Assessing the Effect of Low Calorie Soda Beverages on Primary Tooth Enamel: An *In Vitro* Study

Alexandra Korte */ Matina V Angelopoulou**/Georgios Maroulakos***

Objective: *The purpose of this study was to evaluate the effect of low calorie soda beverages on the enamel of primary teeth.* ***Study Design****: Fifty enamel slabs were prepared from twenty primary extracted teeth and were equally divided into five groups: a) 0.9% NaCl (Control), b) Coca-Cola Classic (Sucrose), c) Diet Coke (Aspartame), d) Zevia Cola (Erythritol), e) Coca-Cola Life (Stevia). Each specimen was exposed to the beverage for a total of sixty minutes. Enamel surface roughness was measured before and after the exposures using a LEXT OLS4000 3D Laser Measuring Microscope.* ***Results:*** *All tested sodas resulted to a statistically significant change on the surface roughness of the enamel (p =.000). However, this effect did not differ significantly between the different treatment groups (p =.103).* ***Conclusions:*** *Both regular soda and low calorie soda containing different commercial sweeteners appear to have an effect on the surface morphology of primary tooth enamel. Thus, it is important to discourage the intake of any type of soda as part of the dietary advice provided in the dental office.*

Keywords: *Low Calorie Soda Beverages, Primary Tooth Enamel, Erosion, Dental Caries.*

INTRODUCTION

Dental caries has been demonstrated to have a multi-factorial etiology, in which three primary factors: the host (saliva and teeth), the micro-flora (plaque) and the substrate (diet) together contribute to the initiation and progression of dental caries[1]. Due to the integral role diet plays in the development of dental caries, the reduction of sugar intake is an important area of focus for prevention provided by dental practitioners. Soda consumption is one of the dietary habits that has been found to increase the risk for dental caries[2]. For this reason, it is important to discourage the intake of soda drinks as part of the dietary advice provided to dental patients. This is even more crucial nowadays as the prevalence of soft drink consumption among children ages 6 to 17 years old has increased from 37% in 1977/1978 to 56% in 1994/1998[3]. Regular soda consumption has shown to increase with age into adolescence, and is higher among families of lower income and education levels[4]. In addition, greater than one-third of adults and 17% of youth in the United States are obese[5] and therefore, efforts to improve diet and lessen sugar consumption have also been of increasing focus amongst medical professionals.

Non-calorie or low-calorie commercial sweeteners have increased in popularity within the last decade as a means to replace the standard sucrose sweetening agent and subsequently address the overwhelming issues with obesity and dental caries. Saccharine, a sulphamide, was the first and most utilized commercial sweetener followed by the introduction of several other sweeteners over time[3]. The Food and Drug Administration (FDA) has now approved a total of five non- or low-calorie sweeteners including aspartame, saccharine, acesulfame potassium, sucralose, and neotame[6]. Furthermore, there are five non- or low-calorie sweeteners that are *generally recognized as safe* (GRAS) by the FDA including sorbitol, xylitol, erythritol, tagatose, and stevia[6].

Over time, many of these commercial sweeteners, including aspartame, stevia, and erythritol have been added to popular soda beverages. Aspartame was discovered in 1965, and approved by the FDA in 1981[8]. It is about 200 times sweeter than sucrose, therefore is used in small amounts, making its caloric addition negligible[7]. Stevia is another popular commercial sweetener that originates from the South American plant, *Stevia rebaudiana*[8]. The active ingredient is a white crystalline material exhibiting sweetness potency 200-300 times greater than sucrose[8]. Stevia is a natural, calorie-free sweetener that is lacking fermentable carbohydrates, and therefore does not contribute to acid production by oral bacteria[8]. Erythritol is a

* Alexandra Korte ,DDS, Pediatric Dentist, Children's Hospital of Wisconsin.
** Matina V Angelopoulou ,DDS, MS, MPH, Assistant Professor, Marquette University School of Dentistry, Division of Pediatric Dentistry.
*** Georgios Maroulakos DDS, MS, Assistant Professor, Marquette University School of Dentistry, Division of Prosthodontics.

