

Modification of a Soft Drink by Adding Calcium Carbonate Nanoparticles to Prevent Tooth Erosion

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Abstract

Statement of Problem: One of the factors in dental erosion is consumption of acidic soft drinks. Although the effects of various additives to acidic soft drinks for the prevention of tooth erosion have been assessed, little data have been published on the possibility of preventing the erosion through soft drinks containing calcium-carbonate nanoparticles.

Objectives: To examine the erosive factors of 7up soft drink and to determine the possibilities of decreasing or preventing the erosion phenomenon of the soft drink containing calcium-carbonate nanoparticles.

Materials and Methods: 7up soft drink was assigned as control and a set of solutions containing 0.04, 0.05, and 0.06 vol % of the nano-particles were assigned as the experimental solutions. The pH, titratable acidity (TA), calcium and phosphorus concentrations and degree of saturation with respect to enamel hydroxyapatite (DS_{En}) were calculated. These parameters refer to assessment of erosive potential of the soft drinks. The erosion potential was evaluated based on the micro-hardness and the structural changes of the tooth surface using scanning electron microscopy (SEM). Data were analyzed using Kruskal-Wallis H test, and Bonferroni-adjusted Mann-Whitney U test.

Results: An increase in the nano-additive content of the solutions increased pH and DS_{En} ; however, it decreased the TA ($P < 0.05$). There was a significant difference between the micro hardness in the control and experimental groups ($p < 0.001$). SEM images revealed less surface erosion of the specimens stored in the higher nano-additive concentrations. The modified drink containing 0.06% nano-additive revealed the highest hardness with no evidence of tooth erosion.

Conclusions: Adding calcium carbonate nanoparticles to soft drinks can be considered as a novel method to reduce or prevent tooth erosion.

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Introduction

In the past few decades, consumption of acidic drinks has increased considerably especially among young people, which can lead to tooth erosion [1]. Tooth

erosion is defined as an irreversible loss of hard tissue due to a chemical process without involvement of microorganisms [1-3]. Most of the investigators believe that one of the fundamental external factors in dental erosion is consumption of acidic foods

and drinks [1]; hence, it is crucial to examine and modify these types of drinks to reduce the risk of dental erosion [4]. In clinical studies [1], drinks with low pH, such as cola-based carbonated drinks, have often been considered as the drinks mostly related to dental erosion. It has also been proposed that the erosive potential of soft drinks may be reduced by some drink modifiers [1]. Other studies have also shown that, based on the calcium hydroxyapatite isothermal solubility, the enamel can be dissolved in the absence of calcium ion, even at pH=8[5]. Rather than pH, there are various parameters which may affect dental erosion, such as concentration of calcium and phosphate ions, degree of saturation with respect to dental enamel hydroxyl apatite (DS_{en}), and the type and the amount of the acid [1-6].

Larsen *et al.* [4] claimed that calcium and phosphorus could be added to orange juice to prevent dental erosion. In addition, they compared the obtained results with those of another study [3] about non-softened enamel surface in contact with yogurt (pH=1.4) due to having the superior concentration of calcium and phosphate. Calcium and phosphate contents saturate the drink with respect to apatite [3]. Eggshell is an inexpensive material that mainly consists of calcium carbonate and can be widely used in the commercial production [7]. Eggshells are mainly made of two parts: The mammillary matrix (i.e., eggshell membrane) consisting of interwoven protein fibers and spherical masses, and the spongy matrix (i.e., calcified eggshell) made of calcium carbonate crystals. The chemical composition of eggshell has been reported in numerous studies with some differences; however, it is generally composed of: calcium carbonate (94 %), magnesium carbonate (1 %), calcium phosphate (1 %), and organic material (4 %) [8-11].

In fact, one way to protect the enamel surface is to neutralize the acid to prevent tooth erosion. In addition, due to other chemical reactions, a protective layer may be formed on the enamel against the acid when nanoparticles are used. In a few previous studies, nano-sized hydroxyapatite (nano-HA) has shown repairing effect on incipient dental caries and it has also been used for sport drink modifications [12-14].

