Compendium of Oral Science Volume 9(1)/2022 Original Article

The Effect of Different Milk Products on Enamel Hardness: An In Vitro Study

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Received: August 09, 2021 Accepted for publication: November 15, 2021

DOI: https://doi.org/10.24191/cos.v9i1.16909

ABSTRACT

Objectives: An in vitro study to assess the effect of different milk products on the hardness of enamel surfaces. Materials and Methods: Extracted primary and permanent teeth incisors (15 deciduous and 15 permanent) were collected and cleaned. The extracted teeth were then divided randomly into five groups (n=6 per group): G1- distilled water (control); G2- fresh milk (cow milk); G3- chocolate flavoured milk; G4- orange flavoured cultured milk; G5 - fruit lassi milk. The 10 days immersion cycles for the test products were performed thrice daily and were interspersed with exposure of the artificial saliva. Measurement of microhardness on the enamel surface microhardness measurement were performed at baseline, 5- and 10day of experimentation using Vickers hardness test machine. The pH of each milk products was also assessed. The data were evaluated with repeated measures ANOVA test. Results: Group 4 had significant reduction in microhardness (p < 0.05) compared to the control group while Group 1 showed significant reduction (p < 0.05) in microhardness compared to all tested samples for day 5 and day 10. While for intergroup comparison, there was no difference in group 3 and group 2. However, there were significant difference between group 4 and group 5 with all other groups on day 10 (p < 0.05). Conclusion: Fresh milk showed to be more effective in increasing the enamel microhardness meanwhile orange culture milk has the highest reduction of enamel surface hardness. Thus, we suggest that milk product with low pH has high tendency in enamel erosion.

Keywords: milk products, extracted teeth, enamel surfaces, microhardness

INTRODUCTION

Current research on the aetiology and prevalence of tooth wear has been remarkably increased (Rios et al 2007; Sun et al 2017; Hedge et al, 2018; Al Khalifah 2020). The four main categories of tooth wear are erosion, attrition, abrasion and abfraction of the tooth (Meurman and Ten Cate, 1996; Warreth et al, 2020). However, among these categories, dental erosion is the major threat for tooth surface loss. WHO reported that the prevalent of oral diseases in the South-East Asian region, affecting 70 to 95% of school-aged children and the vast majority of adults. According to Global Burden of Disease study in 2017, about 3.5 billion of people worldwide are estimated to suffer from oral diseases with caries of permanent teeth being the most common condition (United Nations General Assembly, 2017). Thus, the need for diagnosis and potential treatment for this problem has been a significance challenge for clinicians (Mulic et al, 2012). Dental erosion usually associated with multifactorial condition which is conventionally the causes are divided into "extrinsic" and "intrinsic" factors (Johansson et al, 2012). The extrinsic factor mainly associated with external acidic diet consumed for example citrus fruit juices and some acidic drinks while the intrinsic factors include influx of the acidic stomach, diseases and activities that may enhances dental erosion such as drug abuse, abnormality of salivary gland, diabetes, eating disorder, alcoholism, and others (Johansson et al, 2012). Besides that, modern lifestyle is also known to be one of the contributing factors in dental erosion such as imbalance diets and introduction of various junk foods and fast food with high sugarcontent (Levrini et al. 2014).

