Effects of pH, titratable acidity and calcium concentration of non alcoholic carbonated beverages on enamel erosion: an in vitro study.

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Abstract

Objective: Beverage acidity has been measured routinely using the pH value. However, titratable acidity is thought to be a true indicator of beverage erosive potential. It has also been reported that experimental addition of calcium in beverages can reduce the progression of erosion. This study was carried out to investigate effects the of pH, titratable acidity and calcium concentration of non alcoholic carbonated beverages on enamel erosion of extracted human premolar teeth.
Method: The erosive potential of 13 carbonated beverages and control was characterized based on analysis of pH, titratable acidity, and calcium concentrations. This was followed by ename demineralization tests. Baseline and post-immersion measurements of enamel microhardness were carried out using Vickers microhardness tester. Mean and standard deviation for each parameter was calculated. One way analysis of variance (ANOVA), paired t test, pearson's correlation, and multiple linear stepwise regression analysis were employed for statistical analysis. P values <0.05 were considered statistically significant.
Result: Among the beverages, Pepsi had the lowest pH while Sip-on-appy had the highest pH. Titratable acidity was lowest for Limca and highest for Red Bull. Calcium concentration was lowest in Limca and Sprite and highest in Appy fizz. Statistically significant negative correlation between pH and percentage reduction of enamel microhardness, and between calcium concentration and percentage reduction of enamel microhardness was found. Multiple linear stepwise regression analysis revealed pH as the best predictor for erosive potential.
Conclusion: All beverages have potential for enamel erosion. Beverages with lower pH and less calcium are more erosive.

Key words: Tooth wear, enamel erosion, beverages, enamel microhardness.

Introduction

The term "dental erosion" has been defined as loss of tooth structure by chemical means that do not involve bacteria[1]. A pH of 5.5 is traditionally considered to be the critical pH for enamel dissolution, although loss of mineral may begin at higher pHs [2]. Sources of acids can be endogenous or exogenous and erosive intensity is modified by quality and quantity of saliva [3,4,5]. Acidic food and beverages are the most common extrinsic factors that cause dental erosion[6]. The increase prevalence of erosion lesions reportedly has been associated with increased consumption of soft drinks in last decade[1,7]. The consumption of carbonated drinks is on the increase among children and adolescents and the habit is often carried on into adulthood [4]. Permanent teeth of adolescents are more prone to assault from acids found in beverages due to the porous characteristic of immature enamel and a deficiency of salivary conditioning; as a result, the erosive potential observe in this age group may rise in future[8,9].
The pH or initial pH has been used routinely as a measure of beverage acidity; however, the titratable acidity is thought to be the true indicator of beverage erosive potential[10]. Beverages with high titratable acidity can have a strong buffering capacity and may resist pH changes brought about by salivary actions[11,12]. Soft drink beverages contain several different types of acids, sugars, and modifying agents, all of which could lower the pH and raise the buffering capacity[1].
Supplementation of beverages with calcium has become more common nowadays. This may have a direct impact in dental erosion prevention. It has been reported that the experimental addition of calcium in acidic beverages can effectively reduce the progression of erosion in vitro [13,14] and in situ [15-17].
Hence this study was conducted to investigate effects of pH, titratable acidity and calcium concentration of non alcoholic carbonated beverages on enamel erosion of extracted human premolar teeth.

Materials and Method:

This study tested 13 non alcoholic carbonated beverages (Pepsi, Coca Cola, Thumbs Up, 7Up, Sprite, Fanta, Mirinda, Appy Fizz, Red Bull, Sip on Appy, Bindu Orange, Bindu Jeera, Limca) and tap water as control, in two independent phases. In the phase I, the erosive potential of the beverages and control was characterized based on analysis of pH, titratable acidity, and calcium concentrations. In phase II enamel demineralization tests were done.
Phase I: Characterization of beverages and control
(a) pH measurement:- The pH of each beverage and control

was measured by a calibrated pH electrode. 20 ml of a freshly opened drink and control (all at room temperature) was placed in a beaker and stirred until a stable reading was obtained.

