

The Erosive Potential of Soft Drinks on Enamel Surface Substrate: An *In Vitro* Scanning Electron Microscopy Investigation

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Abstract

Aim: Using scanning electron and light microscopy, this study qualitatively evaluated the erosive potential of carbonated cola beverages as well as sports and high-energy drinks on enamel surface substrate.

Methods and Materials: Beverages used in this study included: Coca Cola Classic®, Diet Coke®, Gatorade® sports drink, Red Bull® high-energy drink, and tap water (control). Extracted human permanent molars free of hypocalcification and/or caries were used in this study. The coronal portion of each tooth was removed and sectioned longitudinally from the buccal to the lingual surface. The crown sections were embedded in acrylic resin, leaving the enamel surfaces exposed. Following finishing and polishing of all surfaces, one side was covered with red nail varnish while the remaining side was exposed to individual beverage immersion for 14 days, 24 hours per day, at 37°C. The specimens were evaluated for enamel surface changes using scanning electron and light microscopy.

Results: Enamel specimens exhibited visual surface changes following immersion in the test beverages with Red Bull® and Gatorade® revealing the most striking surface morphological changes. Specimens subjected to Coca Cola Classic® and Diet Coke® immersion also displayed irregular post-treatment surface morphology.

Conclusions: As verified by microscopic evaluation, all test beverages displayed enamel dissolution in the following order: Red Bull® > Gatorade® > Coca-Cola Classic® > Diet Coke®.

Keywords: Erosion, enamel dissolution, soft drinks, acidogenic

Citation: Owens BM, Kitchens M. The Erosive Potential of Soft Drinks on Enamel Surface Substrate: An *In Vitro* Scanning Electron Microscopy Investigation. J Contemp Dent Pract 2007 November; (8)7:011-020.

Introduction

Non-carious loss of dental hard tissues can be attributed to different etiologies including diagnoses of attrition, abrasion, abfraction, erosion, and/or etiologies that are multifactorial in origin.¹ Attrition is the loss of tooth structure from shearing motions or eccentric forces while in occlusion.² Abrasion is caused by mechanical wear from improper brushing habits or abrasive dentifrices.² Abfraction, as theorized, is a regressive process due to occlusal loading, i.e., tensile and compressive forces from eccentric movements with flexure, fatigue, deformation, and microfracture of the tooth structure.³ Erosion is chemical dissolution of the surface of dental hard tissues by acids without the involvement of microorganisms.⁴

Causative factors for tooth erosion are divided into extrinsic and intrinsic categories. Intrinsic erosion occurs from involuntary gastrointestinal disturbances such as gastroesophageal reflux disease (GERD) and from voluntary regurgitation of gastric acids exhibited by anorexic and/or bulimic individuals.^{5,6} Extrinsic tooth erosion causes include environmental factors, medicaments, lifestyle, and diet.^{5,6}

Dental tooth loss or “toothwear”⁷ can also include multifactorial etiologies. Abrasion can occur more easily in the presence of a tooth surface subjected to the erosive process.⁸ A differential diagnosis discriminating between two or more causes can be problematic in adult patients. However, the predominant cause of tooth loss is usually erosion in children or adolescents.⁹

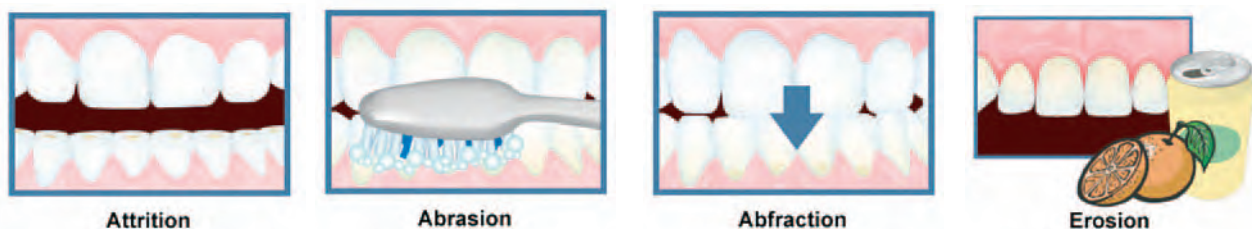
Several studies¹⁰⁻¹⁸ have examined a possible association between dental erosion and the consumption of soft drinks, i.e., carbonated cola beverages and citrus-based fruit drinks. There has also been increased interest in the dental effects of soft drink beverages due to the escalating (an increase of 56%, rising

approximately 2–3% per year) consumption by children and adolescents over the last decade.¹⁹ In 2000, the consumption of soft drinks including carbonated cola beverages, sports drinks, and fruit juices in the United States increased by 500% over the past 50 years.²⁰ The U.S. market alone includes 450 different soft drinks, with a total retail sale over \$60 billion annually.²¹ Americans consume over 50 gallons per person per year, surpassing all other beverages including coffee, milk, alcohol (beer), and bottled water.²⁰



The largest increase in soft drink consumption has occurred among children and adolescents. Forty percent of pre-school children drink more than 250 ml (8.0 ounces) of soft drinks per day, while among 12 to 19-year-old males consumption was 28 ounces per day, and among 12 to 19-year-old females the rate of intake was 21 ounces per day.^{22,23}

Sports drink consumption has also dramatically increased with over \$1.5 billion in sales a year.²³ Although research is incomplete, results from studies²⁴⁻²⁶ evaluating these beverages have shown the destructive effect they have on enamel is even greater than cola-based carbonated beverages.



