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Acidic beverages increase the risk of in vitro tooth erosion

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Abstract

Acidic beverages are thought to increase the potential for dental erosion. We report pH and titratable acidities (i.e., quantity of base required to bring a solution to neutral pH) of beverages popular in the United States and lesion depths in enamel and root surfaces following beverage exposure, and we describe associations among pH, titratable acidity and both enamel and root erosive lesion depths. The pH of 100% juices, regular sodas, diet sodas and sports drinks upon opening, and the titratable acidity both upon opening and after 60 minutes of stirring were measured. Enamel and root surfaces of healthy permanent molars and premolars were exposed to individual beverages (4 enamel and 4 root surfaces per beverage) for 25 hours and erosion was measured. Statistical analyses included two-sample t-tests, analyses of variance with post hoc Tukey's studentized range test; and Spearman rank correlation coefficients. All beverages were acidic; the titratable acidity of energy drinks was greater than regular sodas and diet sodas which were greater than 100% juices and sports drinks ($P < 0.05$). Enamel lesion depths following beverage exposures were greatest for Gatorade® followed by Red Bull® and Coke® which were greater than Diet Coke® and 100% apple juice ($P < 0.05$). Root lesion depths were greatest for Gatorade® followed by Red Bull®, Coke®, 100% apple juice and Diet Coke® ($P < 0.05$). Lesion depths were not associated with pH or titratable acidity. Beverages popular in the United States can produce dental erosion.

Keywords

beverages; erosion; tooth; enamel; root; human

1. Introduction

Dental erosion is defined as the chemical removal of mineral from the tooth structure [1,2]. Erosion is classified as extrinsic (i.e., diet) or intrinsic (i.e., gastro-esophageal) in origin [1-3]. Erosion is typically progressive and results in the wearing away of the exposed tooth surface (i.e., enamel or root surface). Dental caries, on the other hand, is the site-specific acid destruction of tooth surface associated with bacterial fermentation of sugars in the oral cavity.

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Dental erosion is considered a significant oral health concern in European and Middle-Eastern countries. Dugmore and Rock [4] reported a tooth erosion prevalence of 59.7% in a random sample of 12-year-old British children from Leicestershire and Rutland counties participating in a national dental health survey. Erosion prevalence rates of 34% and 26% have been reported for 5 to 6-year-old and 12 to 14-year-old, respectively, boys in Saudi Arabia [5]. Similar results were reported for 5-year-old Irish school children; 47% exhibited some erosion [6]. In the United States, dental erosion has not been identified as a primary oral health concern in children at this time and similar prevalence rates are not available.

Some, but not all, observational studies support an association between acidic beverage (i.e., fruit juices, sodas, sports drinks) consumption and dental erosion. Al-Majed et al [5] reported that the number of permanent maxillary incisors with erosion in Saudi Arabian children was associated with frequency of night beverage intakes and length of beverage time in the mouth. The frequency of fruit and carbonated drink intakes was associated with severe erosion in 5-year-old Irish children [6]. Neither Milosevic et al [7] in British adolescent athletes nor Mathew et al [8] in United States collegiate athletes identified an association between sports drink consumption and erosion.

European investigators have studied acidic foods and beverages as risk factors for enamel erosion with most investigations focusing on acidic beverages [5-11]. Larsen et al [9] of Denmark investigated the *in vitro* erosive potential of soft drinks, mineral waters and orange juices and compared erosion depths to pH and buffering capacity of the beverages. They reported that erosion was minimal in beverages containing a pH above 4.2, but became more evident with pHs decreasing below 4.0. Hunter et al [10] of the United Kingdom examined the *in vitro* susceptibility of permanent and deciduous teeth to erosion by soaking extracted teeth in a low pH fruit drink diluted with mineral water. Increasing length of exposure to the fruit drink was associated with more severe erosion; however, the severity of erosion was not proportional to the length of exposure. Rees et al [11] reported that sports drinks based on acidic fruits popular in the United Kingdom have low pHs, and are erosive when enamel is immersed in the sports drink.

