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Ali, H and Tahmassebi, JF (2014) The effects of smoothies on enamel erosion: An in situ study. International Journal of Paediatric Dentistry, 24 (3). 184 - 191. ISSN 0960-7439

https://doi.org/10.1111/ipd.12058

THE EFFECTS OF SMOOTHIES ON ENAMEL EROSION: AN *IN SITU* STUDY

ABSTRACT

Objectives: To measure, *in vitro*, the pH and titratable acidity (TA) of various soft drinks and to assess the erosive effect of smoothies using an *in situ* model. **Method:** The inherent pH of various soft drinks was measured using a pH meter. The TA was determined by titration with NaOH. An upper removable appliance capable of retaining two enamel slabs was constructed and worn by 14 volunteers. The drinks under test were Innocent® strawberries and banana smoothie and citric acid. Volunteers were instructed to dip the appliance in the test solutions extra-orally five times daily for two minutes each time for 21 days. Measurements of enamel loss were made by surface profilometry and microhardness. **Results:** Diet Coke was the most acidic drink (pH=2.61) while Innocent® mangoes and passion fruit smoothie was the least (pH=3.9). With regard to TA, Innocent® blackberries, strawberries and blackcurrant smoothie had the highest TA requiring 10.8 mol of NaOH to reach pH 7.0 while citric acid required only 3.1 mol of NaOH to reach the same pH value. Surface profilometry and microhardness testing revealed that citric acid caused a statistically significantly greater tooth surface loss compared to smoothie after 21-day pH cycling protocol. **Conclusion:** Smoothies are acidic and have high TA levels. Innocent® strawberries and banana smoothie had an erosive potential to the teeth. However, its erosive effect was significantly less compared to citric acid after 21-day pH cycling protocol using an *in situ* model.

Keywords: enamel erosion, smoothies, citric acid, clinical study

Clinical significance:

1. INTRODUCTION

Tooth wear is recognised as a major problem in both children and adults. The triad of erosion, attrition and abrasion has been known for many years but the contribution of erosion to tooth wear may be increasing. Dental erosion is defined as “The physical result of pathologic, chronic, localised loss of dental hard tissue that is chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement” [1]. It is the loss of tooth substance by acid dissolution of either intrinsic (e.g. regurgitated gastric acid) or extrinsic origin (e.g. acid industrial vapours or dietary components such as soft drinks, pickles, acidic fruits) without the involvement of bacteria

Epidemiological studies have been conducted over the past ten years both in the UK and abroad to elucidate the prevalence for dental erosion. The Children’s Dental Health Survey [2] reported that one-fifth of all 5 year old children show evidence of erosion on one or more of the buccal surfaces of the primary upper incisors; with dentine involvement recognised in 3% of all cases. With regard to older children, i.e. those aged 8 and 15 years, erosion of the permanent incisors was found in 4% and 14% of cases respectively. These results were much higher than the results gathered ten years before. Notably, such an increase may be due to the consumption of soft drinks, which are known to contain acids that can cause erosion [3].

During the recent years, a number of studies have been carried out with the aim of analysing the link between dental erosion development and soft drinks consumption. Such researches have illustrated the belief that dental erosion is positively linked with an increased rate of soft drinks and fruit drinks consumption [4, 5, 6, and 7].

Smoothies are a fruit drink that has been gaining increased popularity in the UK during recent years. For example, in 2010, in the UK, 51 million litres were consumed, meaning every person consumes 0.8 litres per year. Notably, in 2004, the figures stood at 19 million litres, as highlighted by BSDA [8]. Smoothies are made mainly from pureed fruits, and so they are considered healthy owing to their high level of antioxidants, fibres, and vitamins; however, the consumption of smoothies is also viewed as being potentially detrimental to health owing to the high sugar and acid content of such drinks. Considering the drink from a dental perspective, demineralisation may occur as a direct result of consumption, therefore leading to dental erosion and dental caries.

