Original Article

Effect of Popping Chocolate and Candy on Enamel Microhardness of Primary and Permanent Teeth

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¹Dental Materials Research Center, Institute of Health, ²Assistant Professor of Pediatric Dentistry, ³Assistant Professor of Operative and Esthetic Dentistry, ⁴Cellular and Molecular Biology Research Center, Health Research Institute, ⁵Assistant Professor of Clinical Biochemistry, ⁶Student Research Committee, Babol University of Medical Sciences, Babol, Iran **Aims and Objectives:** Dental erosion is a common disease in children. Food diets, due to high amounts of juice, soft drinks, chewing gum, and acidic chocolate, are one of the most important risk factors in erosive processes among children. The aim of this study was to evaluate the effect of candy and chocolate on the microhardness of tooth enamel.

Materials and Methods: Two types of popping candy and one type of popping chocolate were used in this study. Thirty-three healthy permanent premolar teeth and 33 primary incisor teeth (A or B) were selected. Five grams of each popping chocolate or candy was dissolved with 2 ml of artificial saliva. Subsequently, their pH and titrable acidity (TA) as well as microhardness and surface roughness of enamel were examined in the laboratory. Data were analyzed and evaluated Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY through independent *t*-test, paired *t*-test, Tukey test, and ANOVA.

Results: The results of this study showed that only the pH of the candies was below the critical pH of the enamel (5.5) and their TA was B = 0.20 and C = 0.21. The most significant effect on the enamel microhardness of the permanent and primary teeth was by the following types of candy: orange flavor (C), strawberry flavor (B), and chocolate (A), respectively. This difference was significant (P < 0.001) and the surface roughness increased after exposure.

Conclusions: This study showed that popping chocolate and candy reduces microhardness of enamel.

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INTRODUCTION

A fter eruption, three types of acid dissolution may remove tooth minerals: decay, erosion, and acid etching used for retention of resin fill materials.^[1] Loss of dental structure might occur in a variety of forms, including abrasion, attrition, abfraction, demastication, fine cracks, and resorption. Dental erosion is a chemical process where the hard surface is dissolved by acidic processes, and the microorganisms do not interfere with it.^[2]

Dental erosion is a common disease in children. Early erosive damage to teeth may cause severe tooth surface loss, tooth sensitivity, over closure, and poor esthetics. Therefore, early diagnosis of the disease and appropriate preventive measures are important. Awareness of the

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etiological factors of dental erosion is a prerequisite for such actions. In children and adolescents (such as adults), external and internal factors or a combination of them is possible reason for this disease.^[3] When hard dental tissue is exposed to an unsaturated solution, both hydroxyapatite and fluorapatite are dissolved and the teeth become affected by erosion.^[1] At pH below the critical pH of the enamel (pH = 5.5), tooth minerals tend to demineralization while they tend to be remineralized at pH above the critical pH level.^[4]

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Wang *et al.* with evaluation of erosive potential of soft drinks on human enamel reported that the pH values of the soft drinks were below the critical pH value and low pH value causes more surface enamel loss.^[5]

Etiological factors of dental erosion are divided into intrinsic and extrinsic groups: intrinsic factors due to gastric acid that enters the oral cavity through the gastric reflux or vomiting and extrinsic factors due to environmental causes, diet, medications, and lifestyle.^[6] The current diet, due to high consumption of juice, acid beverages, some acidic center-filled chewing gums, and acidic candies, is the most important risk factor for erosive processes among children.^[7-9] The final erosive potential of food and drink depends on the contrast between the chemical properties (pH, total acidity, calcium and the amount of phosphate, and adhesion), biological factors (salivary flow rate, buffering capacity and composition, pellicle formation, tooth composition, and soft-tissue anatomy), and behavioral factors (eating and drinking habits, especially continuous and prolonged ones).^[3] Among these factors, pH, titration acidity, and calcium concentrations are the most important in determining the potential of erosive materials.^[10] Popping candy is producted from a mixture of sucrose, lactose, and corn starch dissolved in water, and then, the solution is melted to the point, it includes 2%-3% water, and then, carbon dioxide (CO₂) is exposed to molten sugar at high pressure (625-675 psi), followed by the mixture being cooled. When the candy is kept inside the mouth, it melts and gas scapes which creates short feelings of entertainment and amusement.[11]