Dental erosion gradually occurs when there is an irreversible dissolution of dental tissue from acidic agents (Levrini et al, 2014). This process does not require the presence of cariogenic bacteria, but it resulted due to acid attack on the enamel surface. Frequent consumption of acidic diet and exposure time may lead to direct removal of hard issues from the enamel surface and causes mineral dissolution and creation of softened layer which later end up with total tissue loss (Babu and Kavyashree, 2015), hence causing permanent loss of dental hard tissue (Lussi et al, 2011). The clinical erosive lesion is smooth, polished, and rounded with the loss of tooth surface characteristics (Mulic et al, 2012). Continuous exposure to acidic solution led to formation of lesion in enamel prism, in which it will affect the interprismatic area. Bulk mineral is centripetally etched away in enamel erosion leaving a partly demineralized softened surface layer, which is prone to mineral deposit after topical fluoride application (Ganss et al, 2001). In dentin, erosive demineralization causes the exposure of outer layer of fully demineralized organic matrix continued by a partly demineralized zone until the sound inner dentin is reached (Buzalaf et al, 2012). Exposure of the underlying dentinal tubules is also the main causes of hypersensitivity, dental pain, reduced lower facial height and this may affect one's appearance (Haghgou et al, 2016). In primary teeth, enamel is less calcified and porous thus it makes it more susceptible to erosion compared to permanent tooth (Lussi et al, 2004). Hence, erosion can be a particular problem for young children as the enamel and the dentin layer of primary dentition are much thinner than those of the permanent teeth (Lussi et al, 2004). Previous study also reported that, there is an interaction between behavioural, chemical and biological factors with the tooth erosion in children and adolescents (Lussi et al, 2011). These factors are known to be crucial in understanding the variation of tooth erosion in certain individual when they are exposed to similar acidic challenges in their diets (Lussi et al, 2004).

The main safety parameter against acid erosion is saliva. Saliva contains calcium, phosphate and fluoride ions, which is needed for remineralization. Remineralization is defined as the replacement of depleted mineral content of bones and teeth (Gupta *et al*, 2009). In addition to that, saliva also has a diluent action against the acids as more than 90% of saliva composition is water. Besides, saliva also eliminates acidic solution by the swallowing action in the oral cavity. Saliva has a buffering capacity to neutralize the acids and involves in the formation of acquired pellicle which acts like a blockade to reduce the contact of acids and the teeth (Nieuw *et al*, 2004). With the addition of milk to saliva, it may increase its protective effect since milk has favourable levels of calcium and phosphate than saliva (Nieuw *et al*, 2004).

It is well known that milk is one of the first carbohydrates that have been introduced to people during their childhood. Milk is known to have many benefits to human and plays a significant role in various ways such as in growth and development of children, for bone health, oral health, metabolic syndrome, and

cardiovascular disease (Muehlhoff et al, 2013). Milk has a good source of nutrients for example calcium, iron, magnesium, phosphorus, potassium, sodium, zinc and others (Muehlhoff et al, 2013). Calcium, phosphorus, and magnesium were known to play an important role in bone health and strengthening the tooth structure. Milk is also found to have components that is good for intellectual development for people especially in teenagers and baby (Clark et al, 2020). Besides minerals, milk also contain water (the percentage depend on the type of milk), fat, protein, and lactose (carbohydrates) (Muehlhoff et al, 2013). Milk contains 4% to 5% of the disaccharide lactose, which can be fermented by oral bacteria. However cariogenic bacteria prefer sucrose as nutrient as it may transform this sugar into polysaccharides to facilities their attachment on tooth surface. Milk also contain casein, which is known to prevents caries through its ability to produce high concentration of phosphate calcium in the plaque structure. This complex can prevent demineralization and initiate remineralization of the enamel (Muehlhoff et al, 2013). Milk can be classified into 3 classes, which are, liquid milk, condensed milk, and dehydrated milk. They also being produced in many types of products such as fresh milk, flavoured milk, fruit-lassi milk, cultured milk and other types of dairy products that contain milk. Some of this milk products contain added sugar and are low in pH. Beside low in pH, acid concentration, exposure time, mineral content, clearance on the tooth surface and calciumchelating properties also should be considered as confounding factors for the erosive effect of the acidic foods (Lussi and Jaeggi, 2008). Some study stated that, milk product such as flavoured milk (Levine, 2001) and yogurt (Lussi et al, 2004) with added sugar does not have significant erosive effect on enamel. They stated that milk-based products may have protective effect against dental caries due to the presence of calcium and phosphate in the solution. However different studies may use different parameters or variables such different type of milk products, the content of the milk products, the pH of the milk products, different immersion time used, as the presence of that may varies from one to another. Therefore, further studies are required to understand the effect of acidic foods with different parameters on enamel surface hardness.