The literature published on the subject provides only very little data concerning the impacts of smoothies consumption on dental erosion. A recent *in vitro* study carried out at Leeds Dental Institute showed that Innocent® smoothies are extremely acidic, and have high titratable acidity. These drinks were found to produce a significant erosive tooth surface loss after the 21-day pH cycling regimen [9]. Although it is known that a number of *in vitro* studies have been conducted in the arena of dental research, such an approach provides only limited information on the erosive potential of drinks owing to the fact that responses to erosion cannot be garnered through non-vital dental tissues. In this regard, biological and environmental aspects, namely food intake, pellicle, saliva, and tooth-brushing, are not always taken into account when an *in vitro* framework is implemented; thus, in this research, an *in situ* framework is adopted with the aim of overcoming the restrictions associated with the *in vitro* approach, and also to consider the oral environmental factors believed to impact dental erosion development.

2. MATERIALS AND METHODS

2.1 *In vitro* study

The properties of various types of soft drinks were investigated by measuring the pH values and the titratable acidity levels. The sample materials chosen for this study were: Innocent® strawberries and bananas smoothie, Innocent® mangoes and passion fruit smoothie, Innocent® kiwis, apples and limes smoothie, Innocent® blackberries, strawberries and blackcurrants smoothie, diet coke, and citric acid 0.3% (positive control). The pH of each sample drinks were checked immediately on opening. The pH measurements throughout the study were made using a pH meter (VWR International Orion, Orion research, UK). The pH electrode was calibrated at the start of each session using standard buffers of pH 4.0 and pH 7.0 and was rinsed thoroughly between uses in order to avoid contamination. One hundred millilitre of the newly opened drink (or freshly prepared) was placed in a beaker and stirred at a rate of 875 rpm until a stable reading was obtained. Three readings were taken of each sample drink to give a mean measurement of the pH of that drink. All tests were carried out at room temperature. The titratable acidity of each sample drinks was tested by placing a 100 ml of each drink in a beaker with a magnetic stirrer continuously moving at the speed on 875 rpm throughout the test. pH value was noted and then 1 mol of sodium hydroxide Na(OH) solution was gradually pipetted until pH of sample drink reached 7.0. The measurement was performed in triplicate and an average value was calculated. Temperature of the drinks was at around 21 ^{o}C.

2.2 *In situ* study

The study received ethical approval from the National Research Ethics Service (NRES) committee of Yorkshire and The Humber-South Yorkshire. All volunteers received verbal and written information concerning the study and provided signed and witnessed consent to participate. The study was a single centre, randomized, two arms cross-over design study. The study was designed, conducted and reported according to the guidelines for good clinical practice. A total of 15 subjects were recruited for the study. Participants had to be aged at least 18 years old, healthy, with no relevant medical history that could interfere with the conduct of the study. In addition, participants had to be dentally fit with a high standard of oral hygiene and without visual signs of untreated caries or periodontal disease. Participants also had to be dentate without removable dental prostheses or fixed or removable orthodontic appliances and sufficient teeth to retain an upper removable appliance clasped by Adam cribs to an upper left and right molar tooth.

Participants were instructed to wear an upper removable appliance capable of retaining two enamel slabs from 8 a.m. to 6 p.m. on each working day for a period of 21 days per study arm. Enamel slabs were derived from intact first or/and second human premolar teeth from and were polished to maximum profiles of 3 mm measured with a profilometer. Enamel slabs were then cleaned with de-ionised distilled water and methanol and covered with nail varnish (red colour, MaxFactor, England, UK) to create a small window of unexposed enamel. Following enamel slabs preparation, they were sent to the Department of Immunology of the University of Liverpool, were they were exposed to gamma radiation (4080 Gy) for sterilisation.

Participants were instructed to immerse the appliances in the test solution (Innocent® strawberries and bananas smoothie, and citric acid) 5 times a day at 8.00, 11.00, 13.00, 15.00 and 18.00 for 2 minutes. The appliances were removed and stored in saline during lunch time, whilst drinking and tooth brushing, and overnight. At the end of the 21-day pH cycling protocol the appliances, containing the enamel slabs, were collected by the study investigator and were assessed using surface profilometry and microhardness testing.

