

Effects of Milk Products on Pepsi Induced Enamel Erosion on Primary Teeth: (An *In Vitro* Study)

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ABSTRACT

Background: Milk contains several nutritious substances. As a result, it will probably reduce erosive effects and give the dental enamel the highest protective benefits following exposure to an acid beverage.

Aims: To assess the effect of milk and milk products on the eroded enamel surface of primary teeth in the present absence sent of fluoride.

Methods: Eight sets of ten newly removed, extremely mobile primary teeth were created, and each group experienced pH cycling for seven days. The teeth were then soaked for three hours at a temperature of 37C in a demineralization solution (Pepsi). Next, they were washed in deionized water. The treatment of each sample is as follows: Group (1): pH cycling + fresh milk (control), Group (2): pH cycling + fresh milk + dentifrice, Group (3): pH cycling + packet milk, Group (4): pH cycling + packet milk+ dentifrice, Group (5): pH cycling + yogurt, Group (6): pH cycling + yogurt+ dentifrice, Group (7): pH cycling + cream, Group (8): pH cycling + cream + dentifrice Each sample's enamel microhardness was assessed at three different times: baseline, after erosive exposure, and following the variable treatment.

Results: Groups 2): fresh milk + dentifrice, Group 4): package milk + dentifrice tooth, Groups 8): cream + dentifrice, & Groups 6): yogurt + dentifrice, respectively, had the most notable effects on the microhardness-hardness of enameled in the primary dentition. Surface microhardness was significantly increased in treated groups with dentifrice compared without treated groups with dentifrice and control group.

Conclusions: The results demonstrated that milk and milk products have a positive effect on teeth structure, these effects due to present of minerals especially the calcium and phosphate ions with the casein fraction and derived from casein (phosphopeptides), also the proteose-peptone fraction. Moreover, milk has synergetic effect with fluoride in protection of teeth agonist erosion.

Keywords: Erosion, Milk, Cream, Yogurt, Fluoridated dentifrice

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INTRODUCTION

Dental enamel is chemically destroyed by acids without the help of microorganisms, which is known as dental erosive wear. Tooth enamel's calcium ions can be replaced by hydrogen ions from acidic solutions, which can lead to the enamel's crystal structure being broken and the start of dental erosion. Erosion can cause teeth to lose their firmness and function as well as severely harm their aesthetics [1].

One of the most widely consumed acidic beverages is Pepsi. Its erosive results are attributed to the phosphoric, citric, and/or citrate acids found in many soft drinks, which can chelate calcium, lessen saliva's ability to act as a buffer, and therefore speed up tooth deterioration. [2] Additionally, eliminating the gas in Pepsi drinks may increase their pH and lessen their capacity for the dissolution of hydroxyapatite [3]. Demineralization is begun by softening the enamel surface, subsequently dissolving enamel crystals incrementally, and leading to the disintegration of tooth structure [4].

As erosion is influenced by various biological and behavioral factors, it is mostly brought on by immediate communication with exogenous or internal acids at low pH. Due to the salivary pellicle's erosion-protective properties and its capacity to re-harden eroded tooth hard tissue via mineral disposal, saliva plays a significant part in the erosion process. Therefore, it is possible that increased calcium and phosphate concentrations, such as those found in milk or its products, facilitate the rehardening of the eroded lesions and thereby have the potential to reduce the loss of dental hard tissue [5].

Milk will probably lessen the erosive effects. After drinking an acidic beverage, teeth enamel was thus most effectively protected by milk and milk product [6]. Milk as well as yogurt is great sources of protein, delivering both organic nitrogen and important amino acids. They also include casein, lipids, calcium, and phosphate, which are all thought to have demineralizing effects. In addition to possessing some degree of antibacterial activity, these substances regulate the pH of plaque and saliva, causing early demineralized lesions to demineralizers [7].