Send all correspondence to:
Matina Angelopoulou
415 E Vine Str #304
Milwaukee, WI 53212
Phone:+1 214 79798898
E-mail: matinangelop@yahoo.gr

four carbon sugar alcohol that is about 70% as sweet as sucrose, and contains considerably less calories by weight[9].

In 2007-2008, the prevalence of beverage consumption containing low-calorie sweeteners increased from 6.1% to 12.5% among children, demonstrating a substantial gain in popularity of the taste and perceived benefits of these sweeteners[10]. With the variety and popularity of sweetened beverages increasing rapidly, it is important to understand their effects on oral health, and more specifically, the teeth.

Extensive research has been done on sugar alcohols including sorbitol and xylitol, with results suggesting both antimicrobial and limited cariogenic properties[11]. However, there is limited research available that directly addresses the erosive/cariogenic potential of other popular commercial sweeteners, especially in soda beverages. The purpose of this study was to evaluate the effect of low calorie soda consumption on the enamel roughness of primary teeth.

MATERIALS & METHOD

Procedure. This was an *in vitro* study that tested the effects of soda solutions on the enamel surface roughness of primary teeth under 3D laser microscope. The study received a notice of determination of not regulated status (872599-1) by the Institutional Review Board of Children's Hospital of Wisconsin, Milwaukee, Wisconsin.

Primary teeth sample collection and preparation

Twenty extracted primary teeth were collected from Children's Hospital of Wisconsin Pediatric Dental Clinic. The primary teeth used for the study were teeth that were extracted due to routine patient care indications, and were not associated with any type of patient identifier. Therefore, the collection of the primary teeth was considered non-invasive and did not require written consent from the parents and/or the child. Exclusion criteria for extracted teeth included primary teeth with large carious lesions affecting the majority of present enamel, and therefore unable to provide a sufficient amount of enamel for the prepared slabs. In addition, extracted primary teeth with visible enamel defects such as enamel hypoplasia or decalcification were excluded. Extracted teeth were disinfected (Cavicide, Metrex Research, LLC, Orange, CA), and stored in a well sealed specimen container with 0.9% NaCl until use for no longer than thirty days. The roots of the teeth were placed in an acrylic resin matrix (Triad Tru-tray, Dentsply Sirona USA, York, PA) and cured (Triad 2000 VLC, Dentsply Sirona USA, York, PA) for two minutes. This provided stabilization of the tooth during enamel slab preparation. Specimens were prepared into enamel slabs using diamond burs with a high speed handpiece and water irrigation. Slabs were prepared to roughly measure about 2 mm x 2 mm x 1 mm. Power analysis determined that 10 specimens per group were required to detect differences at α=.05 and a power of 80%.

Microscopic pre-evaluation of specimens

After slab preparation, all specimens were carefully examined underneath a 3D Laser Measuring Microscope (LEXT OLS4000, Olympus, Tokyo, Japan) under a 20x objective lens. Pre-intervention surface roughness was calculated. S_a was the particular statistical parameter used, which represents an arithmetic average of the three dimensional surface roughness of a specimen. Each slab was measured under the microscope three separate times, and an average S_a was calculated.

Treatment groups

The slabs were randomly assorted into the following treatment/exposure groups: a) 0.9% NaCl (Control), b) Coca-Cola® Classic (The Coca-Cola Company, Atlanta, GA, USA) (Sucrose), c) Diet Coke® (The Coca-Cola Company, Atlanta, GA, USA) (Aspartame), d) Zevia® Cola (Zevia LLC, Los Angeles, CA, USA) (Erythritol), e) Coca-Cola Life™ (The Coca-Cola Company, Atlanta, GA, USA) (Stevia).

