Effect of Some Food Intake on Erosive Beverage Action on Dental Enamel and Cement

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The aim of this study was to assess the effect of some foods consumed previous to an erosive beverage on dental enamel and cement. The study was performed on 40 human teeth unaffected, extracted for orthodontic braces. The teeth were cut to obtain 80 cervical slices including enamel and dentine/cement areas. The slices were randomly divided in four groups. The 20 samples of control group (group 1) were submitted in 72 hours to nine demineralisation cycles (3 cycles/24 hours) by immersion in Pepsi-Cola® for 5 min (32 mL/sample). The samples from other three groups were immersed in cheese extract (group 2), broccoli extract (group 3) and undilluted cow milk (group 4) for 5 min (32 mL/sample) previous to Pepsi-Cola® immersion. For each group, microhardness was measured using digital device CV 400 DAT on 10 samples and surface roughness was determined on another 10 samples by profilometric method using profilometer Taylor Hobson-Surtronic 25. For each sample mean value of Vicker hardness (VH) and mean value of roughness (Ra) were calculated as a result of 5 determinations. In enamel/cement areas the mean value Ra recorded for group 1 was 3,83/3,5 times higher than mean value Ra in study group 4, 2,99/2,95 times higher than mean value Ra in study group 3 and 2,81/3,04 times higher than mean value Ra in study group 2. The mean values of VH of enamel/cement before and after immersion in the tested food varied from 389/68 VH to 235/34 VH in group 4, from 367/64 VH to 354/58 VH in group1, from 382/69 VH to 371/61 VH in group 2 and from 393/63 VH to 381/57 VH for group 3. The consumption of foods like milk, cheese and broccoli before the ingestion of erosive beverages can significantly reduce the erosion of dental enamel and cement.

Keywords: erosive potential, milk, broccoli, cheese, enamel, cement

The dental erosion is a pathological process that consists in chronical loss of dental hard tissues during non-bacterial acid action by electrolytic action or by chelation. The erosion produces the softening and removal of surface layer but also the mineral dissolution of subsurface layer [1]. In this process are implied various factors but most important is represented by the action of extrinsic food acids [2-4] and gastric intrinsic acids [6-8]. Actually, the increase of the consumption of acid foods and erosive beverages has become a major factor in the apparition of erosive loss of dental tissues [4-5].

The potential of some foods and milk to protect the teeth against dental erosion requests an adequate research [9, 10]. The positive effects of milk and cheese on enamel demineralisation produced by acid beverages were analysed in some previous studies [9, 10]. The protective role of these foods was associated with the high content of calcium ions [11, 12]. The calcium and phosphate content represent an important protective factor against erosion, influencing the concentration gradient of local environment of the tooth surface. The addition of calcium and phosphate salts in acid beverages showed promising results regarding the protective effect on hard dental tissues [13, 15]. Some researchers proved that iron can also play an inhibitory effect on erosion processes [11, 12, 16, 17]. The foods that include important iron sources for organism can have a protective role [18, 19]. The protective effect of foods and beverages ingested before the erosive attack was less investigated.

The aim of this study was to assess the effect of some foods supplied previous to the erosive beverage action on dental enamel and cement.

Experimental part

The study was performed on 40 human teeth unaffected, extracted for orthodontic reason. The teeth were cleaned and all traces of soft tissue, blood, bacterial biofilm were removed. The teeth were stored in sodium chloride, at room temperature. The teeth were cut with diamond disc (Extec Corp, Enfield, CT, USA), under water cooling, to obtain cervical slices including enamel and dentine/cement areas. 80 samples having 4mm lenght, 4mm width, 3mm height, were obtained from buccal and lingual faces of the teeth. The slices were covered on half of surface with an acid-resistant varnish applied in two layers. The other half of surface remained uncovered to be submitted to the action of research materials. The covered area represented a benchmark for the changes induced by the erosive agent on the uncovered areas. The slices were randomly divided in four groups.