In the current study, three main objectives were followed: (1) examining the erosive factors of 7up soft drink, (2) determining the critical properties related to the erosion behavior, and (3) identifying the possibilities of decreasing or preventing the erosion phenomenon of the soft drink containing calcium-carbonate nanoparticles. Nanoparticles extracted from eggshells, were added to 7up soft drink as a source of calcium. The importance of using nanoparticles is that they have a higher surface area and consequently higher solubility than larger particles [15]. Hardness tests were performed to determine the hardness of the

enamel surface and compare them with each other. A scanning electron microscope (SEM) was also used to examine surface degradation of the enamel. The null hypothesis was that adding the eggshell nanoparticles to the soft drink in different concentrations would not reduce the dental erosion caused by the soft drink.

Materials and Methods

Nanoparticle Preparation by Milling

The eggshells were washed thoroughly; the organic membranes were removed and dried in air for 2 hours. For primary crushing, electrical milling was used, and then the prepared powder was milled by a high energy planetary ball mill (SEPAHAN-84D Mixer Mill-Isfahan-Iran) at various time intervals and a specified rotational speed. For more information about the eggshell milling, refer to our previous work [16]. X-ray diffraction (XRD) was performed to measure the crystal size and determine the phases of eggshells. The specification of the XRD spectrometer which was used is K_{α} line of copper with the wavelength, $\lambda=1.54056 \text{ \AA}$ (X Pert, Tokyo, Japan). The powder particles were measured using a laser particle size analyzer (LPSA) that works on the basis of dynamic light scattering in which light is scattered from particles, suspended in a fluid (HORIBA- LB550, Tokyo, Japan).

Physiochemical Properties

The soft drink used in this study was 7up, which contained citric acid as one of its components. The drink was divided into four groups, a control solution of pure 7up, and three experimental solutions of the drink containing 0.04, 0.05, and 0.06 Vol. % nano-additives. The pH of the drinks was measured at room temperature with a pH meter. Titratable acidity (TA) was measured by adding a solution of 1M NaOH to 20ml of each experimental solution until the pH reached 7. Both pH and TA were determined three times for each drink. Calcium and phosphorous concentrations in each experimental solution were measured by using atomic absorption spectroscopy. The DS_{en} of each experimental solution was calculated by pH, calcium and phosphorous concentrations and a modified PHREEQ computer program based on the Parkhurst approach [17].

Tooth Preparation

Sixty freshly extracted non-carious permanent human molars were collected from dental clinics. The teeth were stored in normal saline at room temperature immediately after extraction, disinfected, cleaned with an ultrasonic scalar and then stored in 0.1% chloramine T at 4°C until needed. The teeth were first sectioned at the cemento-enamel junction (CEJ) using a water-cooled diamond-bladed saw at high speed in order to remove the root. The teeth were examined by

optical microscope (Leitz-12ME) and the samples with crack, pit, or any abraded lesions were excluded. Then the enamel surface on the lingual and buccal aspect of each crown was sectioned perpendicular to the occlusal surface in order to obtain 2 samples of less than 2mm in thickness. The specimens were embedded in an epoxy casting resin, polished manually with a circular motion on both sides with wet 600, 1000, 1500 and 2000- grit silicon carbide paper until the enamel surface was exposed. The teeth were covered with acid-resistant nail varnish to form an occlusal-half and a gingival half leaving a 3×2mm² central window.

Forty-eight specimens were randomly selected among the prepared samples; the primary microhardness values of all specimens were measured using a Vickers micro-hardness tester (KOOA-MH1-Tehran-Iran). Indentations of a 50grams force for 15 seconds were obtained at three different points with three times of trace diameter apart from each other. Then, twelve specimens were randomly allocated to each group of solution including control (A), and immersed for 24 hours (I) with constant stirring at room temperature. The specimens were washed with distilled water, and second micro-hardness measurement was recorded. The change in Vickers micro-hardness was determined using the following equation:

$$VMH\% = \frac{VMH_1 - VMH_2}{VMH_1} \times 100$$

In order to observe the changes in the structure of the teeth enamel, one specimen was randomly selected from each group and examined under a SEM (LEICA CAMBRIDGE LTD-360-97-01-London, UK) with 5000X.

Statistical Analysis

Levene and Kolmogorov-Smirnov tests were used to assess the homogeneity of variances and normality assumptions. Kruskal-Wallis H test was used for the overall comparison of groups. For pairwise comparisons, Bonferroni-adjusted Mann-Whitney U test was employed. The significant level was set at 0.05. In all statistical analyses, a statistical package (SPSS V.18.0-Chicago-USA) was used.