Although dental erosion has been identified as a significant oral health concern in European countries, it has received much less attention in the United States. Secular trends in beverage habits in the United States including increased consumption of sodas, 100% juice and juice drinks, as well as the introduction of sports drinks and energy drinks, could increase the risk of erosion. Furthermore, the nature of consumption (i.e., sipping for extended periods or concurrent with mouth breathing during athletic training) could increase the opportunity for erosion to occur. Erosion is a gradual process; the secular changes have occurred fairly quickly which may have limited our ability to observe erosive effects of acidic beverages in the population.

Although we have previously described the protection against erosion associated with calcium fortification of 100% fruit juices [12], the erosion potential of beverages popular in the United States has not been thoroughly investigated. We hypothesize that acidic beverages popular in the United States erode both enamel and root surfaces. Knowledge of the erosion potential of popular beverages is important for clinical guidelines regarding beverage consumption practices and development of potentially “safer” beverages. The objectives of this manuscript are to report pH and titratable acidities (i.e., quantity of base required to bring a solution to neutral pH) of beverages popular in the United States, to report lesion depths in enamel and root surfaces following beverage exposure and to describe associations among pH, titratable acidity and both enamel and root erosive lesion depths.

2. Methods and materials

2.1. Design and Samples

The experimental design was descriptive. The *in vitro* erosion potential of representative commercial beverages was evaluated using extracted human permanent teeth [9]. The *in vitro* model was selected because the intact enamel or dentin surface is exposed to the beverage and the model allows for observation of demineralization within a reasonable time period. Different brands of beverages (n=3-4) available in the Iowa City community were purchased at local grocery stores from each of the following categories: 100% juices, regular sodas, diet sodas, sports drinks and energy drinks. Healthy, extracted permanent molars and premolars available from the University of Iowa College of Dentistry were used to evaluate erosion potential.

2.2. Physicochemical Properties

The initial pH and titratable acidity of each beverage was measured using an automatic titrator (Metrohm E512 analog pH meter, Brinkmann Instruments, Incorporated, Westbury, New York 1982). The titratable acidity was measured by adding 1M KOH to 50 ml of each beverage until a pH of 7 was obtained. Each beverage was assayed upon opening (0 minutes); the process was repeated on 3 different containers of each beverage. Also, each beverage was assayed in triplicate after 60 minutes of vigorous stirring to remove all carbonation from the sodas.

2.3. Tooth Preparation

Extracted teeth were disinfected, cleaned of residual debris and stored in a moist environment to prevent drying. Small holes were drilled in the end of the root and floss was used to suspend teeth in the solution. The teeth were painted with red acrylic nail polish except for a small window (1 × 4 mm) of enamel or root surface which was left exposed.

2.4. Beverage Exposure

Teeth (n=4 enamel and n=4 root surface windows per beverage) were randomly assigned to a representative beverage from each category including 100% apple juice, Coke®, Diet Coke®, Lemon-Lime Gatorade® and Red Bull®. Beakers were filled with 250 ml of beverages and the prepared teeth were suspended in the beverages for a total of 25 hours; beverages were replaced every 5 hours.

2.5. Erosion Depth Measurements

Following beverage exposures, the teeth were rinsed, dried, mounted in mandrels with sticky wax, and sliced into 100-150 micron sections (Series 1000 Hard Tissue Microtome, SciFab, Lafayette, Colorado, 1996). A polarized light microscope (Olympus BX-50, Olympus America Incorporated, Center Valley, Pennsylvania, 1996) at magnification 5x and 10x was used to view sections and identify erosive lesions. Three sections from each tooth (i.e., 24 sections per beverage; 12 enamel surfaces and 12 root surfaces) were selected and lesions were photographed (Spot RT Color Video Camera, software version 3.1, Diagnostic Instruments, Sterling Heights, Michigan, 2000). Lesion depths were defined as the average distance between the estimated height of the original tooth structure and the bottom of the demineralized region measured using Image Pro Plus (version 5.1, Media Cybernetics, Incorporated, Silver Spring, Maryland, 2004).

