

EFFECT OF BOVINE AND PLANT-BASED MILKS ON *STREPTOCOCCUS MUTANS* BIOFILM FORMATION, BIOFILM PH LEVEL AND ENAMEL DEMINERALIZATION IN HUMAN PRIMARY TEETH

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Abstract. Various types of milk are given to children but may contribute to dental caries by promoting the growth of the cariogenic bacteria *Streptococcus mutans*. In this study, we aimed to determine level of *S. mutans* biofilm formation, enamel demineralization and pH of the biofilm in vitro using human primary incisor teeth exposed to unsweetened bovine milk and sweetened and unsweetened almond and soy milk products in order to compare their respective risk factors for contributing to caries formation. To determine the level of biofilm formation, we put 200 µl of sterile pooled human saliva in each well of a 96-well polystyrene plate for 24 hours. We then removed the saliva and inoculated each well with 100 µl of *S. mutans* at a concentration of 10⁶ CFU/ml. The studied milks used for this study were: 1) bovine milk (Nongpho[®]), 2) unsweetened almond milk (Almond Breeze[®]), 3) almond milk with 2.7% sucrose (Almond Breeze[®]), 4) unsweetened soy milk (Lactasoy[®]) and 5) soy milk with 7% sucrose (Lactasoy[®]). Each milk product was diluted with sterile deionized water at a ratio of 1:2. We then added 100 µl of each diluted tested milk sample to their respective wells. The study was performed in triplicate for each studied sample. We used Brain Heart Infusion (BHI) with 5% sucrose as a positive control and sterile deionized water as a negative control placing them in their respective wells. The plate was gently agitated and then incubated in 5% CO₂ at 37°C for 24 hours to allow a biofilm to form. The biofilm in each well was stained with 200 µl of 0.05% crystal violet solution for 10 minutes and the optical density (OD) was read to determine the concentration of *S. mutans* biofilm formation. The OD (±standard deviation (SD)) values for the bovine milk, unsweetened almond milk, sweetened almond milk, unsweetened soy milk and sweetened soy milk were: 0.082 (±0.001), 0.076 (±0.026), 1.86 (±0.152), 0.535 (±0.217) and 1.6 (±0.108), respectively. The sweetened plant-based milk

samples (sweetened almond milk and sweetened soy milk) had significantly ($p < 0.05$) greater biofilm formation than the unsweetened plant-based milk samples (unsweetened almond milk and unsweetened soy milk samples) and the bovine milk ($p < 0.05$). There was no significant difference in biofilm formation between the bovine milk and the unsweetened plant-based milk samples so for the pH and demineralization parts of the study, we only compared the bovine milk with the sweetened plant-based milk samples. To assess for enamel demineralization, we cut 40, 4 × 4 mm enamel slab samples from 40 primary human incisor teeth and divided the cut slabs into 5 study groups by the type of solution the enamel slab was immersed in: Group 1: bovine milk ($n = 10$), Group 2: sweetened almond milk ($n = 10$), Group 3: sweetened soy milk ($n = 10$), Group 4 (positive control): 5% sucrose solution ($n = 5$), Group 5 (negative control) deionized water ($n = 5$). Each enamel slab was checked for surface hardness prior to and after being immersed in the respective studied solutions. The immersions occurred for 30 minutes at a time 4 hours apart 3 times a day for 5 consecutive days. The surface hardness readings before and after immersion were compared and the percentage of surface hardness loss (SHL) was calculated. The percents (\pm SD) of SHL in the bovine milk, sweetened almond milk, positive control and negative control were: 73 (± 6), 68 (± 11), 78 (± 5), 83 (± 8) and 73 (± 14), respectively. The percent SHL among the teeth samples exposed to sweetened almond milk was significantly ($p < 0.05$) lower than the percent SHL among the teeth samples exposed to sweetened soy milk and the bovine milk ($p < 0.05$). We measured the pH of biofilm formed on the enamel slab samples exposed to the studied milk samples for 6 days. The pH ranges during Days 1-6 of the biofilm on the bovine milk samples, sweetened almond milk samples and sweetened soy milk samples were: 6.3-6.7, 6.2-6.7 and 5.4-6.6, respectively. We compared the changes in pH between Day 1 and Day 6 for each of these three studied milk samples and found the sweetened soy milk had a significantly ($p < 0.05$) greater pH-decrease than the other groups. In summary, there was significantly greater biofilm formation with the sweetened plant-based milks than the bovine milk and non-sweetened plant-based milks but no significant difference between the bovine milk and non-sweetened plant-based milks. There was a significantly greater percent SHL with the sweetened plant-based milks than the bovine milk and there was a significantly greater drop in the pH of the biofilm with the sweetened plant-based milks than the bovine milk and the drop was significantly greater with the sweetened soy-based milk than the sweetened almond-based milk. We conclude, sweetened plant-based milks increase the risk for primary incisor teeth caries formation greater than bovine milk