Results

Laser Particle Size Analyzer

Figure 1a shows the particle size distribution of the powder made by electrical milling in the range of 1000 to 6000nm. By starting the planetary ball milling after 2 hours (Figure 1b), there was a shift to the left for the diagram and the particle size distribution was in the range of 20 to 80nm. By increasing the time of milling, the diagrams (c-e) shifted to the right representing an increase in the particle size. Thus, the optimum milling time for reaching a nanometer scale was chosen as 2 hours considering the fact that the particle size before planetary ball milling was approximately in the range of 1000 to 6000nm.

XRD Characterization

Figure 2 represents the XRD pattern for eggshells, which verifies the presence of pure calcium carbonate (CaCO₃) phase. Sharp, narrow and pronounced peaks shown in this pattern represent the high degree of crystallinity of the powder. On the other hand, no peak was found showing the presence of compounds apart from CaCO₃ that indicates the high purity of CaCO₃ in eggshells.

Table 1 classifies different concentrations of drinks and represents the mean values of all parameters for each group. Group A showed the lowest pH but it

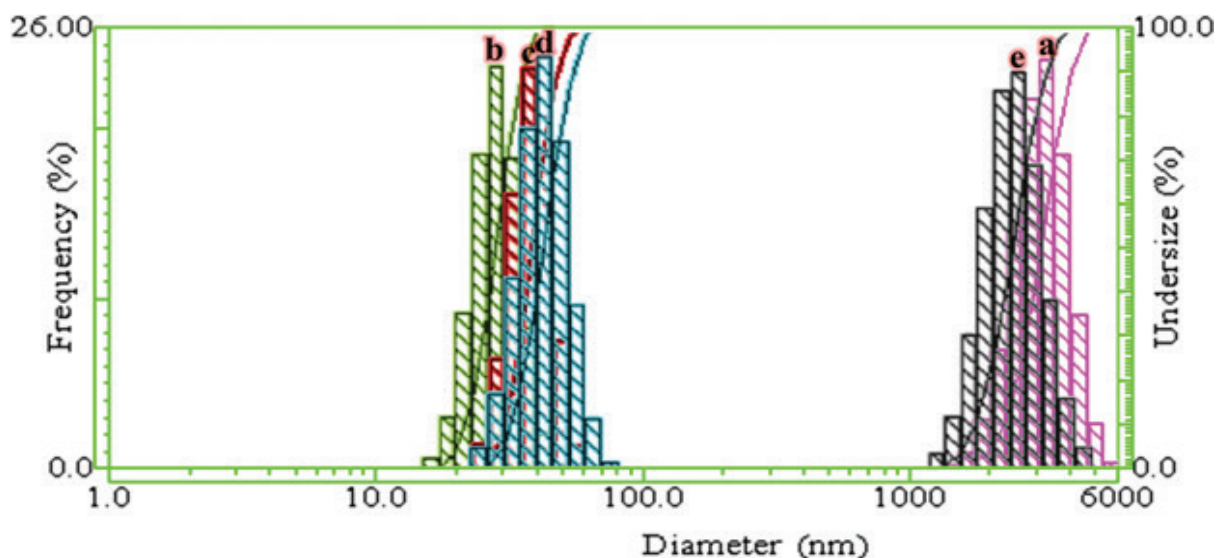


Figure 1: The results of LPSA analysis after immediate electrical milling (a) and after 2 (b), 4 (c), 6 (d), and 8 hours (e) planetary ball milling.

