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Abstract

Objectives
This prospective, randomised *in vitro* study was to investigate the pH and titrateable acidity of fruit smoothie drinks and to assess the effect of these drinks on enamel erosion.

Method
50 enamel slabs were divided into 5 groups which were allocated to the sample solutions groups: Innocent® smoothie strawberries and bananas (SB), Innocent® smoothie mangoes and passion fruit (MP) and Diet Coke. Distilled deionised water (DD) was used as negative control and citric acid 0.3% as positive control. All the slabs were subjected to a 21-day pH cycling regime involving 2 minutes of immersions, 5 times a day with appropriate remineralization periods in between. Measurement of surface loss was assessed using profilometry. Independent sample t-tests were used to compare means.

Results
The titratable acidity for both test smoothies were 3.5-4 times more than that needed to neutralise Diet Coke and citric acid 0.3%. The pH of SB, MP smoothie and Diet Coke was found to be 3.73, 3.59 and 2.95 consecutively. MP smoothie caused the greatest amount of surface loss followed by Diet Coke. Both smoothies were found to cause significant surface loss. MP smoothie resulted in significantly higher surface loss compared with MB smoothie and citric acid 3%.

Conclusion
The smoothies tested were acidic and had high titrateable acidity. They produced a significant erosion of enamel *in vitro.* The results of this study suggest that there should be increased awareness of the erosive effects of smoothies especially as their consumption seems to be on the increase.

Introduction

Over the years, the erosive potential of soft and fruit drinks on the dentition has been the focus of many investigations [Dugmore and Rock, 2004, Tahmassebi *et al*., 2006, Blacker and Creanor 2011, Blacker and Chadwick 2013]. The acid content of these drinks lead to erosion and the sugar content, metabolized by plaque microorganism to generate organic acids will bring about dental caries. While the natural biological process in the mouth can neutralise a single acid attack, frequent use will lead to remarkable detrimental effect [Lussi *et al*., 2004].

In the UK, consumption of shop-purchased smoothies has risen dramatically from 6 million litres in 2001 [Mercer 2007] to 51 million litres in 2010. It is said that the true level of smoothie consumption is not known, as these figures exclude consumption of homemade drinks, as well as, those bought from cafes, coffee shops and juice bars [Blacker et al., 2011]. The promotion of healthy eating has been one of the driving forces behind the continuing rates of the exceptional growth of this industry.

Smoothies are made mainly from pureed fruits, and 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.

In recent years, much of the dietary advices have been encouraging increase in the intake of eating fresh fruit and vegetables, and apparently drinking smoothies is a practical and an easy method in gaining the benefits of fruits. The 5-a-day campaign has also help to encourage intake of this drink. The literature published on the subject provides only very little data concerning the impacts of smoothies consumption on dental erosion. More research needs to be conducted to produce scientific proof of smoothie drinks to guide professionals in giving advice and educating public about the consequences of taking these drinks.

Materials and Methods

The present study was carried out as a pilot study and therefore power calculations were not performed. Sample number was chosen based on studies that had used a similar method but with a different sample of drinks [Abdullah 2009]. There were 50 enamel slabs included in the study, with 10 in each subgroup.

Enamel Slabs

Enamel samples were from sound premolar teeth extracted for orthodontic reasons only. Tissue Bank approval was sought in order to obtain the teeth. Teeth were stored in distilled water and 0.1% thymol (Sigma Aldrich). Crowns were sectioned using water cooled, Diamond Wire Saw, cutting machine (Well®Walter EBNER, CH-2400 Le Loche). The Buccal and palatal surfaces of each crown were removed, and the slabs were prepared according to the relevant standard operating procedure (about 2 mm wide, 2 mm length, and 2 mm depth).