but non-sweetened plant-based milks did not. Further *in vivo* studies are needed to determine if children who drink sweetened plant-based milks have more caries or not.

Keywords: bovine milk, soy milk, almond milk, *Streptococcus mutans*, biofilm, tooth demineralization, primary teeth

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INTRODUCTION

Dental caries is a multifactorial disease resulting from an interaction between acidogenic bacteria, sucrose and host susceptibility (Fejerskov, 2004). In the oral cavity, there exists biofilm, or dental plaque, which can be comprised of more than 800 species of microorganisms living together (Featherstone, 2008). Biofilm is a dynamic community and the microorganism population can shift between healthy and pathological populations when a factor, such as sugar, is introduced (Featherstone, 2008). The ecological plaque hypothesis suggests that a cariogenic oral environment that has a lower pH will have more acidogenic and aciduric microbiota (Marsh *et al*, 2015).

Streptococcus mutans is a microorganism commonly isolated from dental plaque and strongly associated with dental caries (Tanner *et al*, 2016; Tanzer *et al*, 2001; Mitrakul *et al*,

2017; Mitrakul *et al*, 2020; Tantikalchan and Mitrakul, 2022). Not only is *S. mutans* aciduric and acidogenic but it also has the capability to adhere to and deposit on tooth surfaces (Krzyściak *et al*, 2014; Bowen and Koo, 2011). It utilizes sucrose to produce extracellular polysaccharides, which allow them to strongly adhere to the enamel surface in dental plaque. *S. mutans* is also able to generate acid from carbohydrates and tolerate a low pH environment (Tanner *et al*, 2016; Tanzer *et al*, 2001). Over time, more acid is produced as a consequence of sugar fermentation, lowering the pH of the dental biofilm to below 5.5, which is the critical pH threshold below which enamel demineralization can occur (Marsh *et al*, 2015). An ecological alteration in the dental biofilm can cause demineralization of tooth structure and eventually dental caries (Marsh *et al*, 2015). Previous studies have shown an association between *S. mutans* and early childhood caries (ECC), whereby

progressive demineralization of the tooth occurs due to a cariogenic diet, a susceptible host and the population of oral microbiota found among children aged <6 years and these criteria may be used to assess caries risk (Hata *et al*, 2006; Okada *et al*, 2005; Mitrakul *et al*, 2013, Tanner *et al*, 2016; Tanzer *et al*, 2001). Recent studies among Thai children reported the number of *S. mutans* in plaque is greater among children with ECC than among those who are caries-free (Mitrakul *et al*, 2017; Mitrakul *et al*, 2020, Tantikalchan and Mitrakul, 2022).

