

The effects of smoothies on enamel erosion: an *in situ* study

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July 2012

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Dedicated to my family

My mum, dad, sisters and brothers

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ABSTRACT

Aims: To measure, *in vitro*, the pH and titratable acidity (TA) of various soft drinks and to assess the effect of smoothies on erosive tooth surface loss of enamel following a 21-day pH cycling protocol using an *in situ* model. **Methods:** 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 bananas smoothie and citric acid. Participants were instructed to dip the appliance in the test solutions extra-orally five times daily for two minutes for 21-days. Measurements of enamel loss were made by surface profilometry and microhardness. **Results:** Diet Coke was found to be 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 blackcurrants 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. The mean tooth surface loss following exposure to citric acid and Innocent[®] strawberries and bananas smoothie was 28.43 μm (S.D \pm 10.25) and 2.88 μm (S.D \pm 2.13) respectively. Citric acid caused a significantly greater tooth surface loss compared to smoothie. A statistical significant difference (change) in indentation length and enamel microhardness was found before and after exposure to test materials. Citric acid caused significantly greater difference (change) in indentation length and enamel microhardness compared with smoothie. **Conclusion:** Smoothies are acidic and have high TA levels. 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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ABBREVIATIONS

2D	Two dimensional
3D	Three dimensional
%	Percentage
>	Greater than
≥	Great than or equal to
<	Smaller than
µm	Micrometre
µl	Micro millilitre
°C	Degree of Celsius
am	Morning
AFM	Atomic force microscopy
Cl ⁻	Chloride ions
CLSM	Confocal laser scanning microscopy
cm	Centimetre
CRF	Case record form
CSMH	Cross-sectional microhardness
df	Degrees of freedom
DMFT	Decayed , Missing and Filled Teeth
e.g.	example
ESEM	Environmental scanning electron microscopy
g	Gram(s)
Gy	Gray (unit)
h	Hour(s)
H ⁺	Hydrogen ions
IPT	Iodide permeability test
Ka	Acid dissociation constant
KHN	Knoop hardness number
min	Minute(s)
ml	Millilitre
mm	Millimetre
Mol	Mole
N	Number
pH	Acidity
pKa	Acid dissociation constant
pm	Afternoon
PO ₄ ⁻³	Phosphate ions
ppm	Part per million
QLF	Quantitative light-induced florescence
R & D	Research and Development
rpm	Rotation per minute

S.D	Standard deviation
S.E	Standard error
SEM	Scanning electron microscopy
Sig	Significance value
SMH	Surface microhardness
t	t-value
TA	Titrateable acidity
TSL	Tooth surface loss
UK	United Kingdom
URA	Upper removable appliance
USA	United States of America
w/v	Mass concentration
WHO	World Health Organisation
X	Times

1.0 INTRODUCTION

Tooth wear is widely acknowledged as a significant issue amongst both adults and children. It is defined as the surface loss of dental hard tissues other than by dental caries or trauma. Tooth wear is the term used to describe the processes of erosion, attrition, abrasion and abfraction.

Dental erosion is described by ten Cate & Imfeld (1996) 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' (ten Cate & Imfeld, 1996), and may further be described as the loss of tooth substance as a direct result of acid dissolution, which may be either extrinsic or intrinsic in origin.

A number of epidemiological research studies have been carried out during the recent decade, both within and outside of the UK, with the aim of garnering facts regarding the prevalence of dental erosion. With this in mind, it has been found by the Children's Dental Health Survey, conducted in 2003 (Office for National Statistics, 2004), 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 (Office for National Statistics, 1994).

During 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 research studies have illustrated the belief that dental erosion is positively linked with an increased rate of soft drinks and fruit drinks consumption (Millward *et al.*, 1994; Milosovic *et al.*, 1997; Al-Dlaigan *et al.*, 2001a; Dugmore & Rock, 2004a).

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 as highlighted by BSDA (2011). 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 (Sukeri, 2010). Although it is known that a number of *in vitro* studies have been conducted in the area 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.

2.0 LITERATURE REVIEW

The definition, aetiology, mechanism, prevalence, assessment, and approaches of investigating dental erosion will be reviewed in this section. The impacts of soft drinks on dental tissues will be discussed with particular focus on smoothie drinks.

2.1 TOOTH SURFACE LOSS

Tooth surface loss (TSL) is a term referring to the gradual loss of dental hard tissues as a direct result of non-carious causes. The term TSL is commonly used as opposed to a number of other definitions (Smith & Knight, 1984), owing to the fact that other terms, such as tooth wear, for example, are multi-factorial processes wherein one mechanism may control or overshadow, although the overall wear is usually owing to interactions between a number of different wear processes.

Importantly, tooth wear may be as a direct result of abfraction, abrasion, attrition, or erosion, which may occur in isolation or alongside each other (Milosevic, 1998). Such terms are commonly utilised in order to describe particular processes that are linked with the loss of dental hard tissues.

2.2 DEFINITIONS OF TOOTH SURFACE LOSS

2.2.1 Dental attrition

The concept of dental attrition may be described as ‘the physiological wearing away of dental hard tissue as a result of tooth-to-tooth contact with no foreign substance intervening’ (Imfeld, 1996), with the attrition of incisal and occlusal teeth surfaces possibly occurring as a result of teeth-grinding, such as during swallowing, speech, and

when lifting heavy things. Moreover, during mastication, there may also be the attrition wearing of proximal surfaces (Imfeld, 1996).

2.2.2 Dental abrasion

Dental abrasion may be described as, ‘the pathological wearing away of dental hard tissue through abnormal mechanical processes involving foreign objects or substances repeatedly introduced in the mouth and contacting the teeth’ (Imfeld, 1996). With this in mind, it is acknowledged that abrasion may arise as a consequence of obsessive or forceful brushing, biting on hard objects and/or finger nails, detrimental oral habits, or the improper use of dental toothpicks and/or floss. Moreover, it is known that abrasion may also occur through partial dentures’ clasps, as emphasised by Grippo *et al.* (2004).

Furthermore, there is the consideration of work-related abrasion, which may occur amongst various professions, such as tailors or seamstresses, for example, who may cut thread with their teeth, as well as upholsterers and shoemakers, who may also use their teeth to hold nails, and also musicians and glassblowers (Grippo *et al.*, 2004).

Importantly, if it is the incisal surface or occlusal surface of the tooth, that becomes worn, or both, as a result of food bolus-related friction, such wear is then referred to as ‘masticatory abrasion’ (Grippo *et al.*, 2004).

2.2.3 Dental erosion

Dental erosion, as an overall concept, is utilised with the aim of describing the physical outcomes of a chronic, localised, painless, pathologic loss of dental hard tissue, which is known to be chemically eroded from the surface of the tooth as a direct result of chelation and/or acid without the involvement of bacteria (ten Cate & Imfeld, 1996).

It is recognised that dental erosion is commonly caused by acid sources—both intrinsic and extrinsic. The former include vomiting and gastroesophageal reflux; the latter comprise the regular consumption of acidic drinks, foods, medicines, and oral hygiene products, as noted by Imfeld (1996).

2.2.4 Dental abfraction

‘Abfraction’ in the context of dentistry is commonly adopted in regard to a particular type of wedge-shaped defect at a tooth’s cemento-enamel junction (CEJ) (Grippio, 1991). Markedly, such cuts are commonly seen on an individual tooth or those teeth that are not next to one another, which are theorised as being the outcome of eccentrically applied occlusal forces, subsequently resulting in tooth flexure as opposed to being the sole result of abrasion (Imfeld, 1996).

2.3 DENTAL EROSION

Dental erosion is known to be tooth structure loss as a result of acid dissolution, which is known to occur without any direct involvement of bacteria (Lussi, 2006). Importantly, the condition is multi-factorial. Accordingly, in an attempt to help reduce subsequent development, it is fundamental that the condition be identified in its early stages. Moreover, it is essential to establish and analyse the potential risk factors so that preventive strategies may be considered and implemented beforehand.

2.3.1 Aetiology of dental erosion

Common interactions between the tooth's surface and acids may cause dental erosion, with such acids potentially originating from either intrinsic or extrinsic sources. Extrinsic factors are wide-ranging and include dietary, environmental, medicaments and lifestyle agents.

Intrinsic factors

Any disorder or behaviour known to encourage or attract acid from the gastrointestinal tract and subsequently making contact with teeth is recognised as an intrinsic factor. Such an occurrence may arise as a direct result of the regurgitation and reflux of stomach contents, rumination, or vomiting.

Importantly, the occurrence of dental erosion initiated by intrinsic factors and the actions of such has not yet been established; nevertheless, a number of research studies, such as those by Smith & Knight (1984) and Jarvinen *et al.* (1992) suggest that internal

factors play a fundamental role in the development of approximately one quarter of all cases of dental erosion.

Extrinsic factors

Extrinsic factors that are linked with dental erosion are fourfold—dietary, environmental, lifestyle, and medication—and all may act either singly or in combination.

A. Dietary factors

For a great deal of time, it has been acknowledged that acidic drinks and foods are pivotal in dental erosion (Holloway *et al.*, 1958; Hartles & Wagg, 1962). A number of different research studies have been carried out *in vivo*, demonstrating the erosive activities of citric, malic, phosphoric and other acidic ingredients of foods and drinks (Eccles & Jenkins, 1974; Fuller & Johnson, 1977; Asher & Read, 1987; Grobler *et al.*, 1990; Lussi *et al.*, 1997).

Nevertheless, it should be noted that the pH level of dietary foods and drinks alone is not necessarily indicative of whether or not dental erosion will occur. A number of other factors may also play a role, namely behavioural, biological and chemical, all of which will be taken into account in greater detail later on in the literature review.

B. Medication

Any medications known to have a low level of pH and high levels of titratable acidity, and which regularly come into contact or have prolonged contact with the dentition may induce dental erosion. Furthermore, there is evidence to suggest that individuals who

take chewable aspirin are more likely to develop dental erosion than those who merely swallow tablets. With this in mind, Sullivan & Kramer (1983) noted that those children diagnosed as having rheumatoid arthritis and who were under order to take large amounts of aspirin on a daily basis had experienced dental erosion. In contrast, however, those who take aspirin without chewing do not seem to be as prone to dental erosion. In this same vein, a number of other medications have been highlighted as linked with dental erosion, such as vitamin C tablets and iron tonic (James & Parfitt, 1953; Giunta, 1983).

C. Environmental

Any profession known to involve day-to-day exposure to acids means that the individual in such a position is at a greater risk of increased dental erosion as a direct result of environmental factors. Examples of such professionals may include battery factory workers, fertilizer factory workers, galvanizing factory workers, and workers exposed to etching and cleaning processes with the use of acids (Zero, 1996). Moreover, professional wine tasters or staff within the chemical industry, for example, are also known to be at greater risk of experiencing some degree of erosion as a direct result of the increased contact between acid and teeth (Wiegand & Attin, 2007).

D. Lifestyle

Lifestyle, which is known to reference the beverages and foods consumed, further considers the time and frequency at which such consumption occurs, as well as oral hygiene practices, which is viewed as being the most fundamental factor impacting dental erosion development. Essentially, the combination of overzealous brushing and regular consumption of acidic foods and drinks may result in greater risk. A number of

dental procedures, namely tooth whitening and dental prophylaxis, can also be detrimental to enamel if used long-term, particularly when combined with overzealous tooth-brushing (Zero, 1996).

2.3.2 Risk factors for dental erosion

There are a number of different influencing factors in regard to dental erosion. A number of behavioural, biological and chemical factors are known to interact with the surface of the tooth, which may subsequently wear it away if such interaction is long-term. Markedly, the interaction of such risk factors is fundamental, and goes some way to explaining the reasons behind why some individuals show a greater degree of erosion than others, even if exposed to the same levels of acid in their diets (Lussi, 2006; Lussi & Jaeggi, 2008).

A. Chemical factors

There are various chemical factors known to affect and impact food and beverage's erosion capabilities, such as pH, buffering capacity, acid type, temperature, adhesion of the product to the surface of the tooth, calcium concentration, fluoride concentration, phosphate concentration, and chelating properties of the product (Lussi & Jaeggi, 2008).

A number of different research studies conducted *in vitro* and *in situ* have illustrated that beverage and food's acidic pH level does not solely determine its erosive potential; rather, there are a number of others impacting factors, namely buffering capacity, calcium-chelating properties of the product, erosive potential, and mineral contents strongly influence the erosive potential (Holloway *et al.*, 1958; Stephan, 1966; Lussi *et al.*, 1993; Mistry & Grenby, 1993; Bartlett, 2005).

The data available on simple acid solutions and beverages illustrate that the erosion detection threshold is believed to be around a pH level of 5.0. Erosion by liquids with a pH as high as 6.3 can be demonstrated but requires the use of very high area/volume ratios (Parry *et al.*, 2001) or long exposure times (McNally *et al.*, 2006). Moreover, it should also be noted that the pH 5.0 erosion threshold is stated on the basis of empirical observations as opposed to the critical pH, i.e. the pH level whereupon there is the saturation of a solution with regard to hydroxyapatite (Lussi, 2006).

In addition, it is also acknowledged that buffering capacity has a fundamental role to play in impacting erosive potential, with a greater degree of buffering capacity meaning the saliva will take a longer period of time to reach its prior neutral pH level. Undoubtedly, it is known that a solution's buffering capacity has a remarkable impact on the erosive attack when the solution is next to the surface of the tooth and is not replaced by saliva. Markedly, the process of dissolution will be enhanced through a food or drink's higher buffer capacity owing to the fact that there is the need for more ions from the tooth mineral so as to facilitate the acid being rendered inactive for further demineralisation (Lussi, 2006).

In addition, the acid type also affects the erosion rate, with the acid strength dictated by the acid dissociation constant (K_a) values. Importantly, the strength of acids is best described through the pK_a value, which is the negative logarithm of the K_a value. One such example is that of lactic acid ($pK_a= 3.86$), which is known to be much stronger than acetic acid ($pK_a= 4.76$); thus, hydrogen ions are provided more promptly, therefore creating a lower level of pH in the solution.

Temperature can also influence the erosive potential of beverages and soft drinks. Evidences in the literature reported that pH of a solution of weak acid decreases with rising temperature, as dissociation of the acid is more thermodynamically favoured (Hankermeyer *et al.*, 2002). Amaechi *et al.* (1999a) investigated the effect of temperature and exposure time on the dental creation of eroded enamel lesions *in vitro*. Human and bovine enamel were exposed to orange juice at different temperatures (4, 20 or 37°C) for different lengths of time (6 times daily for 5 min on each occasion for 12, 16, 20, or 24 days making a total of 6, 8, 10, or 12 h of exposure to orange juice). It was found that the erosiveness of orange juice was less pronounced at a lower temperature, and increased with an increased exposure time.

In addition, West *et al.* (2000) carried out a study to examine the effect of temperature on enamel and dentine with citric acid. They found that increasing the temperature from 5 to 60°C significantly increase tissue loss for enamel and dentine. In terms of material loss, it has been reported that an increase of temperature of 25°C resulted in loss of approximately 5 µm. Therefore, it is believed that the propensity for soft drinks to cause dental erosion is reduced at lower temperatures (Amaechi *et al.*, 1999a; West *et al.*, 2000).

The liquid's displacement and adhesiveness are other aspects also needing to be taken into account throughout the erosive process, with differences apparent in the potential of beverages to adhere to enamel as a direct result of their thermodynamic properties (Ireland *et al.*, 1995).

Calcium and phosphate contents of a foodstuff or beverages are also essential factors in regard to erosive potential as they are known to impact the concentration gradient in regard to the tooth surface's local environment. Importantly, promising results were garnered through the addition of calcium salts to erosive drinks.

The work of Hughes *et al.* (1999) has demonstrated that if calcium is added to blackcurrant juice with a low pH level, the drink's erosive effect is decreased. A subsequent research was carried out, where a blackcurrant drink with added calcium underwent comparison with standard orange drink, with four 250ml drinks consumed each day throughout the course of 20 working days. It was found through the study that the carbonated blackcurrant drink supplemented with calcium caused less enamel loss when compared with conventional carbonated orange drink (West *et al.*, 2003).

A number of other researchers have also highlighted that phosphate added to acid solutions or erosive drinks results in reduced erosive potential (McDonald & Stookey, 1973; Attin *et al.*, 2003). In contrast, however, the work of Hemingway *et al.* (2006) established no such link between phosphate concentrations and the erosive potential of soft drinks, with the author instead suggesting that such a relationship was improbable owing to the fact that the proportion of total phosphate in the form of PO_4^{-3} ions (which influences the degree of saturation) is extremely low at the pH of erosive drinks.

Nowadays, a number of Ca-enriched sports drinks and orange juices are available on the market, which are recognised as only minimally softening the surface of enamel. With this in mind, another example of a food with a low pH level, i.e. 4.0, but which shows

minimal erosive effort as a result of its high levels of phosphate and calcium content, is yoghurt.

The impact of fluoride in regard to dental erosion is not, as yet, fully understood; theoretically, however, it is believed that there are some protective impacts induced by fluoride which, when present in the mouth throughout the course of day-to-day demineralisation and remineralisation rotations, enables the production of fluorhydroxyapatite or fluoroapatite, both of which are known to have a lesser degree of solubility than hydroxyapatite (Lussi, 2006).

Importantly, there is no clearly defined critical pH for erosion as in the case of caries, owing to the fact that erosion is a multi-factorial process, where a number of other factors might be able to prevent erosion, even at a low pH level. Nevertheless, it should be noted that the critical pH level for the erosion of enamel is approximately 4.5, as highlighted by McIntyre (1992).

B. Biological factors

Biological factors, namely saliva, acquired pellicle, tooth structure and positioning in relation to soft tissues and tongue, are all essential factors to be taken into account in regard to dental erosion development (Lussi, 2006).

It is known that saliva is regarded as one of the most critical of biological factors in the influence of dental erosion prevention owing to its capacity to protect against erosion prior to the attack of acid, owing to the salivary flow rate tending to become augmented

as a direct response to acidic stimuli. Markedly, this would result in the saliva buffering system increasing, which efficiently clears and dilutes acids on the surface of dental features during the erosive occurrence.

Moreover, saliva is also known to be pivotal in the production of the acquired dental pellicle, which is known to have a protective characteristic through acting as a dispersal barrier—sometimes referred to as a ‘perm-selective membrane’—which prevents there from being any direct contact between the tooth’s surface and the acid, thereby decreasing the hydroxyapatite level. Markedly, the pellicle protective level appears to be regulated through its configuration, maturation time, and density (Hannig *et al.*, 2005; Lussi, 2006).