3. RESULTS

3.1 *In vitro* study

Table 1 shows the inherent pH value for each soft drink. The inherent pH was lowest for Diet Coke (pH= 2.61) and highest for Innocent® mangoes and passion fruit smoothie (pH=3.9).

Table 1: Mean inherent pH value for each soft drink

Sample material	pH	S.D
Strawberries and bananas smoothie	3.67	0.02
Mangoes and Passion fruit smoothie	3.9	0.03
Kiwis, apples and limes smoothie	3.75	0.04
Blackberries, strawberries and blackcurrant smoothie	3.81	0.01
Diet Coke	2.61	0.07
Citric acid 0.3%	3.08	0.02

With regard to the titratable acidity, Citric acid 0.3% demonstrated a rapid response to the addition of NaOH, indicating a relatively low titratable acidity. Citric acid required only 3.1 mol of NaOH to bring the pH value to 7.0, whilst Innocent® blackberries, strawberries and blackcurrant smoothie gave the greatest titratable acidity, requiring 10.8 mol Na(OH) to reach the equivalent pH value. Table 2 represents the mean volume of NaOH required to reach pH 7.0 for each soft drink.

Table 2: The Mean volume of NaOH required to reach pH=7.0 for each soft drink

Sample material	Amount of NaOH required to reach pH=7	S.D
Strawberries and bananas smoothie	9.8	0.7
Mangoes and Passion fruit smoothie	9.9	1.1
Kiwis, apples and limes smoothie	7.7	0.7
Blackberries, strawberries and blackcurrant smoothie	10.8	0.6
Diet Coke	3.97	0.1
Citric acid 0.3%	3.1	0.06

3.2 *In situ* study

The subject group consisted of 15 females with a mean age of 31 years, 8 months (S.D±9.42). One participant withdrew consent after 2 days of the acclimatization period as it was difficult for her to cope with the appliance.

The overall distribution of tooth surface loss using surface profilometry after 21-days pH cycling protocol for the SB smoothie and citric acid groups is shown in table 3. The mean surface loss followed by exposure to citric acid was 28.43 µm (S.D±10.25) compared to 2.88 µm (S.D±2.13) following exposure to SB smoothie. Statistical analysis indicates that citric acid group had a significantly higher tooth surface loss compared to the SB smoothie group.

Table 3: Descriptive data on tooth surface loss (µm) after 21-days pH cycling protocol for smoothie and citric acid using surface profilometry

Groups	N	Minimum	Maximum	Mean	S.D
SB Smoothie	28	0.00	7.05	2.88	2.13
Citric acid	28	12.65	56.26	28.43	10.25

Table 4 represents descriptive data on indentation length and enamel microhardness before and after exposure to test materials. In the citric acid group the mean of indentation length and enamel microhardness before the exposure to test material was 66.01 (µm) and 327.64 (KHN) respectively compared with 66.66 (µm) and 325.18 (KHN) for the smoothie group. After the 21-days pH cycling protocol, the mean of indentation length and enamel microhardness was 130.34 (µm) and 85.22 (KHN) for the citric acid group compared with 105.70 (µm) and 124.20 (KHN) for the smoothie group.

A paired sample t-test was carried out to compare the mean differences (changes) in indentation length and enamel microhardness before and after exposure to test materials. The test gave a p value of < 0.05 for both citric acid and smoothie groups. This indicated that there was a statistical significant difference (change) in indentation length and enamel microhardness before and after exposure to smoothie and citric acid.

Table 4: Descriptive data on indentation length (μm) and enamel microhardness (KHN) before and after 21-days pH cycling protocol for smoothie and citric acid

	N	Minimum	Maximum	Mean	S.D
SB smoothie					
Indentation length (μm)					
Pre-exposure (reference area)	28	61.70	70.00	66.66	2.66
Post-exposure (exposed area)	28	78.10	128.00	105.70	13.72
Enamel microhardness					
Pre-exposure (reference area)	28	289.90	389.90	325.18	28.50
Post-exposure (exposed area)	28	91.50	175.60	124.20	21.13
Citric acid					
Indentation length (KHN)					
Pre-exposure (reference area)	28	61.70	70.00	66.01	2.55
Post exposure (exposed area)	28	87.40	146.80	130.34	19.04
Enamel microhardness					
Pre-exposure (reference area)	28	289.90	372.40	327.64	25.55
Post exposure (exposed area)	28	52.40	138.10	85.22	21.34