Bovine milk is a good source of carbohydrates, proteins, fats, vitamins, calcium and phosphate (Huang *et al*, 2019). It is consumed by people of all ages worldwide. Several studies have reported bovine milk contains caries preventive factors, such as casein and fat and also various antimicrobial peptides, such as lactoferrin, lysozyme and peroxide, which may contribute to anti-cariogenicity (Rirattanapong *et al*, 2012; Lee *et al*, 2018; Rölla, 1989; Bowen and Koo, 2011; Huang *et al*, 2019). Lactose, a disaccharide sugar present in bovine milk, is less cariogenic than sucrose (Renyé *et al*, 2004). There is evidence to suggest bovine milk can cause caries if consumed frequently (Huang *et al*, 2019). In some populations, consuming bovine milk is not possible. One reason for this is bovine-product allergies to the protein in bovine milk or lactose intolerance. Other reasons for not consuming

bovine milk include ethical concerns about animal rights, the popularity of plant-based diets and the perception that plant-based milk is healthier than bovine milk (Huang *et al*, 2019; Dashper *et al*, 2012; Bergsson *et al*, 2001). This has led to the increased popularity of plant-based milk, such as soy milk, almond milk, oat milk and pistachio milk. Plant-based milk may be sold as sweetened or unsweetened. However, sucrose is often added to plant-based milk to improve the taste to help it sell better.

Several studies have compared the effect of plant-based milk and bovine milk on *S. mutans* biofilm formation (Bergsson *et al*, 2001; Mandalari *et al*, 2010). They found that plant-based milk produced less biofilm but had a poorer ability to increase the pH in the oral cavity (poorer buffering capacity) compared to bovine milk. When sugar was added to plant-based milk, it resulted in greater biofilm formation compared to bovine milk (Bergsson *et al*, 2001; Mandalari *et al*, 2010). Another study compared milk's ability to demineralize the enamel surfaces of permanent teeth. They reported no significant difference between bovine and plant-based milk (Bergsson *et al*, 2001). A previous study reported soy milk is associated with significantly more *S. mutans*, production of an acidic oral environment due to fermentation and a lower buffering capacity (Dashper *et al*, 2012), suggesting soy milk may be more cariogenic. There are numerous

plant-based milk products available on the market in Thailand but little data is available on the effect of these products on their risk for contributing to caries formation, especially in primary teeth.

In this study, we aimed to determine the level of *S. mutans* biofilm formation, the pH of that biofilm and enamel demineralization caused by unsweetened bovine milk and sweetened and unsweetened almond and soy milk products in order to determine their risk for contributing to caries formation in human primary incisor teeth.

MATERIALS AND METHODS

Bacteria preparation

S. mutans standard strain (ATCC 25175) was grown on a brain heart infusion (BHI) agar plate (Becton, Dickinson and Company, Franklin Lakes, NJ) in 5% carbon dioxide (CO₂) at 37°C for 48 hours. This was then inoculated into fresh BHI broth and grown under the same conditions until it reached a mid-exponential phase (OD_{600 nm}=0.5). The *S. mutans* culture was then diluted to 10⁶ colony-forming units (CFU)/ml.

Saliva preparation

Stimulated pooled saliva was obtained from 3 volunteers who abstained from tooth brushing for at least 6 hours prior to saliva collection.

Saliva production was stimulated by chewing paraffin. The saliva samples from the 3 volunteers were pooled, centrifuged and then diluted to 1:10 with phosphate buffered saline (PBS) and filtered through a 0.22-micron membrane (filter paper, Millipore, St Louis, MO). The stimulated pooled saliva was used to coat the wells of a 96-well polystyrene plate (Corning Inc, Corning, NY) in order to initiate *S. mutans* biofilm formation since the *S. mutans* adheres to the glycoprotein in the saliva.

Artificial saliva was prepared to keep the enamel slabs moist during the enamel demineralization part of the study. The artificial saliva consisted of potassium chloride (0.65 g/l), magnesium chloride (0.058 g/l), calcium chloride (0.165 g/l), dipotassium hydrogen phosphate (0.804 g/l), potassium dihydrogen phosphate (0.365 g/l), sodium carboxymethyl cellulose (2 g/l) and deionized water (1 liter) (Huang *et al*, 2019). It was filtered through a 0.22-micron membrane and kept at 4°C until use.