2.6. Statistical Analysis

Statistical analyses were conducted using SAS for Windows, (version 9.1 SAS Institute Incorporated, Cary, North Carolina). Descriptive statistics were calculated and physicochemical properties are reported as means and standard deviations [13]. The two-sample t-test [14] was used to compare the mean lesion depths between enamel and root surfaces within each

beverage. Differences in physiochemical properties and lesion depths between beverages were investigated using one-way analysis of variance with post-hoc Tukey's studentized range HSD test [14]. Relationships between lesion depths and physiochemical properties were evaluated using the Spearman's rank correlation test [14]. The level of significance was $P < 0.05$.

3. Results

The physiochemical properties of beverage categories are presented in Table 1. All beverages studied were acidic, although the pH of 100% juice was higher than the pH of the remaining beverages. The quantity of base (1 M KOH) required to neutralize the beverages upon opening was highest for energy drinks followed by regular and diet sodas and then 100% juice and sports drinks. The quantity of base required to bring beverages to neutral after 60 minutes of vigorous stirring was again highest for energy drinks followed by regular and diet sodas and then 100% juice and sports drinks.

Lesion depths in enamel and root surfaces following exposure to beverage samples are reported in Table 2. Lesion depths produced in enamel during exposure to Gatorade® were greater than those produced during exposure to Red Bull® and Coke®; these three beverages were more erosive than Diet Coke® and 100% apple juice. Lesion depths produced in root surfaces were greatest for Gatorade®, followed by Red Bull®, Coke®, 100% apple juice, and Diet Coke®. Lesion depths were greater in root than in enamel surfaces following exposure to Red Bull®, Coke®, and 100% apple juice, and less following exposure to Gatorade®. Representative examples of enamel and root lesions following exposure to Coke® are shown in Figure 1.

Lesion depths were not associated with pH in enamel ($r = -0.20$; $P = 0.747$) or root ($r = 0.10$; $P = 0.873$) surfaces; quantity of base required to neutralize beverages immediately upon opening in enamel ($r = 0.00$; $P = 1$) or root ($r = -0.10$; $P = 0.873$) surfaces; or quantity of base required to neutralize beverages following 60 minutes of vigorous stirring in enamel ($r = 0.20$; $P = 0.747$) or root surfaces ($r = 0.50$; $P = 0.391$).

4. Discussion

Our data suggest that beverages available for consumption in the United States have the potential to erode both enamel and root surfaces. The study was conducted in vitro; it is not possible to replicate intra-oral conditions in the laboratory nor is it ethical to conduct such a study in humans. Epidemiologic data suggest that erosion is a significant oral health concern in Europe and Middle-Eastern countries [1,4-7], while limited data are available in the United States. Anecdotal reports do not suggest widespread erosion amongst individuals in the United States.

The erosion following exposure to beverages reported herein in enamel and root surfaces was not related to beverage pH or titratable acidity upon opening or following vigorous stirring, suggesting that neither pH nor titratable acidity can be used to predict erosive potential. Both the concentration and strength of an acid can influence the degree of erosion; the pH is determined by both concentration and strength of the acid in solution. The titratable acidity is the quantity of base required to bring a solution to neutral pH; a higher titratable acidity is consistent with a higher buffering capacity. The pH and titratable acidity of beverages in our study were similar to values reported by Jensdottir et al [15], who also found a correlation between weight loss of eroded tooth sections and both pH and titratable acidity. Beverage compositions vary widely; the milieu of nutrients and nonnutrients likely influences the capacity for erosion [16,17]. Calcium and fluoride have been shown to limit the extent of erosion by saturating the solution and/or altering the solubility of enamel [1,9,12,14,18].