The purpose of this study was to evaluate the surface morphology of enamel specimens subjected to carbonated cola beverages, sports and high-energy drinks, through qualitative analysis using scanning electron and light microscopy.

Methods and Materials

Four test beverages were selected for the study based upon current drinking trends of adolescents in the United States. These beverages included: Coca-Cola Classic®, Diet Coke®, Gatorade® sports drink, and Red Bull® high-energy drink. Tap water was used as a control. The pH and titratable acidity (TA) of each beverage was also measured (Table 1).

Measurement of the pH of each beverage was performed using a pH electrode connected to

a Jenco 3601™ (Jenco Instruments Inc., San Diego, CA, USA) analyzer. The electrode was calibrated immediately prior to measurements using standard buffers of pH 4.0 and 7.0.

Experimental Design

Extracted human permanent molars free of hypocalcification and caries were carefully cleaned of calculus and other debris and stored in a 1% chloramine-T solution (Fisher Chemical, Fair Lawn, NJ, USA) consisting of 12% active chlorine diluted in distilled water. This solution was used for infection control purposes prior to the study. The teeth were then randomly divided into beverage groups for preparation and analysis.

Enamel specimens were prepared by sectioning the crowns from the root surfaces using a diamond bur in a high-speed handpiece with an

Table 1. Beverage Characteristics.

Group	Beverage	Manufacturer	Composition	PH (0-14)	TA (ml of 0.1 M NaOH)
1	Coca-Cola Classic (20 oz)	The Coca-Cola Co. Atlanta, GA, USA	Carbonated water, high fructose corn syrup, caramel color, phosphoric acid, natural flavors, caffeine	2.49	18.3
2	Diet Coke (20 oz)	The Coca-Cola Co. Atlanta, GA, USA	Carbonated water, caramel color, aspartame, phosphoric acid, potassium benzoate, natural flavors, citric acid, caffeine	3.12	20.1
3	Gatorade	The Gatorade Co. Chicago, IL, USA	Water, sucrose syrup, glucose-fructose syrup, citric acid, natural lemon/lime flavors, natural flavors, salt, sodium citrate, monopotassium phosphate, ester gum, yellow dye #5	3.12	14.8
4	Red Bull	Red Bull N.A., Inc Santa Monica, CA, USA	Water, sucrose, glucose, sodium citrate, taurine, glucuronolactone, caffeine, inositol, niacinamide, calcium-pantothenate, pyridoxine HCL, vitamin B12, artificial flavors, colors	3.41	51.9
7	Tap Water	N/A	Water, various minerals	7.11	N/A

air-water spray. The crowns were sectioned from the buccal to lingual surface using a Buehler Isomet™ low-speed diamond saw (Buehler Int., Evanston, IL, USA) cooled with water. The enamel sections were then embedded in acrylic resin in molds with the outer enamel surface exposed. The enamel surfaces were ground using 600 to 2000 grit abrasive paper and polished with diamond paste. Half of the exposed enamel surface was coated with acrylic nail varnish while the remaining surface was left exposed to each beverage tested (Figure 1).

The enamel specimens were stored in opaque plastic containers of each beverage at 37°C for a total of 14 days. The beverages were changed daily. The testing period was a protocol adopted from a study by von Fraunhofer and Rogers¹⁰ for dissolution of enamel in beverage solutions. This protocol was based upon an average daily consumption of 24 ounces (two 12 ounce cans) of soft drink and a residence time in the mouth of 20 seconds (before salivary clearance). This results in an annual exposure of enamel to soft drinks of approximately 90,000 seconds (25 hours) per year. The test period of 350 hours used in the study is comparable to 14 years of normal beverage consumption, a reasonable time period for evaluating the erosion of enamel in adolescents and young adults.

Qualitative Analysis

Qualitative analysis, examining each enamel specimen, was accomplished by a Meiji™ (Meiji-Labax Co., Tokyo, Japan) binocular light stereo-microscope (20x and 40x) and a scanning electron microscopy (JEOL 5510™, JEOL Ltd., Tokyo, Japan) at the conclusion of the 14 day test period. Digital images of the enamel specimens were produced using a Digital Camera for Microscope™ (Hangzhou Scopetek Optp-Electric Co., Ltd., Hangzhou, China). Image retrieval was accomplished by downloading using DirectX 9.0™ (Microsoft Corp., Redmond, WA, USA) and Minisee™ (Hangzhou Scopetek Optp-Electric Co., Ltd., Hangzhou, China) software.