S. mutans biofilm level determination

The 96-well polystyrene plate (Corning Inc, Corning, NY) mentioned above was prepared for biofilm formation by adding 200 µl of sterile pooled human saliva to each well for 24 hours. After that the saliva was removed and 100 µl *S. mutans*

(10^6 CFU/ml) prepared as mentioned above was inoculated into each well. This was done in triplicate for each studied milk. The 5 studied milk products were: 1) bovine milk (Nongpho[®]), 2) unsweetened almond milk (Almond Breeze[®]), 3) almond milk with 2.7% sucrose (Almond Breeze[®]), 4) unsweetened soy milk (Lactasoy[®]) and 5) soy milk with 7% sucrose (Lactasoy[®]). Each studied milk was diluted with sterile deionized water to a milk to water ratio of 1:2. We used brain heart infusion (BHI) with 5% sucrose and sterile deionized water as positive and negative controls, respectively, and added them to their respective wells. The plate was gently agitated and incubated in 5% CO₂ at 37°C for 24 hours to allow biofilm formation. After incubation, the plate was agitated at 200 rpm for 10 minutes on a shaker (Mini-Shaker PSU-2T, Biosan, Rīga, Latvia) and then each well was gently washed with running tap water to remove non-adherent cells. The remaining biofilm in each well was stained with 200 µl per well of 0.05% crystal violet solution for 10 minutes and then the unbound dye was rinsed out with running tap water. The bound dye was extracted by adding 200 µl to each well of a mixture of ethanol and acetone at a ratio of 4:1 for 10 minutes. A 100 µl aliquot of this dyed solution was then transferred to a new 96-well plate and then the optical density (OD) at 575 nm was

measured using a micro-plate reader (Bio-Tek Instruments Inc, Winooski, VT). The amount of absorbance (OD575 nm) indicated the amount of biofilm present in each well.

There was no significant difference in the *S. mutans* biofilm formation between the bovine milk and the unsweetened plant-based milk samples (unsweetened almond milk and unsweetened soy milk) as discussed below in the RESULTS section so we performed the demineralization and pH tests only with the bovine milk, both sweetened plant-based milks and the positive and negative controls. The unsweetened plant-based milks were not included in these parts of the study.

Enamel demineralization testing

For the enamel demineralization test we obtained 40 human primary incisor teeth obtained by normal exfoliation or extracted because of prolonged retention. The minimum number of teeth required for each studied milk group was calculated based on the findings of a previous study (Huang *et al*, 2019) using an $\alpha = 0.05$ with a power of 90%; the calculation was made with nQuery Sample Size Software, Version 6.0 (Statsols, Los Angeles, CA). A minimum of 7 tooth samples was determined to be needed for each group to obtain statistical significance. The 40 caries-free primary incisor teeth were

obtained and stored in 0.1% thymol solution at room temperature. Each tooth was cut off at the cemento-enamel junction. An enamel window was then made by covering the labial middle third of each tooth with a 4 × 4 mm² piece of adhesive tape. Each tooth was then mounted in a resin block. The adhesive tape was removed after the resin was dry and the rest of each tooth was covered leaving only the exposed window where the tape had been. The labial surface of each tooth was then polished using 500, 800, 1000, 2000, 3000, 4000 and 5000 grit sandpaper and then polished with alumina powder until the surface was glossy. An ultrasonic system was then used to remove the residual enamel. Each enamel slab was then sterilized with ultraviolet light (Huang *et al*, 2019). The surface hardness of each enamel slab was measured before demineralization using a micro-hardness tester (FM-ARS 9000, Future-Tech Corp, Kanagawa, Japan). The hardness was tested at 4 different points at least 100 μm apart using 50 grams of force for 15 seconds. After surface hardness testing each tooth was placed in a well of a 12-well polystyrene culture plate (Corning Inc, Corning, NY) containing 2.5 ml sterile pooled human saliva. The plate was then incubated at 37°C for 24 hours to