Each sample group was coded with an identifier on the bottom of a sealed 28- microwell plate. The identifier was non-visible to examiner during microscopic analysis to enable blind measurements of treatment groups during data collection. The enamel slabs in each treatment group were exposed to 2,000 micro-liters of the associated beverage. The slabs were exposed to the different treatments for a total of sixty minutes to simulate the estimated amount of time a child's teeth would be exposed if they were consuming soda over the course of fourteen days[12].

pH measurement. The pH was measured for each of the treatment group beverages. A pH meter (Seven Excellence, Mettler Toledo, Columbus, OH) was used to accurately calculate pH. In addition, pH indicator strips were used to validate correct pH measurements.

Microscopic post-evaluation of specimens

Following the exposure period, each specimen was thoroughly rinsed with distilled water and dried. Each slab was then examined underneath the 3D Laser Measuring Microscope (LEXT OLS4000, Olympus, Tokyo, Japan) to measure post-intervention surface roughness (S_a). Each slab was again measured three separate times and an average S_a was calculated.

Qualitative analysis

Photomicrographs of the enamel slabs were analyzed and the pre-intervention slabs were compared to the post-intervention slabs. The changes seen in enamel surface morphology were subjectively classified by two blinded examiners into three different groups (no change, mild, and moderate) based on visual analysis.

Statistical analysis

The purpose of the statistical analysis was to identify differences between and within the groups according to the objectives stated. Software (SPSS 16.0) was used to conduct the statistical analysis. Statistical significant differences were investigated at a level of $p < .05$. Levene's statistical test was used to evaluate homogeneity of the data. Results indicated that the data was not homogenous and therefore, a non-parametric Friedman statistical test was utilized for comparison of the differences pre- and post-intervention between groups.

RESULTS

***pH measurement*.** The calculated pH measurements of each treatment group are outlined in Table 1. The control group (.9% NaCl) exhibited the highest pH measurement. The Coca-Cola Classic group (sucrose), Zevia Cola group (erythitol), and Coca-Cola Life (stevia) had the lowest or most acidic pH measurements.

Descriptive statistics. Mean surface roughness values for each group pre and post-intervention are summarized in Table 2 and illustrated in Figure 1.

Table 1. pH measurements of each treatment group and control group.

Group	pH	pH Indicator Strips (range)
Control	6.595	6-7
Coca-Cola Classic	2.629	2-3
Diet Coke	3.148	3-4
Zevia Cola	2.771	2-3
Coca-Cola Life	2.754	2-3

Table 2. Descriptive results for surface roughness (S_a) before and after treatment exposures.

Group	N	Mean	25th Percentile	Median	75th Percentile
Control (Pre)	10	6.1229	4.1571	6.077	7.7162
Control (Post)	10	7.7406	5.5038	6.2953	10.6917
Coca-Cola Classic (Pre)	10	8.8125	4.5218	7.6472	13.6564
Coca-Cola Classic (Post)	10	9.1986	6.5942	8.4265	10.7601
Diet Coke (Pre)	10	6.6935	4.9125	6.1077	8.5603
Diet Coke (Post)	10	7.1463	5.707	6.5953	8.7036
Zevia Cola (Pre)	10	10.3355	6.1484	8.751	13.8059
Zevia Cola (Post)	10	10.3581	7.8995	9.4953	13.8696
Coca-Cola Life (Pre)	10	6.6291	5.731	6.8508	7.6895
Coca-Cola Life (Post)	10	7.3096	6.3645	7.1743	8.2891

Inferential statistics. Statistical analysis within the same group showed that all treatment solutions had a statistically significant effect on the surface roughness of the enamel (p =.000). Surface roughness changed the least amongst the Coca-Cola Classic soda (sucrose) and Zevia Cola soda (erythritol) treatment groups. Surface roughness changed the most amongst the Coca-Cola Life (stevia) and Diet Coke (aspartame) treatment groups. However, this effect between the treatment groups did not differ significantly (p = .103).