In general, root surfaces were more susceptible to erosion than enamel surfaces. This is expected because of compositional differences; enamel is approximately 95% mineral and 5% organic material while the root (i.e., cementum and dentin) has a lower mineral and higher organic content [19]. The difference is apparent visually as enamel surfaces are almost completely eroded while the root surfaces retain an organic matrix.

The in vitro nature of the experimental design and artificial time of exposure are study limitations. The in vitro design exposes the tooth to the beverage for a defined time period without consideration of rate of beverage consumption, length of swallow, movement within the mouth during swallowing, clearance by saliva and remineralization potential of saliva. These limitations likely exacerbate the observed erosion potential of all beverages. Moreover, it is not possible to equate experimental conditions to levels of beverage consumption in humans. Lastly, only 5 beverages were studied and it is possible that other specific beverages could have markedly different erosive effects than those tested.

The potential for erosion with consumption of acidic beverages is an important consideration for nutritionists, dentists and physicians counseling patients. Prolonged contact time between the beverage and enamel or root surface increases the opportunity for erosion to occur [20-22]. Salivary flow is stimulated in response to tartness and, under normal circumstances, is thought to clear the acid and limit erosion. However, individuals with limited salivary flow could have increased acid-tooth contact and be at increased risk of erosion. Sanchez et al [23] reported that children with erosion had lower salivary flow rates and buffering capacities than children without erosion. Practices such as “swishing” and “holding” beverages in the mouth prolong acid-tooth contact time and could increase erosion risk. Johansson et al [21] compared potentially erosive habits between Saudi men with high and low indices of dental erosion. They reported that men with erosion consumed twice as much cola-type beverages, held beverages in their mouths 70% longer and were more likely to be mouth breathers than men without erosion [21]. Rios et al [24] reported an association between incisal tooth wear and mouth holding of beverages prior to swallowing in 6-year-olds from Brazil. Although it is logical to encourage tooth brushing immediately following consumption of sugared, acidic beverages, Rios et al reported that brushing enamel immediately after exposure to acidic beverages increased tooth loss [25]. Dental erosion associated with consumption of acidic beverages is a potential oral health concern. Patients with high consumption of acidic beverages, decreased salivary flow, prolonged beverage holding habits, or mouth breathing could be at an increased risk for dental erosion.

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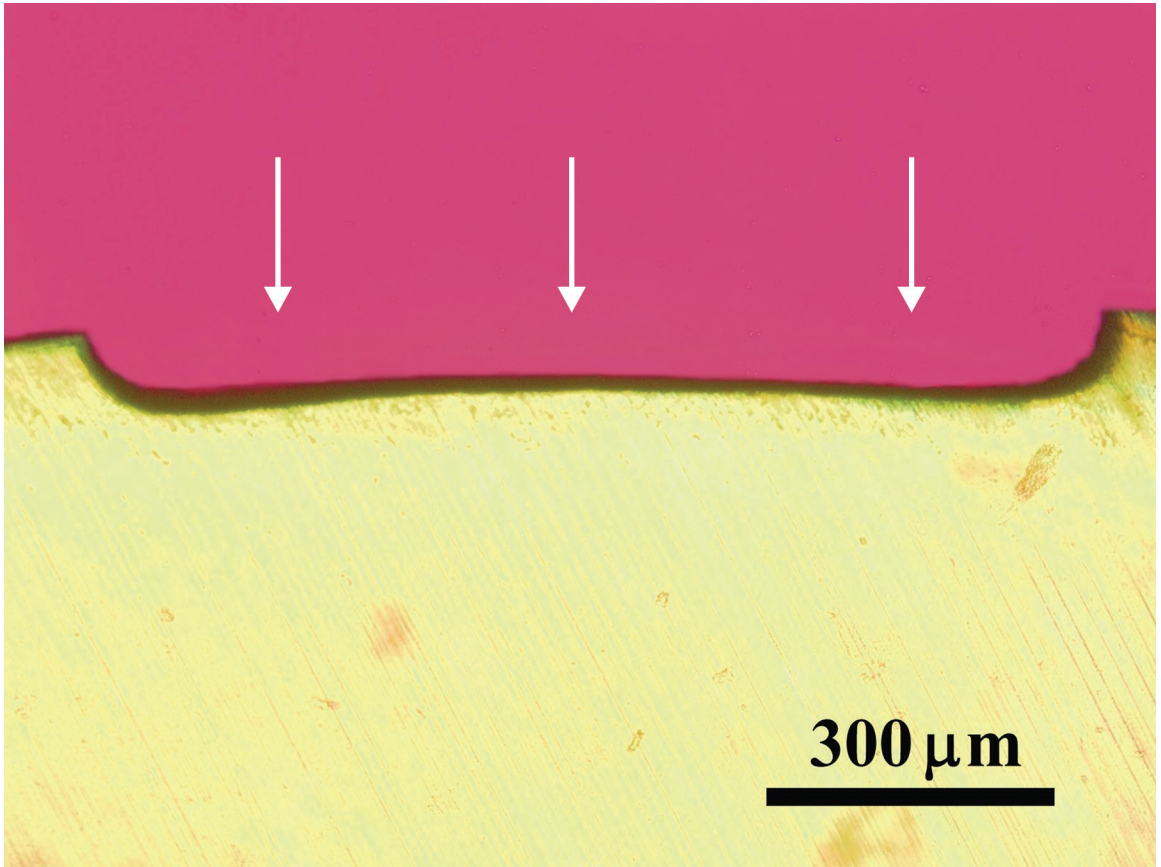
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Abbreviations