There is not many information available on the effect of the selected milk products against the enamel hardness. Therefore, the aim of this study was to investigate the effect of different type of milk products available in the market on enamel surface hardness.

MATERIALS AND METHODS

All materials used

Materials

All teeth were collected from Faculty of Dentistry, UiTM Sungai Buloh and a private clinic in Klang Valley. All milk products were purchased from local supermarket. Materials used to prepare artificial saliva are ethyl-p-hydroxybenzoate 2.0g, sodium carboxymethyl cellulose 10.0g, KCl 0.625g, MgCl₂.6H₂0 0.059g, CaCl₂.2H₂0 0.166g, K₂HPO₄ 0.804g, KH₂PO₄ 0.326g in 1000 mL of deionized water.

Collection of Sample

The present study was conducted in order to assess and compare the effect of different milk products on microhardness surface of enamel and to identify the erosive milk product. Extracted sound deciduous (n=15) and permanent teeth (n=15) were randomly collected from April to September 2017. Milks were randomly selected from a supermarket based on its category, which are plain milk (fresh cow's milk), flavoured milk (chocolate milk), flavoured cultured milk (orange cultured milk) and fruit-based lassi (tropical and mixed Fruits). pH of each milk products was recorded prior further analysis.

Preparation of specimens

Primary and permanent incisors teeth (Total = 30) collected were cleaned using pumice-water slurry and alumina paste with a polishing brush at low-speed handpiece to remove any debris or calculus prior to the study. Primary and permanent teeth's crown were separated from their root and then stored in a container for further analysis. The teeth were fixed at the center of the acrylic plate with beading was and then were placed with the flattest buccal surface facing downwards so that it is parallel with the plate. The teeth had their buccal surface flattened using a grit of 600 and 1200 Al_2O_3 abrasive papers, polished with $0.3\mu m$ alumina paste and felt paper using a water-cooled low speed polishing machine. The specimens were cleaned in deionized water for 10 minutes. The test site was separated by using insulating tape with a 2mm diameter on the buccal surface. Then the tooth and plate were rendered acid-proof by coating them using 2 layers of cosmetic nail polish. While another site was left uncoated. Then, the specimens were stored at 37°C in normal humidity environment in artificial saliva (Mali *et al*, 2015).

Baseline measurement

The assessment of the baseline enamel surface microhardness was done by using Vickers Hardness Machine at Faculty of Mechanical Engineering UiTM Shah Alam. The force applied was 25g with the diamond indenter on the flattest enamel surface at 3 different points and the readings was obtained using Hv unit (Mali *et al*, 2015) (Figure 1.0).

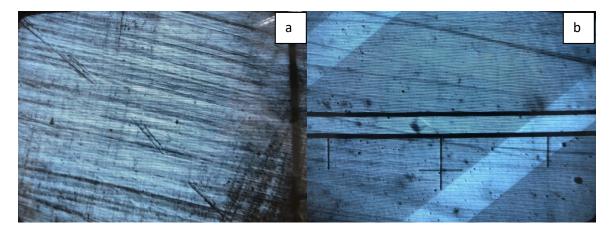


Figure 1: Enamel surface before indentation by the diamond indenter of Vickers hardness machine (a). Enamel surface after indentation by the diamond indenter of Vickers hardness machine (b)

Experimental Group and Immersion Cycle

After the baseline microhardness record, the teeth specimens of 3 deciduous incisors and 3 permanent incisors were randomly selected and were included into 5 groups each respectively. Group 1 (distilled water) as the control group; group 2 (plain milk (fresh cow's milk)); group 3 (flavoured milk (chocolate milk)); group 4 (flavoured cultured milk (orange cultured milk)); and group 5 (fruit-based lassi milk (tropical and mixed fruits)). Each tooth in the group was immersed into 5 mL of their respective solutions for 5 minutes thrice daily interspersed with artificial saliva for 5 days in every 4 hours at 37°C room temperature. Then all teeth were preserved in artificial saliva for further analysis. After the 5th day of analysis, the immersion process was continued for another 5 days.