Mudumba *et al.* 2014 evaluated and compared the change in the microhardness of the enamel after exposure to the acidic center-filled chewing gums in permanent and primary teeth. It was concluded that in all groups exposed to chewing gum, a clear decrease was seen in the microhardness and both types of chewing gum equally erosive.^[7] Davies *et al.* evaluated the erosive potential of a number of commercially available sour sweets *in vitro*. In this study, the erosive potential was

examined by measuring pH and neutralizable acidity as well as the ability of erosion in permanent and primary enamel (using profilometry), and then, they compared the parameters with orange juice as the control group. It was concluded that all sour candies are erosive even some are more erosive than orange juice.^[12] Since there has not been any research on popping chocolates in the oral environment and its effect on permanent and primary teeth, this study evaluates the effect of these chocolates on the microhardness of enamel.

MATERIALS AND METHODS

The present study was carried out in the Dental Material Research Center, Babol University of Medical Science, Babol, Iran, in 2016, and approved by the Ethics Committee of Babol University of Medical Science, Babol, Iran (MUBABOL. REC.1396.11). In this experimental study, two types of popping candy and one type of popping chocolate were used that are commonly available to the public. Popping chocolate and candy compounds are shown in Table 1 according to the factory report.

Before the experiment, each chocolate or candy was crushed with a pestle, and 5 g of each popping chocolate/candy was dissolved in 2 ml of artificial saliva with 40°C.^[10,13] Then, their pH and titrable acidity (TA) as well as microhardness and surface roughness of enamel were examined in the laboratory.

PH DETERMINATION

The pH of the solutions was measured at room temperature by a digital pH meter (Basic 20+, Crison Instruments, SA, Barcelona, Spain). First, the pH meter was calibrated with a measurement error of 0.01 using standard buffers at pH 9.25, pH 7.02, and pH 4. Then, 2 ml of the solution was poured into beakers and the pH meter was placed in. The pH of each sample was measured in triplicate and the mean of the data was recorded.^[14]

DETERMINATION OF TITRABLE ACIDITY

Maguire method was used to determine TA. 0.1 M sodium hydroxide (NaOH) droplets were added to the

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Table 1: Types, compounds, and the manufacturing country of the materials			
Materials	Composition	Manufacturing country	
Merdas popping chocolate	Cocoa powder, sugar, sucrose oil cocoa butter, soya lecithin (E322), dried milk, natural vanilla, salt	Iran	
Orange kick pop candy	Sugar, maltose syrup, carbon dioxide, sodium bicarbonate, artificial flavor, color (E129)	China	
Strawberry kick pop candy	Sugar, maltose syrup, carbon dioxide, sodium bicarbonate, artificial flavor, color (E129)	China	
Hypozalix artificial saliva	per 100 ml, potassium chloride 62.450 mg, sodium chloride 86.550 mg, magnesium chloride 5.875 mg, calcium chloride 16.625 mg, dipotassium phosphate 80.325 mg, monopotassium phosphate 32.600 mg, sorbitol, sodium carboxymethylcellulose, purified water, methyl parahydroxybenzoate (E218), nitrogen	France	

sample by a bort to reach a pH of 5.5 in pH meter. Each sample was titrated 3 times with this method, and the average amount of sodium hydroxide was recorded. The data obtained to calculate the TA are in the following formula:

 $TA = 0.1 \times (\alpha/2 \times 5 \text{ mL})$

 α = volume of sodium hydroxide used to reach pH 5.5/ml

- 5 mL = standard volume for TA calculation
- 0.1 = sodium hydroxide molarity

Since 2 ml of the sample is used, α is divided into 2.

Solutions with TA <0.5 are placed in the low-erosion group, TAs in the range of 0.5-2.5 are placed in the moderate erosion group, and TAs >2.5 are placed in the high-erosion group.^[14]

MICROHARDNESS TEST

We used sample size of 30 permanent teeth (premolar) and 30 primary teeth which selected based on previous study^[15] that were extracted due to orthodontics. The teeth were examined by a stereomicroscope, and the nonhypoplastic, Nonhypocalcification and nonfractioned teeth were selected. The teeth were washed to remove the blood, saliva, and other debris, and they were then cleaned with slurry of pumice and placed in the physiologic serum until the start of the experiment. Subsequently, the samples were fixed to the acrylic resin so that enamel appears. Then, to create a smooth surface, the outer enamel surface of specimens was ground with sandpaper (600 grit and then 1200 grit). Then, the microhardness of the enamel in all specimens was measured with Vickers hardness tester (MHZ, Koopa Company, Mashhad, Iran) using Vickers diamond indenter with 50 g load for 10 s. For each specimen, three different hardness tests were performed and the mean degree was reported. Subsequently, the groups were divided as follows:

- Primary tooth (D)–permanent teeth (P)
- Popping chocolate (A)–strawberry popping candy (B)–orange popping candy (C).