Due to its mineral content of calcium, phosphate and fluoride, saliva can possess a reparative effect on early enamel erosion which is characterised by surface softening and slight subsurface mineral loss (Imfeld, 1996). Several *in vitro* and *in vivo* studies have established that etched and softened enamel is remineralisable on exposure to saliva, therefore it is envisaged that saliva may be capable of remineralising (Albert & Grenoble, 1971; Gangler & Hoyer; 1984; Collys *et al.*, 1993). A study By Amaechi & Higham (2001) with the aim of determining possible remineralisation of early enamel erosion by saliva was carried out. Eroded lesions were produced in bovine incisors by 1 hour immersion in orange juice. Control sections and three experimental slabs were produced from each tooth. The three slabs were assigned randomly to one of three remineralising agents: clarified natural saliva, artificial saliva and remineralising solution. All solutions had a pH of 7.2, a fluoride concentration of 0.022 ppm, and were changed daily. Natural saliva was collected daily from the same individual at the same

time of day. The specimens were exposed to their respective remineralising agents for 28 days. Using microradiography and image analysis, the mineral loss and lesion depth were quantified in sections cut from the control and experimental slabs. The results showed that a significant amount of mineral was gained following exposure to each remineralisation agent. The effect was greatest with remineralisation solution and least with artificial saliva. This study demonstrated that saliva can remineralise early enamel lesions.

The location and positioning of the dental arch's teeth may also impact the overall vulnerability of teeth in regard to erosion, with the upper incisors' surfaces and lower teeth lingual surfaces having higher and lower erosion susceptibility respectively, as noted by Lussi (2006).

C. Behavioural factors

Behavioural factors are also known to play a role in changing the degree to which erosive tooth wear is recognised, with behavioural factors impacting the overall potential of dental erosion to develop, such as through the abusive and unusual consumption of food and beverages, excessive use of tooth bleaching products, frequent dieting with high consumption of citrus fruits and fruit juices as part of weight-reducing plan, healthier lifestyles that may involve frequent consumption of acidic fruits and vegetables, unhealthy lifestyles involving illegal designer drugs, and overzealous oral hygiene practice with abrasive dentifrices (Zero 1996; Zero & Lussi, 2000).

Behaviour may also be significantly impacted by the socio-economic status of the individual, with a number of research studies having previously assessed the link between socio-economic status and dental erosion (Millward *et al.*, 1994; Milosevic *et al.*, 1994). Moreover, Millward *et al.* (1994) noted that the socio-economic status is known to have had a notable impact on the prevalence of dental erosion in the case of 4 year old children, with more severe erosion recognised amongst those of a higher socio-economic status, although also illustrating better oral hygiene when compared with those from lower socio-economic groups.

To sum up, Figure 2.1 provides an overview of the multi-factorial dental erosion inclining factors and aetiologies.

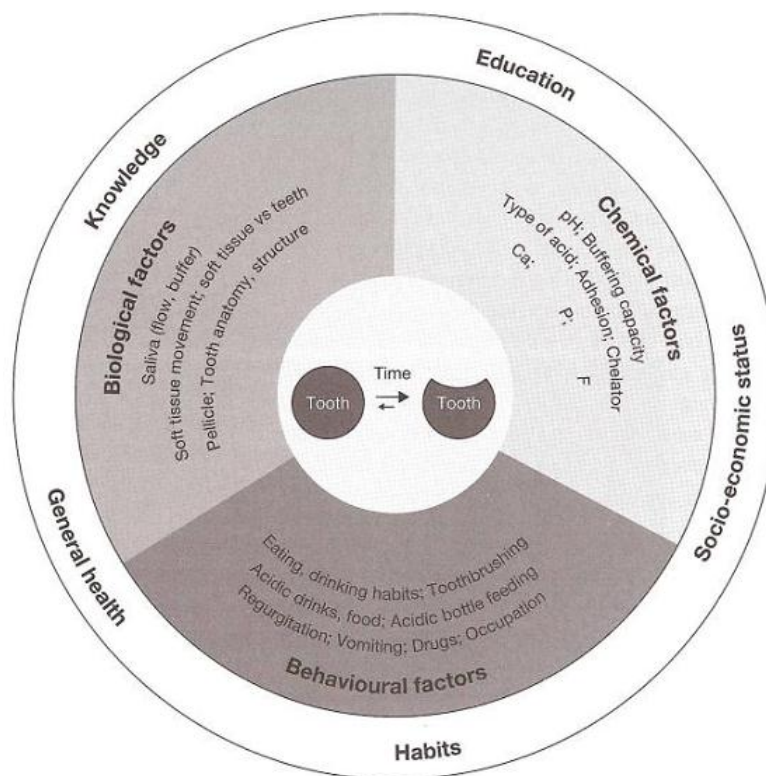


Figure 2.1: Interactions of the different factors for the development of dental erosion (Lussi, 2006)

2.3.3 Mechanism of dental erosion

It is believed that dental erosion arises when the teeth's minerals are dissolved, such as through anions that bind calcium or otherwise via hydrogen ions. The latter of the two are derived from acids as they dissolve in water. The impact of the hydrogen ions' direct attack is the combining of phosphate with carbonate, which subsequently results in the release of crystal surface ions, accordingly creating direct enamel etching, as highlighted by Lussi (2006).

A number of acids, such as citric, may be utilised to discuss the chelating process. In the case of water, citric acid is present as a combination of acid anions, hydrogen ions, and undissociated acid molecules. Importantly, the behaviour of the hydrogen ions is as described above, i.e. through direct attack on the surface of the crystal. The citrate anion may also become adhered with the calcium, subsequently removing it from the surface of the crystal and/or from the saliva (Lussi, 2006).

The erosive process development necessitates that the acid comes into contact with the enamel, thus meaning it is first required to diffuse through the acquired pellicle, with the tooth's surface experiencing a few micrometers mineral loss as a subsequent result, consequently instigating a process known as softening or partial demineralisation (Koulourides, 1968). Moreover, when the softening further develops, the tooth's most superficial layer dissolves, and is ultimately lost completely (Eisenburger *et al.*, 2001a).

The prism sheath area is primarily dissolved, with the prism core subsequently dissolved, which is known to create the honeycomb-like appearance, as noted by Meurman & Frank (1991). Following, the subsurface region's interprismatic area will

experience further mineral loss (Eisenburger *et al.*, 2001b; Lussi & Hellwig, 2001). Such an ions outflow will subsequently induce a rise in the local pH of the tooth substance, as well as in the surface of the liquid in close proximity to the surface of the enamel.

Importantly, a softened tissue's remineralisation is possible following erosion provided there has been no direct etching of the surface. Softened enamel exposure—such as to saliva or remineralisation solution—for sufficient time may result in the regaining of mineral (Koulourides 1968; Collys *et al.*, 1993; Eisenburger *et al.*, 2001a). Nevertheless, once there has been the occurrence of tissue loss, this cannot be reversed to its original form.

2.3.4 Prevalence of dental erosion

It is commonly held that the occurrence of dental erosion is on the increase (Sheiham, 2001), although a number of individuals believe that erosion is being picked up owing to the fact that there is a decline in caries rate. A number of research studies focused on prevalence and incidence have garnered information relating to the occurrence, progression and distribution of erosion. Nevertheless, drawing a contrast between such results is difficult owing to the various examination standards adopted, i.e. the calibration of examiner(s), scoring system, number and site of teeth, etc., as well as the various non-homogeneous groups examined, namely age, gender, number of examined individuals, geographical location, etc.

2.3.4.1 Prevalence of dental erosion in children and adolescents

The very first research to consider the prevalence of tooth erosion within the UK was of the Children's Dental Health Survey of 1993 (Office for National Statistics, 1994). Importantly, the survey emphasised that more than half of 5 and 6 year olds showed some degree of erosion, with almost a quarter involving the dentine. Importantly, tooth tissue loss was the most prevalent in regard to the incisors' palatal surfaces, with more than half (52%) of 5 year olds affected, with only 18% presenting buccal erosion. With regard to the permanent dentition, targeting children aged 11 years and older, approximately 25% showed some degree of erosion, with 2% of teenagers further demonstrating dentine wear. It was suggested via the survey report that such findings may be due to an increase in soft drinks consumption, with such beverages known to contain acids (Office for National Statistics, 1994).

The National Diet and Nutrition Survey (NDNS), which targeted children aged 1½ to 4½ years, established that 29% of all 3½–4½ year olds showed some form of erosion, with 13% erosion involving dentine, as noted by Hinds & Gregory (1995). Moreover, the most recently carried out NDNS study targeting young people aged 4–18 years noted that 42% of 11–14 year olds displayed erosion, with 3% illustrating exposed dentine. However, this research failed to establish a lack of sound relationship between dental erosion and the frequency of intake of either sugary or acid foods (Walker *et al.*, 2000).

It was highlighted through the Children's Dental Health Survey of 2003 that one-fifth of all 5 year olds show some degree of dental erosion in regard to one or more of the primary upper incisors' buccal surfaces, with 3% of cases involving dentine. For those

aged 8 and 15 years, permanent incisor erosion was found in 4% and 14% of cases respectively, with an increase recognised in terms of the number of individuals showing erosion on the lingual surfaces (Office for National Statistics, 2004).

A further study contrasted erosion affecting buccal surfaces of maxillary permanent incisors and palatal surfaces amongst a sample of 11–13 year old children in both the USA and UK. The findings suggest that prevalence is quite similar across both locations, with the USA showing 41% whilst the UK shows 37%. Importantly, lesions were mainly limited to enamel (Deery *et al.*, 2000).

A number of epidemiological studies have been carried out in order to determine the overall prevalence of erosion in children and adolescents. Information garnered through such research studies suggest that the overall prevalence of erosion is between 20% and 70% in the teenage population, with 5% demonstrating severe erosion. Interestingly, erosion was found to be more prevalent amongst males than females. The relationship between erosion and socio-economic status remain controversial (Milosevic *et al.*, 1994; Al-Dlaigan *et al.*, 2001a; Dugmore & Rock, 2004b; Bardsley *et al.*, 2005). Markedly, Table 2.1 provides an overview of such epidemiological studies.

Table 2.1: Epidemiological studies of dental erosion in children and adolescents of the United Kingdom

(O'Sullivan & Milosevic, 2008)

Author	Year of Publication	Age of subjects	Sample size	% with exposed dentine	% with palatal, labial/ dentine exposure	Teeth included	Surfaces
O'Brien	1994	5 12/14	17 061		24 2	U1 ^y incisors U2 ^y incisors	Lab/Pal Lab/Pal
Millward <i>et al</i>	1994	4-5	178	48		All 1 ^y teeth	All
Milosevic <i>et al</i>	1994	14	1035	30%	8	All 2 ^y teeth	All
Jones & Nunn	1995	3	135	17		U1 ^y incisors	Lab/Pal
Hinds & Gregory	1995	1½- 4½	1496	-	8	U1 ^y incisors	Lab/Pal
Bartlett <i>et al</i>	1998	11-14	210	-	2	All 2 ^y teeth	All
Williams <i>et al</i>	1999	14	525	11	1	U2 ^y incisors	Lab/Pal
Walker <i>et al</i>	2000	4-6 7-10 11-14 15-18	363 500 518 345		19 18 3 5	U1 ^y or 2 ^y incisors First 1 ^y or 2 ^y molars	Lab/Pal Occ
Al-Dlaigan <i>et al</i>	2001a	14	418	52		All 2 ^y teeth	All
Dugmore & Rock	2004b	12	1753	3		Incisors & first molars	Lab/Pal Buc/Occ/Lab
Bardsley <i>et al</i>	2004	14	2351	53	10	All anterior teeth & Occ of first molars	Lab/Inc/Pal
Chadwick & Pendry	2004	5 12/15	12698		22 5	U1 ^y incisors U2 ^y incisors	Lab/Pal Lab/Pal

U=upper; 1^y=primary; 2^y=permanent; Lab=labial; Pal=palatal; Occ=occlusal; Buc=buccal; Inc=incisor

2.3.4.2 Prevalence of dental erosion in adults

There has been a number of studies focused on the prevalence of dental erosion involving adolescents and children, however only a few studies have been carried out on adults, possibly owing to the issues associated with recruiting participants for large-scale, unit research studies. Accordingly, a number of studies have garnered adult samples through convenience methods, such as through referred groups or armed forces (Smith & Knight, 1984; Jarvinen *et al.*, 1992). In the UK, for example, only one research has been carried out on the general population with the aim of predicting the overall prevalence of tooth wear in the case of 1,007 dental patients. Markedly, subjects were divided into six respective age groups, as follows: 15–26, 26–35, 36–45, 46–55, 56–65 and >65 years. The findings suggested that, for those in the 15–26 year age group, just under 6% of tooth surfaces had been worn to an unacceptable extent, with the three intermediate groups, i.e. those in the 26–35, 36–45 and 46–55 year age groups, showed values of between 3.37% and 4.62%, whilst the 56–65 year age group showed a figure of 8.19%. Finally, those aged 65 years and older were found to have pathological tooth wear in 8.84% of the surfaces (Smith & Robb, 1996).

2.3.5 Diagnosis of dental erosion

In the context of dental erosion, the clinical detection of such is paramount upon the initiation of dissolution. Nevertheless, currently there is no diagnostic device available for early clinical detection and quantification of dental erosion. Accordingly, one of the most important indicators for dental professionals striving to diagnose erosion is clinical appearance.

The early signs of enamel erosion include a surface lacking in perikymata, which may thus give a shiny, silky appearance. When erosion is more advanced, a number of other morphological changes occur, which may subsequently encourage further surface flattening or enamel concavity development, with the width obviously exceeding its depth (Lussi, 2006).

The early features of erosion on occlusal and incisal surfaces are identical to what has been discussed above, although subsequent development of occlusal erosion results in the cusps rounding, with cusp grooves and incisal edges developing, with restorations also rising above the adjacent tooth's surface level. In more serious cases, the entire occlusal morphology disappears (Lussi, 2006). Furthermore, loss of enamel can lead to dentine exposure with reactionary dentine formation by odontoblasts. The exposed dentine surface may become sensitive to cold and warm foods and to tactile stimuli.

When dental erosion is detected, detailed patient assessment, including in-depth consideration of the patient's history with regard to diet, general health, and habits should be carried out.

2.3.6 Indices to assess dental erosion

Various indices for the clinical diagnosis of erosive tooth wear have been suggested, which more or less are modifications or combinations of the indices published by Eccles (1979) and Smith & Knight (1984).

Notably, all erosion indices comprise criteria for the assessment and measurement of hard tissue loss, and diagnostic criteria with the objective to distinguish erosion from other types of tooth wear. Importantly, the area size impacted is commonly detailed as the size affected in relation to the structure of the sound tooth. The defect and the depth of such are predicted through the use of a dentine exposition criterion; thus, a link between substance loss amount and exposed dentine can then be considered.

The clinical diagnosis of dental erosion indices comprise the following: Eccle's Index (1979), Smith and Knight Tooth Wear Index (TWI) (1984), Modified scoring system of Linkosalo & Markkanen (1985), Aine Index (1993), Erosion Index according to Lussi (1996), Larsen and Westergaard Index (2000), O'Sullivan Index (2000), and the UK National Survey of Children's Dental Health Index (Office of National Statistics, 2004). Such indices are detailed in Appendix 1.

2.3.7 Assessment of progression rate of dental erosion

The development and behaviours associated with dental erosion are problematic to assess. Contrasting clinical photographs of tooth surfaces is one of the tools available to predict possible substance loss over a period of time. The sensitivity and discolouration of the lesion may provide some insight into the tooth surface activity. Moreover, study

casts in addition to the analysis and examination of dental radiology provide a prediction of wear development (Lussi, 2006).

For research purposes, profilometric measurements using acid resistant markers and computed control mapping are tools able to monitor and assess the overall development of dental erosion.

2.3.8 Dental complications of erosion

A number of clinical problems can arise as a manifestation of dental erosion including aesthetics. Severe erosion usually results in enamel fracture, which progresses to shortening of the teeth and loss of occlusal vertical dimension (Linnett & Seow, 2001).

Dentine sensitivity and difficulty in eating are common problems of dental erosion, especially if there is a rapid and progressive rate of erosion. In such an instance of the rapid loss of tooth structure from dental erosion in children with immature teeth, there is a significant likelihood that pulpal inflammation and exposures will be exhibit as a result (Linnett & Seow, 2001).

2.3.9 Investigating dental erosion

A number of assessment approaches have been utilised with the aim of evaluating dental hard tissue loss induced through erosive challenges. The most commonly implemented approaches are discussed in the subsequent sections of this literature review.

A. Scanning electron microscopy

Scanning electron microscopy (SEM) predicts surface alteration following an erosive attack through the use of qualitative measures, classifying the degree of surface alteration severity, which may be carried out on separately adopted scales. In the case of enamel, surface etching and exposure of enamel prisms may be the results of an acid attack due to specimen immersion in erosive solutions. With regard to dentine, acid treatments may create an opening of dental tubules, which may be assessed and analysed in regard to their degree (Meurman *et al.*, 1991).

In specific consideration to environmental SEM (ESEM), there is no need to conduct sample preparation. ESEM also allows examination of samples in wet conditions and without metal or carbon coating.

B. Surface hardness measurements

Microhardness indentation measurements have been used to determine de- and remineralisation effects using the *in situ* model of Koulourides (1966). Microhardness testing measures the resistance of enamel surfaces to indenter penetration and is a function of the degree of porosity of the superficial enamel layer that indicates mineral loss or gain in subsurface lesions (Koulourides, 1971). In this method, a Knoop or

Vickers diamond is positioned on the sample with a well-defined load for a given time, in order to create an indentation in the tooth surface. The indentation length is then determined microscopically (in μm) (Angmar-Månsson & ten Bosch, 1991). The Knoop diamond produces a diamond-shaped indentation whereas the Vickers diamond produces a rectangular-shaped indentation. For an indentation length of 100 μm , the Knoop method penetrates approximately 3.5 μm , whereas the Vickers diamond penetrates approximately 14 μm (Arends & ten Bosch, 1992).

A linear relationship has been demonstrated between the Knoop indentation length and lesion depth (Arends *et al.*, 1980). White (1987) showed a similar relationship for Vickers indentations. However, it has been suggested that this linear relationship is valid only in limited range of lesion depth values (Arends *et al.*, 1980; Zero *et al.*, 1990). Featherstone *et al.* (1983) reported that the length of the Knoop diamond indentation or square root of the hardness number correlated with the mineral content of carious enamel as measured by volume.