Surface Microhardness test

All sample were sent to Faculty of Mechanical Engineering UiTM Shah Alam for microhardness reading using Vickers Hardness Machine. All experimental group were immersed into 5 mL of fresh solution for 5 minutes thrice daily for 5 days. Intermittently, after immersion of the teeth for 5 minutes in the milk products they were washed with distilled water and preserved in artificial saliva with daily change of the solution for 10 days of the analysis.

Preparation of Artificial Saliva

Artificial saliva was prepared according to Macknight-Hane and Whitford (1992) formula. The components consisting of methyl-p-hydroxybenzoate, sodium carboxymethyl cellulose, KCL, MgCl₂.6H₂0, CaCl₂.2H₂0, K₂HPO₄, KH₂PO₄.

a) Methyl-p-hydroxybenzoate (2g) were first dissolved in 800 mL water. b) 20 mL of the solution was stored for further mixing with other chemicals solvent. The remaining solution was kept in refrigerator. c) 200mL of water was boiled and 10g sodium carboxymethyl cellulose was sprinkled on the boiling water and stirred until dissolved. d) Methyl-p-benzoate solution was poured into sodium carboxymethyl cellulose and mixed until they are in gel form. e) 0.625g of KCl was dissolved in methyl-p-hydroxybenzoate solution (item a) and then mixed with methyl-p-benzoate solution (item d). f) 0.059g of MgCl₂.6H₂0 was dissolved in methyl-p-hydroxybenzoate (item a) and the solution was poured in solution (e). g) 0.166g of CaCl2.₂H₂O was dissolved in methyl-p-hydroxybenzoate solution (item a) and the solution was poured in item (f) and mixed the solution. h) 0.804g of K₂HPO₄ was dissolved in item (a) and poured in item (g) and mixed the solution. The pH of the final solution was adjusted to pH 6.75 with KOH.

pH measurement

The pH value of the milk products used for the immersion of the teeth were assessed using a digital pH meter.

Statistical Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) 23.0 software. The analysis was done using a repeated measures ANOVA. Multiple comparison test within group were analysed by assessing the overlapping of confidence interval of one group to another confidence interval in another group to suggest whether there is any significant difference between time and the type of milk products as study parameters.

The ethics of this research were approved by UiTM Research Ethics Committee

RESULTS AND STATISTICAL ANALYSIS

pH value of milk products

The pH of each milk product was measured and tabulated (Table 1). Flavoured cultured milk was found to have lowest pH (3.62), followed by fruit-based lassi (pH 4.16), flavoured milk (pH 6.70) and plain milk (pH 6.75). The pH of distilled water was pH 6.97.

Microhardness analysis

Microhardness of the teeth were assessed at baseline, day 5 and day 10 of the experiment. The mean hardness was least in flavoured cultured milk both for primary and permanent tooth. Meanwhile the mean hardness was maximum in flavoured milk for primary tooth and fresh milk for permanent tooth at day 10 (Table 2).

Both primary and permanent enamel microhardness shows that there is overlapping of confidence interval between plain milk and flavoured milk indicating no significant difference. Meanwhile flavoured cultured milk and fruit-based lassi milk both showed no overlapping of confidence interval between the other groups showing the significant difference on the day 10 of experiment for both primary and permanent teeth.

Table 3 shows the intergroup comparison of surface hardness before and after immersion with different milk products for primary teeth. From the data obtained it shows that there is significant difference between control and flavoured milk, control and fruit-based lassi, plain milk and flavoured cultured milk,

plain milk and fruit-based lassi, flavoured milk and flavoured cultured milk, flavoured cultured and fruitbased lassi from baseline, day 5 and day 10 of the study. Table 4 shows the intergroup comparison of surface hardness before and after immersion with different milk products for permanent teeth. No significant correlation was shown between control and all tested group at baseline however, after day 5 and 10 there were significance different between control and plain milk, control and flavoured cultured milk, control and fruit-based lassi milk. While Table 5 showed the changes in surface hardness between primary and permanent tooth. There is significant difference for surface hardness changes between both type of tooth from baseline, day 5 to day 10 of the study. The significant difference could be due to the surface microhardness before treatment (at baseline) and after first (5th day) and second treatment (10th day). However, there is no difference between the primary and permanent tooth towards different milk products. Figure 2 and 3 shows the changes in surface hardness for both primary and permanent tooth from baseline, day 5 and day 10 of the study.