In control group, 20 samples were submitted in 72 h to nine demineralisation cycles (3 cycles/24 h) by immersion in Pepsi-Cola®, (PepsiCo Inc Company, Romania) for 5 min (32 mL/sample). In the period of time between the immersion in acidic beverage, the samples were mentained in artificial saliva Fusayama- Mayer (pH 7) [20]. The other three groups of samples were immersed in cheese extract (Forgrana, Parmezan Grana Padano, Italia), broccoli extract and undilluted cow milk (Zuzu, S.C.Albalact S.A., România) for 5 min (32 mL/sample) previous to each Pepsi-Cola® immersion. The food extracts were prepared using 250 g of cheese or broccoli submitted to trituration and dilluted in 300 mL deionized water (Ultrapura, SC ROMPACK SRL, România). After each erosive attack the samples were stored in artificial saliva for 18 h. The protective varnish was removed using acetone from

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samples prepared to be investigated regarding microhardness.

For each group, the microhardness assessment was performed on 10 samples and surface roughness was determined on another 10 samples. The profilometric method was performed with profilometer Taylor Hobson-Surtronic 25 (Ultra Precision Technologies AMETEK Inc., USA). The parameters were established as follows: palpation lenght 1.5 mm, wave lenght 0.25 mm, profilometric accuracy 0.4 μm. The profilometric traces were recorded by the stylus movement from the reference area to the area submitted to the erosive attack. For each sample the mean value of surface roughness from 5 determination was recorded. The microhardness was determined using digital device CV 400 DAT (Namicon). The indentations were performed using a load force of 50 g. Minimal distance between two successive indentations was 40μm. For each sample five indentations were performed. For each indentation it was recorded a Vicker hardness number (VHN) that was converted in micro-hardness values (VH). For each sample the microhardness value was obtained as a mean of five measurements. The acceptance criteria of one indentation were as follows: sharp on diagonal edges, uniform diagonally aspect, lack of irregularities in the tested area.

Results and discussions

There were recorded the roughness profiles of the enamel and cement surfaces for control and study groups. Data regarding mean value of a profile deviation from the mean line (Ra) were recorded. Tables 1 present the mean values and standard deviations of Ra to the enamel and cement in control and study groups.

For dental enamel surfaces, mean Ra of samples immersed directly in Pepsi was 3.83 times higher than mean Ra of samples immersed previously to the erosive action in milk, 2.99 times higher than mean Ra of samples immersed previously to the erosive action in broccoli extract and 2.81 times higher than mean Ra of samples immersed previously to the erosive action in cheese extract (table 1).

For dental cement surfaces mean Ra of samples immersed directly in Pepsi is 3.5 times higher than mean Ra of samples immersed previously to the erosive action in milk, 2.95 times higher than mean Ra of samples immersed previously to the erosive action in broccoli extract and 3.04 times higher than mean Ra of samples immersed previously to the erosive action in cheese extract (table 1).

The mean values and standard deviations of VH for enamel and cement areas in control and study groups are presented in table 2.

The mean values of enamel microhardness varied as follows: for control group, with samples immersed only in Pepsi, from 389 VH (before erosive cycles) to 235 VH (after erosive cycles); for study group, with samples immersed in milk before acid attacks, from 367 VH (before erosive cycles) to 354 VH (after erosive cycles); for study group, with samples immersed in broccoli extract before acid attacks, from 382 VH (before erosive cycles) to 371 VH (after erosive cycles); for study group, with samples immersed in cheese extract before acide attacks, from 393 VH (before erosive cycles) to 381 VH (after erosive cycles) (table 2).

The mean values of cement microhardness varied as follows: for control group, with samples immersed only in Pepsi, from 68 VH (before erosive cycles) to 34 VH (after erosive cycles); for study group, with samples immersed in milk before acid attacks, from 64 VH (before erosive cycles) to 58 VH (after erosive cycles); for study group, with samples immersed in broccoli extract before acide attacks, from 69 VH (before erosive cycles) to 61 VH (after erosive cycles); for study group, with samples immersed in cheese extract before acide attacks, from 63 VH (before erosive cycles) to 57 VH (after erosive cycles) (table 2).

The results were submitted to statistically tests ANOVA and Tukey. For enamel surfaces, the results of test F (1037,210 for p 0.0001) show that roughness values varies significantly between study groups. The test Tukey highlighted the existence of significantly differences when compared the roughness mean values between study groups and roughness mean values between study groups and control group (with an exception, the study groups including samples immersed in cheese extract and broccoli extract before erosive attacks) (table 3).

