Destructive effects of citric acid, lactic acid and acetic acid on primary enamel microhardness

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Abstract

Objective: This study aimed to assess the destructive effects of citric acid, lactic acid and acetic acid produced from the fermentation of foods on primary teeth enamel.
Methods: This *in vitro*, experimental study was conducted on 24 sound primary teeth. The teeth were polished with a fine abrasive paper under running water. Tooth pieces measuring 3×4×3mm were cut out of the teeth and stored in 100% humidity until the experiment. The specimens were divided into 3 groups (n=8) and immersed in acetic acid, citric acid and lactic acid, respectively. The enamel microhardness of specimens was measured by Vickers microhardness tester at baseline and 5 and 30min after immersion in the freshly prepared acid solutions.
Results: Repeated measures ANOVA showed that the effect of immersion time on microhardness was significant (p<0.001). Pairwise comparison among 0, 5 and 30 minutes time points using Bonferroni adjustment showed significant differences in microhardness at different time points (p<0.001). Evaluation of the effect of type of acid on microhardness revealed that the microhardness was not significantly different in the three groups of acids (p=0.915). Among the three understudy acids, only the reduction in microhardness from time 0 to 30 minutes was significantly different between lactic acid and acetic acid (p=0.042).
Conclusion: Citric acid, lactic acid and acetic acid were all capable of demineralization and reduction of enamel microhardness. A significant difference existed in the demineralization potential of acids (the highest for lactic acid). However, this effect was more significant early after exposure.
Key words: Acid, Enamel, Fermentation, Hardness, Primary teeth.
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Introduction:

Evidence suggests that the prevalence of tooth erosion is rapidly growing (1). Demineralization of the tooth surface due to acid exposure is characterized by the initial softening of the enamel surface, which varies based on the immersion time and the acids. The thickness of the softened layer in permanent teeth varies from 0.2 to 3 µm (2-6). Continuous layer-by-layer dissolution of enamel crystals occurs afterwards causing permanent loss of tooth structure and leaving a softened layer over the residual tissue. Extensive dentin exposure usually occurs in advanced stages. High consumption of acidic drinks and foods is an important extrinsic factor responsible for erosive tooth wear. Due to lifestyle changes in the recent years, the amount

and frequency of consumption of acidic products have greatly increased (7). The duration and the localization of the acid attack are usually determined by the manner of introduction of dietary acids into the mouth (sipping, sucking, with/without drinking straw) (8-10). Erosion is closely correlated with the frequency as well as the duration of acid attacks; consequently, these factors are important for the adoption of prophylactic measures (11-14). Nocturnal exposure of teeth to acids also leads to erosion because of the lower saliva production at night. Therefore, aside from caries, massive erosive destruction of the tooth structure may occur due to the consumption of sweet acidic drinks, which are commonly and continuously consumed by the infants from bottles during the night. The pellicle acquired is mainly composed of glycoproteins, proteins, lipids and enzymes (15) and is considered an important factor as well. It is assumed that this film protects the tooth from erosion by functioning as a diffusion barrier or a selectively permeable membrane that prevents direct contact between the tooth surface and acids and it has been demonstrated that its basal structure can survive moderate to severe acid exposures (16). Such protective effect has been demonstrated by some in-vitro studies after mild acid challenges (17-19), and to a lesser extent in more severe conditions (20, 21). However, the protection against erosive dissolution is never complete. According to an in situ study, consumption of soft drinks for 20 seconds decreased surface microhardness, although the pellicle structures survived on the tooth surface (22). Millward *et al*. monitored the tooth surface pH of healthy subjects after drinking 1% citric acid. They noted that the pH recovered to >5.5 within 2 minutes at a site adjacent to the palatal surface of the upper central incisor and within 4–5 minutes at the palatal surface of the upper first molar (23). Therefore, the anatomy of the teeth and soft tissues, the movement of the tongue and vestibular mucosa and also the pattern of swallowing can all affect the clearance rate of erosive agents.

Considering the growing prevalence of consumption of acidic foods and soft drinks and its adverse consequences on the teeth, this study aimed to assess the destructive effects of citric acid, lactic acid and acetic acid produced from the fermentation of foods on primary tooth enamel. The null hypothesis tested was that there would be no difference in the magnitude of enamel microhardness reduction as the result of exposure to the understudy three acids.