let the saliva coat the enamel surface in order to obtain the glycoprotein from the saliva to initiate the biofilm formation by the *S. mutans*. After 24 hours the saliva was removed and then 2.5 ml of *S. mutans* cultured in brain heart infusion (BHI) with 5% sucrose was added to each well. The plate was then incubated again in 5% CO₂ at 37°C for 24 hours to allow the biofilm to form on the enamel slab (Fig 1). The 40 enamel slabs were then divided into 5 groups immersed in the studied solutions as follows: Group 1: bovine milk (*n* = 10), Group 2: sweetened almond milk (*n* = 10), Group 3: sweetened soy milk (*n* = 10), Group 4 (positive control): 5% sucrose solution (*n* = 5) and Group 5 (negative control): deionized water (*n* = 5) (Fig 1). Each tooth was immersed in its respective solution for 30 minutes at a time every 4 hours 3 times a day for 5 days. After each immersion was complete each tooth was rinsed twice in normal saline and placed back in its respective well containing artificial saliva (Huang *et al*, 2019). After the 5 days each tooth was briefly rinsed in phosphate buffered saline (pH 7.0). Then the surface microhardness of each slab was measured again and the percentage of surface hardness loss (SHL) was calculated as follows:

$$\% \text{ surface microhardness loss} = \frac{\text{Mean initial SH} - \text{Mean final SH}}{\text{Initial SH}} \times 100$$

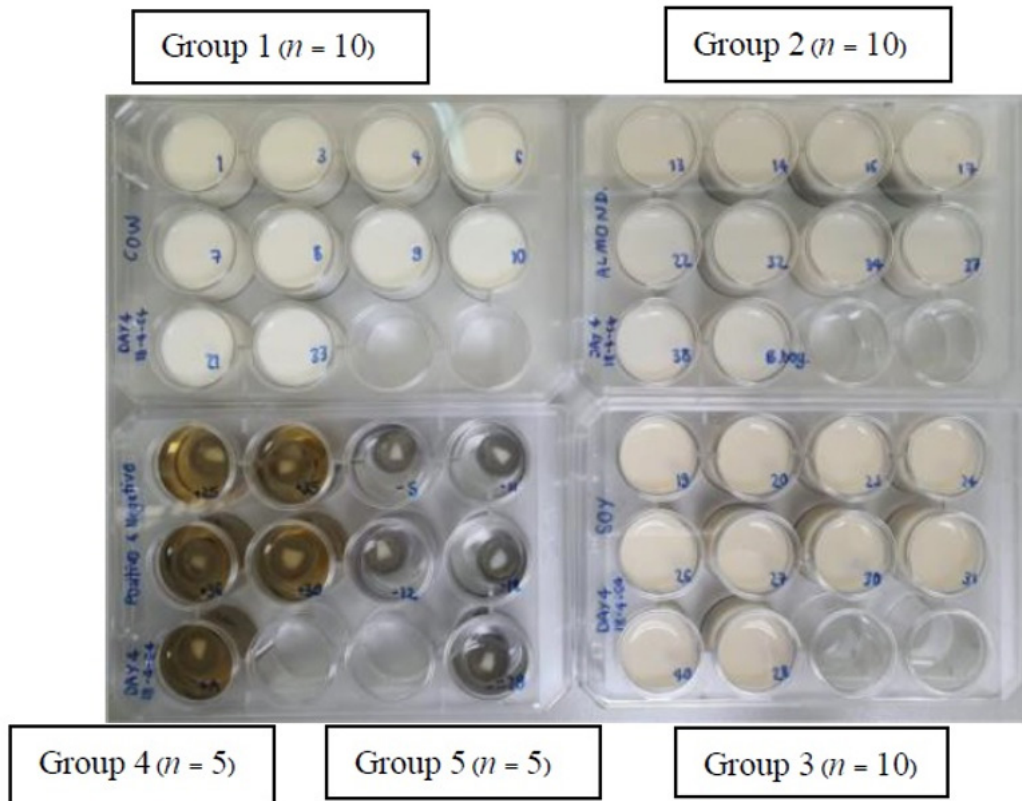


Fig 1 - Enamel tooth samples (N = 40)

Group 1: bovine milk ($n = 10$); Group 2: sweetened almond milk ($n = 10$); Group 3: sweetened soy milk ($n = 10$); Group 4: 5% sucrose solution (positive control) ($n = 5$); Group 5: deionized water (negative control) ($n = 5$)

Biofilm pH testing

The pH of the biofilm on each tooth immersed in its respective solution was measured twice daily (morning and evening) using a glass pH electrode (Mettler-Toledo, Giessen, Germany) and pH meter (Orion A111, St Louis, MO) for 6 days.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) (SPSS Inc, Chicago, IL). We used a Kruskal-Wallis test to determine significant differences between *S. mutans* biofilm formation among tested samples. For enamel

demineralization, the data were presented as means of %SHL for each type of study solution and significant differences among those means were calculated using the analysis of variance (ANOVA) test and the Tukey's test. A p -value <0.05 was considered statistically significant.