One of the major nutritional sources of nutrients for humans is milk. It contains a variety of nutritive components, including fat, lipids (such as essential and non-essential fatty acids), lactose, proteins (such as casein and whey protein), energy, vitamins, and minerals (such as calcium and phosphorus that are good for the teeth [8]. 100 ml of cow milk has about 69 calcium mg in it [9]. It has a great chance of promoting the remineralization of dental enamel [10]. Through the process of remineralizing dental enamel due to its calcium, phosphate, and casein content [11]. Milk's casein protein has the capacity to generate significant amounts of phosphorus and calcium in the plaque's structure, which serves to prevent caries. It can stop demineralization and start remineralization by doing this [12]. Additionally, milk has remineralizing and anticariogenic effects on teeth, which enable it to protect the tooth surface from erosion [13].

One of the dairy products with the greatest distribution is yogurt. It is a coagulated milk product made when milk is fermented by the bacteria Lactobacillus bulgaricus and Streptococcus thermophilous, which results in the production of lactic acid [14]. Yogurt is also an excellent source of calcium and phosphorous and has a higher protein content [15]. Due to the proteolytic activity of microorganisms (LAB activity) contained in the yogurt the natural CPPs content in yogurt is higher than that in milk [16]. Additionally, the fermentation has no impact on the milk's mineral concentration; therefore the yogurt's overall mineral level is unaffected [14].

Yogurt is a strong source of protein, contains significant amounts of calcium, zinc, B - complex, and probiotics, and can be fortified with Vitamin D and extra probiotics that have been linked to improved health. However, it has also been discovered that yogurt extract can help tooth enamel remineralize and even guard against demineralization [17]. Yogurt also contains probiotics, live organisms that have a protective impact against harmful bacteria [18, 19].

One of the main goals of dentistry has been to prevent dental caries and tooth erosion, which has been the

predominant cause of tooth loss in all populations worldwide [20]. In addition to employing fluoride to enhance tooth minerals While fluoride-based remineralization agencies are the core component of remineralizing lesion techniques, there are a number of new remineralization techniques that are available on the market that concentrate or improve deeper remineralization, reduce the potential risks associated with high-fluoride product lines, and influence demineralization over a lifetime [21].

In order to generate ecological variations in biofilm composition, additional elements that reinforce the tooth mineral as significant changes in environmental parameters have been proposed, including diet, dental hygiene practices, and the application of antimicrobials [22]. A variety of food and food components are now recognized as acting possibly as remineralized agents as a result of epidemiological research conducted over the past 10 years that clearly showed a link between diet and health [23, 24]. The aim of the present study is to test *in vitro* the effect of milk and milk products on eroded enamel surface of primary teeth in present and absent of fluoride.

MATERIALS AND METHODS

Eighty freshly extracted highly mobile, free of dental caries, human primary incisors teeth were collected from children between (7-9) years old. Any soft tissue that was still present was manually scaled. Extracted teeth were properly cleaned with tap water and kept deionized with (0.1%) thymol. Coronal teeth are encased in self-curing acrylic resin-filled plastic tubes. It was exposed to various treatment groups on the labial surface. The teeth were divided into 8 groups of ten samples each at random. pH cycling took place in each group for seven days. The teeth were subjected to three hours of demineralization solution (Pepsi) immersion at a temperature of 37C as part of the pH cycling. They were then cleaned with deionized water. The treatment of each sample is as follows: Group (1): pH cycling + fresh milk (control), Group (2): pH cycling +fresh milk + dentifrice, Group (3): pH cycling + packet milk, Group (4): pH cycling + packet milk+ dentifrice, Group (5): pH cycling + yoghurt, Group (6): pH cycling + yoghurt + dentifrice, Group (7): pH cycling +cream and Group (8): pH cycling + cream + dentifrice.

Each sample's enamel microhardness was gauged at baseline, following erosive exposure, and following therapy.

RESULTS

According to the result of current study the pH of the fresh milk (6.9), packet milk (6.7) and cream (6.5). While yogurt's pH (4.3) is below the necessary enamel pH (5.5). As a result of the creation of an eroded lesion, the microhardness of primary tooth enamel significantly differed before and after exposure to a variety of

treatments, according to the study's findings (paired t-test, P 0.001).

According to the findings of the baseline reading, there was no statistically significant difference between both the groups' microhardness levels, which ranged from (264–270) for every group. Additionally, there was no discernible statistical difference in the means of demineralization across the groups.

