ASSOCIATION BETWEEN MILK TEMPERATURE AND MICROHARDNESS OF ENAMEL CARIES

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Abstract. Cow's milk has been found to enhance remineralization of initial caries but it is unclear if the temperature of the milk plays a role in the efficacy of this remineralization. Therefore, the aim of this *in vitro* study was to investigate the effect of cow's milk and buffalo milk temperature on the microhardness of enamel caries. For this study we used 80 bovine teeth and cut each tooth to 8 x 10 mm^2 and placed it in a resin acrylic block. Each tooth was then treated with a demineralizing agent until the enamel had a microhardness of 35-65 VHN. The teeth were then randomly divided into 8 groups (10 teeth per group). Group 1 was a negative control (no treatment) group; Group 2 was a positive control group [treated with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)]; Group 3 was treated with cow's milk at 4ºC; Group 4 was treated with cow's milk at 25ºC; Group 5 was treated with cow's milk at 40ºC; Group 6 was treated with buffalo milk at 4ºC; Group 7 was treated with buffalo milk at 25ºC; Group 8 was treated with buffalo milk at 40ºC. Both the cow's milk and buffalo milk were fortified with calcium. Group 2 was treated for 5 minutes and Groups 3-8 were treated for 2 hours each group, then rinsed with deionized water. The data were analyzed statistically using a paired *t*-test, independent *t*-test, one-way ANOVA and Tukey's test with a 95% confidence interval. After treatment, the mean microhardness values of all the groups were significantly higher (p=0.00) except for the negative control group. The groups treated with cow's milk and buffalo milk at 40°C had significantly higher percentage increases in microhardness than the CPP-ACP treatment group (p<0.05). The mean percentage increases in microhardness values for the cow's milk and buffalo milk at 40°C were higher than the mean percentage increases in microhardness values for the cow's milk and buffalo milk at 4°C (p<0.05). The buffalo milk had a significantly greater increase in the mean microhardness value than cow's milk at 4°C (p=0.00). In conclusion, cow's milk and buffalo milk at higher temperatures had better remineralizing capability than at low temperatures and better than CPP-ACP.

Keywords: buffalo milk, cow's milk, enamel caries, microhardness, temperature

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INTRODUCTION

Dental caries are major public health problem worldwide (Elidrissi and Naidoo, 2016). Remineralization of carious lesions should be conducted early in the disease process. Remineralization of white-spot lesions and carious lesions can be accomplished with agents containing fluoride, bioavailable calcium and phosphate, and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (Nagarathana *et al*, 2015).

Weiss and Bibby (1966) found the solubility of tooth enamel can be reduced *in vitro* with milk. This is thought to be done to the high level of calcium and phosphate in milk (Jenkins and Ferguson, 1966). Reynolds and Rio (1984) reported casein and whey protein in milk significantly reduced caries. Rugg-Gunn *et al* (1985) and Vongsavan *et al* (2010, 2016) found cow's milk fortified with calcium enhanced tooth remineralization. Vongsavan *et al* (2016) found buffalo milk formula increased bovine enamel microhardness.

In our study we wanted to determine if the temperature of the milk had an effect on its ability to remineralize initial enamel caries. The temperature tested were 4ºC and 40ºC to resemble refrigerated and hot milk. The temperature 4ºC was used because it is the safest temperature to store milk (Igumbor *et al*, 2000). The temperature 40ºC was used because it is the ideal warming temperature of milk to preserve milk's nutritional and immunological value (Bransburg-Zabary *et al*, 2015).

Lippert *et al* (2012) reported milk temperature influenced the anticaries potential of milk. However, studies of milk temperature and remineralization of caries in teeth are limited. The aim of this *in vitro* study was to investigate the effect of cow's milk and buffalo milk temperature on microhardness of bovine tooth enamel caries.

MATERIALS AND METHODS

Specimen preparation

We used 80 bovine teeth without wear or caries for this study. We cut the enamel on the labial surface of the teeth to 8x10 mm^2 and embedded it in self-curing acrylic resin. The studied side of each tooth was ground flat using 400, 800, 1200, 2000 and 4000 grit silicon carbide sandpaper sequentially (Buehler, Lake Bluff, IL). The teeth were then stored in deionized water at 4ºC until use.

Artificial caries formation

Artificial caries were formed in the enamel of the teeth by immersing them for 15 hours at 37ºC in the following demineralizing solution: 0.10 M lactic acid, 0.20% Carbopol C907, 4.1 mM $CaCl_2.2H_2O$, 8.0 mM KH_2PO_4 adjusted to a pH of 5.0 using KOH (Lippert *et al*, 2012).

Baseline caries evaluation

The studied teeth surface microhardness after caries lesion formation was examined using a Vickers microhardness indenter (FM-700e Type D; Future-Tech, Tokyo, Japan) at a load of 100 g for 15 seconds (Vongsavan *et al*, 2016). Only teeth with a Vicker Hardness Number (VHN) of 35-65 were used in the study (Vongsavan *et al*, 2016).

Treatment groups

The study teeth were randomly divided into 8 groups of 10 teeth each. Group 1: negative control (no treatment); Group 2: positive control (specimens were treated with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) tooth mousse); Group 3: treated with cow's milk at 4°C; Group 4: treated with cow's milk at 25°C; Group 5: treated with cow's milk at 40°C, Group 6: treated with buffalo milk at 4°C, Group 7: treated with buffalo milk

Table 1
Mean (±SD) and percent change in microhardness values before and after treatment (n=10).