x
times



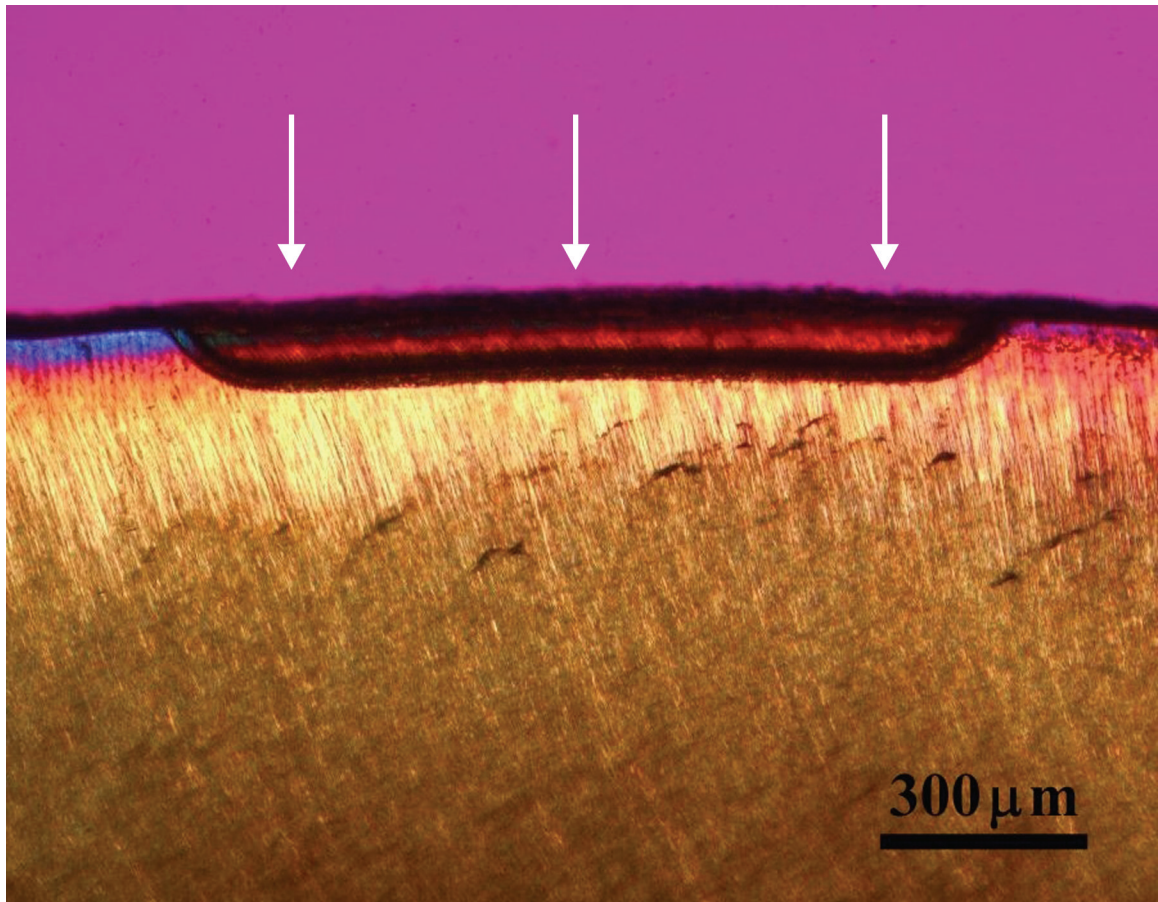


Figure 1.

Enamel or root surfaces were painted with acrylic nail polish to expose windows. The teeth were suspended for 25 hours in Coke®, which was replaced every 5 hours. The teeth were sectioned perpendicular to windows. Sections were viewed using a polarized light microscope and photographed. Representative lesions (arrows) produced in enamel (a) and root (b) surfaces are shown.

Table 1

Physiochemical properties of beverage categories.

Property	Beverage Categories				
	100% Juice ^a	Regular Soda ^a	Diet Soda ^a	Sports Drinks ^b	Energy Drinks ^d
pH					
Titratable Acidity ^c					
0 minutes	3.46 ± 0.08 ^{d,1}	2.65 ± 0.27 ²	2.94 ± 0.17 ³	2.84 ± 0.19 ^{3,4}	2.76 ± 0.31 ^{2,4}
60 minutes	2.56 ± 0.10 ^{d,1}	4.08 ± 0.40 ²	4.15 ± 0.47 ²	2.13 ± 0.37 ¹	6.50 ± 1.13 ³
	2.48 ± 0.08 ^{d,1}	1.07 ± 0.39 ²	1.25 ± 0.80 ²	2.09 ± 0.34 ¹	4.83 ± 0.95 ³

The pH and titratable acidity of beverages were measured using an automatic titrator.

^a n = 4

^b n = 3

^c ml 1M KOH required to bring 50 ml of beverage to neutral pH. "0 minutes" was measured immediately upon opening while "60 minutes" was measured following 60 minutes of vigorous stirring.