In terms of remineralization, there were statistical differences between the groups; the fresh milk group had a higher VHN mean value, followed by the packed milk group, the cream group, and the yogurt group, while the packed milk group had the lowest value. The groups with the largest effects on the microhardness- hardness of enamel in primary dentition were Group 2 (fresh milk

+ dentifrice), Group 4 (packet milk + dentifrice teeth), Group 8 (cream + dentifrice), and Group 6 (yogurt + dentifrice, respectively. Surface microhardness was significantly increased in treated groups with fluoridated dentifrice compared without treated groups with fluoridated dentifrice and control group, Table 1-Table 4.

DISCUSSION

In America, Africa, Asia, Arabic nations, and Europe, dental loss has been documented frequently. Dental erosion has been linked to risk variables such as an acidic diet, a low socioeconomic position, and poor oral hygiene [25].

In a real-world setting, a preventive measure like

Groups	Micro hardness	Mean ± SD	paired t-test	
Control (pH cycling+ fresh milk) —	before	before 232.50 ± 3.536		
	after	319.00 ± 1.414	r < 0.001	
	before	232.50 ± 3.536	— P < 0.001	
ph cycling+ fresh milk +dentiffice	after	337.50 ± 3.536		
nti oveling i nacket milk	before	225.00 ± 4.243	D < 0.001	
рн суспид+ раскет тик	after	313.50 ± 2.121	P < 0.001	
all avaliant analysis mills , doubtining	before	225.00 ± 4.243	D < 0.001	
ph cycling+ packet milk + dentimice	after	329.50.707	P < 0.001	
nH oveling Lyoghurt milk	before	227.50 ± 3.536		
ph cycling+ yognurt milk	after	259.00 ± 1.414	P < 0.001	
nH cycling , yoghurt milk , dontifrico	before	227.50 ± 3.536	D < 0.001	
pH cycling+ yognurt milk + dentifice	after	287.50 ± 3.536	P < 0.001	
pH cycling+ cream	before	225.00 ± 7.071	P < 0.001	
	after	311.00 ± 1.414		
nH oveling , croom , dontifrico	before	before 225.00 ± 7.071		
ph cycling+ cream + dentifice —	after	322.50 ± 3.536	P < 0.001	

Table 2: Mean+ SD (paired t-test) for surface microhardness test for all tested groups after the variable treatment after induction of demineralization.

Groups	Mean <u>+</u> SD	paired t-test
Control (pH cycling+ fresh milk)	319.00 ± 1.054	P < 0.001
pH cycling+ fresh milk +dentifrice	337.50 ± 2.635	P < 0.001
pH cycling+ packet milk	313.50 ± 1.581	P < 0.001
pH cycling+ packet milk + dentifrice	329.50 ± 0.527	P < 0.001
pH cycling+ yoghurt milk	259.00 ± 1.054	P < 0.001
pH cycling+ yoghurt milk + dentifrice	287.50 ± 2.635	P < 0.001
pH cycling+ cream	311.00 ± 1.054	P < 0.001
pH cycling+ cream + dentifrice	322.50 ± 2.635	P < 0.001

Table 3: ANOVA test for the comparison mean values of surface microhardness of baseline, demineralization and in each group.

			ANOVA			
Surface micro	Surface microhardness		df	Mean Square	F	Sig.
	Between Groups	21	7	3	0.706	0.67
baseline	Within Groups	34	8	4.25		
	Total	55	15			
	Between Groups	150	7	21.429	0.922	0.536
demineralization	Within Groups	186	8	23.25		
	Total	336	15			
	Between Groups	8988.438	7	1284.063	211.804	.000*
remineralization	Within Groups	48.5	8	6.063		
	Total	9036.938	15			