Methods:

This *in vitro* experimental study was conducted on 24 sound primary canine teeth. The sample size was calculated to be 24 using Minitab 16 software (Minitab Inc. USA). To enhance measurement of Vickers microhardness, tooth pieces were prepared. These pieces measured 3×4×3mm and were cut out of the buccal surface of teeth with smooth enamel and had two parallel walls. These pieces were cut out using a high-speed hand piece and a long fissure diamond bur. The enamel surface was ground using fine grit sandpaper. The specimens were stored in saline solution at room temperature until the experiment. Acetic acid, citric acid and lactic acid were used in this study. The characteristics of the understudy acids are shown in Table 1.

Table 1-The characteristics of the understudy acids

Acid	pH	Normality	Degree of purity
Acetic acid	3.1	17	99.7%
Citric acid	2.6	0.1	2%
Lactic acid	2.4	10	73.5%

Surface microhardness of specimens was measured at baseline using Vickers microhardness tester (Frank, Germany). The microprobe of this device has a pyramidal cross

section and applies 100g loads at 10s dwell times to the surface. The selection of the amount of load and the dwell time was based on a similar study (24). The microhardness tester is equipped with a 400X magnification lens that enables a clear view of the indentation created by the microprobe. The created indentation has 2 diameters of X and Y that are precisely measured by the device and reported as d1 and d2. The microhardness is calculated using the mean d1 and d2 and the formula below: $D=\frac{d1+d2}{2}$

The microhardness number is inversely correlated with the d value. The greater the d1, d2 and consequently the total d value, the higher the penetration of indenter into the surface and the lower the microhardness number of the object and vice versa. In order to confirm the accuracy of the obtained microhardness value, each specimen was tested 5 times and the mean of all values was reported as the microhardness number. After measuring the baseline microhardness value, specimens were divided into 3 groups of A, B and C (n=8) and immersed in freshly prepared acidic solutions. Group A, B and C specimens were immersed in acetic, citric and lactic acid solutions, respectively. During the experiment, the solutions had a temperature equal to the room temperature (approximately 30°C). After immersion for 5min, the specimens were rinsed with saline solution, dried and their surface microhardness was measured again. After the 2nd measurement, specimens were once again immersed in fresh acidic solutions and after 25 minutes (30 min after the time zero), specimens were rinsed with saline solution, dried and underwent microhardness testing. Therefore, surface microhardness was measured Data were analyzed using repeated measures ANOVA and one-way ANOVA with Tukey's HSD test. $p<0.05$ was considered statistically significant.

Results:

A total of 24 primary teeth pieces in 3 groups were evaluated in this study. The effect of acetic, lactic and citric acid on the mean enamel microhardness based on Vickers scale was investigated and the mean primary enamel microhardness at baseline and 5 and 30 minutes after exposure to acids is shown in Table 2.

Table 2- The mean primary enamel microhardness at baseline and 5 and 30min after exposure to acids

Acid type	mean	T0	T5	T30
Acetic acid	mean	293.93	135.20	102.80
	N	8	8	8
	SD	55.29	41.83	37.91
Citric acid	mean	308.61	140.30	98.02
	N	8	8	8
	SD	40.08	76.14	50.46
Lactic acid	mean	322.13	125.98	72.43
	N	8	8	8
	SD	47.71	38.74	15.73

Overall, acetic acid caused a mean reduction of 159 (28) and 191 (46) at 5 and 30min time points in the primary enamel, respectively; which corresponds to 32 (26) unit reduction during the time interval between 5 and 30min. Citric acid caused a mean reduction of 168 (50) and 211 (43) at 5 and 30min time points in the primary enamel, respectively; which corresponds to 42 (41) unit reduction during the time interval between 5 and 30min.

Lactic acid caused a mean reduction of 196 (43) and 250 (46) at 5 and 30min time points in the

primary enamel, respectively; which corresponds to 54 (41) unit reduction during the time interval between 5 and 30min (Table 3, Diagram 1).