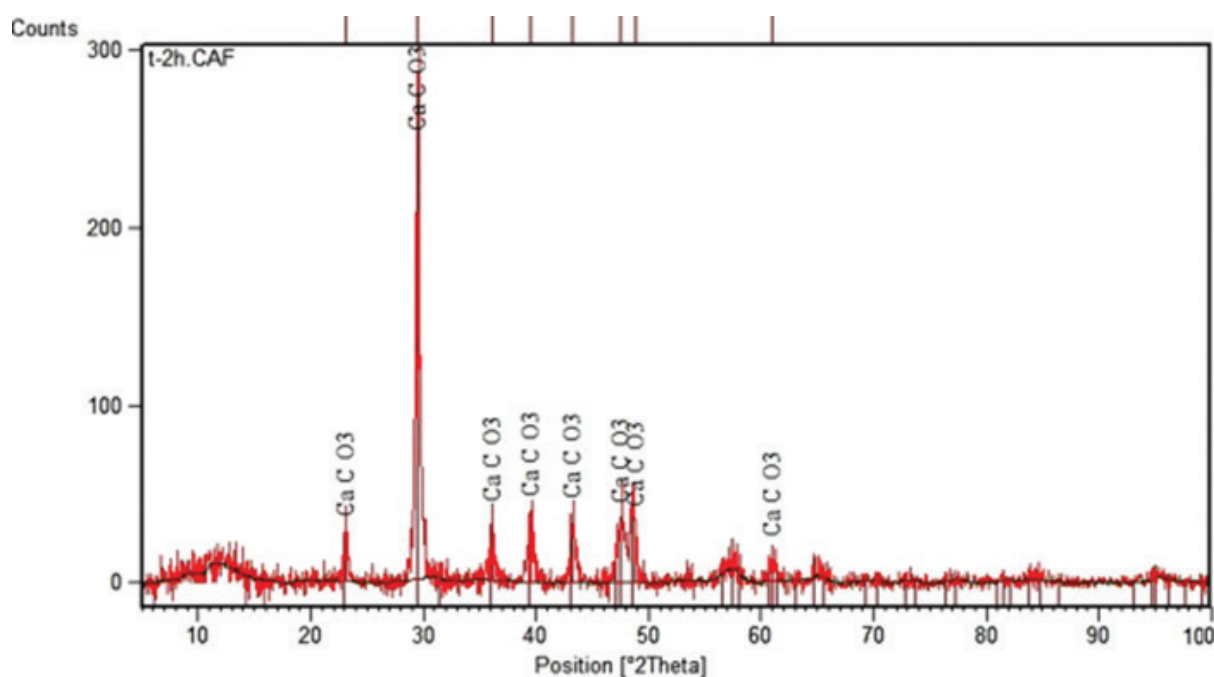


Figure 2: The XRD pattern of eggshell powder after 2 hours planetary ball milling

Table 1: Mean values of pH, Titratable Acidity (TA), Concentration of calcium and phosphorous, and Degree of saturation with respect to hydroxyapatite of the enamel (DS_{En}) for all groups.

Soft Drink (Groups)	pH	TA	Calcium (m mol/lit)	Phosphorous (m mol/lit)	DS_{En}
A	2.96 ^A	0.72 ^A	0.11 ^A	0.003 ^A	0.005 ^A
B ₁	4.37 ^B	0.53 ^B	5.42 ^B	0.050 ^B	0.068 ^B
B ₂	4.73 ^{BC}	0.49 ^{BC}	6.77 ^B	0.062 ^B	0.098 ^B
B ₃	5.10 ^C	0.40 ^C	8.17 ^C	0.072 ^B	0.890 ^C

A: Soft drink with no additive, B1: Soft drink containing 0.04 vol.% additives, B2: Soft drink containing 0.05 vol.% additives, B3: Soft drink containing 0.06 vol.% additive, Different letters indicate a statistically significant difference between the groups in each column

Table 2: Mean±(SD) Vickers micro-hardness of the enamel specimens at baseline, after 24 hours immersion in solutions, and the variation of percentage.

Soft drink (groups)	Baseline	24 hours	(VMH%)	p*
A	325.5±(7.2)	17.3±(4.8)	95 ^a	<0.001
B ₁	329.0±(7.8)	80.7±(18.1)	73 ^b	<0.001
B ₂	323.6±(8.5)	179.3±(26.6)	42 ^c	<0.001
B ₃	322.6±(9.7)	338.2±(38.4)	2 ^d	>0.227

*Kruskal-Wallis H test, Different letters indicate a statistically significant difference between the groups (Mann - Whitney U test)

significantly increased ($P<0.05$) as the concentration of $CaCO_3$ nanoparticles was increased. The highest TA was obtained for the control group (A) and its value significantly decreased ($P<0.05$) by increasing the amount of $CaCO_3$ nanoparticles. By adding more $CaCO_3$, more Ca and P were released in the drink and consequently the concentration of nanoparticles increased significantly ($P<0.05$). The DS_{En} parameters for the experimental groups were 13.41, 19.2 and 174.5 times more than the control group, respectively. There was a significant difference ($P<0.001$) in Vickers micro-hardness (VMH) between the baseline and

experimental groups (Table 2) except for the group B₃ with 0.06 % nano-additive ($P>0.227$). There were significant differences in the percentage of VMH values between the groups ($P<0.001$). There was a decrease of 95, 73, and 42.4 VMH% for the A, B₁, and B₂ groups, respectively; however, it was increased 2 % for the B₃ group.