The enamel slabs were mounted in circular resin blocks of 3 mm thickness. To ensure flatness of the blocks and to make sure no resin covered the enamel surface, a grinding machine was used. Fine grit abrasive papers were used, from 600 grade, to 1200 grade and 2500 grade (Wet or Dry paper, 3M). Minimal enamel was removed. This was easily achieved because the resin blocks were held in rectangular steel blocks which were slightly less than 3 mm deep due to wear. The blocks were then cleaned with methanol to remove any remnants of abrasive paper. Surfaces were polished with 5 µm and 1µm alumina paste. Finally, the enamel blocks were rinsed with DD water. At all times, the slabs were kept moist in DD water in micro-centrifuge tubes and left at room temperature. This was to prevent dehydration of the enamel. Profile of the resin blocks were assessed using surface profilometer (Scantron ProScan 2000).

To achieve flatness of enamel sample, it was ensured that average height to average depth (Rz) range was within 1.0 µm. Enamels that were confirmed to be flat were tested with surface microhardness test. Computer aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark) was used. Indentations were made using a Knoop diamond under 100 g loads for 30 seconds. This was done with care, and the middle area was covered with tape in order not to touch it. The length of indenter penetration was measured by using an image analysis

system. Three indentations were performed and measured, and then the mean was determined.

Slabs with appropriate microhardnes (range of 60-70 μm) were accepted to be included in the study. They were then covered with nail varnish except for the middle area, where a window of 1mm x 2mm was left exposed. Those that were out of range were rejected from the study. Enamel slabs were put in a bag and randomly picked, then placed in a tray with 10 holes. They were secured in position by using adhesive wax. The trays were then put in a bag and randomly picked out and labelled into 5 groups, ready to go through the cycling process. It was not possible to achieve blindness for this study as all the drink samples could easily be differentiated.

Materials

a) Strawberries and bananas smoothie. (Innocent® pure fruit smoothie. London)
b) Mangoes and passion fruit smoothie. (Innocent® pure fruit smoothie. London)
c) Diet Coke® (Coca Cola Company, USA)
d) Distilled deionised water (negative control)
e) Citric acid 0.3%.(positive control)

Testing the drinks for pH and Titrateable acidity

Using a pH meter (VWR International Orion, Orion research, UK) the pH of each sample drinks (100ml) were checked immediately on opening. Tests were done at room temperature with a magnetic stirrer placed in the beaker to mix the sample drinks well at 875 rpm. Between uses, the electrode was rinsed thoroughly to avoid cross contamination. Every day, pH was calibrated using standard buffers of pH 4.0 and 7.0.

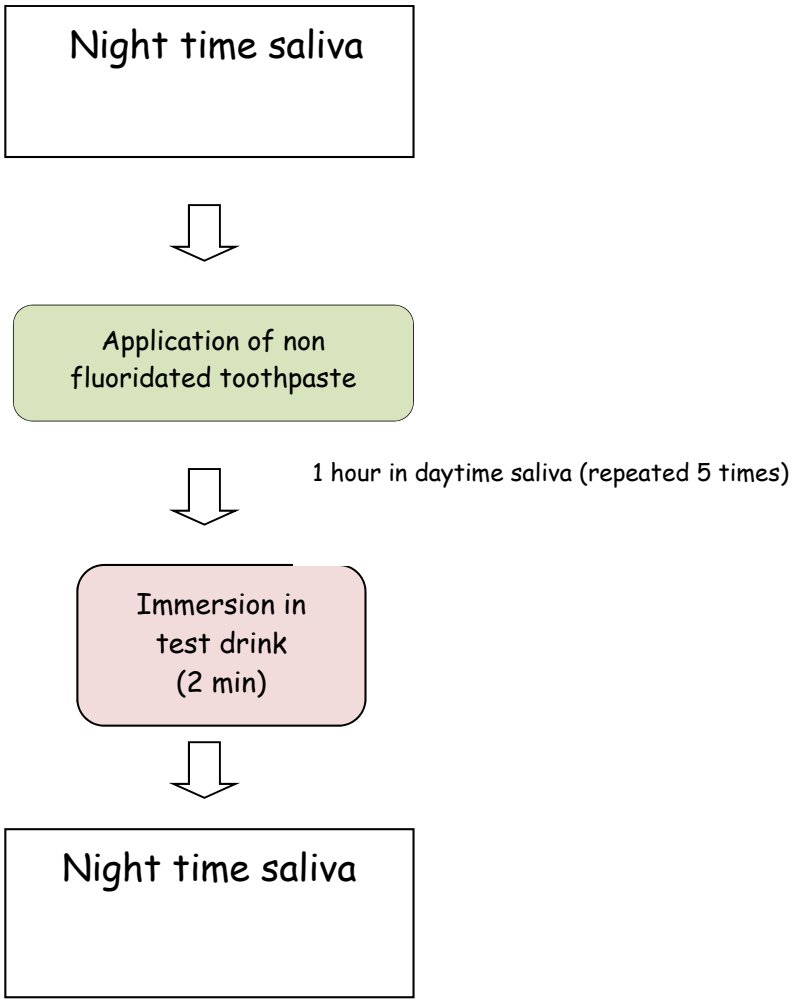
To test the titrateable acidity of the sample material, 100 ml of each drink was put in a beaker with a magnetic stirrer continuously moving at the speed on 875 rpm throughout the test. pH value was noted and then 0.1 μMol sodium hydroxide (NaOH) solution was gradually pipetted until pH of sample drink 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.