Table 3- The mean reduction in the primary enamel microhardness at the first 5 minutes, the first 30 minutes and between 5 to 30 minutes following immersion in the understudy acids

Acid type	mean	T0	T5	T30
Acetic acid	**mean**	158.73	191.13	32.40
	SD	28.37	46.35	26.08
Citric acid	**mean**	168.31	210.58	42.27
	SD	49.85	42.88	41.34
Lactic acid	**mean**	196.15	249.70	53.55
	SD	43.13	45.61	40.73
All three acids	**mean**	174.40	217.14	42.74
	SD	42.78	49.65	36.19

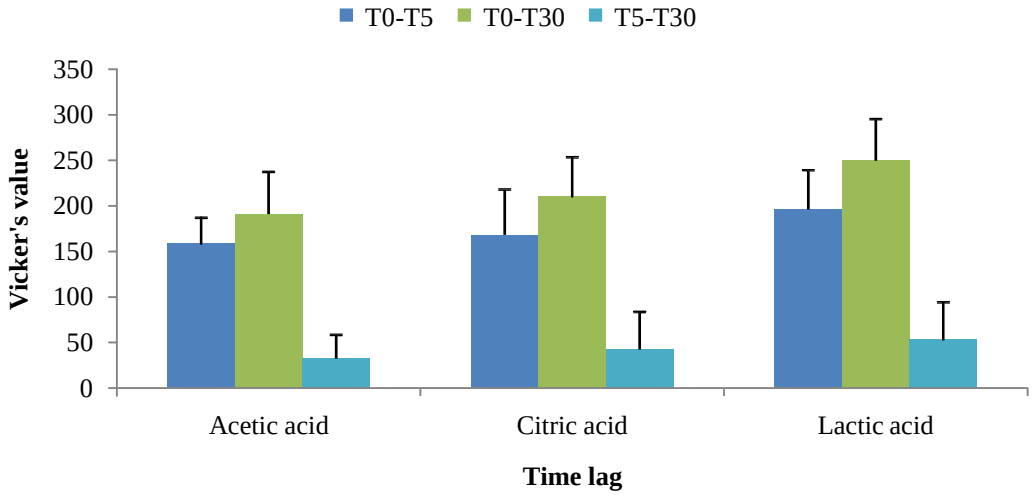

Diagram 1- The mean reduction in the primary enamel microhardness at 5 and 30min following acid exposure

For data analysis using repeated measures ANOVA, first, sphericity assumption was checked for microhardness data by Mauchly's test. This test was not significant and the level of significance calculated for Mauchly's test was equal to 0.58, suggested that the observed matrix have approximately equal variances and equal covariances. The effect of time in repeated measures ANOVA was found to be significant (p<0.001). Pairwise comparison among 0, 5 and 30 minutes time points was done using Bonferroni adjustment. The results showed significant differences in microhardness among all of pairwise time points (p<0.001). The interaction effect of time and type of acid was not significant (p=0.089). Evaluation of the effect of type of acid on microhardness revealed that the microhardness was not significantly different in the three groups of acids (p=0.912). However, when the reduction in microhardness was compared in three time points of the first 5 minutes, the first 30 minutes and between 5 to 30 minutes among the three understudy acids, only the reduction in microhardness from time 0

to 30 minutes was significantly different between lactic acid and acetic acid (p=0.042) (Diagram 2).

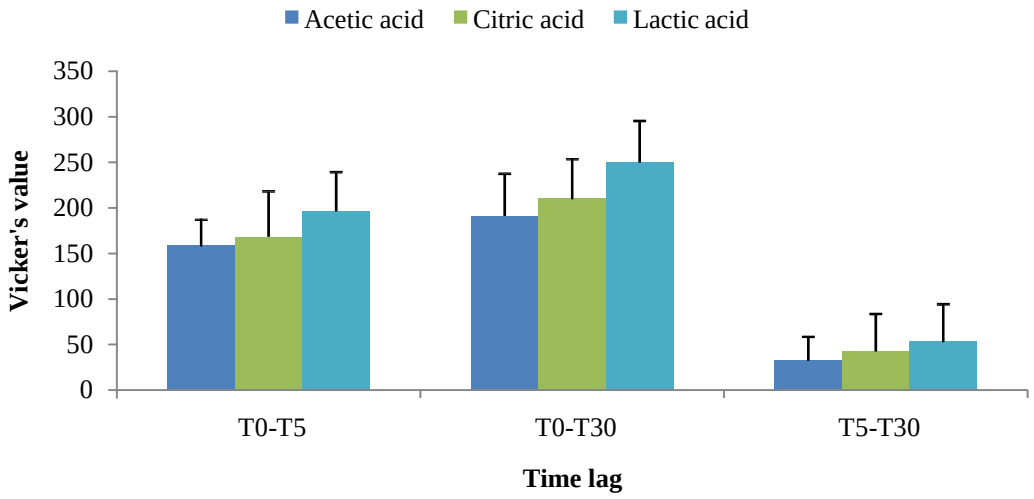

Diagram 2- The mean reduction in microhardness

Discussion:

A trend for greater rate of erosion has already been detected in younger age groups and there are reports regarding 6-50% prevalence of deciduous teeth erosion in preschool children between 2 and 5 years (1). Enamel microhardness reduction due to food fermentation has been extensively discussed and the duration of acid exposure and the pH of acids produced from food fermentation are two important factors responsible in this regard (24, 25). Enamel microhardness reduction is an important sign indicative of the initiation of demineralization.