Two types of microhardness testing are available, namely surface microhardness and cross-sectional microhardness. Surface microhardness (SMH): where a load with a diamond indenter is applied perpendicular to a polished tissue surface. SMH measurements can only give qualitative information on mineral changes when used for the assessment of de- and remineralisation. Moreover, the samples should have flat surfaces according to Arends & ten Bosch (1992). Other factors have been established to affect the indentation length values including lesion shape, mineral redistribution, and protein uptake *in situ*. A linear relationship between indentation length and lesion depth is valid only for a limited range of lesion depth values (Arends *et al.*, 1980; Zero *et al.*,

1990). SMH is a non-destructive technique that allows for a longitudinal study of the same specimen, however it cannot provide details about the subsurface hardness changes or inform about any structural alterations to different sides of the lesion (Featherstone *et al.*, 1983).

Cross-sectional microhardness (CSMH): where the diamond indenter load is applied parallel to the tissue's anatomical surface (Arends *et al.*, 1980). A number of advantages associated with CSMH experiments include the ability to provide indirect evidence of mineral loss or gain as well as the possibility to obtain the mineral profile (volume percentage of mineral as a function of the distance from the outer surface). However, CSMH experiments cannot include the outermost 25 μm of a sample in the measurement (Arends & ten Bosch, 1992).

Some researchers (Jaeggi & Lussi, 1999; Joiner *et al.*, 2004) tried to measure the amount of tooth surface loss caused by the erosive/abrasive challenge using microhardness. The difference between indentation depth before and after abrasion provided a direct measurement of tooth surface loss by abrasion. However, measurement of the amount of surface loss by the erosive attack was not possible because acids removed some substance from the body of the indentation and not only from its surrounding.

The key benefits associated with microhardness determinations include the comparatively low costs, the long-term system experience, and the potential to combine such with a number of other approaches. Nevertheless, there are also drawbacks of using this technique include needing a flat, polished surface and this test only gives

qualitative information on mineral changes, thereby is an indirect measure of enamel loss or gain (Featherstone & Zero, 1992).

C. Surface profilometry

Surface profilometry is a means of measuring surface loss of dental hard tissues. It uses a small metal stylus (20 µm diameter) that scans across the enamel surface at a rate of around 10 mm/min for the acquisition, graphical presentation, evaluation and documentation of surface profiles. In this technique, the enamel surface is divided into two parts, an exposed and a covered part using nail varnish or tape. The sample surface is scanned before and after erosion, and the amount of material loss can be measured from the trace produced. Alternatively, a cast may be made of the eroded enamel surface and the profilometer used to measure the profile of the cast.

More recently, non-contact profilometry has been developed to evaluate tooth surface loss. In this technique, the traditional contact stylus is replaced with white light or a laser, and interferometry is used to build up a map of the surface (Figure 2.2).

The optical profilometer transmits safe white light through a lens that has a spectral aberration built into it. This causes the white light to divide into the full spectral field, and each of the different colour frequencies are focused at a slightly different point through a defined measuring range. When an object is then placed within this range, only one particular colour frequency reflects back from the surface. This information is then passed back into a processor where a spectrometer analyses the signal and converts it into a measurement (Figure 2.3).

Figure 2.2: Schematic description of the basic operational principles of the optical profilometer (Abdullah, 2009)

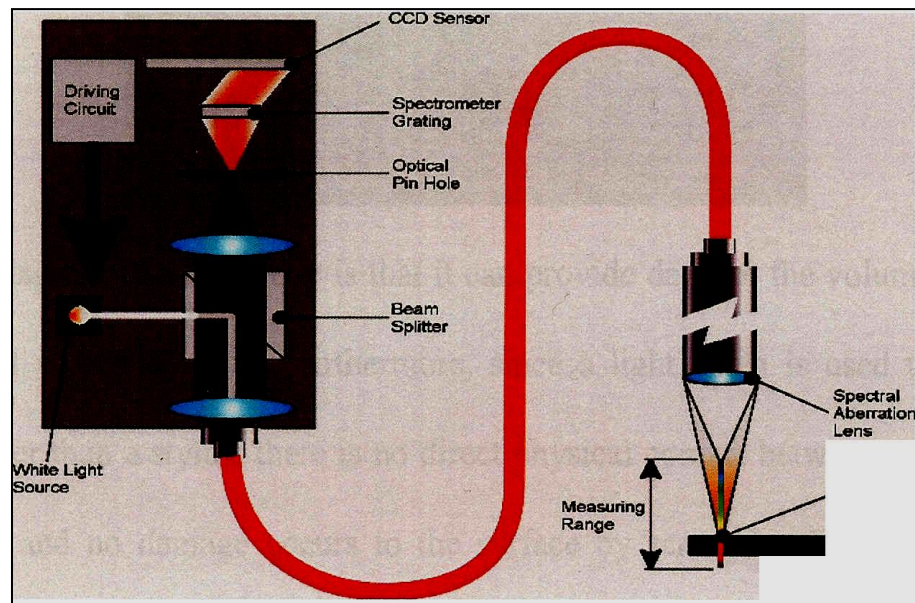
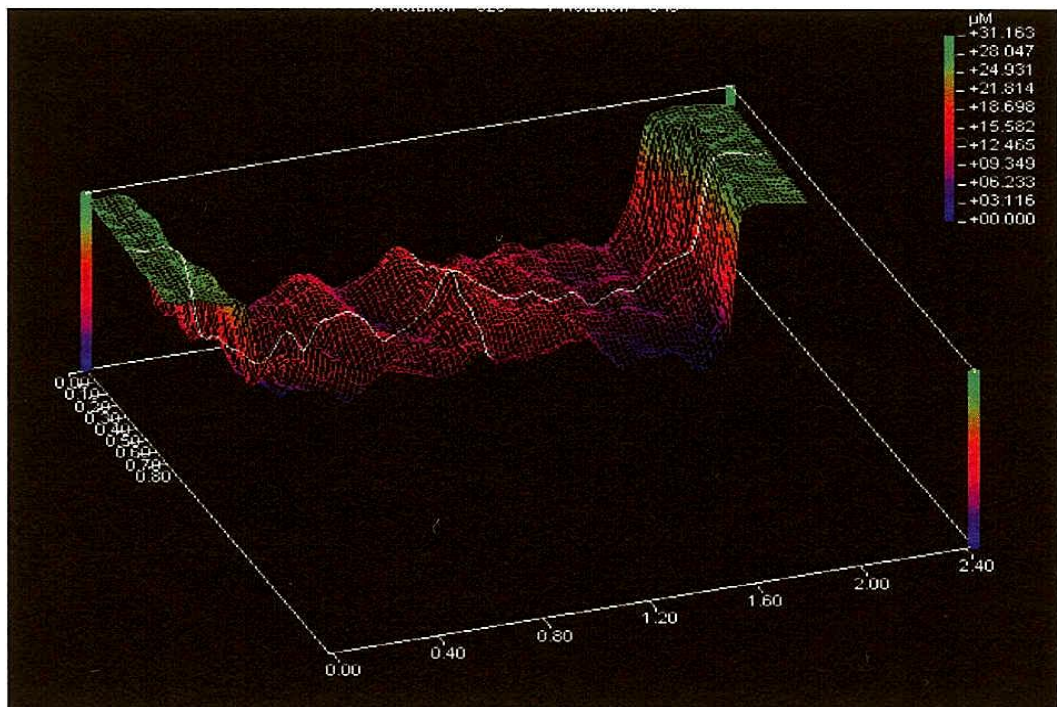


Figure 2.3: Erosive lesion on an enamel slab scanned by an optical profilometer (Abdullah, 2009)



In profilometry studies, polished surfaces are commonly utilised due to the fact that dentine surface and/or native enamel provide an intrinsic coarseness render impossible the detection of small changes as a result of abrasion/erosion. Nevertheless, in natural enamel, the measurement of natural enamel extended depths reaching at least 50 μm of erosive grooves can be measured without there being any call for the surface to be polished (Ganss *et al.*, 2000).

Profilometry is a quick and simple technique that can be used over a relatively large area of enamel. Nevertheless, the sample of enamel needs to be ground flat before utilisation, with the adoption of laser beams not able to create direct contact; thus, no damage will be inflicted upon the surface.

D. Iodide permeability test

Iodide Permeability Test (IPT) was originally devised and introduced by Bakhos *et al.* (1977), and has the main foundation that defined areas of enamel samples are made to soak for a number of minutes in a potassium iodide solution, retrieved from the enamel through the use of millipore prefilter paper discs (Bakhos *et al.*, 1977). The amount of iodide recovered from the discs is established, which helps to give data relating to the enamel's pore volume.

The key benefit associated with IPT is the low costs, which provide a very quick and simple method of screening the effects of various erosive substances on enamel.

E. Chemical analysis of minerals dissolved in the erosive agents

Dental enamel comprises 34-39% m/m (g per 100g) calcium (dry weight) and 16-18% m/m phosphorus (ten Cate *et al.*, 2003). Accordingly, dental enamel dissolution is established through analysing the volume of calcium or phosphate dissolved from the apatite crystals of dental hard tissue; this can provide a potentially feasible approach for examining and estimating dental erosion. The examination of mineral release has been used in various situations, i.e. both *in situ* and *in vitro*, with the use of extra-oral erosive challenge. Moreover, it has also been used *in vivo* (Young *et al.*, 2006), and is appropriate in the context of longitudinal measurements.

The chemical approaches are used to assess erosive dissolution and include the key benefit that they facilitate minor mineral loss (10 µl) detection through the use of unpolished, native tooth samples. However, one limitation is that the erosive challenge cannot take place in the presence of saliva, which would interfere with the analysis. Furthermore, this method does not provide information about possible mineral gain, or about physical and morphological changes.

F. Microradiography

Microradiography is a technique concerned with measuring mineral loss based on the attenuation of X-ray irradiation transmitting dental hard tissue. This approach has been widely implemented in research studies focused on establishing mineral changes as a result of remineralisation and demineralisation (de Josselin de Jong *et al.*, 1987; 1988).

The key benefit associated with microradiography is the fact that this approach enables the simultaneous recognition of the eroded sample's surface loss and demineralisation.

G. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) may be adopted in order to secure high-resolution images, 3D reconstructions, and optical sections through 3D specimens. In those research studies focused on erosion, CLSM is recognised as delivering histotomographic images, thus providing qualitative assessment and the clarification of hard tissue destruction or mineral dissolution. Importantly, CLSM is mainly amalgamated with various other approaches, including microhardness or others, owing to the fact that such images deliver limited information regarding the precise extent of demineralisation (Duschner *et al.*, 1996).

The main benefit of CLSM adoption is recognised as the system's high resolution, which delivers 3D insight into the erosively altered substrate.

H. Quantitative light-induced fluorescence

Quantitative Light-induced Fluorescence (QLF) is commonly implemented in longitudinal examinations of early caries lesions (van der Veen *et al.*, 2000). Nevertheless, this approach has been adopted in two different studies with the aim of analysing erosive lesions, as highlighted by Pretty *et al.* (2003, 2004). Moreover, there has been the validation of QLF in comparison with transverse microradiography, and was found to be an efficient and valuable measure for erosive defect quantification.

I. Atomic force microscopy

Atomic Force Microscopy (AFM) has been implemented in a number of erosion research studies for qualitative approaches comparing dental hard tissue surfaces with

acquired enamel pellicle following exposure to a number of erosive agents (Lippert *et al.*, 2004).

A key benefit of AFM is its potential to perform within ambient conditions (in air or liquids), in addition to within a vacuum environment, thus meaning artefacts may be diminished or altogether eradicated, and enabling serial measurements.

J. Nano-indentation

Indentation is the most widely implemented testing approach with regard to materials' mechanical properties. It is known that nano-indentation provides a great deal of sensitivity, measuring the hardness of materials in small volumes (Oliver & Pharr, 1992).

K. Ultrasonic measurements of enamel thickness

Through ultrasonic pulse-echo measurements, the time periods between the broadcast of an ultrasound pulse on the enamel surface and the echo created through the amelodentinal junction is established (Huysmans & Thijssen, 2000).

This approach offers the benefit of facilitating non-destructive analysis without the need for sample preparation to be too extensive.

2.3.10 Methods for evaluating dental erosion

A number of methodologies have been validated for measuring tooth wear from erosion. Ideally studies should be conducted *in vivo*, using intra-oral measurement of tooth tissue loss. The randomised clinical trial would be the gold standard for this kind of the study. Unfortunately, *in vivo* studies are known for their inability to achieve very precise tooth wear measurements. The main impediment is the low accuracy of available methods for measurements of tooth tissue loss *in vivo* (Huysmans *et al.*, 2011). Other factors include: uncertainty about the pattern of progression, which implies a need for studies of long duration; lack of control over exposure to wear, which makes it difficult to assess the effect of erosion alone, and the need to minimise contact of erosive agents with the dentitions of trial subjects. As a result, *in situ* and *in vitro* models have been developed in attempt to overcome the challenges of *in vivo* studies.

a. *In vitro* models

In vitro models have a number of advantages; they are inexpensive and can be executed over a short period of time. They require fewer staff than *in situ* studies, and do not require participant compliance. However, the main disadvantage of the *in vitro* models is that they cannot replicate the oral environment with all of the biological variation known to influence erosion.

b. *In situ* models

In *in situ* models, specimens of dental tissues are carried in the mouth and are exposed to erosive challenges at pre-determined times, either intra-orally or *ex vivo*. Tissue loss is measured *ex vivo*, allowing the use of accurate analytical methods.

The first *in situ* models used small gold cups (Bunting *et al.*, 1926) or gold plates (Nygaard Østby *et al.*, 1957) to study demineralisation in vital teeth. Few years later, Koulourides & Volker (1964) introduced a new *in situ* model (intra-oral cariogenicity/ICT model) to assess the cariogenicity of different types of foods. They suggested that this model was suitable to determine the ability of topically applied substances to limit tooth decay.

Following the Koulourides & Volker (1964) model, several modified versions have been developed and used widely in various aspects of dental research studies serving as an intermediate step between animal studies and *in vitro* investigations on one hand and clinical or field trials on the other (Manning & Edgar, 1992; Clasen & Øgaard, 1999).

In situ models are particularly suited to assess erosion by beverages and the possibility that numerous agents can deliver protection in regard to a number of erosive challenges. They enable the entire process of erosion to be monitored over time in a more or less entirely natural environment comprising pellicle development, routine oral care, and saliva flow.

Overall, *in situ* models use two approaches:

a) Removable appliances with intermittent or continuous presence in the mouth

With intermittent application, the removable appliances are worn by participants only under supervision, e.g. during office hours, but are removed from the mouth during out-of-office time, including night times (West *et al.*, 2003; 2004). The main advantages of this removable model are that the participant's compliance can be monitored by the trial

co-ordinator, and strict standardised conditions of treatment can be imposed. In addition, the variation in participant's behaviour can be reduced.

In comparison, when removable appliances are continuously present in the mouth, participants may be asked to remove the appliances during certain times e.g. eating, drinking or brushing, but otherwise to wear them at all times. In this model, participant's compliance can be compromised, but a more realistic model of erosion is achieved because overnight exposure to oral conditions incorporates the possible beneficial effect of remineralisation into the model (Abdullah *et al.*, 2006; Laheij *et al.*, 2010).

b) Fixed appliance with continuous presence in the mouth

Fixed appliances are attached to participant's teeth for a short period of time to overcome problems of compliance (Amaechi *et al.*, 2000; Amaechi & Higham, 2001; Amaechi *et al.*, 2010). These appliances are continuously present in the mouth allowing the specimens to be exposed to all the daily processes occurring within the oral cavity. When these models are used to assess the erosive potential of an agent, upper and lower soft mouth-guards with a window exposing only the specimen to the agent can be used to protect the participant's teeth from direct contact with the agent.

2.4 SOFT DRINKS

Soft drinks are non-alcoholic beverages, usually containing a flavouring agent and water. Many of these beverages are sweetened by the addition of sugar. They may also contain ingredients such as caffeine and fruit juice. The British Soft Drinks Association (BSDA) state that soft drinks are viewed as carbonated drinks, fruit juices, smoothies and bottled waters, including sports and energy drinks, and still and dilutable drinks (BSDA, 2011).

The consumption of soft drinks has experienced a significant increase during recent years in economically developed countries, as has been established by the BSDA through its annual consumer researches. Importantly, in 2010, it was found that 14,585 million litres of soft drinks were consumed, equating to approximately 235.1 litres per person (BSDA, 2011). Table 2.2 provides an overview of such data.

Table 2.2: UK soft drinks consumption, 2004–2010 (BSDA, 2011)

UK soft drinks consumption, 2004 - 2010							
Year	2004	2005	2006	2007	2008	2009	2010
Million litres	13510	13565	13985	13865	13725	14005	14585
% change	-1.4	+0.4	+3.1	-0.9	-1.0	+2.0	+4.1
Litres per person	224.8	224.5	230.3	227.1	223.6	226.9	235.1
Value, £ million	11955	12155	12525	12595	12720	13120	13880
% change	-1.4	+1.7	+3.0	+0.6	+1.0	+3.1	+5.8
Value per litre, £	0.88	0.90	0.90	0.91	0.93	0.94	0.95

Source: Zenith International

Figure 2.4 indicates that carbonated drinks are the most popular consumed drink in the United Kingdom, with consumption rising from 98.8 litres in 2009 to 103.1 litres per person in 2010 (see Table 2.3).