Product	рН	Contents*
Distilled water	6.97	water
Plain Milk	6.75	100% Fresh cow's milk
Flavoured milk (chocolate milk)	6.70	Constituents of milk, sugar, milk fat, cocoa, food conditioners and colouring and flavouring substances.
Flavoured cultured milk (orange cultured milk)	3.62	Water, sugar, skimmed milk powder, polydextrose, fermented milk (citric acid, skimmed milk powder, water, lactobacillus), lactic acid, soy bean fibre, stabilizer, flavouring, sodium citrate, steviol glycosides, colouring, tartrazine and dimethylpolysiloxane
Fruit-based lassi milk (tropical and mixed fruits)	4.15	Sugar, Fruit Juices Mix [(Apple, Pineapple, Orange, Banana, Lemon, Apricot, Mango, Guava, Grape, Passion Fruit, 5%), Colourings, Flavourings, Citric Acid, Potassium, Sorbate], Milk Solids (Cow Milk), Stabiliser, Dextrin, Citric Acid, Flavourings, Mixed Live Culture (Lactobacillus acidophilus, Bifidobacterium lactis, Streptococcus thermophiles). Contains Stabiliser as permitted food conditioner. Contains permitted flavourings and colourings. All additives are of plant or synthetic origin.

Table 1. pH of each milk products

*Detail content was taken from the label of the milk products

	Products	Day	Mean	Std. Error	95% Confidence Interval	
Tooth					Lower	Upper
					Bound	Bound
Primary	control - distilled water	0	194.813	10.334	171.789	217.838
		5	194.880	9.886	172.854	216.906
		10	194.910	7.986	177.116	212.704
	Plain milk	0	213.510	10.334	190.485	236.535
		5	224.280	9.886	202.254	246.306
		10	266.870	7.986	249.076	284.664
	Flavoured milk	0	251.347	10.334	228.322	274.371
		5	263.600	9.886	241.574	285.626
		10	279.610	7.986	261.816	297.404
	Flavoured cultured milk	0	176.510	10.334	153.485	199.535
		5	156.413	9.886	134.387	178.440
		10	151.607	7.986	133.813	169.400
	Fruit-based lassi milk	0	247.363	10.334	224.339	270.388
		5	229.460	9.886	207.434	251.486
		10	217.293	7.986	199.500	235.087
Permanent	control - distilled water	0	367.993	9.589	346.628	389.359
		5	368.220	8.851	348.499	387.941
		10	370.757	7.662	353.684	387.829
	Plain milk	0	369.223	9.589	347.858	390.589
		5	381.067	8.851	361.346	400.787
		10	394.687	7.662	377.614	411.759
	Flavoured milk	0	344.203	9.589	322.838	365.569
		5	356.470	8.851	336.749	376.191
		10	380.193	7.662	363.121	397.266
	Flavoured cultured	0	352.090	9.589	330.724	373.456
	milk	5	288.823	8.851	269.103	308.544
		10	271.617	7.662	254.544	288.689
	Fruit-based lassi milk	0	359.013	9.589	337.648	380.379
		5	342.947	8.851	323.226	362.667
		10	325.513	7.662	308.441	342.586

Table 2: Mean microhardness of the teeth subjected to the tested milk products with time