(b) Titratable acidity:- 20 ml of each beverage and control was placed in a beaker, titrated with 0.10 M sodium hydroxide (NaOH) added in 0.25 ml increments and stirred until the pH reached 5.5 (measured by calibrated pH electrode). Values were expressed as amount of NaOH required (in ml).

(c) Calcium concentration:- 20 ml of each beverage and control was placed in a beaker. Calcium concentration was measured by Orion 9320BN calcium electrode. Values were expressed in ppm.

Five samples of each beverage and control were analyzed for all of the above measurements.

Phase II: Enamel demineralization test

(a) Demineralization process:- Human premolar teeth which had been extracted for orthodontic reasons were used to examine the effects of beverages and control on enamel. Teeth had been sterilized in formalin immediately after extraction. They were rinsed with tap water, cleaned with pumice and preserved in formalin until they were used.

Teeth selected for study did not have any restoration(s), and did not show any defects such as dental decay or enamel hypoplasia.

Human saliva used in experiment was collected from 20 healthy volunteers. Inclusion criteria were (1) no medication since last one month (2) No oral or systemic disease.

Each tooth was placed nto a cylindrical acrylic mould (approx 1" diameter, 1 centimeter thick), exposing the facial s urface. The specimen were alternately immersed manually, 5 seconds each, in 20 ml of beverage or control and human saliva for 30 cycles at room temperature. Total soaking time was 300 seconds. After the soaking sequence was completed the specimen was rinsed with distilled water, blotted dry and subjected to post immersion microhardness testing.

(b) Measurements of enamel microhardness:-

Baseline and post immersion measurements of enamel microhardness were done using Vickers indenter attached to a microhardness tester. A load of 500 gms was applied for 15 seconds and VHN (Vickers Hardness Number) was calculated.

For each beverage and control hardness test was conducted on fifteen specimens.

Ethical clearance for the study was obtained from ethical committee of the institution and informed consent was taken from the volunteers (for premolar teeth a nd saliva collection).

Statistical Analysis: Mean and standard deviation for each parameter was calculated. One way ANOVA was used for multiple group comparison. VHN of the baseline and post immersion were compared using paired t test. Correlations of pH, Titratable acidity, and calcium concentration with percentage reduction of enamel hardness were calculated using Pearson correlation analysis. Multiple linear stepwise regression analysis was employed to analyze the association of independent variables with percentage reduction in enamel microhardness. P values <0.05 were considered statistically significant.

Table 1. Mean and Standard Deviation (SD) of pH, titratable acidity, and calcium concentration of carbonated beverages

SI NO	Beverages	pH (Mean±SD)	Titratable Acidity (ml, Mean±SD)	Calcium Concentration (ppm,Mean±SD)
1	Pepsi	2.65±0.026	2.17±0.06	240.00±0.00
2	Coca Cola	2.89±0.010	1.90±0.10	160.00±0.00
3	Thumbs Up	2.82±0.015	2.77±0.06	320.00±80.00
4	7 Up	3.48±0.006	2.17±0.06	400.00±80.00
5	Sprite	3.47±0.015	1.80±0.10	80.00±0.00
6	Fanta	3.22±0.006	2.43±0.06	133.33±46.19
7	Mirinda	2.96±0.023	3.20±0.10	586.67±46.19
8	Appy Fizz	3.68±0.015	2.40±0.10	640.00±0.00
9	Red Bull	3.78±0.006	5.83±0.06	133.33±46.19
10	Sip on Appy	4.27±0.006	2.10±0.10	560.00±0.00
11	Bindu Orange	3.37±0.00	1.80±0.10	480.00±80.00
12	Bindu Jeera	3.34±0.006	2.33±0.06	480.00±80.00
13	Limca	2.79±0.006	1.63±0.06	80.00±0.00
14	Control	6.87±0.15	0.13±0.06	960±80.00
	ANOVA	F=1830.925 p<0.001,S	F=723.195 p<0.001,S	F=73.406 p<0.001,S