Dono	ndent Variable	dent Variable		Std. Frror		Sig	95% Confidence Interval	
Depe	indent variable		(I-J)	Sta. I		Sig.	Lower Bound	Upper Bound
			2	-18.500^{*}	2.462	0	-24.18	-12.82
			3	5.5	2.462	0.056	-0.18	11.18
		4	-10.500*	2.462	0.003	-16.18	-4.82	
	1	5	60.000*	2.462	0	54.32	65.68	
		6	31.500*	2.462	0	25.82	37.18	
			7	8.000 [*]	2.462	0.012	2.32	13.68
			8	-3.5	2.462	0.193	-9.18	2.18
			1	18.500 [*]	2.462	0	12.82	24.18
			3	24.000 [*]	2.462	0	18.32	29.68
			4	8.000*	2.462	0.012	2.32	13.68
		2	5	78.500*	2.462	0	72.82	84.18
			6	50.000*	2.462	0	44.32	55.68
			7	26.500 [*]	2.462	0	20.82	32.18
			8	15.000 [*]	2.462	0	9.32	20.68
			1	-5.5	2.462	0.056	-11.18	0.18
			2	-24.000*	2.462	0	-29.68	-18.32
			4	-16.000*	2.462	0	-21.68	-10.32
		3	5	54.500*	2.462	0	48.82	60.18
		-	6	26.000*	2.462	0	20.32	31.68
				2.5	2.462	0.34	-3.18	8.18
			8	-9.000*	2.462	0.006	-14.68	-3.32
			1	10 500*	2 462	0.003	4 82	16.18
			2	-8 000*	2 462	0.012	-13.68	-2 32
			3	16.000*	2.462	0.012	10.32	21.68
		4	5	70.500*	2.462	0	64.82	76.18
			6	42 000*	2.462	0	36.32	47.68
			7	18 500*	2.462	0	12.82	24.18
				7.000*	2.402	0.022	1 32	12.68
Pominoralization	LSD	5	1	F0.000*	2.402	0.022	65.69	E4 22
Remmeralization				-60.000	2.402	0	-03.08	-54.52
			2	-78.500	2.402	0	-64.16	-72.82
				-34.300	2.402	0	-00.18	-48.82
			4	-70.500	2.402	0	-70.18	-04.82
				-28.500	2.402	0	-54.10	-22.02
			/	-52.000	2.402	0	-57.08	-40.52
			8	-63.500	2.462	0	-69.18	-57.82
			2	-31.500	2.462	0	-37.18	-25.82
			2	-50.000	2.462	0	-55.68	-44.32
		6	3	-26.000	2.462	0	-31.68	-20.32
	6	4	-42.000	2.462	0	-47.68	-36.32	
		5	28.500	2.462	0	22.82	34.18	
		/	-23.500	2.462	0	-29.18	-17.82	
		8	-35.000*	2.462	0	-40.68	-29.32	
			1	-8.000	2.462	0.012	-13.68	-2.32
			2	-26.500*	2.462	0	-32.18	-20.82
		3	-2.5	2.462	0.34	-8.18	3.18	
	7	4	-18.500*	2.462	0	-24.18	-12.82	
		5	52.000 [*]	2.462	0	46.32	57.68	
		6	23.500*	2.462	0	17.82	29.18	
		8	-11.500*	2.462	0.002	-17.18	-5.82	
			1	3.5	2.462	0.193	-2.18	9.18
			2	-15.000*	2.462	0	-20.68	-9.32
			3	9.000*	2.462	0.006	3.32	14.68
		8	4	-7.000*	2.462	0.022	-12.68	-1.32
			5	63.500*	2.462	0	57.82	69.18
			6	35.000*	2.462	0	29.32	40.68
			7	11.500*	2.462	0.002	5.82	17.18
	Dunnett t	2	1	18.500°	2.462	0	10.4	26.6

Table 4: Multiple Comparisons.

	3	1	-5.5	2.462	0.221	-13.6	2.6
	4	1	10.500*	2.462	0.013	2.4	18.6
(2 -:	5	1	-60.000*	2.462	0	-68.1	-51.9
(2-sided) ^a	6	1	-31.500 [*]	2.462	0	-39.6	-23.4
	7	1	-8	2.462	0.053	-16.1	0.1
	8	1	3.5	2.462	0.597	-4.6	11.6

*. The mean difference is significant at the 0.05 level. a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

lowering the erosional capability of an acidic drink alone appears to be less reliant on patient adherence or behavior. The goal of this study was to alter a popular acidic beverage's erosive potential while also supplying calcium and fluoride in accordance with the required dietary requirement. According to Vieira, et al. [6] researches, extensive dental treatments will be necessary because of the general increase in the frequency of dental erosion. It is crucial to emphasize and instruct people on how to reduce these risks in order to reduce the number of therapy patients will require in the future because of a healthy diet.