Ethical consideration

This study was approved by the Ethics Approval Review Board, Faculty of Dentistry and the Faculty of Pharmacy, Mahidol University (MU-DT/PY-IRB 2020/015.2205).

RESULTS

Levels of *S. mutans* biofilm formation

The OD (\pm standard deviation (SD)) values for *S. mutans* biofilm formation in bovine milk, unsweetened almond milk, sweetened almond milk, unsweetened soy milk, and sweetened soy milk were 0.08 (± 0.00), 0.08 (± 0.03), 1.86 (± 0.15), 0.54 (± 0.22) and 1.60 (± 0.11), respectively (Table 1). There were no significant differences in *S. mutans* biofilm levels between the bovine milk and the unsweetened plant-based milk

Table 1

Optical density at 575 nanometer (OD_{575 nm}) after staining the biofilm with 0.05% crystal violet solution

Experiment and control groups	OD _{575 nm} Mean \pm SD
Positive control (Brain Heart Infusion broth with 5% sucrose)	1.28 \pm 0.16
Negative control (Deionized water)	0.06 \pm 0.00
BM	0.08 \pm 0.00
UA	0.08 \pm 0.03
SA	1.86 \pm 0.15
US	0.54 \pm 0.22
SS	1.60 \pm 0.11

BM: bovine milk; SA: sweetened almond milk (with 2.7% sucrose); SD: standard deviation; SS: sweetened soy milk (with 7% sucrose); UA: unsweetened almond milk; US: unsweetened soy milk

samples (unsweetened almond milk and unsweetened soy milk). The sweetened plant-based milk samples had significantly ($p<0.05$) more biofilm formation than the unsweetened plant-based milk samples and bovine milk ($p<0.05$) (Table 1, Fig 2).

Enamel demineralization

The percent SHL (\pm SD) results for the bovine milk, sweetened almond milk and sweetened soy milk were 73.1 (\pm 5.8), 68.3 (\pm 11.1) and 78.1 (\pm 5.3), respectively. The percent SHL in

the sweetened almond milk was significantly less than the sweetened soy milk ($p<0.05$) and bovine milk ($p<0.05$) (Fig 3).

pH of *S. mutans* biofilm

The pH ranges for the bovine milk samples, sweetened almond milk and sweetened soy milk during days 1-6 were: 6.3-6.7, 6.2-6.7, and 5.4-6.6, respectively. The sweetened soy milk had a significantly ($p<0.05$) greater pH-decrease than the other groups (Table 2).