Qualitative analysis. In this qualitative analysis, the control group had no visual change on the enamel (Figure 2). Coca-Cola Classic soda (sucrose) (Figure 3) and Zevia Cola soda (erythritol) (Figure 4) treatment groups were found to have mild change. Coca-Cola Life (stevia) (Figure 5) and Diet Coke soda (aspartame) (Figure 6) treatment groups were found to have moderate surface change.

Figure 1. Boxplot of change in surface roughness amongst the five treatment groups (1 = Control Pre; 2 = Control Post; 11 = Diet Coke Pre; 12 = Diet Coke Post; 21 = Coca-Cola Classic Pre; 22 = Coca-Cola Classic Post; 31 = Zevia Cola Pre; 32 = Zevia Cola Post; 41 = Coca-Cola Life Pre; 42 = Coca-Cola Life Post)

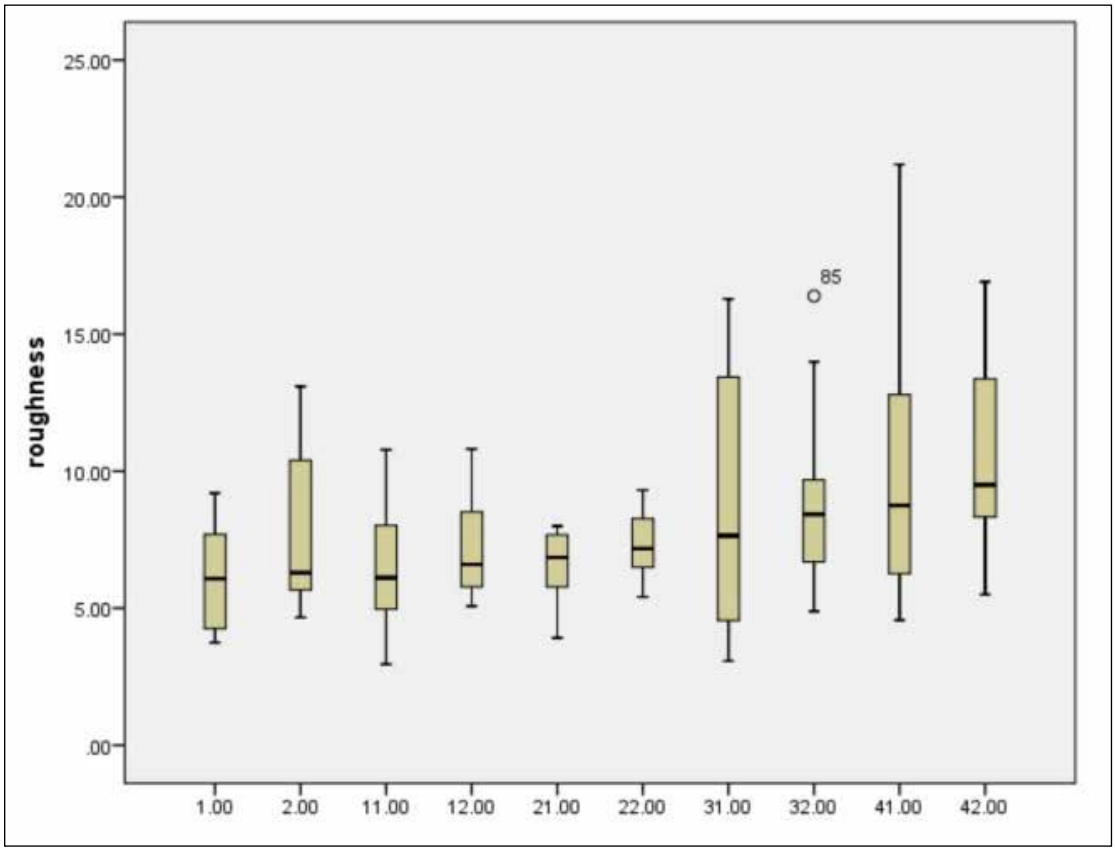


Figure 2. Photomicrographs of enamel slabs before (a) and after (b) exposed to .9% NaCl (600 x 600 µm).