These were titles as DA-DB-DC-PA-PB-PC. Each group included 10 teeth.^[15]

All specimens were exposed to a solution containing 5 g of popping chocolate/candy dissolved in 2 mL of saliva twice a day for 5 min in 5 days. After each exposure, specimens were washed into distilled water for 20 s and then immersed in the artificial saliva until the next stage of the test. Artificial saliva was changed daily.^[15] Subsequently, retests were carried out with the Vickers hardness test. For each specimen, hardness was measured in triplicate and the average was reported. Before the

start of the experiment, one sample of each group was sent to atomic force microscopy (AFM) (Easyscan2 Flex) to evaluate surface roughness, and topography was prepared. After the experiment, topography was made from the same sample [Figures 2 and 3].

Results

The results of this study showed that there was a significant difference between the microhardness of the permanent and primary tooth enamel before and after the exposure to popping chocolate and candy (paired *t*-test, P < 0.001) [Table 2].

The most significant effect on the microhardness of enamel in permanent and primary teeth was orange candy (C), strawberry candy (B), and chocolate (A), respectively. This difference was significant (P < 0.05) (ANOVA) [Table 3].

The results of Tukey test showed that there is a significant difference between effects of chocolate and candy in both

 Table 2: Comparison of the microhardness before and

after effect of chocolate and candies in each group (<i>n</i> =10) (paired <i>t</i> -test)			
Groups	Hardness	Mean±SD	P (paired t-test)
PA	Before	332.50±32.79	< 0.001
	After	263.40±37.14	
PB	Before	337.90±42.873	< 0.001
	After	234.10±26.480	
PC	Before	336.60±27.26	< 0.001
	After	231.20±35.62	
DA	Before	328.20±38.27	< 0.001
	After	261.20±38.01	
DB	Before	34.90±13.39	< 0.001
	After	231.70±21.58	
DC	Before	349.50±18.46	< 0.001
	After	237.50±12.30	

SD=Standard deviation

Table 3: Comparison of effect of candy and chocolat	e on
the microhardness of the permanent and primary to	eth

enamel				
Dentition	Туре	ΔVHN (unexposed-exposed), mean±SD		
Permanent teeth	А	69.10±38.53ª,A		
	В	103.80±27.16 ^{b,D}		
	С	105.40±17.47 ^{b,E}		
Deciduous teeth	А	67.00±32.50 ^{a,A}		
	В	111.20±17.73 ^{b,D}		
	С	112.00±15.87 ^{b,E}		

The difference in lower case indicates a significant difference between chocolate and candy, The difference in capital letters indicates that there is a significant difference between the average microhardness of the permanent and primary teeth with the effect of each candy or chocolate. VHN=Vickers hardness number, SD=Standard deviation permanent teeth and primary teeth, but there is no significant difference between effects of the candies [Table 3].

The results of independent *t*-test showed that there was not a significant difference between the mean of microhardness with the effect of popping chocolate (A) (P = 0.89), strawberry popping candy (B) (0.48), and orange popping candy (C) (P = 0.38) (0.48) in permanent and primary teeth [Table 3].

Only the pH of the candies (strawberry and orange flavor) reached the critical pH of enamel (5.5), and thus, only their TA was calculated [Table 4].

The findings from AFM are as follows:

The results of AFM have shown that Ra (index showing the surface roughness) has increased in all groups. Hence, surface roughness has increased [Figure 1].

DISCUSSION

Different studies on the prevalence of dental erosion in children and adolescents have shown increased risk of teeth erosion with frequent use of candy.^[16] The aim of this study was to evaluate the effect of two types of popping candy and popping chocolate: orange Kick pop candy, strawberry Kick pop candy and Merdas popping chocolate on the microhardness of extracted human permanent and primary enamel tooth. The results showed that only the pH of the candies was below the critical pH of enamel, and in all groups, surface roughness has increased and also showed a reduction in the hardness of the enamel tooth after exposed to solutions containing candies or chocolate.