In a recent study, we explored the associations between whole fresh milk, packet milk, cream and yogurt dietary intake with and without the use of fluoridated dentifrice and the risk of demineralization among a teeth samples of Muslin children (7-9 years old). The microhardness of tooth enamel prior to and after exposure to varied treatments after the production of demineralization was significantly different, according to the current study (paired t-test, P 0.001).

It is common knowledge that drinking acidic beverages frequently can dissolve enamel since their pH is below a crucial level. These soft drinks' acidic ingredients, such as citric, phosphoric, and carbonic acid, can cause a pH as low as 2.6, which has the ability to erode surfaces [26]. Although the immediate effects of these drinks on dental enamel are modest, the extended interaction of enamel surface and soft drinks raises the likelihood of enamel demineralization [27].

The findings of the current study concur with those of Shroffa, et al. (2018) [28] who came to the conclusion that tooth erosion from carbonated soft drinks was considerable. In India, the majority of the popular drinks drank by kids and young adults had erosive potential. We advise drinking milk or its products after each and every meal that contains acidic drinks since they have the greatest protective effects on dental enamel when they are exposed to acidic beverages. Since milk and dairy products contain calcium, phosphate, and various protein structures, they can also be seen from a wider dental viewpoint as having additional health advantages. Functional foods have a variety of effects, including a variety of antibacterial activities, a decrease in the acidogenicity of dental biofilms, an impact on adhesive qualities, and improved remineralization [29].

Dairy products' potential to lessen enamel demineralization has been linked to a number of different pathways. 3 mechanisms were suggested by Levine in 2001 [30]. First, milk proteins might adhere to the enamel surface and prevent enamel demineralization; second, milk fat might adhere to the enamel surface and play a protective role; and third, milk enzymes might play a role in slowing the growth of bacteria that cause acidogenic plaque.

In a previous article, Herod, 1991 [31] also said milk and cheese could lessen the overall effect of metabolic acids and could aid in restoring enamel that is damaged during eating. According to the findings of this study, protective mechanisms may include buffering, salivary stimulation, a decrease in bacterial adhesion, a decrease in enamel demineralization, and/or the encouragement of calcification by casein and ionic Ca and P. In particular, calcium and phosphorus are released from milk and milk products, and as their concentrations in tooth plaque rise, a common-ion action prevents demineralization and encourages remineralization [12].

Yogurt and milk, two dairy products that are great sources of protein and organic nitrogen, are dairy products. They also include casein, lipids, calcium, and phosphate, which are all thought to have remineralization effects [20]. A high source of protein, yogurt is therefore rich in calcium, zinc, Vitamin b, and probiotics. It can also be fortified with Vitamin D and extra probiotics that have been linked to improved health [32].

According to the current study, the fresh milk group had a higher VHN mean value, which was trailed by the packed milk grouping, then the cream group and yogurt group had the lowest value. Additionally, the following groups had the greatest effects on the microhardness- hardness of the enamel in primary dentition: Group 2): fresh milk Plus dentifrice; Group 4): package milk + dentifrice; Unit 8): cream + dentifrice; and Group 6): yogurt + dentifrice. When drank right after consuming acids, cow milk can help prevent tooth erosion. Owing to casein, calcium, & phosphate, it has been demonstrated to improve dental enamel remineralization [33].

Cow milk's calcium and phosphorus are crucial during remineralization to promote enamel microhardness. Additionally, milk is a biological emulsion made primarily of casein, a protein made up of 1,000 protein molecules coupled to calcium and phosphate [34]. Milk is an excellent substitute because it can stop tooth erosion after acid exposure and because it can be consumed right away. In addition to increasing the amount of organic material and mineral deposits on the surface of the enamel, milk also serves as a self - purification agent that forms a protective coating. This layer is connected to casein adsorption on the top of the enamel to decrease its dissolved hydroxyapatite crystal and prevent ions discharge [11]. Beverages with a high calcium content exhibit increased remineralization and decreased erosion development. The introduction of calcium may change the fundamental characteristics of beverages, such as flavor and color, in addition to modifying the potency of erosion. Therefore, it is recommended to consume milk or other liquid that has a natural source of calcium [33].