Results

The results of this study showed the effects of different soft drink beverages on enamel surface substrate through qualitative microscopic evaluation. Light microscopic images at 20x and

40x magnification revealed exposed enamel surfaces following immersion in Coca-Cola Classic (Figure 2) and Diet Coke (Figure 3).

The specimens immersed in Coca-Cola Classic® showed a more uniformly roughened tooth surface compared to the Diet Coke® surface. A dark residue was also examined on the Coca-Cola Classic® exposed surface. The specimens immersed in Gatorade® and Red Bull® (Figures 4 and 5) revealed extensive enamel dissolution with an exposed dentin substrate, as opposed to the carbonated cola beverage exposed surfaces that showed irregular, roughened, “wavy” patterns in enamel only.

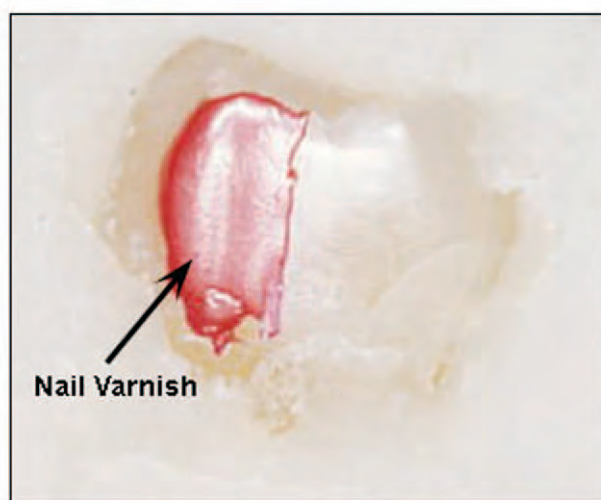


Figure 1. Representative enamel specimen embedded in white acrylic resin. Note one side of specimen is covered with a coating of red nail varnish.

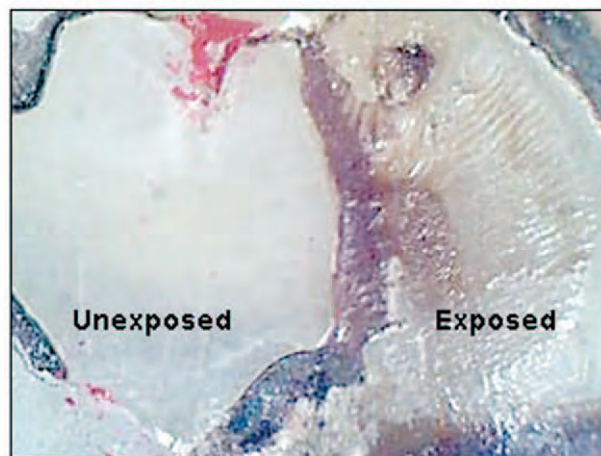


Figure 2. Photograph of specimen following immersion in Coca-Cola Classic at 20x magnification.

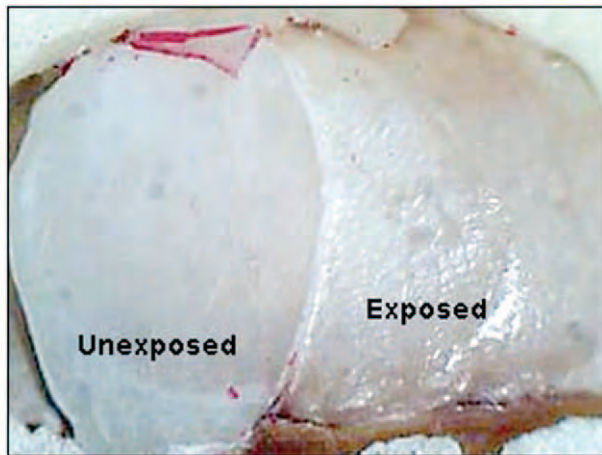


Figure 3. Photograph of post-immersion specimen (Diet Coke) at 20x magnification.