Table 4: Findings on determining the pH and titrable acidity of candy and chocolate				
Туре	Mean±SD		Erosion group	
	pН	ТА		
A	6.30±0.01	-	-	
В	5.36 ± 0.02	0.20 ± 0.0	Low	
С	5.33±0.01	0.21±0.0	Low	

TA=Titrable acidity, SD=Standard deviation

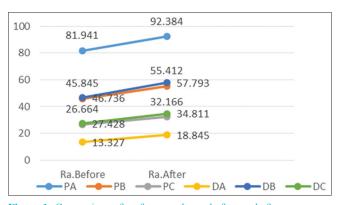


Figure 1: Comparison of surface roughness before and after exposure

Hardness is, in fact, the resistance of a substance or surface to the indentation or penetration, which is an important mechanical property for matter. The material's resistance to wear or friction or erosion with water or any substances generally increases with hardness. This means that the enamel with more mineral content has less wear than dentine.^[17] Regarding the fact that, due to the exposure of the tooth structure to acidic solutions, first, the microhardness of the tooth structure decreases, and then, the surface tissue will be lost as a part of the erosion process.^[18] Therefore, we measured the permanent and primary enamel teeth hardness by vickers after their were effected by candy and chocolate. Hardness testing with Vickers is a standard method for evaluating the hardness of materials, especially hard materials, nonhomogeneous and prone to cracking like tooth enamel.^[17]

In previous studies, hardness testing was carried out by a Vickers hardness test device for each sample applying 50 g load for 10 s. In this study, 50 g load was applied for 10 s to determine the hardness of the teeth before and after the exposure. The results of this study showed that there was a significant difference between the two groups in the pre- and postexposure to chocolate and candies with different flavors, i.e. the microhardness of enamel after chocolate and candy consumption decreased, which is in line with the study of Mudumba *et al.*^[7] and Bolan *et al.*^[15] and Wagoner *et al.*^[19] illustrating the effects of candy and chocolate on reducing the acidity of the saliva and consequently the erosion of the enamel.

It has been shown in some studies that permanent enamel is more resistant to erosion compared to primary enamel. ^[20,21] Hunter *et al.* showed that there was a significant difference between primary enamel and permanent enamel teeth after 15 days of orange juice consumption, but in this study, the effect of these chocolates and candies did not show significant difference in reducing the microhardness between permanent and primary teeth, which is also in line with the results of Bolan et al.^[22,15] Furthermore, Carvalho et al. studied the effects of nine different dietary substances on permanent and primary teeth enamel and surface hardness of the teeth after their immersion for 2 and 4 min; it was shown that there was no significant difference between the reduction of hardness in permanent and primary teeth after immersion.^[23]

pH and TA affect the potential of the erosive material.^[24] In this study, the pH of the orange candy (pH = 5.33) and strawberry candy (pH = 5.36) is lower than the critical pH of the enamel, which is 5.5, and their pH was also lower than the pH of the chocolate (pH = 6.30). In Lazzaris *et al.*^[10] studies, in 2015, when they examined the erosive potential of commercially available candies

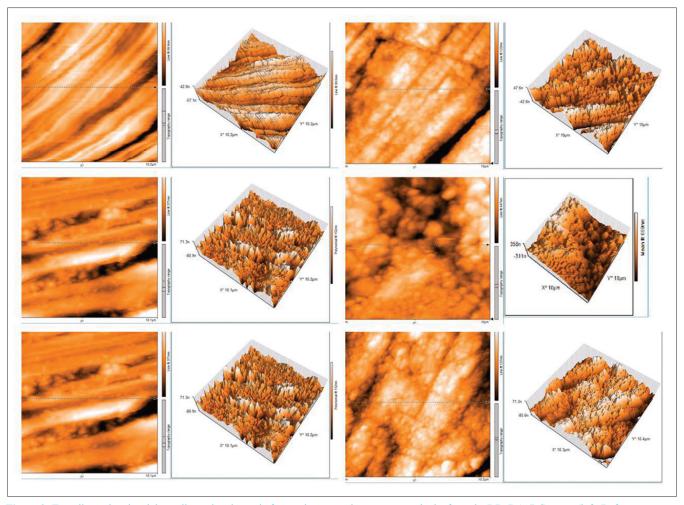


Figure 2: Two-dimensional and three-dimensional atomic force microscopy images, respectively, from the DB, DA, DC group (left: Before exposure, right: After exposure)