^d Means ± SD. Means within a row with different superscript numbers are significantly different. Statistical test was one-way analyses of variance with post-hoc Tukey's studentized range (HSD) test; $P < 0.05$.

Table 2
Enamel and root surface lesion depths following exposure to select beverages.

Lesion Depth (μm)	Beverage				
	100% Apple Juice	Coke®	Diet Coke®	Gatorade®	Red Bull®
Enamel	$57 \pm 6^{a,1}$	92 ± 6^2	61 ± 4^1	131 ± 8^3	100 ± 5^2
Root	$77 \pm 3^{a,1}$	101 ± 3^2	66 ± 2^3	118 ± 5^4	111 ± 4^5
Enamel vs. Root, <i>P</i> -value	0.001 ^b	0.040 ^b	0.074 ^b	0.035 ^b	0.012 ^b

Windows in enamel or root surfaces were exposed to beverages for 2.5 hours with beverages replaced every 5 hours. Teeth were sectioned following beverage exposure and lesion depths were measured.

^aValues are means \pm SD. Within group comparisons; means within a row with different superscript numbers are significantly different. Statistical test was one-way analyses of variance with post-hoc Tukey's studentized range (HSD) test, $P < 0.05$.

^bBetween group comparisons; *P* -values for enamel vs. root lesion depth comparisons using the two-sample t-test.