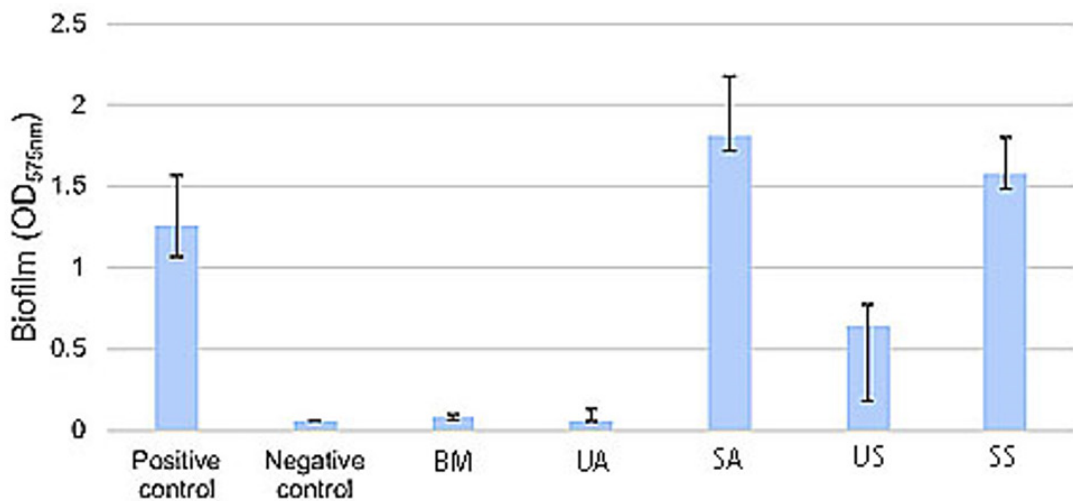


Fig 2 - Biofilm optical density at 575 nanometer (OD_{575 nm})

Note: Vertical bar represents standard deviation.

BM: bovine milk; SA: sweetened almond milk (with 2.7% sucrose); SS: sweetened soy milk (with 7% sucrose); UA: unsweetened almond milk; US: unsweetened soy milk

Table 2
pH value of *Streptococcus mutans* biofilm on the primary teeth during enamel demineralization Days 1-6

Treatment	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	07:30	18:00	07:30	18:00	07:30	18:00	07:30	18:00	07:30	18:00	07:30	18:00
DI	6.7	6.1	6.6	6.1	6.6	6.1	6.7	6.1	6.6	6.6	6.2	6.7
5S	6.5	5.7	6.6	5.6	6.2	5.7	6.4	6.0	6.5	5.8	6.6	6.6
BM	6.7	6.4	6.7	6.4	6.4	6.3	6.4	6.4	6.6	6.6	6.6	6.5
SA	6.7	6.2	6.6	6.6	6.5	6.9	6.5	6.9	6.6	6.7	6.7	6.5
SS	6.6	5.7	6.5	6.0	6.4	5.9	6.4	5.7	6.6	5.4	6.4	6.4

BM: bovine milk; DI: deionized water (negative control); SA: sweetened almond milk (with 2.7% sucrose); SS: sweetened soy milk (with 7% sucrose); 5S: 5% sucrose (positive control)

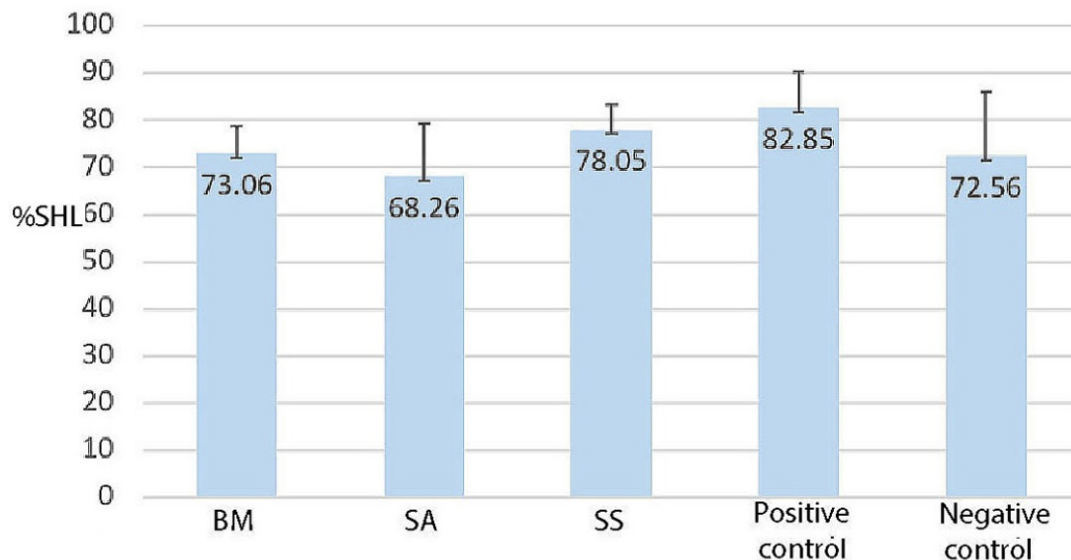


Fig 3 - Percent surface hardness loss (SHL) of studied teeth after demineralization

Note: Vertical bar represents standard deviation.