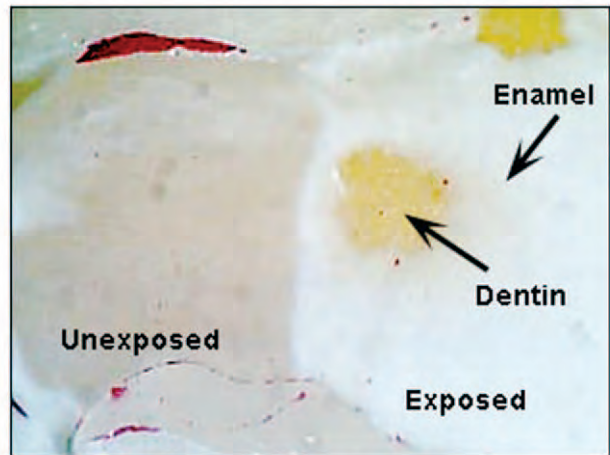


Figure 4. Photograph of specimen (20x) following immersion in Gatorade. Note white, chalky enamel substrate with area of dentin visible.

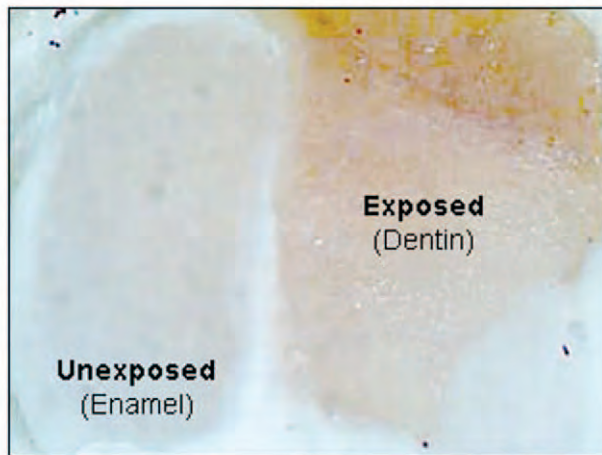


Figure 5. Photograph of specimen (20x) revealing post-immersion surface from Red Bull® with almost totally denuded enamel substrate down to the dentin surface.

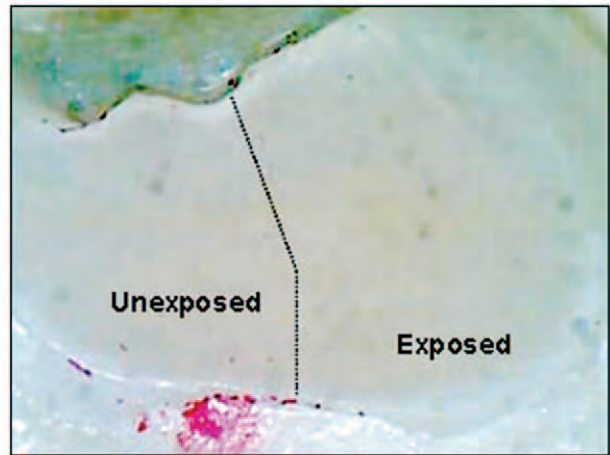


Figure 6. Photograph (20x) showing enamel surfaces before and after immersion in tap water, with no visible differences displayed.

Also, Gatorade® and Red Bull® immersion imparted a dull white, chalky appearance to the enamel surfaces, while the surfaces exposed to the cola beverages revealed a somewhat “shiny” appearance. The enamel specimen immersed in tap water (control) showed no differences between the exposed and unexposed surfaces (Figure 6).

Scanning electron microscopy revealed images of the post-immersed enamel specimen surfaces. Figures 7A and B reveal the “wavy”, roughened appearance from the Coca-Cola Classic® exposed surfaces with Figure 7C revealing an unexposed, unaffected enamel surface. The striations noted on this surface were produced from toothbrush marks during removal of the nail varnish residue.

This result was unexpected, and viewing from a greater magnification (100x) showed the damage rendered (potentially) to healthy enamel substrate simply by soft toothbrush bristles. Figures 8A and B show exposed enamel surfaces following immersion in Diet Coke®.

The Gatorade® specimen in Figure 9A shows a remarkable difference between exposed and unexposed tooth surfaces. There is extensive dissolution on the unprotected enamel surface revealing dentin surface substrate. Residue particles are also visible on the exposed side of the enamel specimen.

Figures 9B and 9C offer a closer view (100x and 250x magnification) of the Gatorade® specimen.

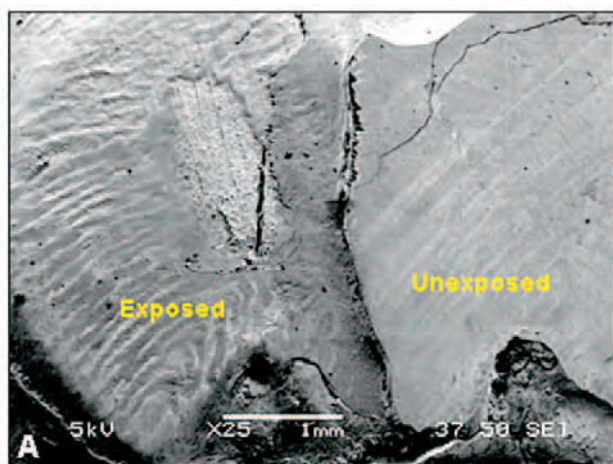


Figure 7A. SEM photograph 25x magnification revealing both surfaces (exposed vs. unexposed) following immersion in Coca-Cola Classic®.