Baseline	5 th day	10 th day	
G1 vs G2 (NS)	G1 vs G2 (S)	G1 vs G2 (S)	
G1 vs G3 (S)	G1 vs G3 (S)	G1 vs G3 (S)	
G1 vs G4 (NS)	G1 vs G4 (S)	G1 vs G4 (S)	
G1 vs G5 (S)	G1 vs G5 (S)	G1 vs G5 (S)	
G2 vs G3 (S)	G2 vs G3 (S)	G2 vs G3 (NS)	
G2 vs G4 (S)	G2 vs G4 (S)	G2 vs G4 (S)	
G2 vs G5 (S)	G2 vs G5 (S)	G2 vs G5 (S)	
G3 vs G4 (S)	G3 vs G4 (S)	G3 vs G4 (S)	
G3 vs G5 (NS)	G3 vs G5 (S)	G3 vs G5 (S)	
G4 vs G5 (S)	G4 vs G5 (S)	G4 vs G5 (S)	

Table 3. Intergroup comparison of surface hardness between 5 groups, at baseline, 5th day,and 10th days for primary teeth.

G1: Distilled water (control); G2: plain milk; G3: flavoured milk; G4: flavoured cultured milk; G5: fruit-based lassi milk; S = Significant; NS = Non-significant.

Table 4. Intergroup comparison of surface hardness between 5 groups, at baseline, 5 th day,
and 10 th days for permanent teeth.

Baseline	5 th day	10 th day	
G1 vs G2 (NS)	G1 vs G2 (S)	G1 vs G2 (S)	
G1 vs G3 (NS)	G1 vs G3 (NS)	G1 vs G3 NS)	
G1 vs G4 (NS)	G1 vs G4 (S)	G1 vs G4 (S)	
G1 vs G5 (NS)	G1 vs G5 (S)	G1 vs G5 (S)	
G2 vs G3 (S)	G2 vs G3 (S)	G2 vs G3 (NS)	
G2 vs G4 (NS)	G2 vs G4 (S)	G2 vs G4 (S)	
G2 vs G5 (NS)	G2 vs G5 (S)	G2 vs G5 (S)	
G3 vs G4 (NS)	G3 vs G4 (S)	G3 vs G4 (S)	
G3 vs G5 (NS)	G3 vs G5 (ŇŚ)	G3 vs G5 (S)	
G4 vs G5 (NS)	G4 vs G5 (S)	G4 vs G5 (S)	

G1: Distilled water (control); G2: plain milk; G3: flavoured milk; G4: flavoured cultured milk; G5: fruit-based lassi milk; S = Significant; NS = Non-significant.

Taath	Dev	Maan	Ctd Francis	95% Confidence Interval		
Tooth	Day	Mean	Std. Error	Lower Bound	Upper Bound	
Primary	0	216.709	6.784	202.813	230.605	
	5	213.727	9.574	194.114	233.339	
	10	222.058	12.462	196.532	247.584	
	0	355.171	6.784	341.275	369.067	
Permanent	5	344.313	9.574	324.701	363.926	
	10	344.617	12.462	319.090	370.143	