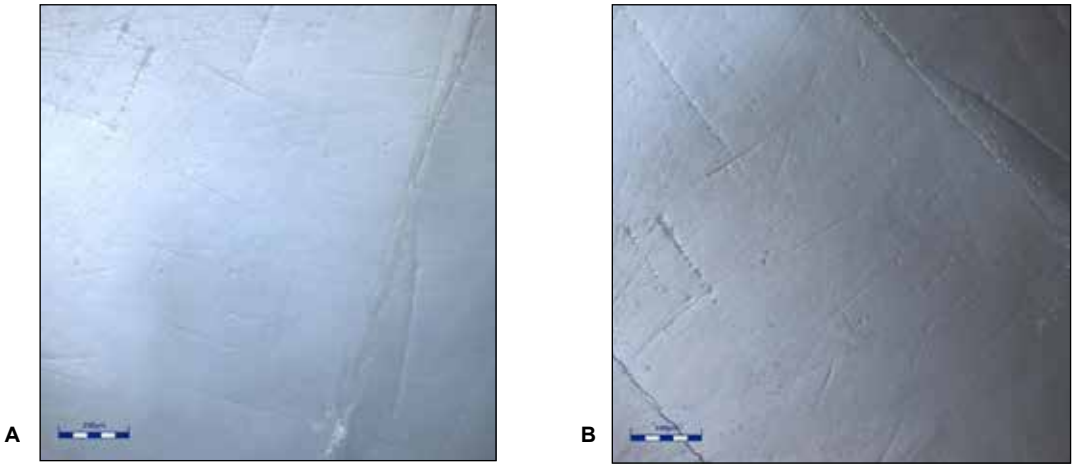


Figure 3. Photomicrographs of enamel slabs before (a) and after (b) exposure to Coca-Cola Classic containing sucrose (600 x 600 µm). Arrows indicate examples of locations with significant textural change.

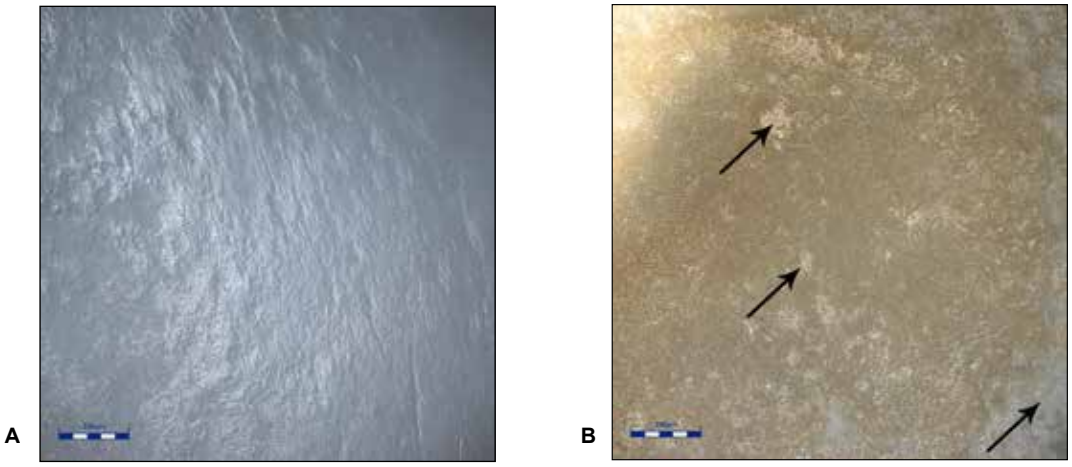


Figure 4. Photomicrographs of enamel slabs before (a) and after (b) exposure to Zevia Cola containing erythitol (600 x 600 µm). Arrows indicate examples of locations with significant textural change.