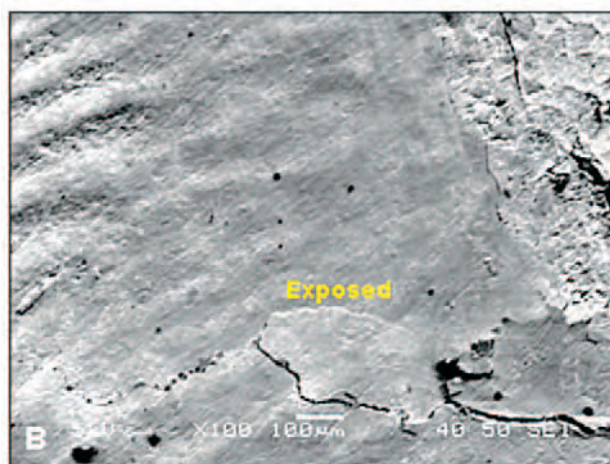


Figure 7B. SEM photograph (100x) of exposed enamel surface. Note “wavy” enamel surface characteristic.

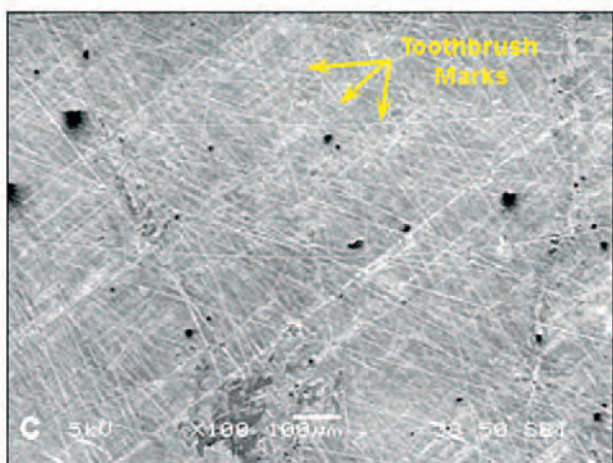


Figure 7C. SEM photograph of unexposed surfaces (Coca-Cola Classic). Note striations (toothbrush marks) on the exposed surface.

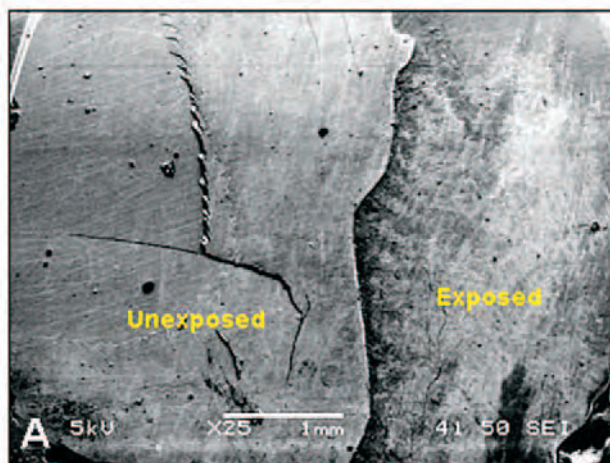


Figure 8A. SEM photograph (25x) showing both surfaces following immersion in Diet Coke.

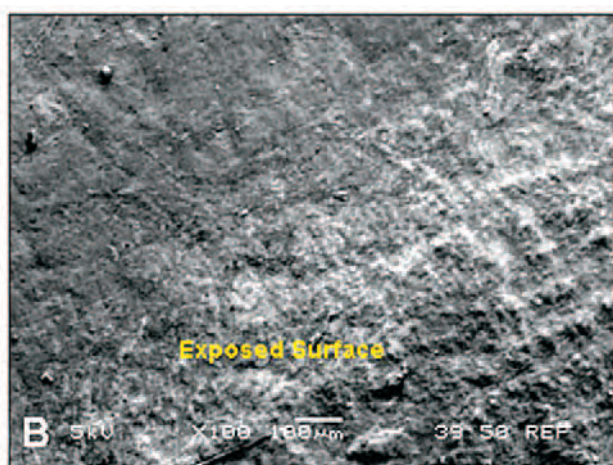


Figure 8B. SEM photograph (100x) of exposed surface following emersion in Diet Coke.