Group	Mean VHN ±SD		Percent change
	Before treatment	After treatment	
No treatment	52.67±8.29	52.49±8.30	-0.47±1.47[a]
CPP-ACP	50.17±6.68	59.86±7.26*	18.84±5.80[b,c]
Cow's milk at 4ºC	55.78±2.92	62.03±4.30*	11.11±3.44[b]
Cow's milk at 25ºC	56.67±6.27	71.42±6.90*	26.55±11.11[c,d]
Cow's milk at 40ºC	50.65±5.34	65.09±7.66*	28.48±6.34[d]
Buffalo milk at 4ºC	43.84±4.54	52.40±6.26*	19.66±6.05[b,c]
Buffalo milk at 25ºC	45.28±6.84	56.10±7.40*	24.25±5.22[c,d]
Buffalo milk at 40ºC	49.94±4.53	64.34±6.43*	28.84±5.16[d]

*Indicates significant difference in microhardness between before and after treatment. Different letters indicate a significant difference in the percent change in microhardness among the groups. VHN, Vicker's hardness number; SD, standard deviation.

at 25°C and Group 8: treated with buffalo milk at 40°C. The cow's milk used for the study was Anlene™ concentrated calcium milk (Mary Anne Dairy Products, Bangkok, Thailand). The buffalo milk used for the study was Murrah™ organic milk (Murrah Dairy, Chachoengsao, Thailand). Group 2 was treated for 5 minutes. Groups 3-8 were treated for 2 hours. After treatment the teeth were then rinsed with deionized water and blotted dry (Vongsavan *et al*, 2016). All the specimens were again examined for microhardness and the VHN value was recorded.

Statistical analysis

A paired t-test was used to compare the results before and after treatment for each group. An independent t-test was used to compare the percent changes in microhardness values of the different types of milk at each temperature. The one-way ANOVA and Tukey's tests were used to compare the percent changes in microhardness values among groups with a 95% confidence interval. The formula used to calculate the percent change was:

$$\frac{\text{VHN value after treatment} - \text{VHN value before treatment}}{\text{VHN value before treatment}} \times 100$$

If the result of the above calculation was a positive number then this was the percent increase and if the result was a negative number then this was the percent decrease.

RESULTS

Prior to treatment the mean VHN values for each group ranged from 35-65 VHN (Table 1). After treatment, the mean microhardness values for all the groups were significantly higher (p=0.00) except for the negative control group. The groups treated with cow's milk and buffalo milk at 40°C had significantly higher percentage increases in microhardness than the teeth in the CPP-ACP group (p<0.05). The

Table 2
Percent change in the mean (±SD) microhardness values after treated with cow's milk and buffalo milk at each temperature (*n*=10).

Temperature	Percent change in the mean ± SD VHN	
	Cow's milk	Buffalo milk
4ºC	11.11±3.44	19.66±6.05*
25ºC	26.55±11.11	24.25±5.22
40ºC	28.48±6.34	28.84±5.16

* indicates a significant difference in the percent change in the mean microhardness values between cow's milk and buffalo milk. VHN, Vicker's hardness number; SD, standard deviation.

mean percent increases in microhardness values for the teeth treated with the cow's milk and buffalo milk at 40°C were significantly higher than the teeth treated with the cow's milk and buffalo milk at 4°C ($p<0.05$). The buffalo milk treated teeth had a significantly higher percentage increase in microhardness values than cow's milk at 4°C ($p=0.00$)(Table 2).

DISCUSSION

The cow's milk used in our study (Anlene™) contained 4 times the amont of calcium than regular cow's milk (800 mg per 110 ml). The buffalo milk used in our study (Murrah™) contained twice the amount of calcium regular cow's milk (354.89 mg per 180 ml).

Our finding that the studied cow's milk and buffalo milk contributed to remineralization of enamel is similar to a study by Vongsavan *et al* (2010, 2016) who studied these 2 same brands of milk and found them to contribute to remineralization of tooth enamel. Cow's milk and buffalo milk are composed of casein, calcium and phosphate (Vongsavan *et al*, 2010). Casein in milk has been reported to significantly reduce the extent of tooth caries (Reynolds and Rio, 1984) and calcium and phosphate can enhance reminelization of caries (Vongasavan *et al*, 2010). Other studies have also reported the remineralization effect of milk on tooth enamel (McDougall, 1977; Mor and Rodda, 1983).

In our study, we found milk at 40°C was better at enhancing remineralization than milk at 4°C. This may be due to a higher mobility of calcium and phosphate ions at a higher temperature (Mallanoo and Stokes, 1977).

Buffalo milk contains more casein and whey protein than cow's milk (Khedkar *et al*, 2016). Reynolds and Rio (1984) reported casein and whey protein in milk significantly reduced caries. In our study, buffalo milk increased microhardness of teeth significantly more than cow's milk at 4ºC in spite of using cow's milk with twice the amont of calcium as the buffalo milk used.

In our study, higher temperatures of the studies cow's milk and buffalo milk were more effective at enhancing remineralization than lower temperatures. Further studies are needed among human teeth to determine if this same effect is present on human teeth.

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