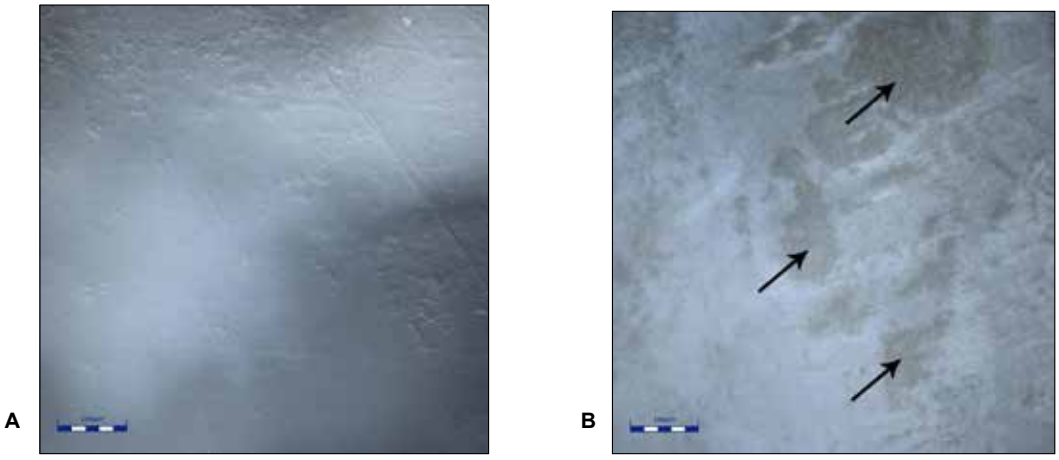


Figure 5. Photomicrographs of enamel slabs before (a) and after (b) exposure to Coca-Cola Life containing stevia (600 x 600 µm). Arrows indicate examples of locations with significant textural change.

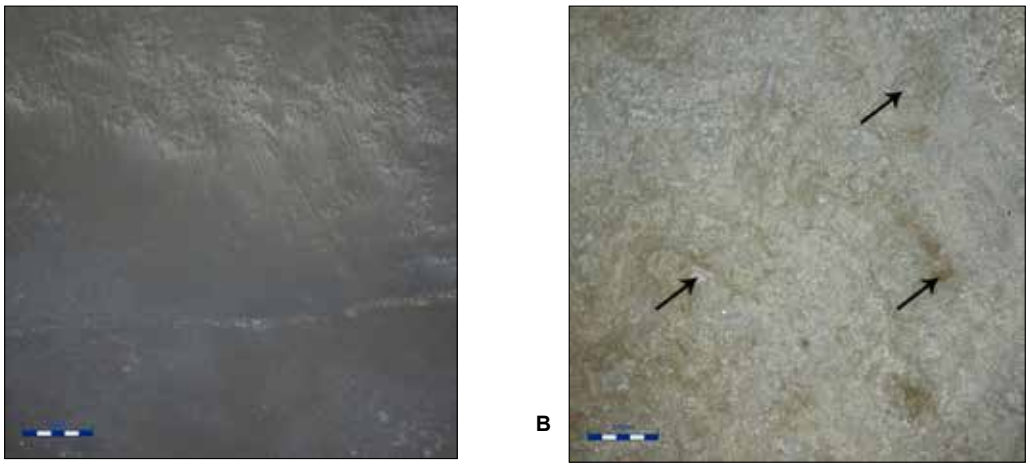
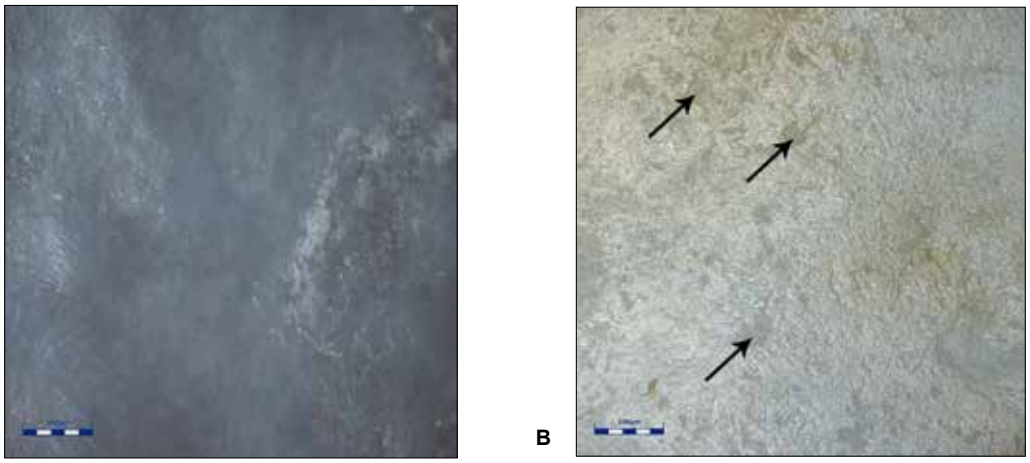