**Figure 2.4: UK soft drinks sectors, 2010
(BSDA, 2011)**

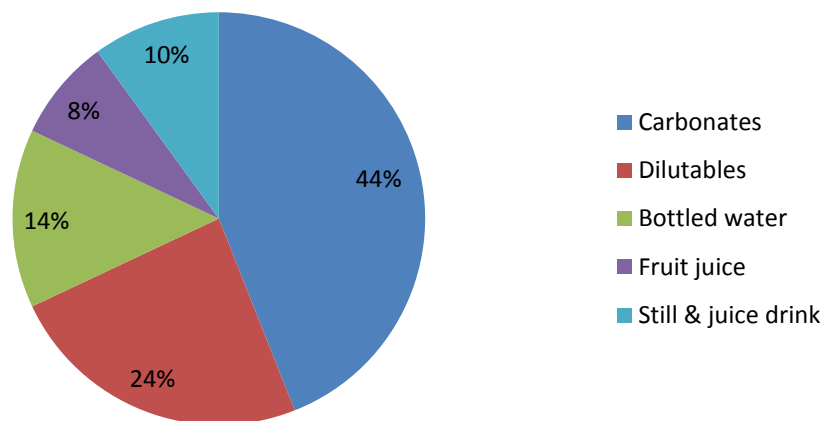


Table 2.3: UK carbonates consumption, 2004-2010 (BSDA, 2011)

UK carbonates consumption, 2004 - 2010							
Year	2004	2005	2006	2007	2008	2009	2010
Million litres	6195	6015	5875	5810	5920	6100	6400
% change	-5.1	-2.9	-2.3	-1.1	+1.9	+3.0	+4.9
Litres per person	103.1	99.6	96.8	95.2	96.4	98.8	103.1
% of all soft drinks	45.9	44.3	42.0	41.9	43.1	43.6	43.9
Value, £ million	6930	6795	6755	6850	7120	7515	8000
% change	-1.8	-1.9	-0.6	+1.4	+3.9	+5.5	+6.5
Value per litre, £	1.12	1.13	1.15	1.18	1.20	1.23	1.25

Source: Zenith International

With regard to fruit juices (including smoothies) consumption, there has been a decline in 2009 (see Table 2.4), most likely related to reduced disposable income due to current economy. However, in 2010, fruit juices consumption registered 3.1% volume growth with consumption rising from 18.6 litres in 2009 to 19.0 litres per person in 2010.

Table 2.4: UK Fruit juice consumption, 2004–2010 (BSDA, 2011)

UK fruit juice consumption, 2004 - 2010							
Year	2004	2005	2006	2007	2008	2009	2010
Million litres	1040	1120	1210	1230	1190	1145	1180
% change	+3.0	+7.7	+8.0	+1.7	-3.3	-3.8	+3.1
Litres per person	17.3	18.5	19.9	20.1	19.4	18.6	19.0
% of all soft drinks	7.7	8.3	8.7	8.9	8.7	8.2	8.1
Value, £ million	1540	1675	1820	1830	1760	1670	1760
% change	+0.7	+8.8	+8.7	+0.5	-3.8	-5.1	+5.4
Value per litre, £	1.48	1.50	1.50	1.49	1.48	1.46	1.49
Source: Zenith International							

The majority of young people drink soft drinks. The national diet and nutrition survey (NDNS) conducted in 2000 demonstrate that high calorie soft drinks were the largest contributor by weight to the total weight of sugary foods consumed. The mean daily consumption of carbonated drinks was 337g, which is equivalent to just over a can a day, and 239g for boys and girls aged 15 to 18 years respectively (Walker *et al.*, 2000).

2.4.1 Effect of consumption of soft drinks on dental erosion

A number of different factors are known to play a role in the erosive potential of soft drinks, such as the drink's buffering capacity and its pH level. Notably, a number of drinks may contain various different acids, some of which may be derived through natural sources and therefore considered inherent, whilst others may be additives and preservatives (Tahmassebi *et al.*, 2006).

A number of research studies have emphasised a link between the consumption of soft drinks and dental erosion, with the aetiological role of the consumption of soft drinks in regard to dental erosion development evidenced through the conduction of animal studies, case reports, clinical trials, epidemiological studies, experimental clinical studies, and experiments, etc.

a) Clinical trials

A research study was carried out by Tomas in 1957 concerned with evaluating the impacts of the daily ingestion of numerous amounts of acidic beverages in terms of microscopic and macroscopic changes in the maxillary anterior teeth's labial surface of a number of dental hygiene students and dental students. Markedly, the subjects were divided into groups of 20, and were asked to drink orange juice, grapefruit juice and carbonated cola, with the numbers further subdivided into groups of five according to whether they were to drink 6, 12, 18 or 24 ounces of the juice or carbonated beverages per day. There was also a control sample of 10 students, who notably refrained from ingesting all forms of citrus fruits and carbonated beverages. The research study established that those consuming cola, grapefruit juice or orange on a daily basis for a

six-week period showed signs of erosion on their labial incisors, with such a finding most prevalent amongst those drinking grapefruit juice (Tomas, 1957).

In addition, Stabholz *et al.* (1983) contrasted the ultrastructure of exfoliated primary teeth of children who consumed 100ml of orange beverage on school days for 12–18 months with teeth from children who did not receive any beverage at school. As a result, it was found that the teeth of those children who had consumed the orange showed slight demineralisation.

b) Epidemiological studies

Epidemiological research studies have garnered a great deal of valuable data concerning the link between acidic drinks and dental erosion, with Lussi *et al.* (1991), for example, conducting a survey with the aim of establishing the prevalence of dental erosion in an adult population in Switzerland. Markedly, 391 randomly selected adults from two age groups (26–30 and 46–50) were examined in regard to the severity and frequency of erosion on all tooth surfaces. Moreover, data relating to diet, general health and oral health habits were gathered through subject interviews. The findings emphasised a strong, significant link between dietary habits, such as the consumption of fruit juices, citrus fruits or other fruits, with erosion (Lussi *et al.*, 1991).

In a further study carried out by Millward *et al.* (1994), the prevalence of dental erosion was examined amongst 101 English children aged 4–16 years, with the role played by diet in regard to dental erosion assessed, subsequently highlighted that the erosion prevalence of the population stood at 80%. Moreover, it was further noted that there was

a strong link between erosion severity and the consuming of carbonated beverages, fruit drinks and fruit juices.

Another study was carried out by Al-Dlaigan *et al.* (2001b) with the aim of establishing a relationship between dental erosion and the dietary intake of 418 British children aged 14 years. The results showed that over 80% of teenagers regularly consumed soft drinks but approximately half of these children had a relatively low weekly consumption. A strong, significant correlation between the consumption of soft drinks, carbonated beverages and fresh fruits with the prevalence of dental erosion was noted.

c) Experimental clinical studies

Experimental clinical research studies have emphasised that the consumption of, or rinsing with, acidic beverages markedly decreases the pH level of oral fluids, with this finding strongest in regard to grapefruit juice (Imfeld, 1983). Enamel slab experiments have also illustrated that there is the softening of enamel within an hour of exposure to cola, although such a situation can be reversed through exposing the teeth and mouth to milk or cheese, which has a pH-neutralising effect (Gedalia *et al.*, 1991a; 1991b).

d) Animal studies

Animal frameworks have been widely implemented with the aim of evaluating acidic and soft drinks' erosive effects, with Stephan (1966) and Holloway *et al.* (1958) establishing that fruit drinks cause erosion to teeth. However, as a result of the differences in the approaches of drinking and the differences in salivary flow and composition, there are clearly problems in inferring or generalising the results, and applying them to human studies.

e) Case reports

A number of case reports have suggested the erosive effects associated with soft drinks consumption, with Eccles & Jenkins (1974), for example, describing 26 individual instances of erosion believed to be linked with excessive consumption of acidic beverages. In addition, dental erosion as a direct result of excessive consumption of fruit-flavoured drinks has also been witnessed in the case of children, i.e. those aged 9–16 years (Asher & Read, 1987). Moreover, single case reports have highlighted that the regular and frequent consumption of cola, or holding cola in the mouth, can also be linked with high erosion (High, 1977; Guggenheimer & Schneider, 1980).

f) Experiments *in vitro*

In vitro experiments add further substance to the clinical evidence regarding the link between soft drinks consumption and erosion. Larsen & Nyvad (1999) examined the erosive potential of soft drinks, mineral waters and orange juices *in vitro*, contrasting erosion depths to beverage pH and buffering capacity, and further reporting that erosion is minimal in those beverages containing a pH above 4.2, although the situation was clearer when the pH decreased below 4.0. Importantly, the vulnerability of the deciduous and permanent to erosion has been analysed *in vitro* through taking extracted teeth and soaking them in a low-pH fruit drink diluted with mineral water. Severe erosion was witnessed following the increase of time of exposure to the fruit drink; nevertheless, erosion severity was not proportional to the exposure period (Hunter *et al.*, 2000).

Moreover, Owens & Kitchens (2007) conducted an *in vitro* study to compare the erosive potential of carbonated cola beverages as well as sports and high-energy drinks

on enamel surface substrate. Extracted human premolars were submerged in regular Coca-Cola, Diet Coke, Gatorade sports drink, Red Bull high energy drink, and tap water (control) for 14 days, 24 hours per day, at 37 °C. Teeth were evaluated for enamel changes using scanning electron and light microscopy. It was found that all test beverages eroded enamel, the erosion caused by Red Bull and Gatorade being significantly greater than the erosion caused by the other two. The authors ascribed the high level of adamantine dissolution caused by Gatorade and Red Bull to the high concentration of refined carbohydrates (sucrose and glucose) contained in these, and to the chelating properties of the sodium citrate contained in Red Bull.

g) *In situ* studies

West *et al.* (1998) created an intra-oral appliance, comprising enamel samples palatally, with the aim of examining the impacts of the consumption of orange juice on enamel. Ten individuals were involved in the research, and were advised that the intra-oral appliance needed to be worn during the hours of 9 am till 5 pm every day for a period of 15 days. Moreover, under supervision, the subjects were required to consume either 250ml of orange juice or 250 ml of water over a period of 10 minutes at 9 am, 11 am, 1 pm, and 3 pm. The appliance was removed for 1 hour over lunch (12-1 pm) and stored in saline. At the end of each day study the samples were taken from the appliances and were tested for enamel loss using surface profilometry. The findings showed a great deal more erosion on the enamel specimens when the individuals consumed orange as opposed to merely water. Moreover, this same experiment was carried out *in vitro*, with orange juice again found to be more erosive than water; indeed it was in the order of 10 times that produced *in situ*. The following year, in 1999, this same experiment was carried out by Hughes *et al.* and West *et al.* with both parties subsequently confirming

the technique's reproducibility, and delivering further information regarding the erosiveness of fruit drinks on the enamel of human teeth.

In addition, Rios *et al.* (2009) conducted a 14 days *in situ*, crossover study to compare the erosive potential of a light version of cola drink to the regular version. The study concluded that the light cola version caused a 5-fold decrease in wear compared to the regular cola. The probable influencing factors were said to be the difference in pH, the better adhesion of regular coke to enamel surfaces or the presence of the amino acid phenylalanine from the hydrolysis of aspartame in the presence of saliva.

2.4.2 Smoothies and fruit drinks

Smoothies are fruit juice drinks, comprising crushed whole fruits, served chilled, and are sometimes further blended with yoghurt or ice. The consistency is thick and almost milkshake-like, and may also contain milk.

Smoothies first became popular during the 1960s, at which time the USA experienced a resurgence of macrobiotic vegetarianism. Smoothies sales have increased from 47 million litres in 2009 to 51 million litres in 2010. This means that each person consumes approximately 0.8 litres of smoothies each year. Table 2.5 provides an overview of such data.

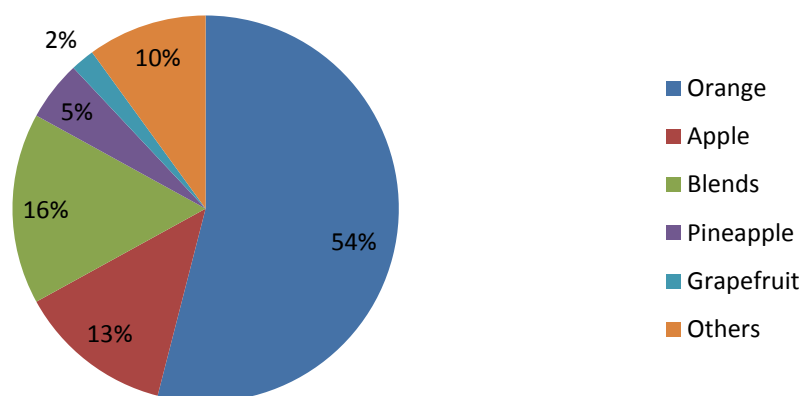
Table 2.5: UK smoothies consumption, 2004–2010 (BSDA, 2011)

UK smoothies consumption, 2004 - 2010							
Year	2004	2005	2006	2007	2008	2009	2010
Million litres	23	35	55	79	64	47	51
% change	+15.0	+52.2	+57.1	+43.6	-19.0	-26.6	+8.5
Litres per person	0.4	0.6	0.9	1.3	1.0	0.8	0.8
% of all soft drinks	0.2	0.3	0.4	0.6	0.5	0.3	0.3

Source: Zenith International

There are many varieties of smoothies, mainly differing in their content. They can consist of 100% fruits but also can have add-ins such as honey, nuts, green tea, herbal supplements, chocolate, coffee, soda or even alcohol depending on personal preference. The BSDA consumer research showed that orange juice is the most popular choice among different types of fruits used in smoothies, followed by apple for single flavour or part of blended choice (Figure 2.5).

Figure 2.5: Choice of fruit juice flavours by UK consumer market 2010 (BSDA, 2011)



In the UK, ‘Innocent’ is the market leader in smoothies where they claimed that their smoothies contain 100% fruits and do not contain sugar, water or preservatives. Market reports have shown that consumers drink smoothies for health reasons and to fulfil their 5-A-day requirements of consuming at least five portions of fruit and vegetables every day.

2.4.3 5-A-Day campaign

The world health organisation (WHO) recommended an intake of 400g of fruit and vegetables per day (WHO/FAO, 2003), in order to reduce the risk of deaths from chronic diseases and some cancers. Without doubt, fruits and vegetables are good for health due to their high content of vitamins, minerals, fibres and antioxidant agents. The Department of Health introduced a 5-A-Day campaign to encourage people to eat a combination of at least 5 portions of fruit and vegetables every day. One medium-sized piece of fruit or vegetable, or a portion of 80 g, counts as one of the 5-A-day portions (Department of Health, 2010).

Drinking smoothies and fruit or vegetable juices were among the suggested steps helping to achieve the recommended intake. A glass of 150 ml of unsweetened 100% juice counts as a portion. Smoothies, however, may count for more than one portion due to their combinations of homogenised fruit and juice. Most single servings of commercially available smoothies, made only of fruit, contain at least one portion of whole crushed fruit (80 g), plus one portion of juice (150 ml), or contain in excess of 80 g of whole crushed fruit with the remainder as juice (Ruxton, 2008).

The public promotion of the 5-A-Day campaign point out damage to the teeth very briefly but clearly there was no mention of possible erosion development that may arise as a result of consumption of 5 portions of fruits and vegetables every day.

2.4.4 Benefits and issues of drinking smoothies

Smoothies are a blend of fresh crushed fruits; these drinks are an outstanding source of vitamins, antioxidants, polyphenol and fibre. It was noted by Ruxton *et al.* in 2006, that the juices of fruit and vegetables seem to present equivalent health benefits to whole fruits and vegetables, most likely because of the similar antioxidant and/or polyphenol content. Apart from their high nutritional value, smoothies have excellent health benefits and can be made with ease specifically for dietary purposes including detoxification, weight loss, muscle gain, remedy, and so forth.

The Juice and Smoothie Association (JASA) describes smoothies as a meal in a cup that doubles up as a quick snack or dessert. Smoothies have an average of 180 calories making it a great replacement of a meal. However, having add-ins such as honey, chocolate and nuts may cause the calories to rise. The overall energy intake per day will be very high if smoothies are drank excessively or taken to complement a meal, without compensating for the extra energy intake (Juice and Smoothie Association, 2010).

On the market, there are smoothies that claimed that they consist of 100% fruits only. However, there are some that contain high levels of sugar to improve the taste and attract the consumer. There are several dental health concerns over the occurrence of acids and intrinsic sugars found in fruits used to make smoothies. These are likely to promote demineralisation, which could cause dental erosion and caries.

Few studies have been carried out focusing on the result of smoothies causing dental erosion. A recent *in vitro* study by Sukeri (2010) focused on the properties of smoothies and their erosive potential was carried out. Two groups of 50 enamel slabs were categorised into two groups which consisted of brushing and non-brushing. These were then sub-categorised under the names of the sample products which included: de-ionised distilled water, Diet Coke, Innocent[®] mangoes and passion fruit smoothie, Innocent[®] strawberries and bananas smoothie, and citric acid 0.3%. Each enamel slab was subjected to a 21-day pH cycling regimen involving two minutes of immersion, five times a day with adequate time in between for remineralisation to take place. An assessment of surface loss was performed using the method of surface profilometry. Their results revealed that both of the smoothies used in their study were acidic and also had high levels of titratable acidity. Furthermore, both drinks generated considerable surface loss, similar to the damage caused by contact with Diet Coke. Citric acid 0.3% and Innocent[®] strawberries and bananas smoothie caused considerably less surface loss in comparison to Innocent[®] mangoes and passion fruit smoothie. Insignificant surface loss was noted between non-brushing and brushing categories

Many *in vitro* studies have been conducted in the area of dental research, however 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. Thus, in this research, an *in situ* framework was 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.

The purpose of the present study was two-fold. Firstly, to investigate, *in vitro*, the potential dental effects of different types of soft drinks including smoothies and Diet Coke by measuring their pH and titratable acidity levels and secondly, to assess the effect of smoothies on enamel erosion with the use of an *in situ* model.

2.5 Aim of the study

The aim of the present study was two-fold. Firstly, to investigate, *in vitro*, the potential acidity of different types of soft drinks including smoothies and Diet Coke by measuring their pH and titratable acidity levels and secondly, to assess the effect of smoothies on erosive tooth surface loss of enamel following 21-day pH cycling protocol using an *in situ* model.

The objective of the *in vitro* study is:

- To investigate the pH and titratable acidity of various types of soft drinks including smoothies and Diet Coke.

The objectives of the *in situ* study are:

- To assess the effect of a commonly consumed smoothie on erosive tooth surface loss of enamel after a 21-day pH cycling protocol using an *in situ* model.
- To compare the effect of smoothies on erosive tooth surface loss of enamel with citric acid 0.3% after a 21-day pH cycling protocol using an *in situ* model.

The null hypothesis for the study:

- There is no difference between the surface loss caused by smoothies and citric acid 0.3%.

3.0 MATERIALS AND METHODS

In this section, the methodology used to measure, *in vitro*, the pH and titratable acidity of the sample materials is described. In addition, the methodology adopted for the current *in situ* study is explained including preparation of enamel slabs and *in situ* appliances.