SEM Evaluation

Figure 3 shows the SEM images of the tooth enamel surface of all groups. Figure 3.a shows an acid-eroded surface that conceives clear honeycomb

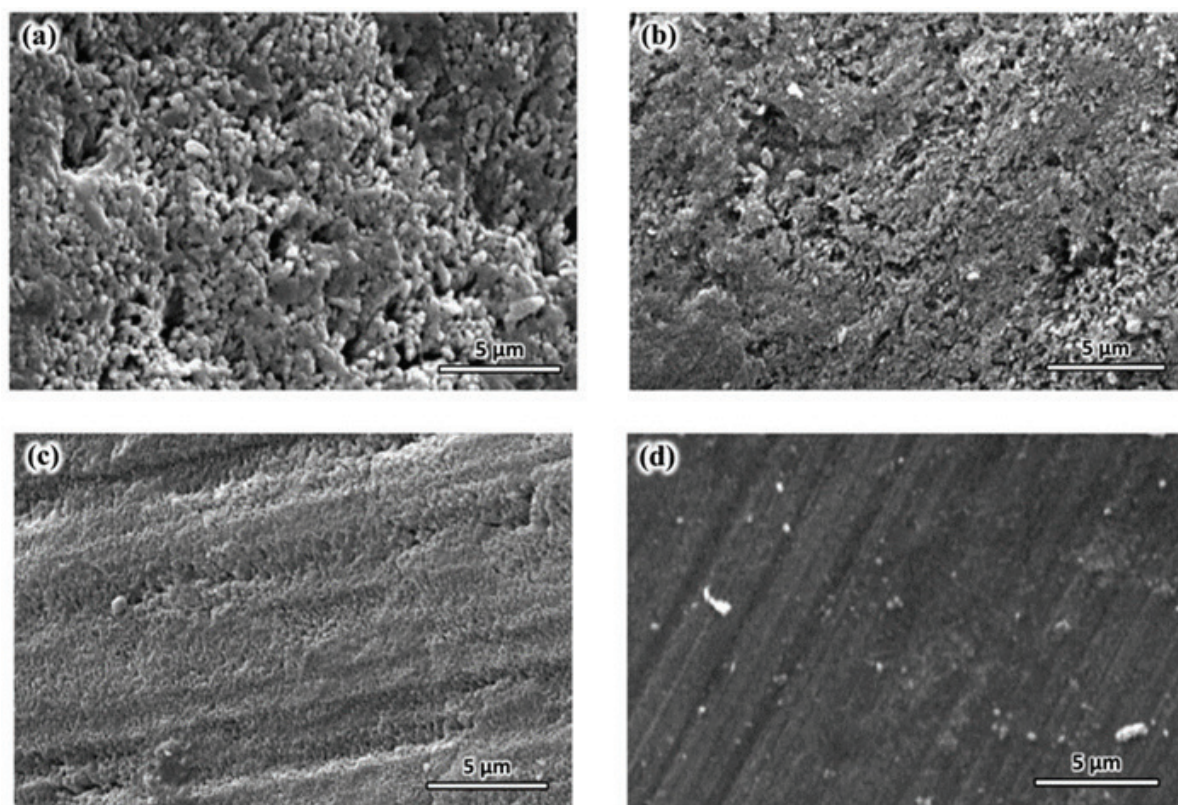


Figure 3: The SEM images of the enamel surface after subjected to pristine drink (a) and 0.04, 0.05, and 0.06vol% nano CaCO₃ (b-d, respectively).

structure, which indicates the powerful etching effect of untreated drinks on the enamel surface. In the group with 0.04 % nano-additive (Figure 3b), the amount of erosion and depth of the holes were a little less than those in the control group. The depth of holes in the 0.05 % nano-additive specimen (Figure 3c) was less than that of the two previous groups; no damage was observed in the 0.06% nano-additive group (Figure 3d).

Discussion

In this in-vitro study, we focused on the properties of the 7up drink in relation to its erosive potential in comparison with its modification by adding CaCO₃ nanoparticles. The potential of dental erosion caused by 7up drink was decreased as the nano-additive concentration was increased. As a result, the null hypothesis was rejected. Jendottir *et al.* [1] have reported that modification of drinks by adding tricalcium phosphate can reduce the potential erosion of the enamel. They tested the experimental drinks containing 4 and 8g tricalcium phosphate in one liter of drink [1]. Min *et al.* have also investigated the effect of adding nano-sized hydroxyapatite to a sport drink to prevent dental erosion [12]. They concluded that higher contents of nano-sized hydroxyapatite in their tested drink had a better ability to prevent dental erosion.