Table 5. Comparison between primary and permanent tooth and time

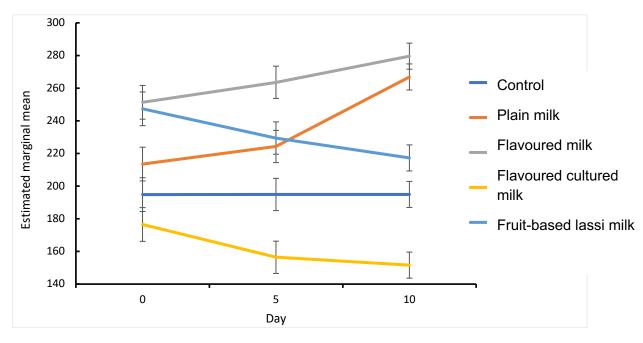


Figure 2: Milk products versus time for primary teeth

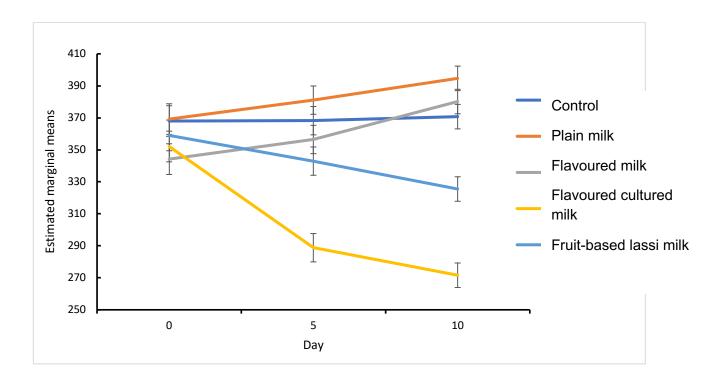


Figure 3: Milk products versus time for permanent teeth

DISCUSSION

Excessive consumption and continous exposure of acid beverages on the tooth surface leads to partial demineralization of the tooth. Demineralisation become even worse if the patient is suffering with low salivary flow rate. In the current study, significant reduction of tooth surface hardness occurred in flavoured cultured milk and in fruit-based lassi milk as shown in Table 2. This is due to low pH in both products which is pH 3.62 and pH 4.15 for flavoured cultured milk and fruit-based lassi milk, respectively. This indicated that milk product with low pH has disrupted mineralization process and lead to enamel erosion. Previous study stated that, the frequency of exposure of drinks with low pH is directly related to the progression of the dental erosion (Marcell *et al*, 2014). When tooth enamel exposed to acids whether it is intrinsic or extrinsic, it could be subject to erosion, in this case it can be as the drinks used in the current study.

In the current study there is an increase enamel surface microhardness after continous immersion in plain milk and flavoured milk (Table 2). Thus, the finding suggest that high mineral content in plain milk such as calcium and phosphate as reported by previous study (Muehlhoff *et al*, 2013) could be responsible for the remineralization process and tooth integrity. Previous study stated that consuming milk immediately after consumption of potentially erosive drinks could decrease the progression of dental erosion (Haghgou *et al*, 2016). Increased concentration of calcium and phosphate prevent the dissolution of the hydroxyapatite crystal, hence inhibits the erosion process (Haghgou *et al*, 2016). Beside that, milk pH (pH 6.75) which is near to neutral also help in remineralization process (Haghgou *et al*, 2016). This is clearly shown in the current study (Graph 1 and 2) that fresh milk and chocolate flavoured milk with higher pH has increase in microhardness for both primary and permanent tooth from baseline, day 5 and day 10. However, milk products with low pH such as flavoured cultured milk and fruit-based lassi milk showed reduction in enamel microhardness from baseline to day 10 of the experiments for both primary and permanent tooth. Thus, suggest that lower pH milk products does affect the enamel hardness of the tested tooth.

Study by Tabari *et al*, (2017) stated that primary enamel is more likely to undergone erosion when compared with permanent enamel. In the present study it was found that there is significant different between primary and permanent tooth within time from baseline, day 5 and day 10. It showed that primary tooth has lower mean enamel surface microhardness compared with permanent teeth as shown in Table 5.

This could be due to the difference in the mineral content in both primary and permanent tooth. A study stated that significant reduction in microhardenss of primary compared to permanent tooth are basically due to the thickness and mineralization of the tooth (Tabari *et al*, 2017). Previous study stated that the amount of calcium and phosphorus are higher in permanent teeth when compared with primary teeth (Oliveira *et al*, 2010). They also reported that the primary tooth enamel are thinner, softer and more prone to fracture when compared to permanent teeth.

CONCLUSION

In conclusion, we found that flavoured cultured milk and fruit-based lassi milk may reduce the tooth enamel surface hardness that may cause tooth erosion, while plain milk and flavoured milk increase the enamel hardness. This finding eventually give some information and educate patient to choose the type of milk that could beneficial to their teeth. Besides that it also may give an awareness to patient that eventhough some commercialised milk products can have good health effects, they should note that some acidity of the milk products could also negatively effect their teeth.

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