3.1 *IN VITRO* STUDY

The properties of various types of soft drinks including smoothies and Diet Coke were investigated by measuring the pH values and the titratable acidity levels. The soft drinks chosen for this study were:

- Strawberries and bananas smoothie (Innocent[®] pure fruit smoothie, London)
- Mangoes and passion fruit smoothie (Innocent[®] pure fruit smoothie, London)
- Kiwis, apples and limes smoothie (Innocent[®] pure fruit smoothie, London)
- Blackberries, strawberries and blackcurrants smoothie (Innocent[®] pure fruit smoothie, London)
- Diet coke[®] (Coca Cola Company, USA)
- Citric acid 0.3% (positive control)

The nutritional information of each soft drink is displayed in Appendix 2.

3.1.1 Measurement of inherent pH

The inherent pH of each sample drink was measured 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.

3.1.2 Measurement of titratable acidity

To measure the titratable acidity, sodium hydroxide NaOH with the concentration of 1 mol/l was prepared in the lab by dissolving 40 g of NaOH in 1L of distilled water. The titratable acidity of each sample drink 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 (NaOH) solution was gradually pipetted until the pH of the sample drink reached 7.0. The measurement was performed in triplicate and an average value was calculated. The temperature of the drinks was around 21 °C.

3.2 *IN SITU STUDY*

This *in situ* study was a single centre, randomised, two arms cross-over design study. The study was designed, conducted and reported according to guidelines for good clinical practice (note for guidance on good clinical practice, 1996).

3.2.1 Ethical approval

Ethical approval of this study was sought from the National Research Ethics Service (NRES) committee of Yorkshire and The Humber-South Yorkshire (REC reference number: 11/YH/0228, Appendix 3). Following this, the study received approval from the Leeds Research and Development Directorate (R&D) in order for it to be conducted at the Leeds Teaching Hospital Trust (LTHT R&D number: DT11/9967, Appendix 4).

The Principal Investigator ensured that this study was conducted in full conformance with the laws and regulations of the country in which the research was conducted and the Declaration of Helsinki/Venice/Tokyo/Hong Kong/South Africa (1996).

A participant's information sheet was given to potential participants in the study. This sheet provided details of the procedure and method of the study and gave an explanation of the potential risks and benefits. Participants had to give an informed written consent prior to participation. The participant's information sheet and consent form are presented in Appendix 5 and 6 respectively.

3.2.2 Recruitment and selection of participants

Potential participants were invited to take part in the study via posters placed on noticeboards of Leeds Dental Institute and University of Leeds (Appendix 7). Interested participants received an information sheet and they were given a minimum period of seven days before being invited to the first visit. Prior to commencement of any study-related activity, the investigator obtained written (signed and dated by the subject) informed consent from each individual participating in this study following an adequate explanation of the aims, methods, objectives and potential hazards of the study. The investigator also explained to the subjects that they were completely free to refuse to enter the study or to withdraw from it at any time.

Participants recruited in the study had to fulfill the following inclusion and exclusion criteria:

Inclusion criteria

- i. Adults of either gender aged at least 18 years old.
- ii. Good general health.
- iii. Dentate subjects without removable dental prostheses or fixed or removable orthodontic appliances.
- iv. Subjects with sufficient teeth to retain an upper removable appliance with U clasps on the upper first permanent molars.
- v. Free from visual signs of untreated caries or periodontal disease.
- vi. Subjects with un-stimulated whole salivary flow rate ≥ 0.25 ml/min and a stimulated whole salivary flow rate ≥ 0.8 ml/min.
- vii. Informed consent obtained by the volunteers.

Exclusion criteria

- i. Volunteers with current/recurrent disease that could affect the oral cavity or interfere with the dental examination and/or wearing the oral appliance.
- ii. Severe physical, psychiatric and medical disorders requiring treatment or making the participants unlikely to give informed consent or to cope with the procedures requiring by the study protocol.
- iii. Antimicrobial therapy within 14 days prior to screening or during the study.
- iv. Antibiotic treatment within 28 days prior to screening or during the study.
- v. Use of medication causing reduced salivary flow rate.
- vi. Dental disease requiring treatment in the short or long term.
- vii. Oral surgery/extraction 6 weeks prior to screening or during the study.
- viii. Wearing of prostheses/orthodontic appliances that could affect study procedures.
- ix. Known or suspected intolerance/hypersensitivity to study materials closely related to compounds/ingredients that will be used in this study.
- x. Women pregnant or intending to become pregnant or lactating.
- xi. Participation in another clinical study within 30 days of screening and during the study.

3.2.2.1 Screening of participants

A subject screening record and a Case Report Form (CRF) were used to document the screening evaluation along with any reason for failure (Appendix 8).

At the screening visit, a CRF was completed for all subjects and the following evaluations were performed:

- **Demographics:** The investigator recorded each subject's date of birth, gender and race in the CRF.
- **Medical History:** The medical history of each subject was checked and the investigator reviewed the inclusion/exclusion criteria and ensured the subject's eligibility to enter the study.
- **Oral examination and DMFT measurement:** All subjects received a dental examination to determine the DMFT, using BASCD criteria. The results of the oral examination were recorded in the CRF as either normal or abnormal with any abnormalities being described.
- **Salivary flow rate:** A referenced salivary flow rate (an un-stimulated whole salivary flow rate ≥ 0.25 ml/min and a stimulated whole salivary flow rate ≥ 0.8 ml/min) was measured to ensure that a standard remineralisation effect of the saliva of all volunteers was achieved. The subjects were seated in a quiet, comfortable position, with their head tilted forward so that saliva collected in the front of the mouth. The subject was asked to swallow to clear their mouth of any residual saliva. This action marked the start of a five minute saliva collection period. During this five minute period the subject was not permitted to swallow any saliva but was required to spit or dribble any excess saliva into a graduated collection bottle to measure the salivary flow rate.

For the stimulated collection, subjects chewed on gum base for one minute. After one minute the subjects were instructed to swallow any pooled saliva. They then chewed the gum base for two minutes during which time they emptied any pooled saliva into a collection tube.

During the saliva collection period the subjects were not permitted to drink, chew or speak. An audible alarm sounded after five minutes to indicate the end of the saliva collection period. Subjects were required to spit all remaining saliva collection into the saliva collection bottle for measurement.

- Measurements made for *in situ* oral appliances: Upper and lower impressions with alginate were taken for each subject. Natural bite was also recorded in wax. The impressions and wax bite were disinfected before being transported to the lab.

3.2.2.2 Participants withdrawal criteria

Subjects had the right to withdraw from the study at any time and for any reason. The investigator also had the right to withdraw subjects from the study in the event of inter-current illness, adverse events, treatment failure after a prescribed procedure, protocol deviations, administrative reasons or other reasons. It was understood by all concerned that an excessive rate of withdrawals could render the study underpowered; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts were made to complete and report the observations as thoroughly as possible. A complete final evaluation at the time of the subject's withdrawal was made with an explanation of why the subject was withdrawing from the study.

If the reason for removal of a subject from the study was an adverse event or an abnormal laboratory test result, the principal specific event or test was also recorded on the case report form '(CRF)'. A description of the 'stopping rules' or 'discontinuation criteria' for individual subjects were described.

3.2.3 Sample size determination

Statistical advice was sought and the sample size was calculated by using data from a previous Master's thesis (Sukeri, 2010) at the Leeds Dental Institute. Stata version 11 was used to determine the sample size. Assuming on a medium effect size of 0.5, significance level 0.05, power 80%, it was found that the study required 10 participants.

Fifteen participants were screened to ensure that at least 10 subjects would successfully complete the study. Each participant wore an upper removable appliance containing two enamel slabs. The study consisted of two legs and each leg lasted for 21-days. Therefore, a total number of 60 enamel slabs were required for the whole study.

3.2.4 Teeth selection

Approval from the Leeds Dental Institute Tissue Bank was obtained (Tissue Bank application number: 120711/HA/68, Appendix 9) for collection of teeth fulfilling the following inclusion and exclusion criteria.

Inclusion criteria

- i. Intact first or/and second, upper and lower premolars (cariou lesions not present).
- ii. First or second premolars extracted for orthodontic reasons under general or local anaesthesia in Leeds Dental Institute.
- iii. Extracted from healthy children and adults.

Exclusion criteria

- i. Premolars from children and adults with medical conditions.
- ii. First or/and second, upper and lower premolars with signs of caries, trauma, erosion, restorations or any malformation.

3.2.5 Enamel slabs

Enamel slabs were prepared from teeth fulfilling the inclusion and exclusion criteria mentioned above.

a) *Preparation of enamel slabs*

Enamel slabs were from human premolars. All teeth were stored in distilled water and 0.1% thymol (Sigma Aldrich, thymol 98%) at room temperature. Teeth were then cleaned using a spoon excavator and a toothbrush with a pumice powder and stone to

remove any soft tissue remnants. All teeth were screened by transillumination and transmitted light using low-power microscopy for the detection of cracks (Leitz, Wetzlar®, Germany), caries or any malformations. Suitable teeth were selected for the study.

b) *Cutting and grinding the enamel slabs*

Each tooth was mounted in 'green stick' impression compound (Kerr, UK) on plates. After this, crowns were sectioned using water cooled, Dimond Wire Saw, cutting machine (Well®Walter EBNER, CH-2400 Le Loche, Figure 3.1). The buccal and palatal surfaces of each crown were removed, and the slabs were prepared from these surfaces so that each enamel slab was approximately 2mm x 2mm x 2mm in size.

Enamel slabs were then mounted in circular resin blocks of 3 mm thickness (Stycast; Hitek Electronic materials, Scunthrope, UK). This was achieved using a rectangular steel block which has a circular hole of 3 mm depth. 600 grade fine grit abrasive paper (Wet or Dry paper, 3M) followed by 1200 and 2500 grade were used respectively to grind enamel surfaces after mounting in resin to the same thickness as the hole in the steel block. The slabs were then cleaned with methanol to remove any remnants of abrasive paper. Surfaces were then polished with 5 µm and 1 µm alumina paste. Thereafter, these slabs were cleaned with de-ionised distilled water and methanol and then covered with nail varnish (red colour, MaxFactor®, England, UK) except for a small window that was left exposed. Figure 3.2 shows an illustration of enamel slab preparation.

Figure 3.1: The cutting machine

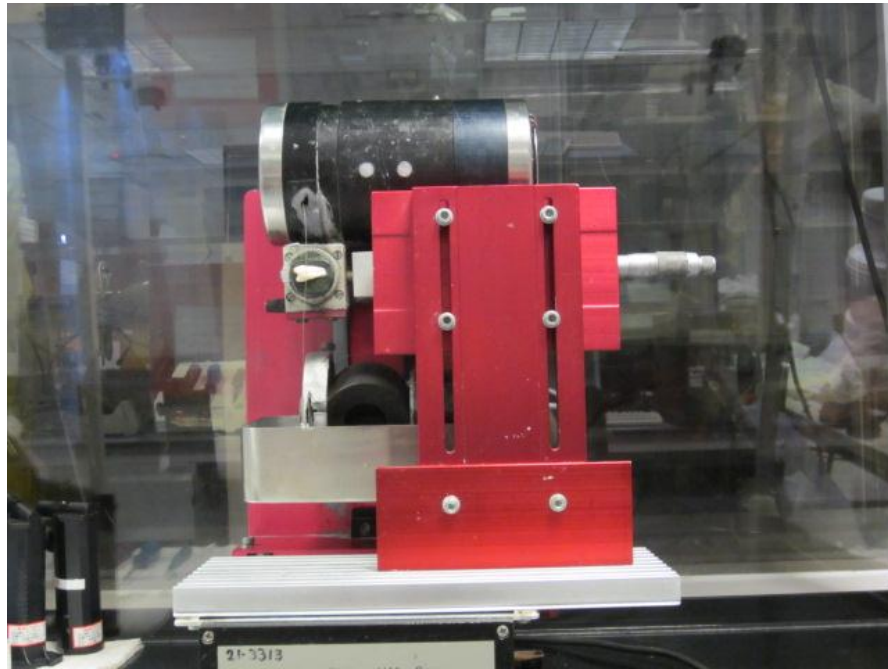
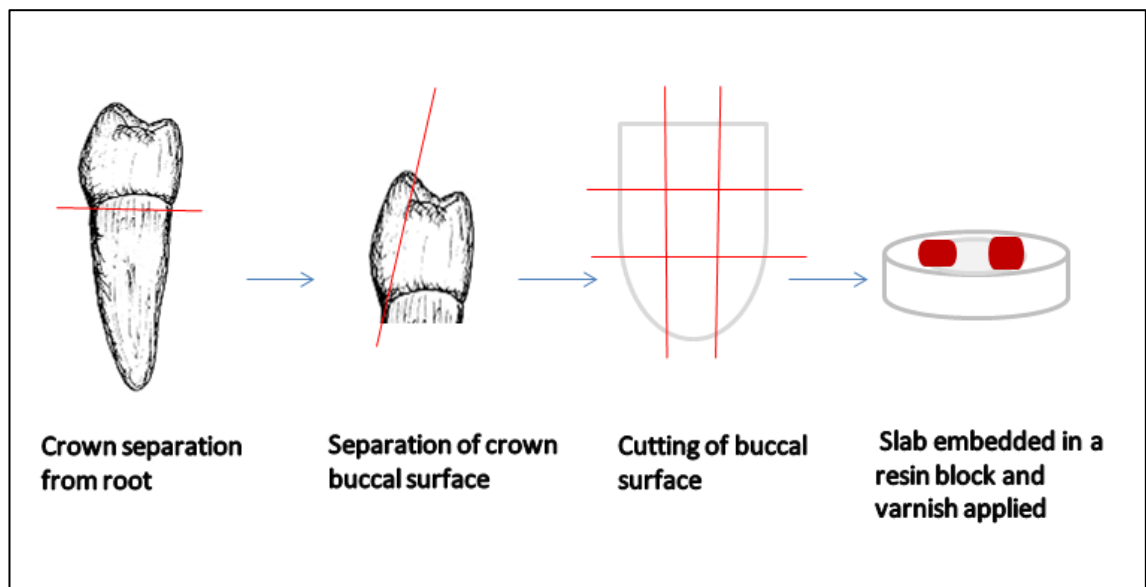


Figure 3.2: Illustration showing enamel slab preparation



c) *Sterilisation of enamel slabs*

Once the enamel slabs were prepared, they were placed in a micro-centrifuge tube and immersed overnight in sodium hypochlorite (12% w/v available Cl⁻), which was pipetted into each tube using a disposable squeeze pipette and fume cupboard. Each enamel slab was then rinsed thoroughly with de-ionised water and immersed and agitated in phosphate buffered saline (pH 7.4) in new micro-centrifuge tubes for a second night. The enamel slabs were then transferred to a tube containing 0.1% thymol and distilled, de-ionised water solution and were sealed with parafilm (Parafilm M, SPI, USA) to prevent leakage of the thymol solution and dehydration of the enamel. They were then sent to the Department of Immunology of the University of Liverpool, where they were exposed to gamma radiation (4080 Gy). This level of exposure provides sterilisation without altering the structural integrity of the enamel slabs.

3.2.6 Test methods

The following tests were used for each enamel slab to ensure eligibility of the enamel slab to be included in the study.

a) Surface profilometry

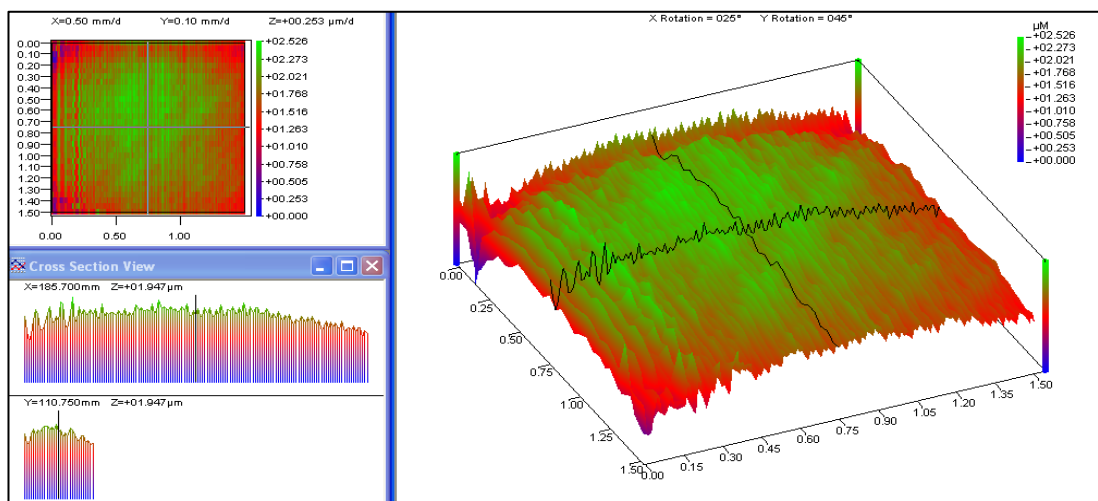
To ensure flatness of enamels, the profile of the resin blocks were assessed using surface profilometry (Scantron ProScan 2000, Figure 3.3). The average height to the average depth range of each slab should be $\pm 1.0 \mu\text{m}$. Figure 3.4 illustrates an image of a flat enamel slab. The measurement was achieved by placing the sample on a key stage on the Scantron ProScan and using a 150 mm height of the camera as standard. The step size used was 0.01 mm. After scanning, the average height to the average depth range (Rz) of five lines (2D) was measured. The lines were located at 0.1 cm, 0.4 cm,

0.7 cm, 1.0 cm, 1.4 cm from the edge of the slab. The mean of those lines was calculated.

Figure 3.3: The surface Profilometry (Scantron Proscan 2000)



Figure 3.4: Image of a flat enamel slab, at baseline, as viewed using surface profilometry scan

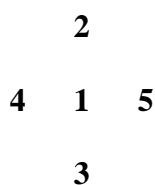


b) Microhardness testing

Flat enamel slabs were then tested with the Knoop microhardness test. Microhardness testing measure the resistance of enamel surfaces to indenter penetration and is a function of the degree of porosity of the superficial enamel layer. Microhardness of enamel slabs was assessed using computer-aided Duramin indenter machine (Struers A/S, DK 26-10, Denmark, Figure 3.5).

The indentations were made using a Knoop diamond under a 100 g load for 15 seconds (Zero *et al.*, 1990). The depth of indenter penetration was measured by means of an image analysis system. The length of the indenter was measured in micrometre using computer software that calculates the indentation length (μm) and microhardness value (KHN) after identifying the border of the indentation.