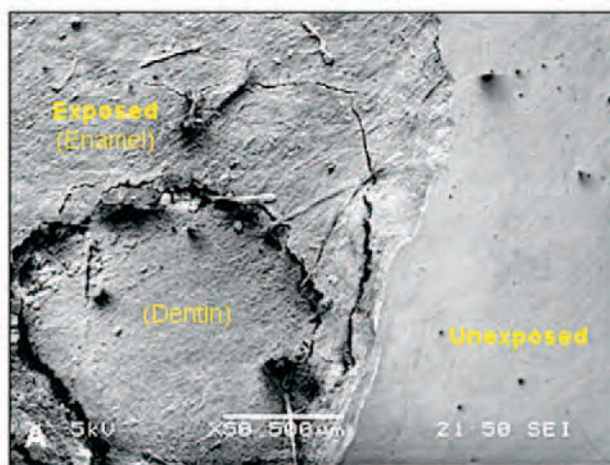
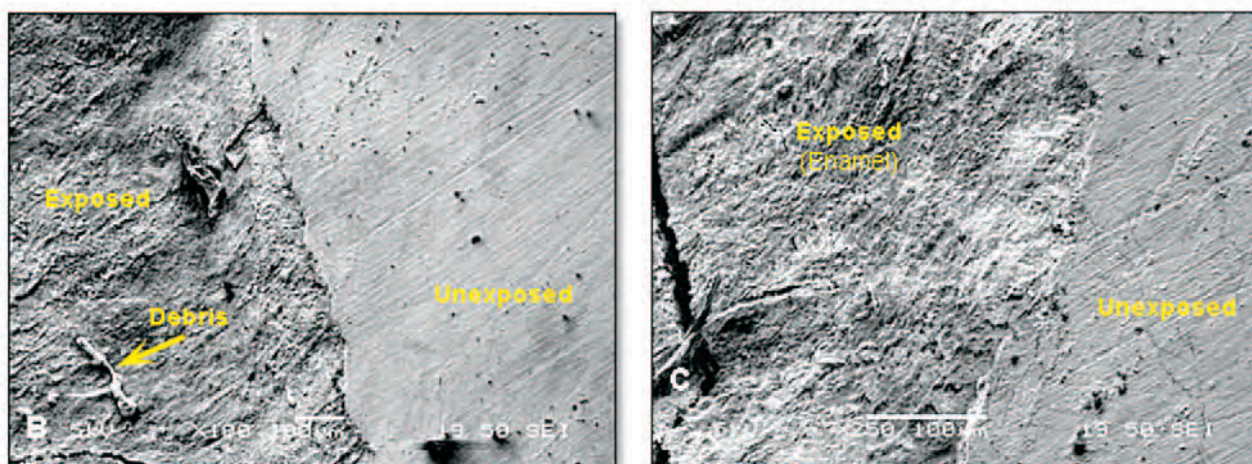


Figure 9A. SEM photograph (50x) revealing both surfaces following immersion in Gatorade®. Note enamel erosion to dentin substrate.



Figures 9B-C. SEM photographs (100x/250x) showing progressively higher magnifications of both exposed/unexposed (Gatorade®) enamel surfaces.

With extreme enamel dissolution visible, an abrupt ledge was noted between unexposed and exposed surfaces. Surface cracks and a residue coating were also visible. Drastic enamel dissolution from immersion in the Red Bull® high-energy drink with remaining surface residue particles were also distinguishable at progressively higher magnifications (Figures 10A through D).

A specimen immersed in tap water had minimal or no visible differences between the exposed/unexposed surfaces except for toothbrush striations (Figures 11 A and B).

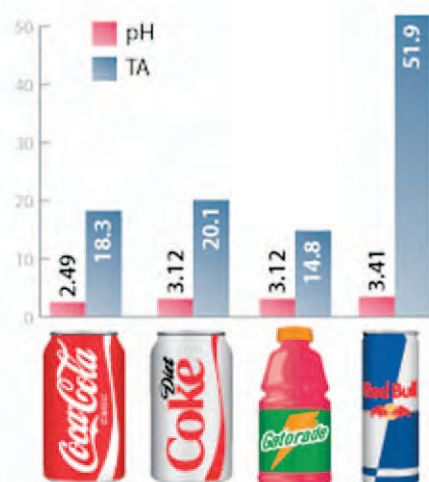
Discussion

Visual Results

The visual results reported in this study indicate carbonated cola beverages, sports and high-energy drinks cause changes in enamel surface morphology. These results could be extrapolated to a clinical diagnosis of dental erosion. The findings in this study are in agreement with other *in vitro* studies¹⁰⁻¹⁸ in which enamel dissolution from different soft drinks, especially carbonated cola beverages, were reported. Research by von Fraunhofer et al.²⁶ has indicated sports and high-energy drinks or beverages containing higher concentrations of citric acid are even more aggressive in this regard.