Our results demonstrated that acids produced from food fermentation namely acetic, lactic and citric acid decreased enamel microhardness and initiated the process of demineralization of primary teeth under in-vitro conditions. The effect of acid exposure in reduction of microhardness during the 30 minutes was significant between acetic and lactic acids. This reduction in microhardness may be attributed to the methodology of our study since the teeth were exposed to a fresh acid solution for the 2nd time at 5min time point. Thus, the solution was no longer saturated with hydroxyapatite (HA) crystals. This finding was in accord with the results of Liu, *et al.* in 1988 although they used calcium ion selective microelectrodes (Ca-ISME) for the assessment of enamel microhardness; while, we used Vickers hardness tester (26).

Primary teeth enamel is mainly secreted and matured before birth and thus, the effect of environmental factors on its development is minimal. As the result, primary enamel is smoother than permanent enamel and has less developmental defects (27). Racial, ethnic, cultural, economical and environmental factors can all influence enamel microhardness after birth (28).

Grobler in 1994 evaluated the erosive effect of honey on the enamel and found that despite its acidic pH, it caused no erosion due to its calcium and phosphorous content (29). Despite the dissolution of tooth structure in a pH less than the critical pH (5.5), demineralization depends on the amount of fluoride and calcium ions in the environment as well and the erosion associated with a low pH caused by acidic foods

can be somehow compensated by the addition of calcium, phosphorous and fluoride to the environment (30).

Patel, *et al.* in 1987 reported that the concentration of acid is an important factor in enamel microhardness reduction and weak acids usually have no effect on enamel dissolution (31). In our study, lactic acid caused a greater reduction in microhardness compared to citric acid and particularly acetic acid and the difference in this respect at 30min time point was statistically significant.

The acidity of lactic acid is lower compared to other acids (under similar conditions in terms of temperature, pH and pressure) and therefore, it has lower reactivity. However, it reacts with enamel HA and produces apatite lactate salt that significantly compromises the strength and resistance of enamel HA. This phenomenon explains the greater effect of lactic acid on the enamel. This effect was also observed in our study and over time, the difference in this respect between lactic acid and the other two became more significant. Another reason for greater microhardness reduction as the result of exposure to lactic acid is its lower pH and subsequently greater reactivity.

It should be noted that the saliva protects the teeth from acid attacks by neutralizing acids and washing away the leftover food. We evaluated the effect of duration of acid exposure in our study; however, the protecting effect of saliva was not considered and needs to be evaluated in future studies. Hannig in his studies in 1999, 2003 and 2004 (16, 32, 33) evaluated the protective potential of salivary pellicle and its survival against demineralization and concluded that the salivary pellicle can protect the enamel surface against acid attack and erosion to some extent. He also demonstrated that the longevity of pellicle had no impact on its protective potential.

It is clear that if an acid cannot cause significant demineralization under in-vitro conditions, it definitely cannot cause significant demineralization in the oral environment in presence of protective agents and the saliva (34). Fluoridated water, neutral gels and fluoride varnishes and mouth rinses can significantly improve enamel resistance, decrease tooth hypersensitivity following acid exposure and prevent extension of lesions (35).

The best method to prevent erosion is to eliminate the causative agent and correct the dietary habits. Low acid foods are generally suggested for children. Based on our results, foods producing less lactic acid following fermentation are safer for children and cause less erosion or caries. Since the nutritional and oral hygiene habits are formed early in life, childhood is the best time to form healthy dietary and hygienic habits.

Conclusion:

The best method to prevent erosion is to eliminate the causative agent and correct the dietary habits. Low acid foods are generally suggested for children. Based on our results, foods producing less lactic acid following fermentation are safer for children and cause less erosion or caries. Since the nutritional and oral hygiene habits are formed early in life, childhood is the best time to form healthy dietary and hygienic habits.

Conflict of Interest: "None Declared"

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