The overall distribution of the differences (changes) in indentation length and enamel microhardness after 21-days pH cycling protocol for smoothie and citric acid is shown in table 5. The mean difference (change) in indentation length and enamel microhardness followed exposure to citric acid was 64.33 µm (S.D±19.47) and -242.42 KHN (S.D±33.66) respectively compared to 39.04 µm (S.D±13.88) and -200.97 KHN (S.D±29.24) following exposure to SB smoothie. Statistical analysis showed that citric acid group caused signinficantly greater difference (change) in indentation length and enamel microhardness compared with SB smoothie group.

Table 5: The differences (changes) in indentation length (µm) and enamel microhardness (KHN) after 21 days pH cycling protocol for smoothie and citric acid

Groups	N	Minimum	Maximum	Mean	S.D
Indentation length:					
SB smoothie	28	9.20	64.50	39.04	13.88
Citric acid	28	20.3	101.30	64.33	19.47
Enamel microhardness:					
SB smoothie	28	-247.40	-120.90	-200.97	29.24
Citric acid	28	-302.10	-181.10	-242.42	33.66

4. DISCUSSION

The aim of this *in vitro* study was to assess the potential acidity of various soft drinks by measuring their pH and titratable acidity levels. Diet coke was chosen for this study because its consumption has increased among adolescents who have become more weight conscious [10]. Many studies have found an association between carbonated drinks, especially carbonated cola drinks with dental erosion [11, 12, and 13]. The association between erosion and Coke drinks was well established. In fact, these drinks have been used in many studies to create erosive lesions [14, 15].

The method used in this study to determine the titratable acidity has been employed previously in many studies [16, 17 and 18] and is known to give a realistic measure of buffering capacity of drinks by quantifying the amount of alkali required to bring the pH to a chosen value. Various end points have been used in previous studies from pH 5.5 to 10. The definition of the exact value of pH below which enamel dissolution may occur is controversial [19], since in the mouth it is the degree of undersaturation with respect to tooth mineral that is the crucial point. The end point chosen, therefore, for this study was pH 7.

The results of this *in vitro* study demonstrated that all smoothies tested were acidic, registering pH values well below the critical pH 5.5, at which decalcification occurs. Generally, all smoothies had a higher pH than Diet Coke (pH=2.61) and citric acid (pH=3.08). However their titratable acidity was 3-4 times more than both Diet Coke and citric acid. Innocent® blackberries, strawberries and blackcurrant smoothie required the largest amount of sodium hydroxide (10.8 mol), thus is the most resistant drink to a pH change up to neutralization (pH 7.0).

It is important to note that smoothies are viscous drinks due to their high content of fibres. The fibre content, in smoothie drinks, varies from 10% in the strawberries and bananas sample up to 19% in the kiwis, apples and limes smoothie. During the process of measuring titratable acidity, a magnetic stirrer was used to the speed of 875 rpm in order to mix the sodium hydroxide well. It was noted that the pH meter took a longer time to come to a reading, as it needed a stable reading before giving a measurement. This property of the smoothies may have influenced the amount of tooth surface loss.

In situ studies are being widely carried out in dental research as they simulate the natural oral processes better than animal or *in vitro* studies without being as time consuming or costly as *in vivo* studies. Furthermore; *in situ* studies allow for better control of the study subjects and improved compliance compared with *in vivo* studies as the latter last longer [20]. Many studies have investigated the erosive potential of foodstuffs and beverages, but different protocols have been used. Various *in vitro* studies have immersed teeth in different types of acidic challenges and using different time durations (usually a prolonged period of time). Although these methods provided information on the erosive potential of these drinks, it exaggerated the erosive effect due to the absence of modifying influence of saliva.