Acid Content of Beverages

In general, two methods to quantify the acid content of a beverage are the pH or initial acidity



and the TA or buffering capacity. Beverages with lower pH values generally have greater erosive effects on tooth structure; however, a measurement of the TA level is a more accurate method for detecting the erosive potential in a given beverage.²⁷⁻³⁰ The pH is a measure of the hydrogen ion concentration, while TA is the total number of acid molecules and determines the actual hydrogen ion availability for interaction with the tooth surface.³¹ The greater the buffering capacity, the longer time it will take for saliva to restore the pH value (salivary clearance).³²

Carbonated cola beverages, sports and high-energy drinks have been reported to have a low pH and a high buffering capacity. They are sweetened with highly refined carbohydrates and contain additional additives which together with sugar substitutes can contribute to enamel

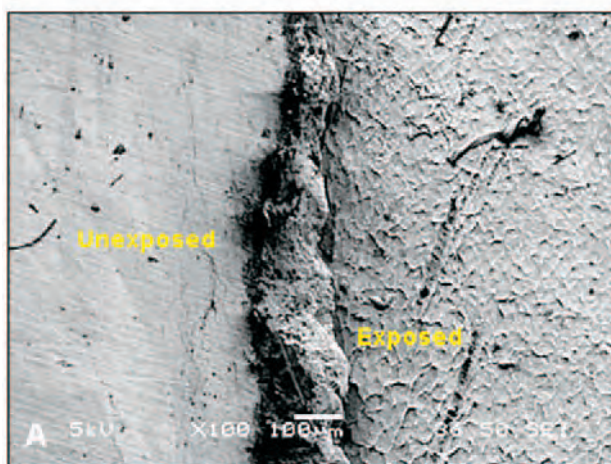


Figure 10A. SEM photograph (100x) showing exposed and unexposed surfaces following immersion in Red Bull®.

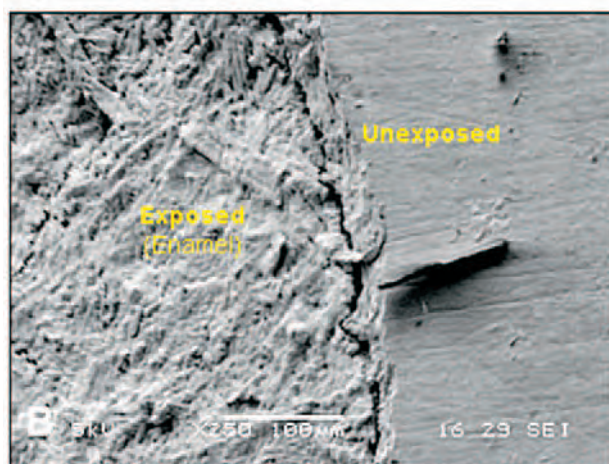


Figure 10B. SEM photograph (250x) showing exposed and unexposed enamel surfaces.

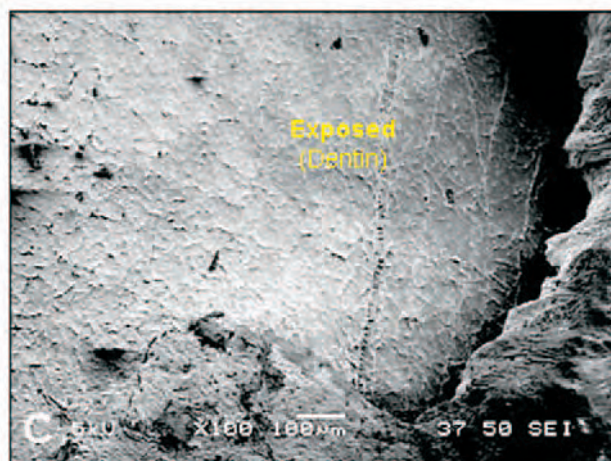


Figure 10C. SEM photograph (100x) of exposed dentin surface.