The best potential to increase enamel hardness is seen in cow milk. It has the potential to be the main recommendation to the general population in order to prevent teeth surface damage by acidic drinks and to take precautions [9].

Because milk and yogurt have many of the same ingredients, it is reasonable to assume that both milk and yogurt use the same protective mechanisms against dental caries. Temperature, the length of time that milk is exposed to teat, how much light it is exposed to, and storage conditions are all factors that affect how milk is processed. The types and bacteria strains used in the fermentation process have an impact on the final composition of yogurt [35].

Yogurt often contains more protein than milk because non-fat dry milk is added throughout manufacturing and concentration, which raises the protein concentration of the finished product Yogurt, is also a great provider of phosphate and calcium. The total nutrient composition of yogurt is unaffected since fermentation has no impact on the mineral composition of milk. Additionally, since yogurt has a lower pH than milk does, the majority of the calcium in it is in the form of ions [35].

It's interesting to note that yogurt has a higher concentration of CPPs than milk because yogurt microbes have proteolytic activity [16]. CPPs in yogurt could have a remineralizing impact in vitro when combined with a demineralizing agent, according to the findings of a study by Ferrazzano et al. (2008) [15]. This preventive effectiveness isn't a result of the dental enamel being strengthened; rather, it results from the suppression of demineralization. When protective physiological processes are insufficient, CPPs offer a reliable preventive measure versus early demineralization of enamel, despite the fact that they are not a therapy strategy. Butterfat from unpasteurized cow's milk is used to make the cream. A large amount of B2 (riboflavin), which promotes bodily health, is present in fresh cream and heavy cream. These minerals include calcium and vitamin A. contains phosphate, potassium, magnesium, vitamin D, and vitamin C as well [36]. For this reason, calcium, phosphorus, and casein in milk products blocked demineralization and increased tooth remineralization. Foods high in calcium and phosphate could also enhance tooth remineralization. The ability of casein derivative products, like Casein PhosphoPeptides (CPP), to stabilize calcium and phosphate in their amorphous, non-crystalline condition, commonly referred as to amorphous calcium phosphate, makes them an alternate remineralizing agent (ACP). The remineralization of enamel is increased by CPP-ACP because it promotes calcium and phosphate adherence to micro porosity [37].

According to the findings of the current study, treated groups using dentifrice had considerably higher surface microhardness than untreated groups using dentifrice and the control group. Additionally, using fluoride toothpaste doesn't seem to have much of an impact on restoring enamel minerals lost after consuming an acidic beverage. It's crucial to understand that, despite fluoride's ability to help remineralize enamel, using toothpaste on teeth that have already been damaged by an acidic meal will have the opposite impact (26). Fluoride added to milk, according to studies, can greatly prevent the demineralization of enamel [38]. When the pH is balanced and there are enough phosphate and calcium ions present in the local environment, remineralization takes place. As a result, partially dissolved apatite crystals can be rebuilt. Remineralization must be accelerated or dehydration must be slowed down to regain the natural equilibrium. Early enamel lesions may remineralize, increasing their resistance to additional acid challenges, especially when improved remineralization therapies are used [39].

CONCLUSION

In summary, it is known that milk and milk products have a positive effect on teeth structure; this effect is the due presence of minerals especially the calcium and phosphate ions with the casein fraction and derived from casein (phosphopeptides) and also protease'speptone fraction. Moreover, milk has a synergetic effect with fluoride in the protection of teeth agonist erosion.

Research limitations

The latest study had the drawback of only looking at main incisor teeth. It is suggested that in upcoming studies, the influence of these milk and milk products should be done on the extracted hidden posterior teeth. In addition, current study was accomplished *in vitro*, neglectful the oral environment and effect of normal saliva as mineral contain, pH of the saliva and buffer capacity.

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