The protocol used for this study was similar to the protocol used in a previous study [21]. This was developed at the University of Leeds and is a slightly modified version from the method used by Amaechi *et al.* [14] who introduced a cyclic model to produce dental erosion lesions using simple *in vitro* technique. Lesion were produced by immersing the teeth in aliquots of 20 ml of orange juice at regular intervals six times per day (to stimulate drinking at breakfast, midday, lunch, late afternoon, dinner & bedtime) for 5 minutes on each occasion giving a total of 30 minutes daily exposure to orange juice. The six times dipping for 5 minutes immersion was thought to be an overestimation of the real situation therefore, five times dipping for 2 minutes immersion was used instead.

The findings of this study were aimed to determine the erosive effect of Innocent® strawberries and bananas smoothie and compare it to citric acid using an *in situ* model. Citric acid was used as a positive control as it is the major organic hydroxyl acid found in fruit juices and soft drinks. In addition, citric acid can be easily prepared for *in vitro* or *in vivo* studies. Beverages would commonly have concentration of round 0.3% citric acid in their ready to drink juices [22] therefore this percentage was chosen for this study.

Comparison of the erosive effect of smoothie with citric acid using surface profilometry and microhardness testing demonstrates that there was a significant difference in tooth surface loss between the two groups. Surface profilometry measurements revealed that citric acid caused a greater tooth surface loss compared with smoothie. Microhardness testing showed a statistical significant difference (change) in indentation length and enamel microhardness before and after exposure to smoothies and citric acid. The results also revealed that citric acid caused significantly significant greater difference (change) in indentation length and enamel microhardness compared with smoothie group.

Innocent® strawberries and bananas smoothies is acidic, It had a higher pH (pH=3.7) than citric acid (pH=3.1) and its titratable acidity was 3 times more than citric acid. The present study showed that tooth surface loss after exposure to Innocent® strawberries and bananas smoothie was not related to pH or titratable acidity, suggesting that neither pH nor titratable acidity can be used to predict erosive potential. Lussi [23] reported that the pH and the titratable acidity, do not readily explain the erosive potential of food and drink. Other chemical factors are important and need to be considered such as the mineral content of food and drinks, temperature, type of acid as well as the ability of any of the components to complex or chelate calcium and remove it from the mineral surface. Besides, these chemical factors several others including the components of saliva and the flow rate of saliva have an impact on dental erosion *in vivo*. The degree of saturation with respect to the tooth mineral, hydroxyapatite and fluorapatite also strongly influence the erosion outcome. All of the above factors have to be taken into account to explain or even predict to some extent the influence of foods and beverages on dental hard tissue.

The relevance of saliva on the erosion process could be better illustrated by a comparison between in vitro and in situ erosion models. Comparison of the current study results with those obtained from the *in vitro* study conducted by Sukeri [9] leads to the predictable conclusion that investigating erosion in a laboratory setting grossly overestimated the amount of surface tissue loss that might be expected *in situ*. Thus, there was a dramatic difference between the loss of enamel due to smoothies exposure in the two environments. This can be explained in part since there was no protection for the enamel *in vitro*, whereas pellicle formation occurring *in vivo* would afford some benefit [24]. The enamel slabs *in vitro* had no opportunity for remineralisation, nor was the effect of the smoothies limited by the buffering capacity of saliva. Moreover, the smoothies had total contact with the specimens *in vitro*, whereas *in situ* the specimens were exposed to a passing acid fluid mixed with saliva.

Several studies reported that there was a greater protection against erosion *in situ* compared with the *in vitro* environment. There may be several reasons for this, which include the chemical composition of the protecting saliva, the quantity of saliva protecting the specimens, the presence of organic layers covering the specimens and the potential effect of fluoride *in situ* [25, 26]. In addition, it has been reported that saliva collected or prepared for *in vitro* studies may undergo a number of changes which reduce its protective ability against acid degradation of tooth tissue. These changes include a reduction in the degree of phase buffering [27] and protein breakdown [28].

In summary, the present study concluded that smoothies are acidic and have high titratable acidity. Innocent® strawberries and bananas smoothie had an erosive potential to the teeth. However, its erosive effect was significantly less compared to citric acid after 21-days pH cycling protocol using an *in situ* model.

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