The indents on the slabs were tested as follows: middle, up, down, left, right to form a cross:

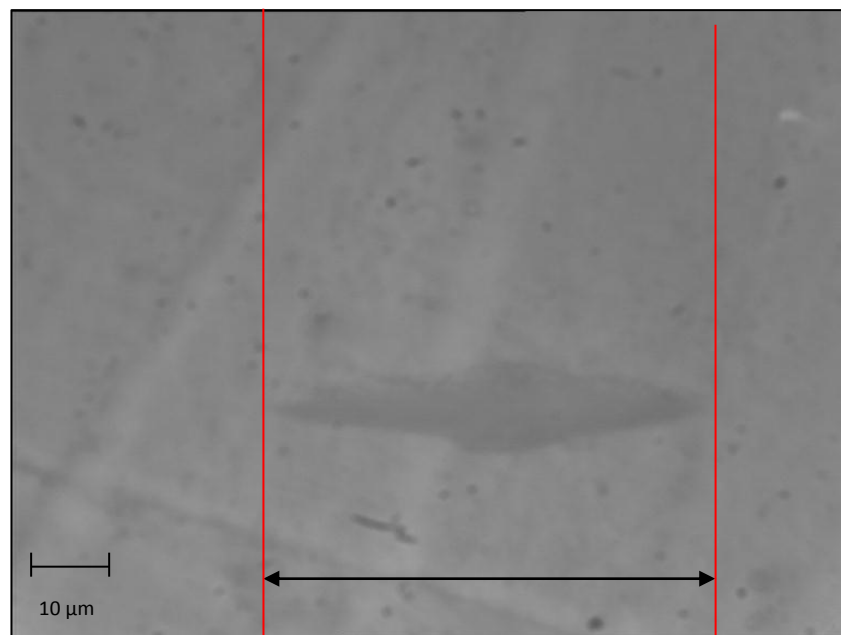


Five indentations, spaced 50 μm apart, were made for each slab and the mean was determined. The initial surface microhardness of the enamel slabs was measured in order to exclude from the study slabs with very soft enamel or slabs where areas with exposed dentine were present. The length of the enamel indent before any exposure was usually about 60-70 μm and the microhardness was 290-396 KHN (Figure 3.6). Slabs whose enamel microhardness was not within this normal range were excluded from the study.

Figure 3.5: The computer-aided Duramin indenter machine



Figure 3.6: Image of a normal diamond-shaped indentation on the surface of enamel slab, at baseline, as viewed under the microscope. The microhardness is assessed by the length of the indentation.

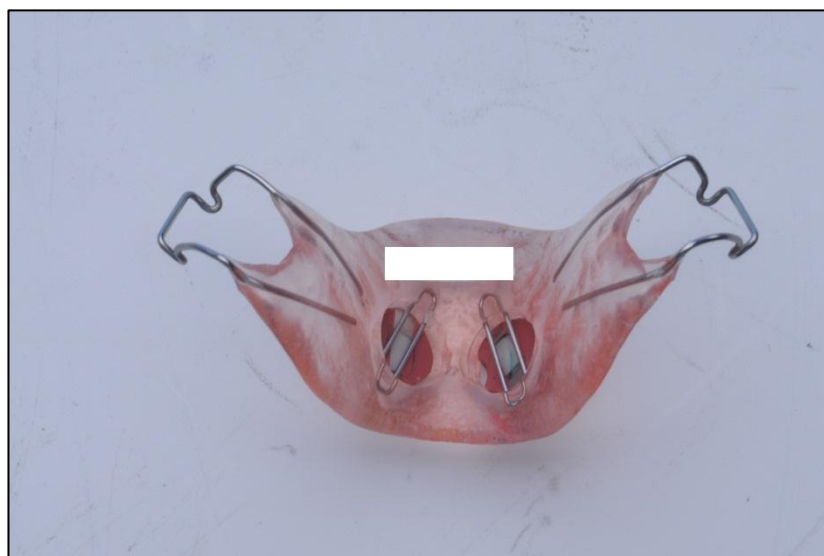


Indentation length

3.2.7 The upper removable appliance

An upper removable appliance with U clasps on the upper first permanent molars and acrylic plate on the palatal surface was used for this study. Two enamel slabs were secured in the palatal plate of the appliance. The slabs were assigned to the midline and were secured with sticky wax (Brendent, Germany). The slabs were protected from the effect of the tongue using arched wires leaving a space of 1 mm between the wire and the slab (Figure 3.7). Participants were instructed to wear the appliances from 8.00 to 18.00 h on each working day for a period of 21-days per study leg. The appliances were removed and stored in saline during lunch time, whilst drinking and tooth brushing, and overnight. Participants were instructed to immerse the appliances in the test solutions 5 times a day at 8.00, 11.00, 13.00, 15.00 and 18.00 h for 2 minutes. 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.

Figure 3.7: The upper removable appliance



3.2.8 Randomisation and blindness

Randomisation and blindness was carried out for the enamel slabs. Enamel slabs were coded and randomised using a table of random numbers, and were then randomly allocated to each participant. The codes were kept with another member of staff and this was not revealed at any time to the investigator. When the slabs were analysed with surface profilometry or microhardness, the investigator did not know which subject or group the slab belonged to, making the analysis completely blind.

3.2.9 Intra-examiner reproducibility

To carry out the intra-examiner reproducibility, the study investigator randomly re-tested 10% of the enamel slabs with surface profilometry and microhardness testing at the end of the study. Intra-examiner reproducibility was measured using the Bland-Altman plot.

3.2.10 Sample materials

a) *Citric acid 0.3%*

Citric acid 0.3% was prepared fresh every day by the participants. A pre-weighed 1.5g of citric acid crystals were measured by the investigator and supplied in plastic bottles. Five hundred ml bottles of mineral water (Buxton[®] natural mineral water) and pyrex glass bottle which had an indication line at the level for water to be added were provided. The participants were advised to pour the 1.5g of citric acid crystals into the pyrex glass bottle (up to line) and shake it well to make sure that the crystals had dissolved. In addition, participants were provided with a dipping pot to dip the

removable appliances extra-orally in citric acid. This pot had an indication line (50 ml) to standardise the amount of citric acid used for each dipping.

b) *Innocent® strawberries and bananas smoothie*

Participants were supplied with 250 ml bottles of Innocent® strawberries and bananas smoothie and dipping pots with an indicator line (50 ml) to standardise the amount of smoothie used for each dipping. The participants were advised to keep the smoothie bottles in a refrigerator and to use one bottle per day.

3.2.11 Study protocol

This *in situ* study was a single centre, randomised, two arms cross-over design study.

The protocol of the study is demonstrated in Table 3.1.

Table 3.1: Study protocol

<p>Screening and recruitment period</p>	<p>1 week</p>	<ul style="list-style-type: none"> • Screening of participants (medical history, DMFT, salivary flow rate). • Recruitment of participants and signing consent form. • Scale and polish. • Taking upper impressions. • Preparation of upper removable appliances, containing two enamel slabs. • Fitting of upper removable appliances
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1st arm of the study	3 weeks	<ul style="list-style-type: none"> • Wearing upper removable appliances for 21-days. • Immersion of upper removable appliances in smoothie 5 times a day for 2 minutes for a period of 21-days. • Collection of upper removable appliances at end of 21-days pH cycling protocol. • Removal of enamel slabs in the laboratory.
2nd arm of study	1 week	Wash-out period before starting the 2 nd leg
	3 weeks	<ul style="list-style-type: none"> • Wearing upper removable appliances for 21-days. • Immersion of upper removable appliances in citric acid 5 times a day for 2 minutes for a period of 21-days. • Collection of upper removable appliances at end of 21-days pH cycling protocol. • Removal of enamel slabs in the laboratory. • In the laboratory: enamel slabs were tested using surface profilometry and microhardness.

Participants were given supplies for one week at a time. Therefore, participants attended the study site for each study leg as follow:

- 1st visit: Fitting of appliance.
- 2nd visit (at day 2): Checking the fit of appliance.
- 3rd visit (at day 7): To give supplies for 1st week and to check the appliance
- 4th visit (at day 14): To give supplies for 2nd week.
- 5th visit (at day 21): To give supplies for 3rd week.
- 6th visit (at day 29): To collect dipping diary and appliance.

3.2.12 Study periods

a) Acclimatisation period

Prolonged use of *in-situ* oral appliances could potentially cause some discomfort to the participants. As a result, a try-in period with the *in-situ* oral appliance of 2-7 days was commenced prior to commencement of the study. During the acclimatisation period, participants tried to wear their appliances at all times (except when eating, drinking, or brushing their teeth, and overnight).

For this study any product put into the oral cavity, including chewing gum, flossing or using a breath freshener or mouthwash was considered as eating and drinking. If the participants experienced discomfort, they were asked to return to the study site for the appliance to be adjusted. They then continued with the acclimatisation period.

b) Wash-out period

One week wash-out period was commenced between the two arms of the study. During this period, participants returned to normal daily routines and without wearing the upper removable appliances. They were provided with a standard toothbrush and standard fluoride toothpaste. Participants were only permitted to use the standard toothbrush and toothpaste supplied. At the end of the wash-out period, participants returned to the study site for fitting the appliances and to start the 2nd leg of the study.

c) Follow-up period

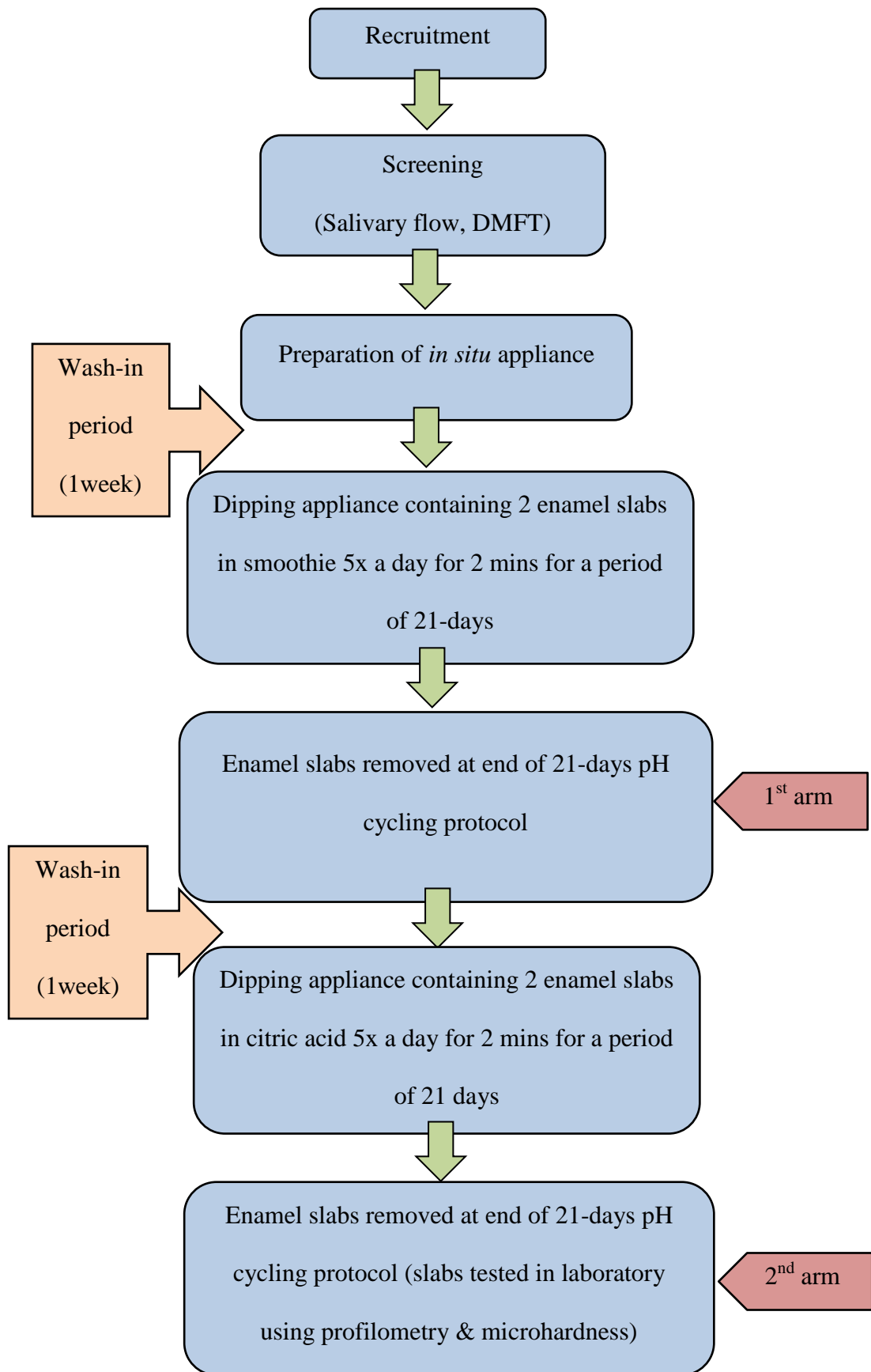
Participants attended for a follow-up visit within 14 days of the final assessment day. The visit included a brief medical interview, oral examination and optional application of commercially available topical fluoride gel or remineralisation treatment (Tooth Mousse/Duraphat).

3.2.13 Compliance

The volunteers' compliance was checked using the following methods:

1. Each volunteer had a CRF to monitor and record each step throughout the study.
2. Dipping diary which was checked every week at volunteer visits (Appendix 8).
3. The used smoothies and citric acid bottles were collected and the remnants of each bottle were measured and recorded on the product disposal form.

Flow chart of the study (Figure 3.8)



3.2.14 Analysis of data

After the 21-day pH cycling protocol had been completed, enamel slabs were rinsed with de-ionised distilled water and air dried. The nail varnish was removed using acetone and the slabs were then measured using surface profilometry and microhardness testing. Collected data were compiled into excel sheets and statistical analysis was carried out using the SPSS statistical package for Windows version 19 (SPSS Inc. Illinois). A significance level of $p < 0.05$ was adopted.

The following statistical methods were carried out:

- Checking for normality of data: All continuous data were checked for normality using the Shapiro-Wilk test. If the p value is more than 0.05 then the data are normally distributed however, if the p value is less than 0.05 then the data cannot be modelled by a normal distribution.
- Descriptive statistics: Descriptive statistics such as means, maximum and minimum values as well as standard deviations were computed using SPSS.
- Homogeneity (equality) of variances was tested using Levene's test. P value of more than 0.05 indicates that equal variances are assumed whilst p value of less than 0.05 indicates that equal variances are not assumed.
- Independent samples t-test and paired t-test were used to compare means of parametric data.
- Intra-examiner reproducibility was assessed using the Bland Altman plot.

4.0 RESULTS

This section represents the results of the study, in the same order as described in the materials and methods section in order to facilitate reading.

4.1 IN VITRO STUDY RESULTS

4.1.1 Inherent pH measurement

Inherent pH and standard deviation values are shown in Table 4.1. The inherent pH was lowest for Diet Coke (pH=2.61) and highest for Innocent[®] mangoes and passion fruit smoothie (pH=3.9).

Table 4.1: 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	3.90	0.03
Kiwis, apples and limes smoothie	3.75	0.04
Blackberries, strawberries and blackcurrants smoothie	3.81	0.01
Diet Coke	2.61	0.07
Citric acid 0.3%	3.08	0.02

4.1.2 Titratable acidity

The titratable acidity of soft drinks was determined by monitoring the pH after addition of NaOH. The amount of NaOH necessary to change the pH of 100 ml of the soft drink to pH 7.0 was calculated. Table 4.2 represents the mean volume of NaOH required to reach pH 7.0 for each soft drink.

The citric acid 0.3% (positive control) 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 blackcurrants smoothie gave the greatest titratable acidity, requiring 10.8 mol NaOH to reach the equivalent pH value.

Table 4.2: 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 blackcurrants smoothie	10.8	0.6
Diet Coke	3.97	0.1
Citric acid 0.3%	3.1	0.06

4.2 IN SITU STUDY RESULTS

4.2.1 Recruitment and selection of participants

Fifteen healthy adult females participated in the study. One participant withdrew consent after 2 days of the acclimatisation period as she found it difficult to cope with the appliance. The mean age of participants was 31 years, 8 months (S.D±9.42) and their mean DMFT was 2.73 (S.D±1.79). The mean un-stimulated and stimulated salivary flow rates were 0.71 ml/min (S.D±0.24), and 0.97 ml/min (S.D±0.2) respectively (see Table 4.3).