Figure 6. Photomicrographs of enamel slabs before (a) and after (b) exposure to Diet Coke containing aspartame (600 x 600 µm). Arrows indicate examples of locations with significant textural change.



DISCUSSION

Both regular soda and low calorie soda containing different commercial sweeteners appear to have an effect on the surface morphology of primary tooth enamel; however the difference between each treatment group was not statistically significant.

The methodology used was based on the study of Giacaman *et al* who showed the cariogenic potential of commercial sweeteners on enamel in an experimental biofilm caries model. This *in vitro* study exposed bovine enamel slabs to different commercial sweeteners in tablet or powder form and measured pre and post-intervention surface micro-hardness to evaluate enamel demineralization[6]. Our study investigated similar sweeteners, however did not use the sweeteners in their powder or tablet form, but rather within popular soda beverages which is the most common method of intake among the pediatric and adolescent populations. Also, our study is the first to specifically focus on primary human teeth, as previous studies have used bovine enamel slabs[6]. Therefore, the results are more applicable in regards to the effects of soda consumption on primary tooth enamel. Treatment exposure times were based on the calculation used by Kitchens *et al* ,They investigated the effect of carbonated beverages on the erosion characteristics of dental enamel[12]. It was estimated that the annual exposure of enamel to soft drinks is about 90,000 seconds (25 hours) *per* year, which equals to 30 minutes *per* week[12]. Therefore, our study implemented an exposure time of 60 minutes to simulate an estimated amount of time a child's teeth would be exposed to soda over the span of fourteen days.

A 3D microscope was used as the primary measurement tool. In the past, profilometer has been used to observe enamel surface changes caused by various tooth whitening modalities[13]. A 3D microscope would provide more accurate data, since a profilometer's stylus diameter cannot measure all micro asperities of a surface.

Results from this study suggest that both regular soda and low calorie soda containing different commercial sweeteners have an effect on the surface morphology of primary tooth enamel. This finding is in agreement with previous studies that have found commercial sweeteners to have some effects on enamel[6, 12]. In the present study no difference between the treatment groups was found statistically significant. This finding is in agreement with the results of Kitchens' study that found no difference between groups on the erosive effects of different beverages. However, Giacaman *et al* reported that commercial sweeteners cause less enamel demineralization in comparison to sucrose[6].

Temperature has been reported to affect the degree of dissolution of enamel surface structure. Specifically, higher temperatures result in an increased solubility and diffusion coefficient rate of ions in aqueous solution through enamel[12]. Other study designs have utilized an incubator which immerses the enamel specimens in at 37 ^{o}C to simulate an oral environment. In this study, the enamel specimens were exposed to the different solutions and stored at room temperature which may have lessened the magnitude of effects on enamel surface morphology.