and their acidity, they also determined that the pH of the candies with different flavors varies, and the pH of strawberry candy, cherry candy, and orange candy was below the critical pH of the enamel. Furthermore, in the study of Leelavathi and Chaly,^[9] the pH of the lollipops with different flavors was measured, which is consistent with this study. In this study, candy and chocolate exposure to the teeth and it was shown that the reduction of microhardness was higher in orange candy, strawberry candy, and chocolate, respectively. Although the pH of the chocolate did not reach the critical pH of the enamel, it decreased its microhardness because, in addition to the pH, frequency, the time of exposure, the type, and concentration of acid affect on erosion.^[15]

In this study, as some studies^[25,26] imitate the saliva-induced oral environment, artificial saliva was used as control group and intermediate medium. In the Davari *et al.* studies, the physiological serum was used. ^[27] In Leelavathi studies, the volume of 10 mL was used to dissolve the candy, but in this study, according to the

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amount of stimulate saliva released per minute, which was $1-2 \text{ mL}^{13}$, the solution was considered as a 2 mL.

Different studies applied different regimens to immerse the samples in their investigated solutions.^[28]

The longer exposure time with acid has been reported 10–60 min and the shorter exposure time in range 1–4 min, so, given that most of the studies considered 5 min of exposure in the conditions of *in vitro*,^[7] specimens in our study, following Bolan *et al.*,^[15] were exposed to acid for 5 min.

In dental erosion, enamel crystals are dissolved layer-to-layer.^[29] Therefore, to understand the early stages of the dissolution in enamel, it is necessary to precisely measure the mineral loss. In this study, AFM was used to determine the surface changes caused by the popping chocolate and candies. AFM gives images with high contrast and high resolution of the surface.^[30] In this study, one sample was selected from each group and the Ra (index showing the surface roughness) was

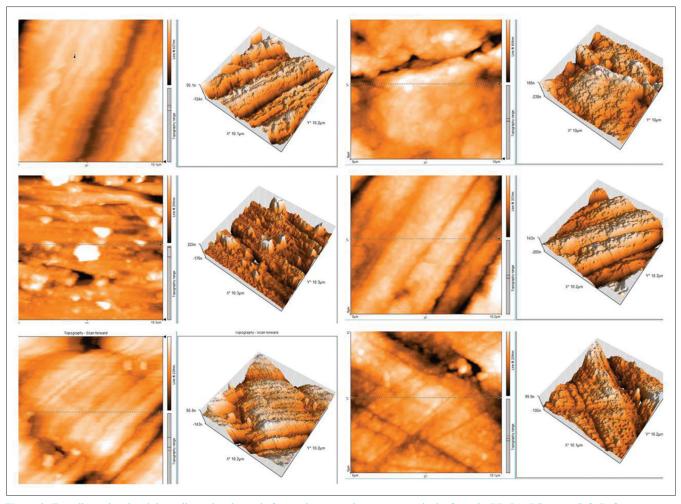


Figure 3: Two-dimensional and three-dimensional atomic force microscopy images, respectively, from the PB, PA, PC group (left: Before exposure, right: After exposure)

reported and compared with Ra after exposure, which was consistent with the study of Poggio *et al.*^[31] in which after demineralization with an acidic substance such as Coca-Cola, there was an increase in surface roughness.

One of the limitations of this study was that only the permanent premolar teeth and primary incisors had been investigated. It is suggested that in later studies, the effect of these candies and chocolate should be performed on the extracted hidden teeth.

This study was also performed *in vitro*, and it is suggested that to construct closer conditions, studies should be performed as clinical examination in oral environment by measuring the pH of the saliva.

CONCLUSIONS

This study showed that orange Kick pop candy, strawberry Kick pop candy, and Merdas popping chocolate, especially orange Kick pop candy, reduce the microhardness of enamel and consequently lead to teeth erosion. Given the access of children and adolescents to the types of chocolates and candies examined in this study, it is suggested that families apply more control over children and adolescents for their consumption. Furthermore, it is suggested that they wash their mouth with water after taking the candies and chocolates. Toothbrushing is not immediately recommended because the enamel softened by acid is easily removed using toothbrush and toothpaste.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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