Protocol/regime for pH cycling

The trays containing the 50 enamel slabs were randomly divided into one of the 5 intervention groups, 10 enamel slabs per each group. Slabs were immersed under static conditions for 2 minutes each time, 5 times daily in fresh 50 ml aliquots of sample drinks for 21 days (figure 1). A gap of 60 minutes was left between immersions, where slabs were placed in daytime saliva. Overnight, slabs were kept in night time saliva. All this was carried out at room temperature. The two smoothies and the Diet Coke used during the immersion were used chilled (around 15^{o}C) and the DD water and citric acid 0.3% were kept at room temperature.

Before and after immersion with the sample drinks, the slabs were rinsed with DD water. Fresh artificial saliva was changed after each immersion: one hour before starting the pH cycling regime and one hour after the final immersion.

Figure 1: Flowchart representing 21-day regimen



Results

pH and titrateable acidity

Sample materials were analysed for their pH and titrateable acidity. Table 1 shows that the smoothies are acidic in nature. Diet Coke had the lowest pH level at 2.95 followed by SB smoothie at 3.59 and MP smoothie at 3.73. Both smoothies have a high titrateable acidity. The amount of sodium hydroxide (NaOH) needed to neutralise the smoothies were 3.5 to 4 times more than the amount needed for Diet Coke and citric acid 0.3%.

Table 1. Properties of sample materials

Sample materials	**pH**	**Titrateable acidity**
SB smoothie	3.73	10.83 µMol
MP smoothie	3.59	11.60 µMol
Diet Coke	2.95	2.87 µMol
Deionised distilled water	n/a	n/a
Citric acid 0.3	3.60	3.17 µMol

Table 2. Result of Pearson's correlation between surface loss and pH as well titrateable acidity

pH	Pearson correlation Sig. (2 tailed)	-0.09 0.77
Titrateable acidity	Pearson correlation Sig. (2 tailed)	0.59 0.04

Pearson's Correlation coefficient was determined to see associations between surface loss with pH and titrateable acidity. It is shown in Table 2 that there was a positive correlation r = 0.59 with titrateable acidity which was statistically significant ($p<0.05$). However, a value of 0.59 is considered only a fair/medium association.

Test of normality of the data

Shapiro–Wilk test was used to check the normality of the data. This test showed that all groups had p>0.05, thereby the measurements from this study were of normal distribution. Therefore, parametric tests could be used for all groups (table 3).

Table 3. Shapiro Wilk test result for all groups

Groups	Statistics	df	Sig.
SB smoothie	0.91	10	0.31
MP smoothie	0.89	10	0.15
Diet Coke	0.90	10	0.24
DD water	0.85	10	0.05
Citric acid	0.93	10	0.41