Studying SEM images (Figure 3) in this

investigation also indicates that increasing the amount of additive decreased the dental erosion that occurred due to acid content of the drink. Min *et al.* have presented some SEM micrographs in their report [12], showing that the drink which contained more nano-sized hydroxyapatite caused less tooth erosion. Different parameters of drinks such as pH, concentration of calcium and phosphate ions, degree of saturation with respect to hydroxyapatite, TA, and the type and amount of acid had different effects on dental erosion [1-6]. The most important and influential parameter for preventing or decreasing the erosion seems to be the mineral content of the drink [18,19].

In other words, based on previous studies, in the lowest pH, if the mineral content and degree of saturation with respect to hydroxyapatite has a sufficient value, erosion could be decreased [20]. Due to releasing and saturating calcium ions in the drink, citric acid would be unable to chelate with the calcium of the enamel. Therefore, calcium ion may penetrate into the enamel surface and form a protective layer against the acid [1,6,20]. Barbour *et al.* showed that there is a linear direct relationship between the pH of the drinks and hardness of tooth enamel [21]. Similarly, here by increasing the pH due to the increase of nanoparticles concentration, microhardness was increased linearly. Based on previous studies, DS_{En} parameter had a critical effect on tooth

erosion [4]. Larsen et al reported no erosion effect on the surface of the tooth in contact with orange juice with $DS_{ES}=3.42 \times 10^1$; while for orange juice with $DS_{ES}=4.52 \times 10^2$ the tooth enamel was greatly eroded [4].

In this study, there is a significant VMH% difference for all of the groups and tooth enamel erosion rate was clear in the SEM micrographs; these results confirm the previous report. This pattern of decreased erosion due to increasing DS_{En} and the concentration of calcium and phosphate ions in the drinks is consistent with the previous reports [1,6,18,20]. TA shows the total value of acid in the drink which is defined as another factor for dental erosion and attributes it to the stability of acidic environment in the mouth. Phosphate ions released from nanoparticles, trap the hydrogen actions and this reaction may be the reason of decreasing TA by increasing the amount of nanoparticles. In other words, drinks containing additive may last longer in order to recover the neutral pH in the mouth in comparison with drinks without additive [12].

The drink used for the present study showed the most erosive effect in comparison with other edible acids which could possibly be related to the COOH groups in it. Citric acid has three COOH groups for each molecule and dental erosion occurs due to the joining and chelating the acidic group to the calcium of the enamel surface [12]. In fact, destruction of dental mineral tissue has an important role in dental erosion and decreases the hardness of the enamel [4,12]. In this study, by increasing the amount of additive and saturating the calcium ion in the solutions, percentage changes of Vickers micro-hardness reduced to a lower value. In addition, the increase of VMH % for group B₃ could be attributed to its protective role due to penetration into the enamel surface [22]. The reduction in the amount of erosion in drinks B₁ and B₂ is related to the release of calcium and phosphate ions in the drinks that prevents the citric acid to bond to the enamel calcium.

The importance of using nanoparticles is that they have a higher surface area and consequently higher solubility than larger particles [15]. In this study, eggshell was used not only as a source of calcium, but also as an inexpensive material. In a previous study [12] on the modification of sport drinks by adding hydroxyapatite (HA), there was a slight amount of precipitation in both 0.10 and 0.25% nano-HA groups, and it might have been caused by the large number of ions in the solution such as sodium, potassium, and chloride. However, in this study there was no amount of precipitation in any of the solutions. In regards to the drink's taste, no change was noticed due to the increase of pH and the calcium concentration by adding low additive values containing 0.04, 0.05, and 0.06 vol. % of the nano-particles.

The main limitation of the current study was

increasing the solution pH that could lead to deterioration of the soft drink sooner than that of the pristine one. Therefore, improving the usage time of the solutions is suggested to be considered in future studies.

Within the limitation of this study it, was concluded that the most critical and influential parameter for preventing or decreasing the erosion is the mineral content of the drink. Thus, the erosion potential of the drink used here effectively decreased with the increase of nano-additive concentration, and even more effectively dental erosion was inhibited by adding 0.06 % nano-additives.

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Conflict of Interest: None declared.

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