BM: bovine milk; SA: sweetened almond milk (with 2.7% sucrose); SS: sweetened soy milk (with 7% sucrose)

DISCUSSION

In our study, there was no significant difference in the levels of biofilm formed in bovine milk and the unsweetened plant-based milk samples but sweetened plant-based milk samples had significantly more biofilm formation than the unsweetened plant-based milk samples and the bovine milk. Our findings are similar to a previous study where sucrose was added to plant-based milk (almond and soy

milk) and a large amount of biofilm was formed (Lee *et al*, 2018). Another study of sweetened almond milk found it resulted in greater *S. mutans* biofilm formation than chocolate cashew milk, unsweetened flax milk and bovine milk (Huang *et al*, 2019).

In our study, there was no significant difference in biofilm formation among unsweetened almond milk, unsweetened soy milk and unsweetened bovine milk. A previous study reported unsweetened

soy milk had greater *S. mutans* biofilm formation than unsweetened almond milk and bovine milk (Lee *et al*, 2018).

As far as we know, this is the first study in Thailand of SHL in primary teeth after exposure to different types of milk. In our study, the percent SHL in the teeth exposed to sweetened almond milk was significantly less than the teeth exposed to sweetened soy milk or bovine milk. However, a previous study reported sweetened almond milk caused greater enamel demineralization than bovine milk (Huang *et al*, 2019). Our study was the first published study to report the effect of soy milk on enamel demineralization so we were not able to compare our results with those of other studies. A previous study reported soy milk has a low ability to increase pH level in the environment (lower buffering capacity) when compared with bovine milk (Dashper *et al*, 2012). Because of this, enamel exposed to soy milk might remain in an acidic environment longer than enamel exposed to bovine milk, resulting in more demineralization with the soy milk than the bovine milk.

In our study, the biofilm pH levels in the teeth exposed to the sweetened almond milk and bovine milk were significantly higher than the pH of the biofilm on the teeth exposed to sweetened soy milk. Our results differ from those of a previous study that reported finding sweetened almond

milk had the lowest pH, sweetened soy milk had a higher pH and bovine milk had a still higher pH (Lee *et al*, 2018). A reason for this difference could be differences in the sugar components of the studied milks. Evaporated cane juice may be present but not listed as a sugar. It is listed as a juice concentrate, although it has a high sugar content. A previous study reported soy milk had a lower pH buffering capacity than bovine milk (Dashper *et al*, 2012). Previous studies have reported that bovine milk, including sweetened bovine milk, has a better pH buffering capacity than soy milk (Bowen and Pearson, 1993; Giacaman and Munoz-Sandoval, 2014; Munoz-Sandoval *et al*, 2012; Prabhakar *et al*, 2010). This suggests lower risk for caries with bovine milk than soy milk.

A limitation of this study was that it involved a single species of bacteria, which might not reflect the complex flora of the mouth. Another limitation was that it was an *in vitro* study and thus did not look at actual outcomes of caries formation.

In summary, we found no significant difference in the level of *S. mutans* biofilm formed between bovine milk and unsweetened plant-based milk (unsweetened almond milk and unsweetened soy milk). However, both the sweetened plant-based milk samples tested had significantly greater biofilm formation than the bovine milk and unsweetened plant-based milk

samples. Sweetened soy milk caused greater enamel demineralization than sweetened almond milk and bovine milk. Sweetened soy milk had a significantly lower pH than sweetened almond milk and bovine milk. We conclude that sweetened plant-based milks have a greater risk for producing caries. Further in vivo studies are needed to determine if these in vitro studies actually reflect an increase in caries among children who consume sweetened plant-based milks and if changing to unsweetened plant-based milks would result in few caries.

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CONFLICT OF INTEREST DISCLOSURE

All authors declare no conflict of interest.

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