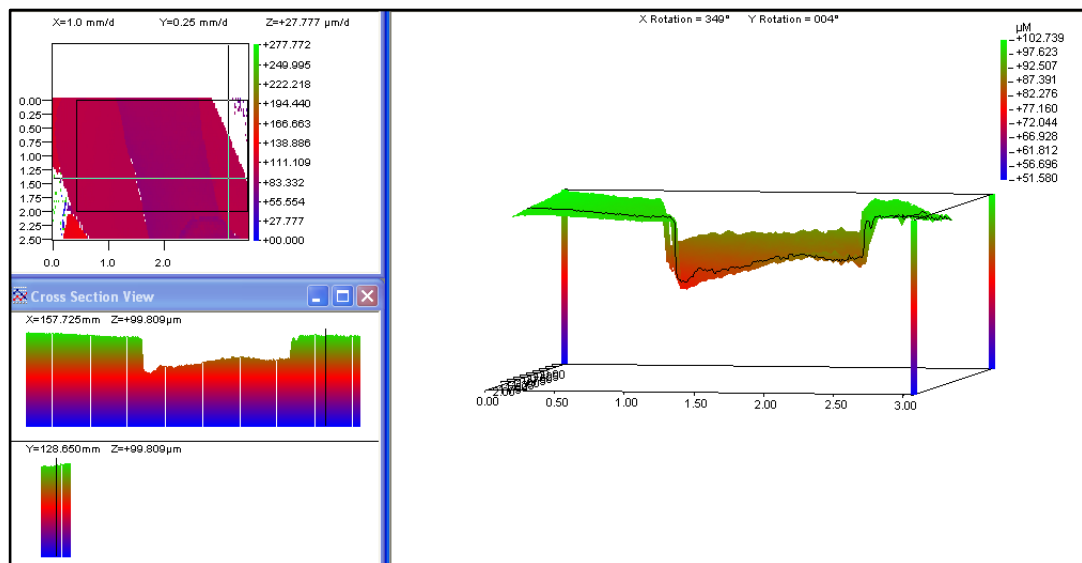
Table 4.3: Study group characteristics

	N	Minimum	Maximum	Mean	S.D
Age (years/months)	15	22.10	59.10	31.8	9.42
DMFT	15	0.00	7.00	2.73	1.79
Salivary flow rate (ml/min)					
Un-stimulated	15	0.40	1.10	0.71	0.24
Stimulated	15	0.80	1.50	0.97	0.20

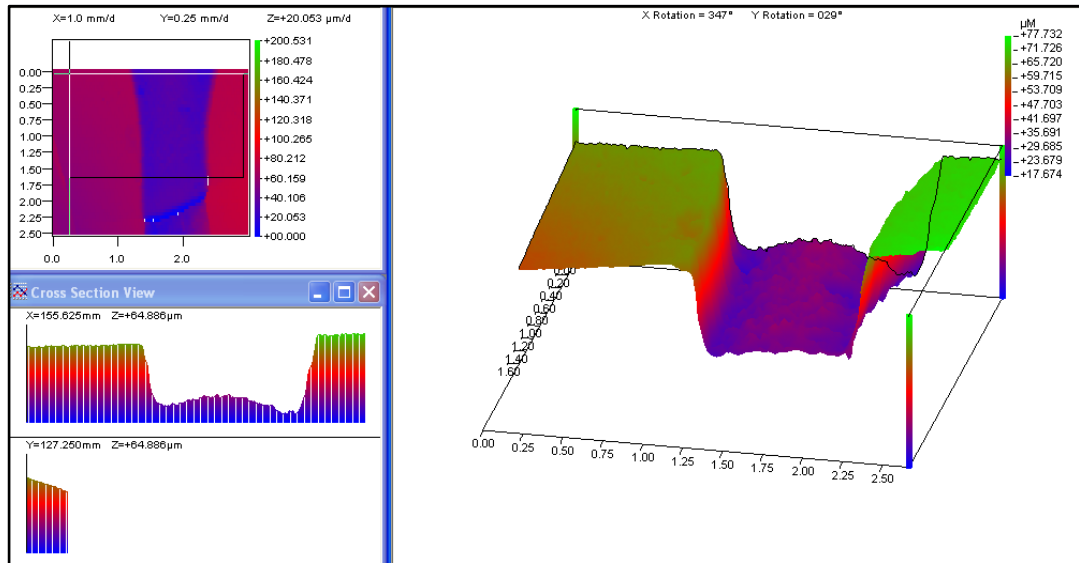
4.2.2 Surface profilometry results

Tooth surface loss was represented by differences in heights of tooth surface by using the area covered by nail varnish as a reference area. The Proscan machine has a function of ‘3 Point Step Height’, where it is ideal for measuring height differences between two areas on an unlevelled or difficult to level scans. Figures 4.1 and 4.2 demonstrate presentation of surface profilometry scans. The software was used to calculate the average height for the three selected areas. The first two areas were averaged and differences between this value and the third area value were calculated and displayed. This was repeated three times for each slab and the mean for each sample was used.

**Figure 4.1: Presentation of a surface profilometry scan
(Example of smoothie group)**



**Figure 4.2: Presentation of a surface profilometry scan
(Examples of citric acid group)**



4.2.2.1 Test of normality of tooth surface loss data obtained from surface profilometry

Shapiro-Wilk test was used to check the normality of tooth surface loss data obtained from measurements using surface profilometry. The test showed that both the test (SB smoothies) and control (citric acid) groups had p values > 0.05; thereby the measurements from this study were of a normal distribution (Table 4.4). Therefore, parametric tests were used for both groups.

Table 4.4: Normality test of tooth surface loss data obtained from surface profilometry

Groups	Statistics	df	sig.
SB smoothie	0.944	28	0.136
Citric acid	0.946	28	0.159

4.2.2.2 Overall distribution of tooth surface loss after 21-days pH cycling protocol for smoothie and citric acid

Table 4.5 and Figure 4.3 demonstrate the overall distribution of tooth surface loss after 21-days pH cycling protocol for the test (SB smoothie) and control (citric acid) groups. The mean tooth surface loss followed by exposure to citric acid was 28.43 μm (S.D \pm 10.25) compared to 2.88 μm (S.D \pm 2.13) following exposure to SB smoothie. The citric acid group had a greater mean tooth surface loss compared to the smoothie group.

Table 4.5: 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

Figure 4.3: Distribution of tooth surface loss after 21-days pH cycling protocol for smoothie and citric acid

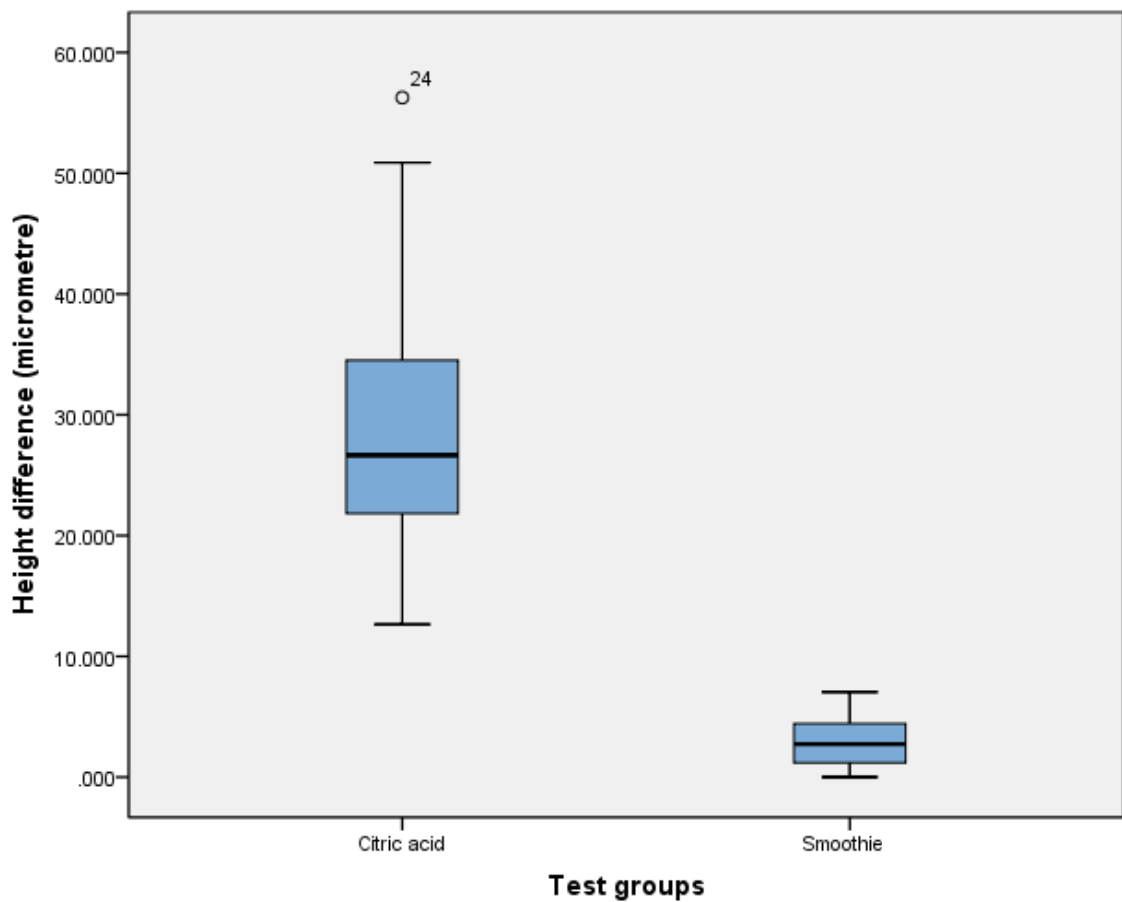


Figure 4.3 also represents an outlier in the citric acid group that was noted. This outlier refers to slab number 24 which had a tooth surface loss value of 56.26 (μm). When slab was checked for further details, it was noted that the participant to whom this slab belonged had dipped the appliance in citric acid for 15 minutes instead of 2 minutes on one occasion.

4.2.2.3 Comparison of the mean differences of tooth surface loss between smoothie and citric acid

An independent samples t-test was used to identify differences in tooth surface loss between the test (SB smoothie) and control (citric acid) groups. Before the t-test was carried out, Levene's test was performed to test homogeneity (equality) of variances. The test gave a p value of 0.00 indicating that equal variances were not assumed.

The independent samples t-test gave a p value of < 0.05 , indicating that there was a statistical significant difference in tooth surface loss between the two groups at p value < 0.05 (Table 4.6). The citric acid group had a significantly higher tooth surface loss compared to SB smoothie group. Therefore, the null hypothesis of this research is rejected.

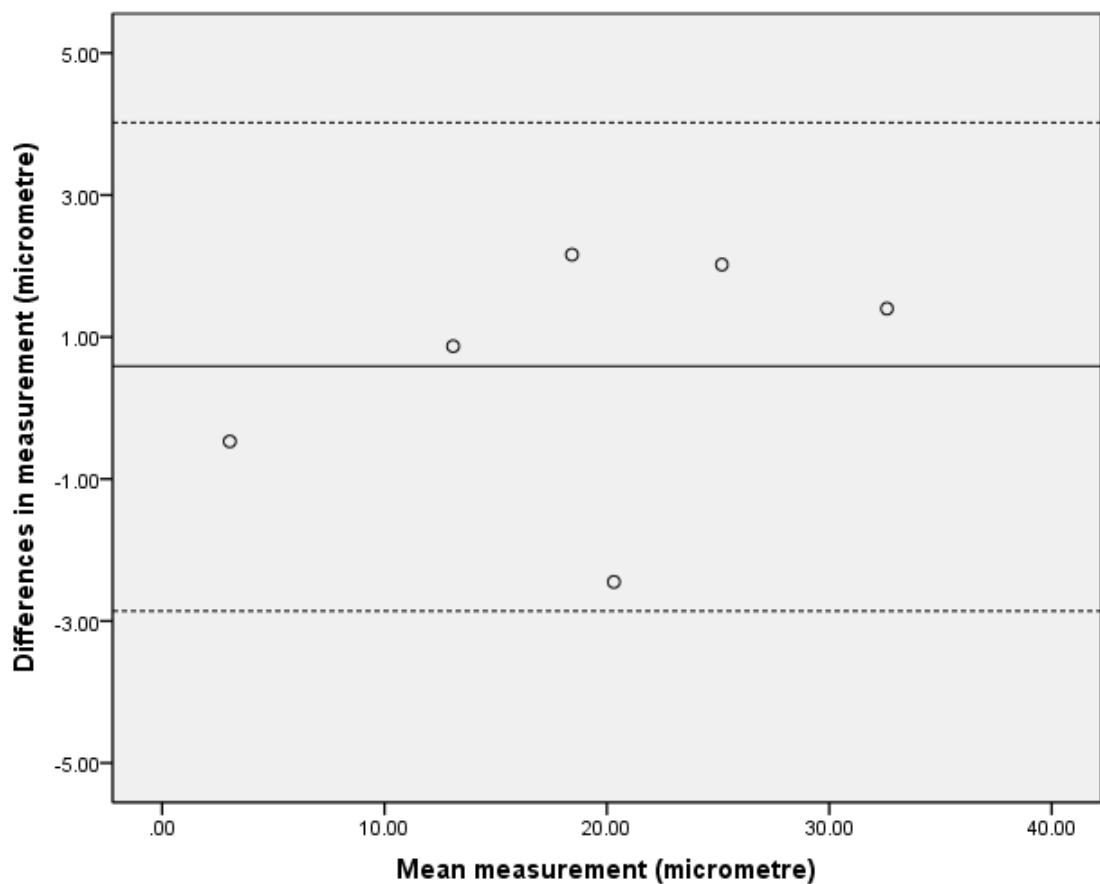
Table 4.6: Comparison of the mean differences of tooth surface loss (μm) between smoothie and citric acid using an independent samples t-test

Groups	df	Sig.	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
					Lower	Upper
SB smoothies vs. citric acid	29.34	0.00	25.55	1.98	21.50	29.59

4.2.2.4 Intra-examiner reproducibility for surface profilometry

Ten percent of 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 (Figure 4.4). The mean of the differences or the bias was 0.58 μm . The 95% limits of agreement (4.05, -2.87) included 0, interpreting that this variation was not clinically important and therefore the intra-examiner reproducibility was considered acceptable.

Figure 4.4: Bland-Altman plot for intra-examiner reproducibility for surface profilometry



4.2.3 Microhardness results

Loss of enamel microhardness was measured by the resistance of enamel to the penetration of indenter. In this study, enamel microhardness was measured with a Knoop diamond indenter. Hardness indentations were used to quantify the amount of tooth surface loss of enamel by placing indentations and measuring their lengths using computer software that calculates the indentation length (μm) and microhardness value (KHN) (see Figures 4.5 & 4.6). These measurements were carried out before and after the 21-days pH cycling protocol. This was performed three times for each slab and the mean for each sample was used.

Figure 4.5: Images illustrating indentation length before and after 21-days pH cycling protocol (Example of smoothie group)

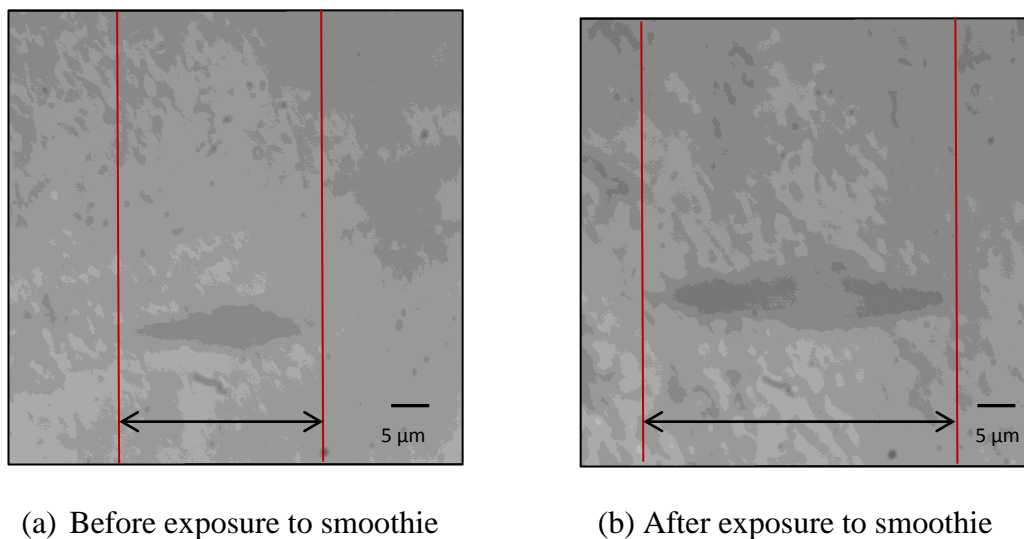
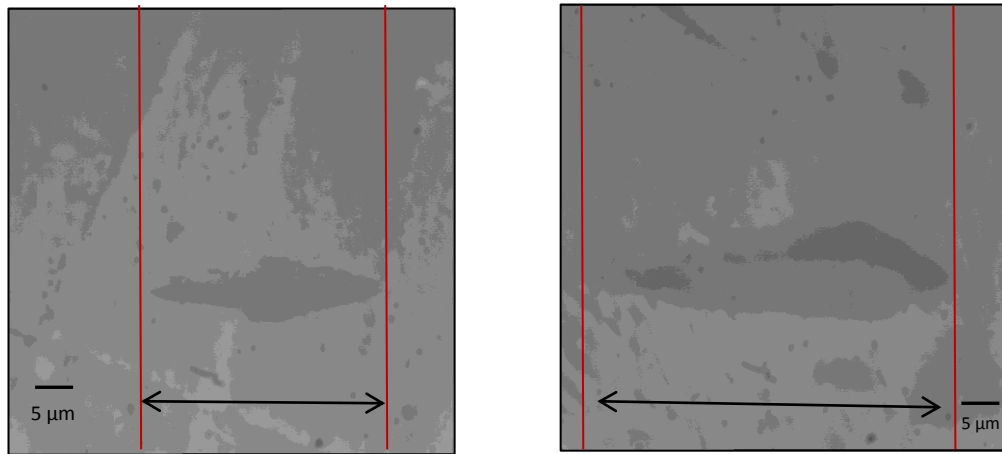


Figure 4.6: Images illustrating indentation length before and after 21-days pH cycling protocol (Example of citric acid group)



(a) Before exposure to citric acid

(b) after exposure to citric acid

4.2.3.1 Test of normality of indentation length and enamel microhardness data obtained from microhardness testing

Shapiro-Wilk test was used to check the normality of indentation length and enamel microhardness data obtained from measurements using microhardness testing. The test showed that both the test (SB smoothie) and control (citric acid) groups had p values > 0.05; thereby the measurements from this study were of a normal distribution. Therefore, parametric tests were used for both groups (Table 4.7).

Table 4.7: Normality test of indentation length and enamel microhardness data obtained from microhardness testing

Groups	Statistics	df	Sig.
<u>Indentation length</u>			
SB smoothie	0.936	28	0.088
Citric acid	0.964	28	0.443
<u>Enamel microhardness</u>			
SB smoothie	0.94	28	0.13
Citric acid	0.95	28	0.28

4.2.3.2 Overall distribution of indentation length and enamel microhardness before and after 21-days pH cycling protocol for smoothie and citric acid

Table 4.8 represents descriptive data on indentation length and enamel microhardness before and after exposure to test materials (citric acid and SB smoothie). In the citric acid group the mean of indentation length and enamel microhardness before the exposure to test material was 66.01 μm (S.D \pm 2.55) and 327.64 KHN (S.D \pm 25.55) respectively compared with 66.66 μm (S.D \pm 2.66) and 325.18 KHN (S.D \pm 28.50) for the smoothie group. After the 21-days pH cycling protocol, the mean of indentation length and enamel microhardness was 130.34 μm (S.D \pm 19.04) and 85.22 KHN (S.D \pm 21.34) for the citric acid group compared with 105.70 μm (S.D \pm 13.72) and 124.20 KHN (S.D \pm 21.13) for the smoothie group.

Table 4.8: 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					
<u>Indentation length</u>					
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
<u>Enamel microhardness</u>					
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					
<u>Indentation length</u>					
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
<u>Enamel microhardness</u>					
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

4.2.3.3 Comparison of the mean differences in indentation length and enamel microhardness before and after 21-days pH cycling protocol for smoothie and citric acid

A paired-samples 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 at p value < 0.05 (Table 4.9).