For cement surfaces, the results of test F (1619,402 for p 0.0001) show that roughness values varies significantly between study groups. The test Tukey highlighted the existence of significantly differences when compared the roughness mean values between study groups and roughness mean values between study groups and control group (with an exception, the study groups including samples immersed in cheese extract and broccoli extract before erosive attacks) (table 4).

The study aimed to assess the potential of some foods to influence the erosive action of an acid beverage on the dental enamel and cement. The study used three periods of acid aggression to simulate the consumption of foods and beverages accordingly to daily meals (breakfast, lunch, dinner). The artificial saliva was used to store the samples between erosive attacks to simulate the real conditions in oral cavity. The saliva is responsible for the formation of acquired pellicle, a physical barrier with protective role against acid attacks. This barrier avoids the direct contact between the acids and tooth surface, reducing the hydroxyapatite dissolution. The protection of tooth surface by the acquired pellicle is sustained by the literature data [21, 22]. This *in vitro* study that did not used the acquired pellicle, proved that its absence can increase the effects of erosive attacks on the dental enamel and cement.

	Milk	Broccoli	Cheese	Control
Mean Ra for enamel surfaces (μm)±SD	0.3902±0.079	0.4998±0.007	0.5317±0.055	1.4988±0.027
Mean Ra for enamel surfaces (μm)±SD	0.5304±0.033	0.6308±0.019	0.6116±0.010	1.8615±0.091

Table 1
MEAN Ra AND STANDARD DEVIATIONS (SD) FOR ENAMEL AND CEMENT SURFACES IN CONTROL AND STUDY GROUPS

	Milk	Broccoli	Cheese	Control
Mean values of VH for enamel areas±SD	360.52±0.049	376.54±0.012	387.03±0.052	289.02±0.047
Mean values of VH for enamel areas ±SD	61.58±0.052	65.4±0.032	60.92±0.037	38.92±0.031

Table 2
THE MEAN VALUES AND STANDARD DEVIATIONS OF VH FOR ENAMEL AND CEMENT AREAS IN CONTROL AND STUDY GROUPS

Multiple Comparisons

Roughness Crown (Enamel)

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
milk	Broccoli	-0.109600*	0.022659	**0.000**	-0.17063	-0.04857
	Cheese	-0.141500*	0.022659	**0.000**	-0.20253	-0.08047
	control	-1.108600*	0.022659	**0.000**	-1.16963	-1.04757
broccoli	milk	0.109600*	0.022659	**0.000**	0.04857	0.17063
	cheese	**-0.031900**	0.022659	**0.503**	-0.09293	0.02913
	control	-0.999000*	0.022659	**0.000**	-1.06003	0-.93797
cheese	milk	0.141500*	0.022659	**0.000**	0.08047	0.20253
	broccoli	**0.031900**	0.022659	**0.503**	-0.02913	0.09293
	control	-0.967100*	0.022659	**0.000**	-1.02813	-0.90607
control	Milk	1.108600*	0.022659	**0.000**	1.04757	1.16963
	broccoli	0.999000*	0.022659	**0.000**	0.93797	1.06003
	cheese	0.967100*	0.022659	**0.000**	0.90607	1.02813
milk	Broccoli	-0.109600*	0.022659	**0.000**	-0.17063	-0.04857
	Cheese	-0.141500*	0.022659	**0.000**	-0.20253	-0.08047
	control	-1.108600*	0.022659	**0.000**	-1.16963	-1.04757
broccoli	milk	0.109600*	0.022659	**0.000**	0.04857	0.17063
	cheese	**-0.031900**	0.022659	**0.503**	-0.09293	0.02913
	control	-0.999000*	0.022659	**0.000**	-1.06003	0-.93797
cheese	milk	0.141500*	0.022659	**0.000**	0.08047	0.20253
	broccoli	**0.031900**	0.022659	**0.503**	-0.02913	0.09293
	control	-0.967100*	0.022659	**0.000**	-1.02813	-0.90607
control	Milk	1.108600*	0.022659	**0.000**	1.04757	1.16963
	broccoli	0.999000*	0.022659	**0.000**	0.93797	1.06003
	cheese	0.967100*	0.022659	**0.000**	0.90607	1.02813