*S= Significant

Table 2. Effect of incubation with beverages on the microhardness of enamel

SI NO	Beverages	Pre treatment (Mean±SD)	Post treatment (Mean±SD)	Paired t test (P value)
1	Pepsi	295.67±7.5	180.33±4.93	0.000,S
2	Coca Cola	291.67±5.51	178.33±1.53	0.000,S
3	Thumbs Up	292.33±5.03	193.67±3.51	0.000,S
4	7 Up	296.67±9.02	234.67±7.02	0.000,S
5	Sprite	293.00±4.00	210.00±3.00	0.000,S
6	Fanta	292.67±4.93	212.33±3.79	0.000,S
7	Mirinda	296.33±6.66	121.67±5.13	0.000,S
8	Appy Fizz	297.00±6.56	291.67±6.11	0.004,S
9	Red Bull	292.00±6.00	231.67±4.51	0.000,S
10	Sip on Appy	297.33±3.21	295.00±3.61	0.020,S
11	Bindu Orange	294.67±7.02	235.33±5.51	0.000,S
12	Bindu Jeera	291.00±7.81	232.33±6.66	0.000,S
13	Limca	296.33±8.62	181.33±5.51	0.000,S
14	Control	301.00±2.65	301.00±2.65	1.000,NS

S=Significant, NS=Not significant

Table 3. Percentage reduction in microhardness of enamel.

SI NO	Beverages	% Reduction in microhardness (Mean±SD)
1	Pepsi	39.00±0.74
2	Coca Cola	38.85±0.65
3	Thumbs Up	33.75±0.07
4	7 Up	20.89±0.06
5	Sprite	28.33±0.04
6	Fanta	27.45±0.08
7	Mirinda	28.23±0.12
8	Appy Fizz	1.79±0.17
9	Red Bull	20.66±0.13
10	Sip on Appy	0.78±0.20
11	Bindu Orange	20.14±0.06
12	Bindu Jeera	20.16±0.14
13	Limca	38.81±0.40
14	Control	0.00±0.00

ANOVA
F=6155.73, p <0.001, Significant

Table 4. Stepwise multiple linear regression analysis with percentage reduction of enamel microhardness as dependent variable

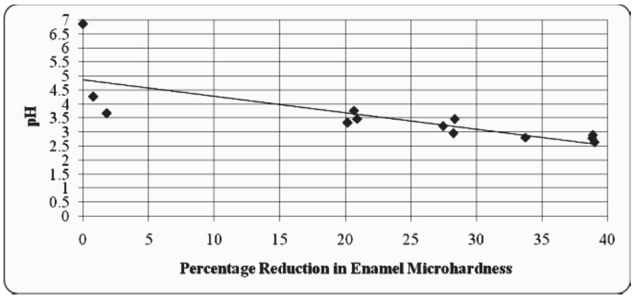
Model	R	R^2	ANOVA F value	P value
1	0.765(a)	0.585	56.275	p<0.001, Significant
2	0.827(b)	0.684	42.157	p<0.001, Significant
3	0.852(c)	0.725	33.405	p<0.001, Significant

a. Predictors: (constant), pH
b. Predictors: (constant), pH, Calcium Concentration
c. Predictors: (constant), pH, Calcium Concentration, Titratable acidity