To investigate potential confounding variables, the pH of the distilled water that was utilized to rinse the specimens was measured. A pH indicator strip measured a pH within a range of 5 to 6 which reportedly is accurate for distilled water[14]. Distilled, de-ionized, and tap water cannot be considered as pure water since their exposure to air causes CO_2 gas to begin dissolution into it, leading to the formation of carbonic acid[14]. The slight difference in the surface roughness measurements of the enamel specimens serving as the controls could be attributed to the slight acidic nature of the distilled water that was used to rinse the specimens. Furthermore, it may have had an effect on the magnitude of change in surface roughness amongst the other test groups.

The *in vitro* experimental design and simulated time of exposure are also limitations of this study. The *in vitro* methodology exposed the teeth to the treatment groups for a specific time period without consideration for rate of soda consumption, movements within the mouth during swallowing, neutralization by saliva, and remineralization potential of saliva. Therefore, clinical application of the results should be acknowledged with caution. Other limitations include the potential lack of complete flatness to each sample, and the inability to measure the exact same points on each sample before and after.

In the future, implementation of a longer exposure time in addition to incubation of the enamel slabs at 37 ^{o}C may help increase the validity of the results. Furthermore, an *in vitro* design using saliva would increase the clinical applicability of the results.

CONCLUSION

Overall, the results clearly suggest that sodas containing low calorie sweeteners do have an effect on primary tooth enamel. However the magnitude and difference of that effect in comparison to conventional soda beverages necessitates further investigation.

Despite the limitations of this study, soda beverages containing low calorie sweeteners were found to affect primary teeth enamel and for this reason it is important to discourage the intake of any type of soda as part of the dietary advice provided in the dental office.

REFERENCES

1. West NX, Joiner A. Enamel mineral loss. J Dent 42 Suppl 1: S2-11, 2014.
2. Sohn W, Burt BA, Sowers MR. Carbonated soft drinks and dental caries in the primary dentition. J Dent Res 85: 262-6, 2006.
3. French SA, Lin BH, Guthrie JF. National trends in soft drink consumption among children and adolescents age 6 to 17 years: prevalence, amounts, and sources, 1977/1978 to 1994/1998. J Am Diet Assoc 103: 1326-31, 2003.
4. Han E, Powell LM. Consumption patterns of sugar-sweetened beverages in the United States. J Acad Nutr Diet 113: 43-53, 2013.
5. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 311: 806-14, 2014.
6. Giacaman RA, Campos P, Munoz-Sandoval C, Castro RJ. Cariogenic potential of commercial sweeteners in an experimental biofilm caries model on enamel. Arch Oral Biol 58: 1116-22, 2013.
7. Shankar P, Ahuja S, Sriram K. Non-nutritive sweeteners: review and update. Nutrition 29: 1293-9, 2013.
8. Goyal SK, Samsher, Goyal RK. Stevia (Stevia rebaudiana) a bio-sweetener: a review. Int J Food Sci Nutr 61: 1-10, 2010.
9. Roberts MW, Wright JT. Nonnutritive, low caloric substitutes for food sugars: clinical implications for addressing the incidence of dental caries and overweight/obesity. Int J Dent 2012: 625701, 2012.
10. Sylvetsky AC, Welsh JA, Brown RJ, Vos MB. Low-calorie sweetener consumption is increasing in the United States. Am J Clin Nutr 96: 640-6, 2012.
11. Gupta C, Prakash D, Gupta S, Goyal S. Role of Low Calorie Sweeteners in Maintaining Dental Health. Middle-East Journal of Scientific Research 11: 342-346, 2012.
12. Kitchens M, Owens BM. Effect of carbonated beverages, coffee, sports and high energy drinks, and bottled water on the in vitro erosion characteristics of dental enamel. J Clin Pediatr Dent 31: 153-9, 2007.
13. Kwon SR, Kurti SR, Oyoyo U, Li Y. Effect of various tooth whitening modalities on microhardness, surface roughness and surface morphology of the enamel. Odontology 103: 274-9, 2015.
14. Kulthanan K, Nuchkull P, Varothai S. The pH of water from various sources: an overview for recommendation for patients with atopic dermatitis. Asia Pac Allergy 3: 155-60, 2013.