*. The mean difference is significant at the 0.05 level

Table 3

THE RESULTS OF TUKEY TEST FOR COMPARISONS OF ROUGHNESS ENAMEL SURFACES IN CONTROL AND STUDY GROUPS

Multiple Comparisons

Roughness Root (Cement)

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
milk	broccoli	-0.100400*	0.022376	**0.000**	-0.16066	-0.04014
	cheese	-0.081200*	0.022376	**0.005**	-0.14146	-0.02094
	control	-1.331100*	0.022376	**0.000**	-1.39136	-1.27084
broccoli	milk	0.100400*	0.022376	**0.000**	0.04014	0.16066
	cheese	0.019200	0.022376	**0.826**	-0.04106	0.07946
	control	-1.230700*	0.022376	**0.000**	-1.29096	-1.17044
cheese	Milk	0.081200*	0.022376	**0.005**	0.02094	0.14146
	broccoli	-0.019200	0.022376	**0.826**	-0.07946	0.04106
	control	-1.249900*	0.022376	**0.000**	-1.31016	-1.18964
control	Milk	1.331100*	0.022376	**0.000**	1.27084	1.39136
	broccoli	1.230700*	0.022376	**0.000**	1.17044	1.29096
	cheese	1.249900*	0.022376	**0.000**	1.18964	1.31016

*. The mean difference is significant at the 0.05 level.

Table 4

THE RESULTS OF TUKEY TEST FOR COMPARISONS OF ROUGHNESS CEMENT SURFACES IN CONTROL AND STUDY GROUPS

The hydrogen ions that contact the tooth surface play an important role in the erosive potential of beverages by the initiation of enamel prisms dissolution. In the beginning of the erosive processes the characteristic SEM aspect is „honeycomb" [23, 24]. Further new quantities of neionized acid will difuse in the interprismatic enamel and the mineral dissolution will persist in the subsurface areas [25-27]. Further the ions will migrate to surface and local pH will increase both in the liquid layer in contact with enamel and in the enamel surface [27].

The study results showed that milk, cheese and broccoli have the ability to reduce the erosive effects when supplied previous to the acid aggression similar results were obtained by a previous study [28].

The calcium ions play an essential role in the prevention and stopping of acid dissolution of dental structure. Numerous studies proved the preventive role of calcium ions and phosphoproteins from milk against erosive attacks [29], as well as the reparatory effect in the enamel remineralisation [30, 31], when milk is consumed immediately after the acid aggression. Also this study proved the protective effect of milk even when is consumed after the acid erosive aggression. The milk contains significant quantities of calcium and phosphate ions. The milk phosphate is linked by casein and serine.

The presence of calcium and phosphate ions linked to casein help to maintain casein to a stable formula [32]. A previous study proved the role of case into the reduction of hydroxyapatite dissolution [14]. This protective role was explained by the adsorption of case into the hydroxyapatite surface, with effect in stabilization of the crystals surfaces and inhibition of the ions migration [14]. Also the protective role of casein as barrier against acid diffusion increases in the presence of acquired pellicle [33]. The protective role of milk is also due to the content of proteins and fats that act like a physical barrier and limit the acid aggression [34]. In the case of cheese, there were proposed a few hypothesis regarding the inhibitory effects of hard dental tissues dissolution as well as the stimulation of remineralization processes: the ability to neutralize the acidity due to the products resulted from proteins metabolism, content of casein, calcium lactate, calcium ions, phosphate ions [35]. Similar results were obtained in a previous study that investigated the effect of cheese uptake before the action of citric acid on bovine enamel samples [36].

The protective mechanism of iron ions against acid dissolution is not completely understood. Previous studies proved the existence of protective effect of iron ions against erosive processes, when these ions were added in the acid beverages [15, 37]. It is possible that the formation of a slight acid-resistant layer of iron oxide on the enamel surface to represent a protective barrier. Also the precipitation of iron phosphate can influence the surface ions stabilization [38-45]. The formation of this surface layer can reduce the contact of acidic beverage with enamel in next acidic attacks and can avoid further enamel dissolution.

Conclusions

The consumption of foods like milk, cheese and broccoli before the ingestion of erosive beverages can significantly reduce the erosive effect on dental enamel and cement. The surface roughness and the decrease of enamel and cement hardness are smaller when the consumption of investigated foods was made before the acid aggression.

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