Figure 2. Distribution of surface loss of all sample groups

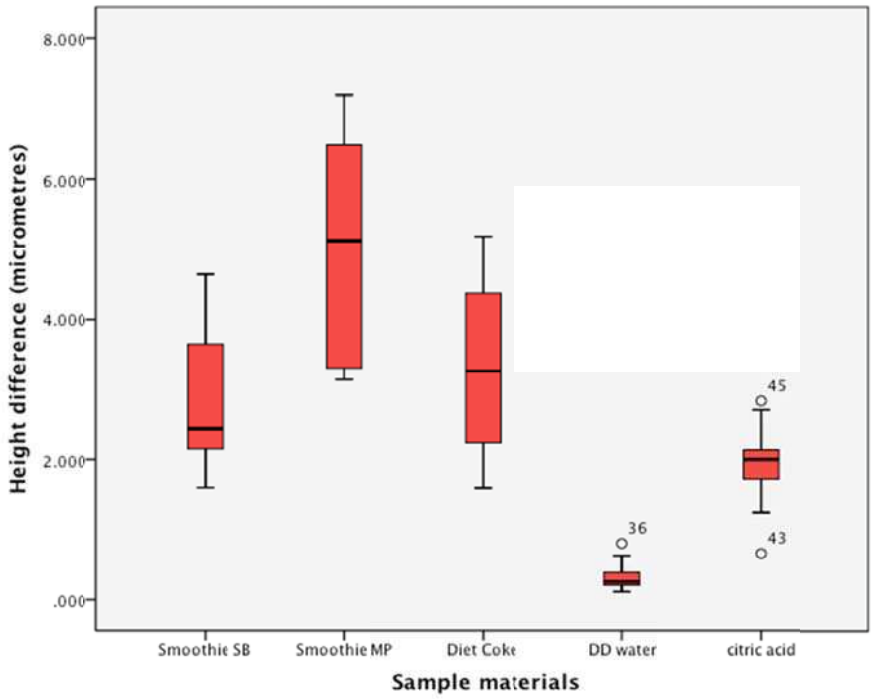


Figure 2, shows the distribution of the enamel surface loss by separate sample materials. MP smoothie has caused the largest change in surface loss, followed by Diet Coke and SB smoothie. DD water which was the negative control for this study showed very minimal volume loss.

Differences between sample groups

The differences between each group were compared. Multiple comparisons were conducted with Bonferroni correction in order to identify where the differences lay. Table 4 shows the result of independent samples test of SB smoothie or MP smoothie against other sample materials.

Table 4. Results comparing mean differences of surface loss experienced by control and test groups using independent samples t-tests.

Groups		Mean Difference	SD	95% Confidence Interval of the Difference		Sig (2tailed)
				Lower	upper	
SB Smoothie Vs	SB smoothie	-	-	-	-	-
	MP smoothie	-2.22	1.61	-3.46	-0.97	0.00
	Diet Coke	-0.57	0.97	-1.66	0.53	0.29
	DD Water	2.49	0.22	1.82	3.14	0.00
	Citric acid	0.89	0.63	0.12	1.65	0.03
MP Smoothie vs	SB Smoothie	2.22	0.97	0.97	3.46	0.00
	MP Smoothie	-	-	-	-	-
	Diet Coke	1.65	1.34	0.26	3.04	0.02
	DD Water	4.70	0.22	3.63	5.78	0.00
	Citric acid	3.10	0.63	1.96	4.25	0.00

Comparing smoothies against other sample materials

Comparing SB smoothie against DD water, P value was lower than 0.01 which was the level of significance after Bonferroni correction. Therefore, the difference was statistically significant. Confidence interval was from 1.82 to 3.14 and does not include zero. In comparison with water there was significantly more surface loss by SB smoothie.

When comparing Diet Coke and SB smoothie, the p value was 0.29, indicating there was no significant difference between the two groups at 5 % level. The mean difference in surface loss was 0.57 μm with a 95 % confidence interval of -1.66 to 0.53. As this confidence interval includes zero, we could confidently state that there was no difference between SB smoothie and Diet coke. No significant difference was seen when comparing SB smoothie with citric acid as well, which was the positive control.

On comparing MP smoothie to DD, MP smoothies caused significantly more surface loss after the erosive challenge. There were also significant differences when comparing MP smoothie with SB smoothie and citric acid 0.3%. But no statistical significant difference was found when comparing MP to Diet coke, the p value was > 0.01 and therefore the enamel loss by MP smoothie was not significantly different to the surface loss attained by erosive challenges with Diet Coke.