Result

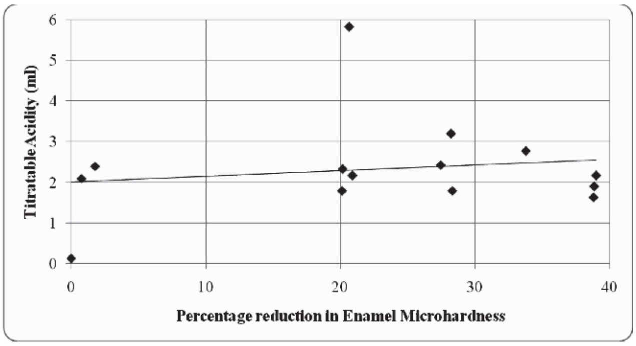
Table 1 shows mean and standard deviation of pH, titratable acidity, and calcium concentration of carbonated beverages and control.
pH was lowest for Pepsi (2.65 ± 0.026) and highest control (6.87 ± 0.15). Among the beverages it was highest for Sip-on-appy (4.27 ± 0.006). Titratable acidity was lowest for control (0.13 ± 0.06). Among the beverages titrable acidity was lowest for Limca (1.63 ± 0.06 ml) and highest for Red Bull (5.83 ± 0.06). Calcium concentration was lowest in Limca and Sprite (80.00 ± 0.00 ppm) and highest in control (960 ± 80). Among the beverages it was highest for Appy fizz (640.00 ± 0.00).
Table 2 shows effect of incubation with beverages and control on the microhardness of enamel.
Post immersion enamel microhardness was significantly less than base line values in all the beverages. There was no change in control group.
Table 3 shows percentage reduction in microhardness of enamel.
Lowest reduction in microhardness was in Sip on appy (0.78 ± 0.20) and highest was in Pepsi (39.00 ± 0.74).
Stepwise multiple linear regression analysis, which was executed to estimate the linear relationship between the dependent variable (percentage reduction of enamel microhardness) and various independent variables revealed that the best predictors in descending order are pH, Calcium Concentration, titratable acidity (Table 4).
Figure 1 shows correlation between pH of beverages & control and percentage reduction of enamel microhardness.
Statistically significant, negative correlation was found between pH of beverages & control and percentage reduction of enamel microhardness.
Figure 2 shows correlation between titratable acidity of beverages & control and percentage reduction of enamel microhardness.
Correlation between titratable acidity of beverages & control and percentage reduction of enamel microhardness was statistically not significant.
Figure 3 shows correlation between calcium concentration of beverages & control and percentage reduction of enamel microhardness.
Statistically significant, negative correlation was found between calcium concentration of beverages and control and percentage reduction of enamel microhardness.

Figure 1. Correlation between pH of beverages & control and percentage reduction of enamel microhardness



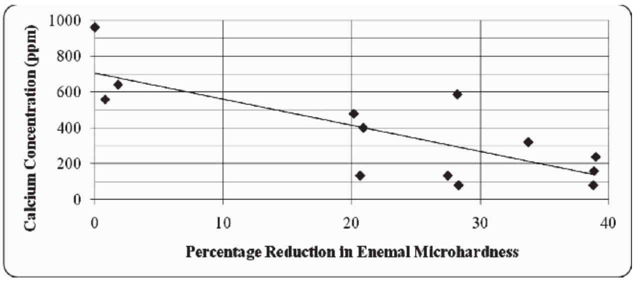
Pearson Correlation Coefficient (r)= -0.765, p =0.000, significant

Figure 2. Correlation between titratable acidity of beverages & control and percentage reduction of enamel microhardness



Pearson Correlation Coefficient (r)= 0.157, p =0.321,not significant

Figure 3. Correlation between calcium concentration of beverages & control and percentage reduction of enamel microhardness



Pearson Correlation Coefficient (r)= -0.760, p =0.000, significant

Discussion

Studies among children and adults have shown that as lifestyles have changed through the decades, the total amount and frequency of consumption of acidic foods and drinks have increased [18,19] . Nowadays children's daily food selections are excessively high in discretionary or added sugar. Sweetened drinks including carbonated soft drinks constitute the primary source of added sugar in the daily diet of children. In addition to the caloric load, these drinks pose a risk of dental erosion because of their acidity [18,20] . In a sample of 987 preschool children of Saudi Arabia, consumption of vitamin C supplements, carbonated drinks and fruit syrup from a f eeding bottle at bed or naptime were related to erosion [21]. An in vitro study showed that both primary and permanent teeth were equally susceptible to erosion.[22] Excessive consumption of acidic candies combined with a low salivary buffering c apacity may aggravate erosive lesions[18].

