the treatment groups using the length of time and milk immersion as variables. However, the enamel surface hardness differed significantly using the same variables. There were considerable differences between the groups of teeth immersed in soy formula for five minutes and those immersed in cow formula for ten minutes and between groups of teeth immersed in soy formula for five minutes and those immersed in soy milk formula for ten minutes. The results agree with those reported by Yendriwati et al.,<sup>8</sup> who observed that the longer and the more frequently the enamel was exposed to calcium and phosphorus contained in drinks, the more minerals were incorporated into the enamel, increasing its hardness.

The increase in enamel surface roughness is the result of increased porosity.<sup>15</sup> When remineralisation occurs, calcium and phosphate ions repair the enamel damage

caused by demineralisation.<sup>16</sup> The formed hydroxyapatite crystals reduce the interprismatic gap and enamel microporosity, which smoothens the surface of the porous part and decreases the surface roughness.<sup>17</sup> After five minutes, the average reduction in enamel surface roughness after immersion in cow formula milk was not different compared to that of using soy formula milk. However, after ten minutes, the average reduction in enamel surface roughness after immersion in cow formula milk was lower than that using soy formula milk.

In the demineralisation process, hydroxyapatite crystals dissolve and release calcium and phosphate ions. This process results in larger interprismatic gaps and reduces the enamel surface hardness. When remineralisation occurs by incorporating calcium and phosphorus ions, hydroxyapatite crystals are regenerated and fill the voids. According to several studies, the crystals formed are amorphous and differ from previous hydroxyapatite crystals. This may increase the surface hardness of the remineralised enamel, but it changed the hydroxyapatite crystals.<sup>18</sup> The study showed that there were significant differences after immersing the teeth in soy formula for five and ten minutes. The longer immersion allows the integration of more calcium and phosphorus ions, which leads to a higher degree of the remineralisation.

Milk prevents the demineralisation process according to several mechanisms. Milk protein can be adsorbed onto the enamel surface and inhibits the enamel demineralisation. In addition, milk fat that is adsorbed onto the enamel surface may act as a protection, and enzymes in the milk can reduce the acidogenic stage of plaque bacteria.<sup>12</sup> Milk contains proteins that bind calcium and phosphate ions. The remineralisation process starts with the attachment of milk proteins to the enamel surface. The calcium and phosphorus ions in milk can diffuse into the enamel subsurface.<sup>19</sup> The ions can occupy the empty HA crystal space because of demineralisation and initiate the remineralisation by forming hydroxyapatite crystals.<sup>17,20</sup> The newly formed hydroxyapatite crystals fill the interprismatic gaps of the enamel. This process decreases the roughness and increases the surface hardness of the enamel.<sup>14</sup> The following is the general chemical equation for the demineralisation and remineralisation process:  $8 H^+ + (Ca_{10}(PO_4)_6(OH)_2) \rightarrow 6 (HPO_4)^{2-} + 10 Ca^{2+} + 2 H_2O$  (demineralisation);  $10 Ca^{2+} + 6 (H_2PO_4)^{2-} + 14 OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2$  (remineralisation).

The demineralisation process results in the damage of the HA crystals, which starts with a bond formation between the ( $\text{PO}_4^{3-}$ ) ion and the free  $\text{H}^+$  ion, causing the dissolution of the apatite crystal. The  $\text{H}^+$  ion binds to an  $\text{OH}^-$  ion to form  $\text{H}_2\text{O}$  and it binds to a  $\text{PO}_4^{3-}$  ion to form  $\text{HPO}_4^{2-}$ . When in contact with the ionic acid,  $\text{HPO}_4^{2-}$  turns into  $\text{H}_2\text{PO}_4^-$ .<sup>17</sup> The diffusion of calcium and phosphorus ions into the enamel surface can initiate the remineralisation.<sup>18</sup> HA crystals can undergo atomic replacement, which leads to a change in the chemical formula HA. It can be concluded that teeth immersed in cow formula and soy formula for five and ten minutes might not decrease the roughness of the enamel surface, but it might increase the hardness of it. The hardness of the enamel surface of teeth immersed in soy formula for ten minutes was higher than that immersed in soy formula for five minutes.

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