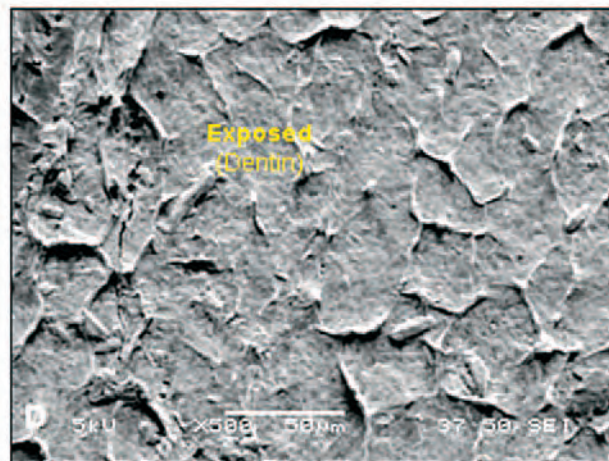
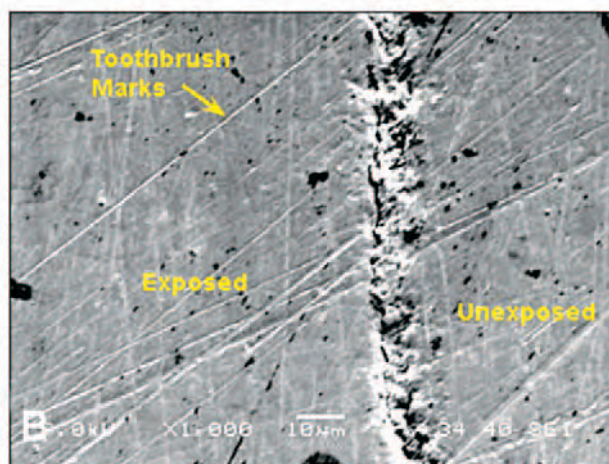
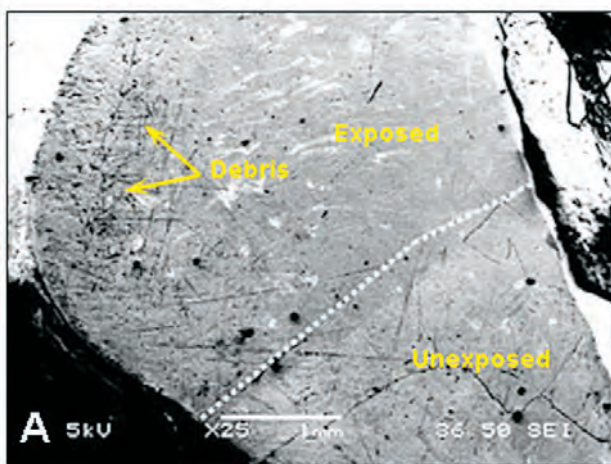


Figure 10D. SEM photograph (500x) of exposed dentin surface.



Figures 11A-B. SEM photographs (25x/1000x) of specimen following immersion in tap water. No surfaces changes are visible; however, surface striations are remarkable at 1000x magnification.

surface dissolution.^{10-18,27,33} Other factors involved with enamel surface dissolution and clinical erosion include the type, concentration, and amount of acid; chelating properties of the beverage ingredients; exposure frequency; duration of the exposure and temperature.^{32,34-40}

The results from the present study indicate beverage pH, TA, composition, exposure period, and possibly temperature (although immersion in different beverage temperatures were not conducted in the present study) were causative factors regarding enamel surface dissolution. Although the pH of the carbonated cola beverages (Coca-Cola Classic® and Diet Coke®) was lower than the Gatorade® and Red Bull® drinks, the dissolution patterns of the exposed enamel surfaces (carbonated beverages) was not as severe as compared to the other beverages. Carbonated beverages start with a lower pH but do not require as much titration as non-carbonated, fruit-based drinks containing multiple refined carbohydrates.¹⁴



The Red Bull® and Gatorade® drinks showed the greatest degree of enamel surface dissolution. Possible explanations for the higher degree of enamel dissolution as evidenced by Gatorade® and Red Bull® is the addition of high concentrations of refined carbohydrates (sucrose and glucose) by the manufacturer which promotes greater degrees of acid production and, in turn, higher buffering capacities. In addition, beverages containing citric acids have the ability

to irreversibly chelate (bind) calcium at higher pH values with the net effect of an accelerated loss of calcium from tooth structure, thus, maintaining the pH of the beverage below the threshold level (pH of 5.5) for erosion of enamel to occur.^{38,41} Red Bull® contains sodium citrate (sodium salt of citric acid), a buffering agent which can possibly aid in maintaining the pH levels in soft drinks. Citrates can also bind to calcium, promoting increased titratable acidity levels.

Since consumption of these soft drinks includes younger age groups, a great deal of attention has been focused on the susceptibility of permanent teeth to erosion and the intake of soft drinks. Immature teeth are porous and are more easily dissolved by acids until “conditioned” from continual exposure to salivary ions, causing enamel to become harder and less penetrable to acid assault.^{33,42}

The study protocol attempted to realistically simulate an adolescent-to-young adulthood experience of beverage consumption by immersing the specimens in the beverages studied for a realistic period of time. This clinical model probably provided a worst-case scenario but could extrapolate to possible clinical findings of a direct relationship of time (several years) and enamel destruction from beverage exposure. Furthermore, this study only considered chemical erosion, possibly understating possible concurrent tooth wear from other etiologic sources such as abrasion and attrition.

Conclusion

All beverages (Coca-Cola®, Diet Coke®, Gatorade®, and Red Bull®) tested showed varying amounts of enamel dissolution as verified by light microscope and SEM. The degree of enamel dissolution was recorded from greatest-to-least listed as: Red Bull®>Gatorade®>Coca-Cola Classic®>Diet Coke®. Although the experimental model was severe, results could be extrapolated to a clinical diagnosis of dental erosion.

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