Consumption of carbonated beverages in form of sports drink to meet the demand of energy and water is coupled with high episodes of dental erosion[18,23].

The result from this study shows that carbonated beverages vary in their erosive potential. The dissolution of enamel occurs at a critical pH of about 5.5 the beverage tested, in this study have the potential to erode enamel due to their comparatively low pHs. Stepwise multiple linear regression analysis revealed that pH is the best predictor for erosive potential. Pearson correlation coefficient shows inverse relation between pHs of beverages and percentage reduction in enamel microhardness. This support the fact that pH directly reflects its erosive potential on the teeth[24,25] and beverages or food stuffs with lower pH have greater erosive effect[6,7].

In present study correlation between titratable acidity of beverages and percentage reduction in enamel microhardness was not significant. Titratable acidity better characterizes the erosive potential during longer exposure times[26,27]. This could be the possible explanation for the results as total test duration in present study was only 5 minutes. Beverages with high titratable acidity compete with natural buffering characteristics of saliva and resist the pH changes. The greater the titratable acidity, the more time it takes for saliva to restore pH values. These activities cause a prolonged period of oral acidity and in turn increase the erosive potential. For these easons, the titratable acidity is generally considered more important than pH in terms of a beverage's erosive potential[1].

Compared to other drinks tested, Red Bull required significantly more titration of NaOH to reach final pH level, which was similar to the study conducted by Owens (2007)[1].

Calcium was found to be effective in reducing erosion potential. Pearson correlation coefficient shows inverse relation between calcium concentration of beverages and percentage reduction in enamel microhardness. This result was in keeping with previous studies conducted by Hara and Zero(2008)[14], and Hughes et al.(2000)[28]. This can be explained by Law of Mass Action, where the initial presence of the reaction products of enamel demineralization tends to decrease the extent and rate of erosive reaction[14].

Dietary acids differ in their ability to cause erosion. Phosphoric acid is thought to be very erosive compared to organic hydroxyl acids such as citric, malic and lactic acids at the comparable pH and concentration. Phosphoric acid is the cheapest acidulant available. Manufacturers use this acid in a number of consumer products, particularly cola drinks.

In their in vitro study West et al. (2000)[29] found that loss of enamel and dentin increased with rising temperature. If the acidic soft drinks are consumed at low temperature, which is often the case, less erosion should occur[29]. However, this area needs further exploration for clarity and clinical relevance.

In a study, Parry et al. (2001)[30] investigated the erosive effects of various till and sparkling mineral waters together with a number of comparative soft drinks. Though sparkling mineral waters showed slightly greater dissolution than still mineral waters, these levels of dissolution were very low.

Authors concluded that these mineral waters represent alternatives to more erosive acidic beverages[30].

During consumption, food or drink make contact only shortly with the tooth surfaces before it is washed away by saliva. In previous studies, substrates usually contacted acidic foodstuffs for a prolonged period of time or did not account for the role of saliva[6,7,31]. Salivary protective effect plays a major role in moderating the extent of erosion in the mouth[6]. This study was designed to simulate the washing effect of saliva by cyclic specimen immersion. Other protective effects of saliva such as buffering capacity, acquired pellicle or remineralization could not be simply reproduced in vitro.

Conclusion

All the beverages have potential for enamel erosion. pH is the best predictor for erosive potential followed by calcium concentration of the beverage. Beverages with lower pH and less calcium are more erosive compared to others.

Recommendations

The public should be made aware about the hazardous effects of beverages on teeth. By increasing pH, lowering acid concentration and adding calcium, beverages of reduced dental erosivity can be formulated. However, furthe r studies are needed to study other factors that pertain to the erosive potential of a beverage including type of acid, chelating properties, temperature, inclusion of modifying agents and protective action of saliva.

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