Intra-examiner agreement

A sample of 10 enamel slabs were randomly selected and re-measured. The differences between the two readings were plotted to the average of the two readings in the Bland-Altman Plot. Mean of the differences or the bias was 0.15 μm. Bias was considered quite small, indicating good agreement between the readings. The 95% limits of agreement (-0.09, 0.398) included 0, interpreting that the measurement were likely to agree on certain enamel slabs.

Discussions

Smoothies are a relatively new type of soft drinks compared with fruit juices, however their sales have soared in recent years. A survey has shown that 37% of consumers occasionally or regularly drink smoothies [You Gov, 2008]. In the UK, “Innocent” is the market leader in smoothies where they claimed their smoothies contained 100% fruits and did not contain sugar, water or preservatives. The Innocent smoothies contain 10-18% fibres and count as two of the 5-a-day portion [Ruxton, 2008] . Innocent’s top selling smoothies are the

‘strawberries and bananas’ and the ‘mangoes and passion fruit’ which were the test materials used for this research.

Pure fruit and vegetable juice offer similar health benefit as having whole fruits and vegetables [Ruxton *et al*., 2006] . Undoubtedly, their content of antioxidants, polyphenol and fibres are good for health. On the other hand, health concerns from drinking smoothies include the high energy and sugar content as well as the effect of caries risk from non-milk extrinsic (NME) sugars present in the juices/smoothies. Both concerns were shown to have no higher than the impact and risks of having two portions of whole fruits [Ruxton, 2008, Hussein *et al*., 1996, Beighton *et al*., 2004]. Smoothies contain acids from fruits, and theoretically this may lead to dental erosion. There are only limited studies in the literature relating smoothies to erosion [Blacker and Chadwick 2013].

Carbonated drinks dominate the sales of soft drinks in UK (BSDA, 2010) . In 2009, there was consumption of 98.3 litres per person. Coca Cola® is a widely known example of carbonated soft drink produced by Coca Cola Company, and the Diet Coke® is a popular variation of it. The reason Diet Coke was included in this study was because the consumption of this drink has increased among adolescents who have become more weight conscious [Mintel, 2008]. The association between erosion and Coke is so well established, that it has been used to create artificial erosive lesions in many studies [Amaechi *et al*., 1999b, Hooper *et al*., 2007].

In the current study, an *in vitro* model was used as it has several advantages over *in vivo* studies. The *in vitro* model allows for several experimental variables to be controlled as well as flexibility of the study design [Koulourides and Chien, 1992]. *In vitro* studies have low operation costs [White, 1992] and are highly controlled allowing single experimental parameters to be investigated [ten Cate, 1994] . In addition, highly sensitive detection and analytic techniques, as used in the present study, can be applied to measure the TSL.

On the other hand, the *in vitro* model does have some limitations. Dental tissue is non-vital and thus cannot reproduce biological responses to erosion. Moreover, the specific interactions of dental hard tissue and saliva, as well as the relationship between sample surface area and fluctuations in the volume and composition of oral fluids cannot be adequately simulated *in vitro* [ten Cate, 1990] . Also other factors such as rate of consumption and manner of

swallowing, which would have influence on enamel erosion cannot be duplicated in the *in vitro* model.

Different studies have immersed teeth in various types of acidic challenges and using different time durations, usually at prolonged periods of time. Therefore, their results were exaggerated as there was no modifying influence of saliva [Amaechi *et al.*, 1999b] introduced a model to produce dental erosion lesions using simple *in vitro* technique. Lesions were produced by cycling teeth in aliquots of 20 ml of orange juice, at regular intervals six times per day (5 minutes every time). The six times of exposure was to simulate drinking at breakfast, midday, lunch, late afternoon, dinner and bedtime. Immersion was done at room temperature and the bovine teeth were kept in artificial saliva in between immersion and at night. The experiment was carried out in such way for 24 days, giving a total of 12 hour exposure [Amaechi *et al.*, 1999b]. The 5 minute exposure was chosen because there was an observation that pH of saliva and its calcium phosphate saturation returned back to baseline after 5 minute rinse with citric acid rinse [Bashir and Lagerlof, 1996]. This study concluded that the technique used was suitable to mimic the conditions *in vivo* and can be used in looking at various parameters on dental erosion.

The method used for this study was from a protocol developed at the University of Leeds as used in a study by Abdullah [2009]. The protocol is slightly modified from the method used by Amaechi [1999b]. The six times dipping of 5 minutes emersion was thought to be an overestimation of real life situation. Therefore, a protocol consisting of 5 times of dipping with 2 minutes of immersion each time was used. Two types of artificial saliva were used. The daytime saliva was used for remineralisation and the night time artificial saliva was used for maintaining the minerals. The slabs were immersed in 50 ml fruit solution each time. This amount was chosen as it is believed that most consumers will drink about one bottle of smoothie per day [Mintel, 2008]. So, 5 exposures were thought to represent a bottle of 250 ml of smoothie every day.

Tooth wear measurement in this study was represented by difference in height, using unexposed areas as a reference point. The three point height difference was performed by using the laser profilometry software. Profilometry is method of choice to measure loss of surface. This technique is simple, quick and allows measurement of surface loss of a large area with a high precision. This method is also widely used in many studies, allowing comparisons.

Fruit juices contain weak acids which exist in solution as either undissociated molecules or in dissociated form; pH measures the hydrogen ion (H^+) concentration and titrateable acidity (TA) measures concentration of all H^+ ions, both free and bound to undissociated acids and anions. Generally, the smoothies have a higher pH than the Diet Coke (2.95) and Citric acid (3.60). However their TA was 3.5-4 times more than both Diet Coke and Citric Acid. For water, theoretically the pH of pure water (H_20) is 7.0 at 25^{o}C, but when exposed to the carbon dioxide in the atmosphere this equilibrium results in water becoming slightly acidic with time. Water also does not have buffering capacity; the pH reading shoots up even with very small amount of NaOH. Hence, no reading for pH and titrataeble acidity for DD water was done.

There have been debates on whether pH or TA is a better predictor of a drink's erosive potential. pH measures H^+ concentration while TA determines the actual hydrogen ion availability for interaction with tooth surface [Boulton, 1980] . Some studies have shown that pH was a better predictor and some believed that TA was more important [Edwards *et al.*, 1999, Cairns *et al.*, 2002]. There have been suggestions that pH is a good predictor for the first few minutes of erosion challenge, whereas the TA better characterizes the erosive potential during longer exposure times [Jensdottir *et al.*, 2006]. This was agreed by Hannig who believed that when there is short term exposure of enamel to acidic environment, pH and type of acid impose a bigger effect on erosion rather than titrateable acidity [Hannig *et al.*, 2005a]. In this present study, there was a positive correlation to TA (r = 0.59) but the value shows fair/medium association and should be treated with suspicion that there are other variables influencing the association. Generally, it is agreed that pH and TA alone do not readily explain the erosive potential of food and drinks.

It was noted that smoothies are viscous drinks, containing a lot of fibres compared to the other sample drinks. Mangoes and passion fruit smoothie had 18% fibre and the strawberry and banana smoothie had 15% of its content consisting of fibres. During the process of titrating, a magnetic stirrer had to be used to the speed of 875 rpm in order to mix the NaOH well. It also took the pH meter a longer time to come to a reading, as it needed a stable reading before giving a measurement.

When comparing smoothies with Diet Coke, it was found that erosion from both smoothies were not significantly different to the erosion caused by the Diet Coke. In other words,

drinking smoothies will cause almost the same amount of erosion as when slabs were exposed to Diet Coke.

Conclusions

This study showed that Innocent Strawberries and Bananas smoothie and Innocent Mangoes and Passion fruit smoothies are acidic and have a high titrateable acidity. Both smoothies caused significant surface loss/erosion to enamel after 21-day pH cycling regimen. Innocent Mangoes and Passion fruit smoothie cause significantly more surface loss compared with Innocent Strawberries and Bananas smoothies. Both smoothies caused surface loss/erosion to enamel similar to the effect of Diet Coke after the 21-day pH cycling regimen.

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Conflict of Interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper

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