Table 4.9: Comparison of the mean differences in indentation length (μm) and enamel microhardness (KHN) before and after 21-days pH cycling protocol for smoothie and citric acid using a paired-sample t-test

Groups	t	df	Sig.	SE Mean	95% Confidence Interval of the Difference	
					Lower	Upper
<u>Indentation length</u>						
SB smoothie	14.88	27	0.000	2.62	33.65	44.42
Citric acid	17.47	27	0.000	3.68	56.77	71.88
<u>Enamel microhardness</u>						
SB smoothie	36.36	27	0.000	5.52	189.63	212.31
Citric acid	38.10	27	0.000	6.36	229.36	255.47

4.2.3.4 Overall distribution of differences (changes) in indentation length and enamel microhardness after 21-days pH cycling protocol for smoothie and citric acid

Table 4.10 and Figures 4.7 & 4.8 demonstrate the distribution of the differences (changes) in indentation length and enamel microhardness after 21-days pH cycling protocol for smoothie and citric acid. The mean difference (change) in indentation length and enamel microhardness followed exposure to citric acid was 64.33 μm (S.D \pm 19.47) and -242.42 KHN (S.D \pm 33.66) respectively compared to 39.04 μm (S.D \pm 13.88) and -200.97 KHN (S.D \pm 29.24) following exposure to SB smoothie.

Table 4.10: The differences (changes) in indentation length (μm) and enamel microhardness (KHN) after 21-days pH cycling for smoothie and citric acid

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

Figure 4.7: Distribution of the differences (changes) in indentation length after 21-days pH cycling protocol for smoothie and citric acid

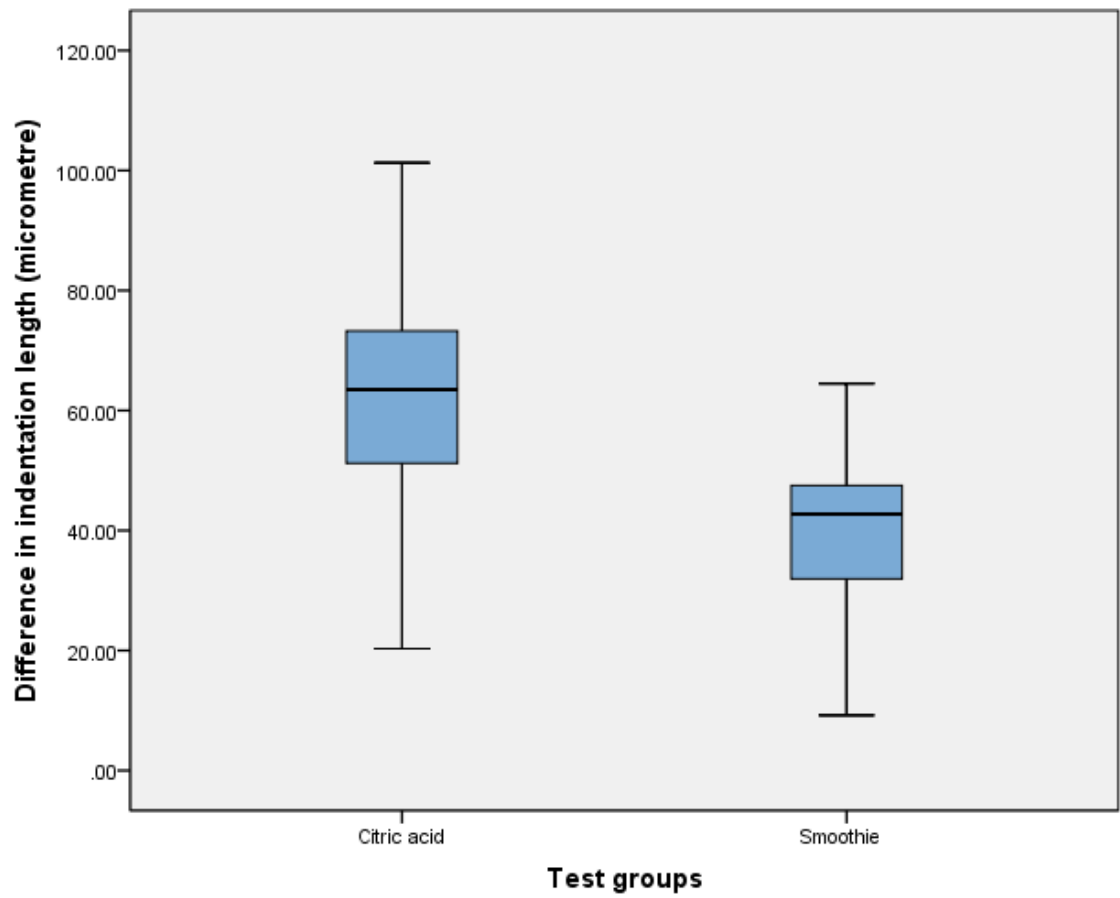
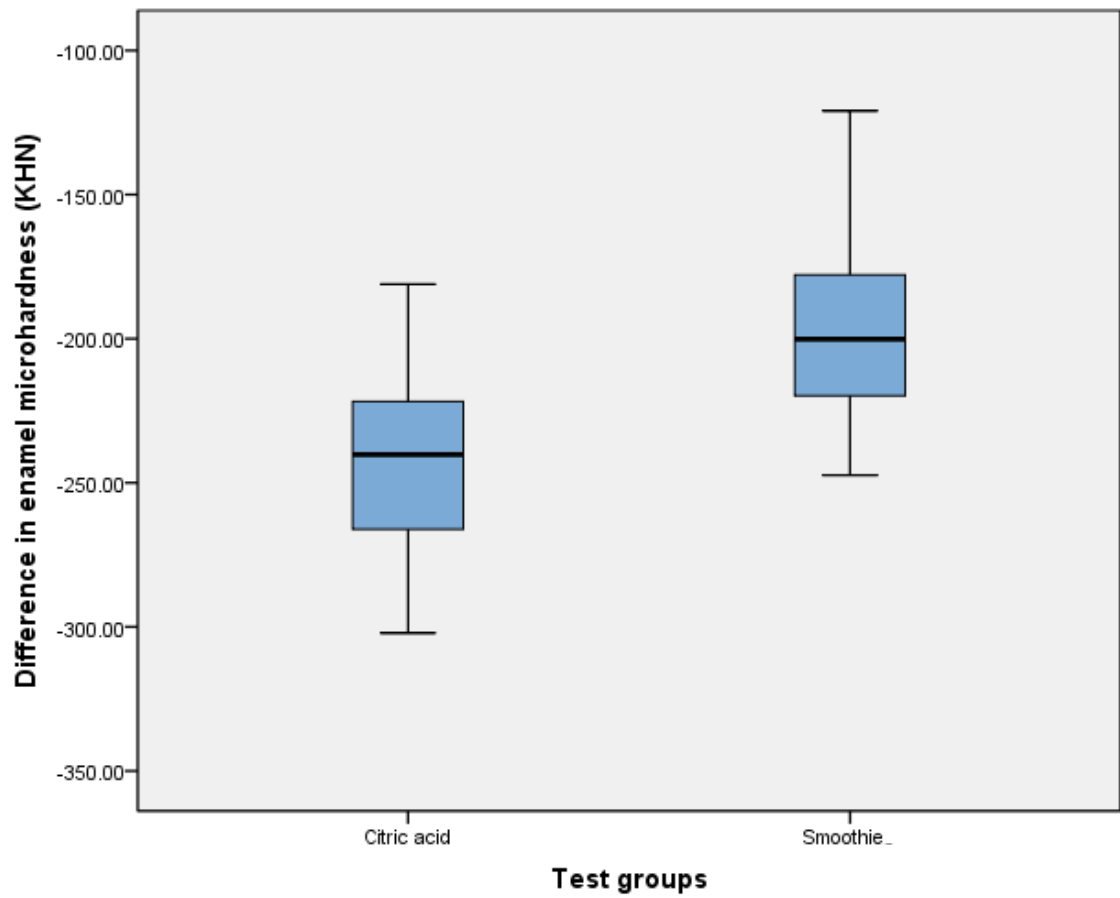


Figure 4.8: Distribution of the differences (changes) in enamel microhardness after 21-days pH cycling protocol for smoothie and citric acid



4.2.3.5 Comparison of the mean differences in indentation length and enamel microhardness between smoothie and citric acid

An independent samples t-test was used to compare the mean differences in indentation length and enamel microhardness between the test (SB Smoothie) and control (citric acid) groups after the 21-days pH cycling protocol. Before the t-test was carried out, Levene's test was performed to test homogeneity (equality) of variances. The test gave a p value of > 0.05 , indicating that equal variances were assumed.

The independent t-test gave a p value of < 0.05 , indicating that there was a statistically significant difference in indentation length and enamel microhardness between the two groups at p value < 0.05 (Tables 4.11 & 4.12). Citric acid group caused significantly greater difference (change) in indentation length and enamel microhardness compared with SB smoothie group.

Table 4.11: Comparison of the mean differences in indentation length (μm) between smoothie and citric acid using an independent samples t-test

Groups	df	Sig.	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
					Lower	Upper
SB smoothies vs. citric acid	54	0.00	25.29	4.52	16.23	29.59

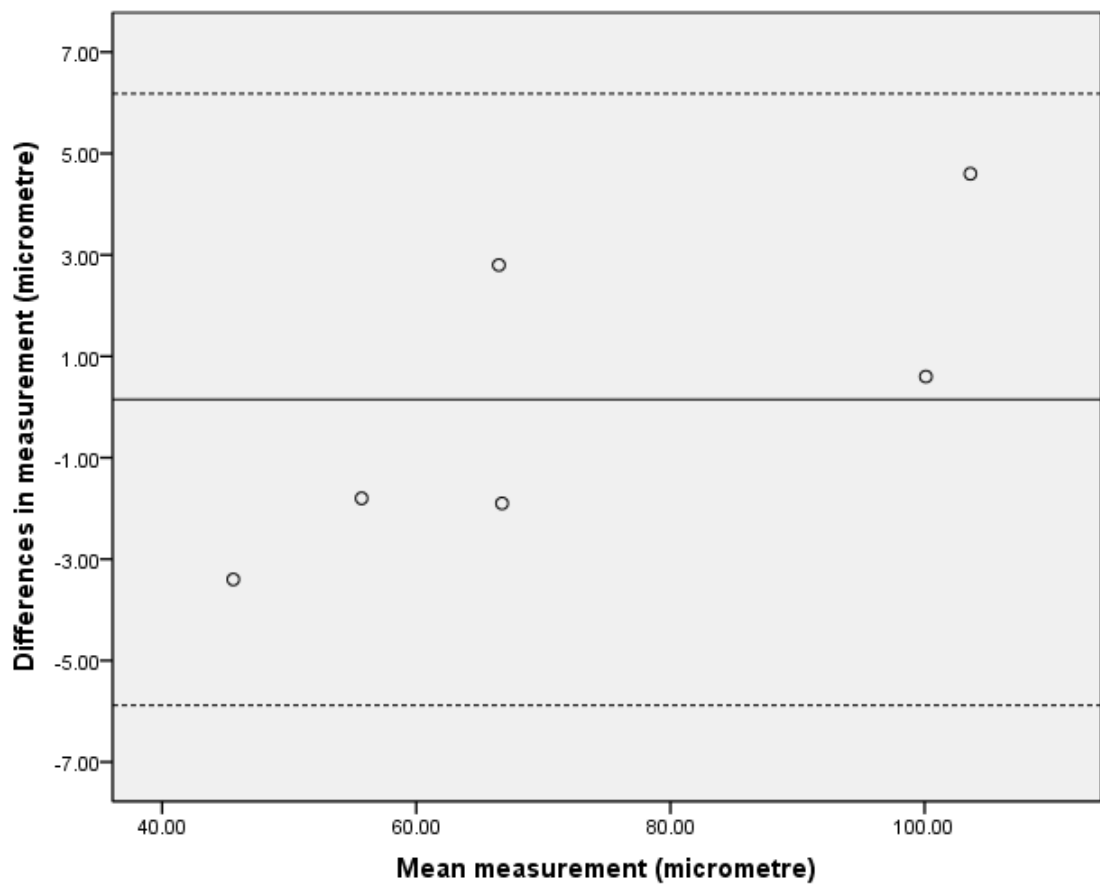
Table 4.12: Comparison of the mean differences in enamel microhardness (KHN) between smoothie and citric acid using an independent samples t-test

Groups	df	Sig.	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
					Lower	Upper
SB smoothies vs. citric acid	54	0.00	-41.44	8.42	-58.34	-24.54

4.2.3.6 Intra-examiner reproducibility for microhardness testing

The intra-examiner reproducibility was tested using the Bland Altman plot (See Figure 4.9). The mean of the differences or the bias was 0.15 μm . The 95% limits of agreement (6.18, -5.88) included 0, interpreting that this variation was not clinically important and therefore the intra-examiner reproducibility was considered acceptable.

Figure 4.9: Bland-Altman plot for intra-examiner reproducibility for microhardness testing



5.0 DISCUSSION

Smoothies are drinks made with pure crushed fruits, and may also be blended with yoghurt or milk. Industry data suggest that fruit juice consumption increased year-on-year from 2004 to 2007, but decreased in 2008 and 2009 as consumers traded down to cheaper alternatives during the recession. However, in 2010, consumption of fruit juices registered 3.1% volume growth to reach 1.18 million litres (BSDA, 2011). Sales of smoothies, which saw large increases from 2004 to 2007, also decreased significantly in 2009 due to the VAT increase and rising living costs. Yet in 2010, smoothies registered a strong recovery, up to 8.5% in volume to reach 51 million litres, meaning that each person consumes approximately 0.8 litres of smoothies each year (BSDA, 2011).

In the UK, “Innocent[®]” is the market leader in smoothies. Innocent’s top selling smoothies are the “strawberries and bananas” and the “mangoes and passion fruit”. Therefore, Innocent[®] strawberries and bananas smoothie was chosen as the test product for this research.

The Department of Health introduced a campaign to encourage the intake of at least five portions of fruits or vegetables per person per day in order to help reducing the risk of some cancers, heart disease and many other chronic conditions (Department of Health, 2010). Fruit juices and smoothies contain vitamins, such as vitamin C, antioxidants, polyphenols and fibres depending on the fruits used. Epidemiological and intervention studies suggested that there is some evidence that the consumption of fruit and vegetable juices may reduce the risk of coronary heart disease (Ruxton *et al.*, 2006).

Drinking smoothies and fruit juices were among all suggested steps helping to achieve the recommended intake of five portions of fruit and vegetables a day recommended by the Department of Health. A glass of 150 ml of unsweetened fruit juice can count as one portion, and smoothies that contain at least 150 ml of fruit juice as well as 80 g of crushed/pulped fruit can count as two portions (Ruxton, 2008). The public promotions of the 5-A-day campaign mentioned damage to the teeth as a result of consumption of 5 portions of fruits and vegetables every day very briefly. There was no clear mention of possible erosion damage.

Searching through Medline and Pubmed databases resulted in a small number of papers on smoothies. Most of these papers were related to issues such as food supplements and food processing technique. There was no published study to examine the effect of smoothies on dental erosion. One Master's research study conducted at Leeds Dental Institute investigated the properties of smoothies and assessed their effect on dental erosion using an *in vitro* model. This study showed that smoothies are acidic and caused significant erosion after 21-days pH cycling regimen (Sukeri, 2010).

In vitro studies are extremely useful to demonstrate the propensity characteristics of an erosive substance but cannot replicate the oral environment with all its biological variations. *In situ* models are useful to overcome the limitations of the *in vitro* method by taking into account the oral environmental factors affecting the development of dental erosion. Therefore, the aim of the present study was two-fold, firstly to investigate, *in vitro*, the potential acidity of different types of soft drinks including smoothies and Diet Coke and secondly to assess the erosive potential of a commonly consumed smoothie using an *in situ* model.

5.1 *In vitro* study

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 included in this *in vitro* study because its consumption has increased among adolescents who have become more weight conscious (Mintel, 2008). Many studies have emphasised a link between carbonated drinks, especially carbonated cola drinks with dental erosion (Jensdottir *et al.*, 2006; Owens & Kitchens, 2007, Rios *et al.*, 2009). The link between erosion and Coke drinks was well established. These carbonated drinks were used in many studies to create erosive lesions (Amaechi *et al.*, 1999c; Hooper *et al.*, 2007).

The inherent pH and the titratable acidity of smoothies were measured in this *in vitro* study to investigate the potential erosive effect of these drinks. pH measures the hydrogen ion (H^+) concentration whereas titratable acidity (TA) measures the concentration of all H^+ ions, both free and bound to un-dissociated acids and anions. There has been a debate on whether pH or TA is a better predictor of the erosive potential of a drink. Some studies showed that the pH was a better predictor (Rugg-Gunn *et al.*, 1998; Hemingway *et al.*, 2006) whereas other studies reported that titratable acidity was more important (Meurmann & ten Cate, 1996; Edwards *et al.*, 1999; Cairns *et al.*, 2002). Rugg-Gunn *et al.* (1998) noted that the pH is a good predictor of the erosive potential of drinks for the first few minutes of erosion challenge, whereas the titratable acidity characterises the erosive potential during longer exposure times.

The method used in this study to determine the titratable acidity has been employed previously in many studies (Birkhed, 1984; Lussi *et al.*, 1993; Touyz, 1994) 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 (Duggal *et al.*, 1995), 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 blackcurrants smoothie required the largest amount of sodium hydroxide (10.8 mol), thus is the most resistant drink to a pH change up to neutralisation (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. The pH meter notably took a longer time to come to a reading when measuring the titratable acidity of smoothies, as a stable reading was required before giving a measurement. This property of the smoothies may influenced the amount of tooth surface loss.

These results are noteworthy as the low pH values and high titratable acidity levels reflect that smoothies are acidic and consequently could all contribute to the development of dental erosion.

5.2 *In situ* study

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 (Zero, 1995).

Intra-oral models involve less subjects and use *in vitro* measurement techniques which are very sensitive resulting in observation of the desired effect in much less time than when conducting an *in vivo* study. However, an *in situ* study can only be considered as an intermediate step between *in vitro* or animal and *in vivo* studies and should not be overestimated against *in vivo* studies and results should be carefully extrapolated (Manning & Edgar, 1992).

In *in situ* studies, specimens of dental tissue are carried out in the mouth via wearing an appliance and are exposed to erosive challenges or other treatment at pre-determined times, either intra-orally or *ex-vivo*. Tooth surface loss is measured *ex-vivo*, allowing the use of accurate analytical methods. Therefore, *in situ* studies provide an accurate method of measuring tooth surface loss over time in a more or less completely natural environment of saliva flow, pellicle development and routine oral care.

5.2.1 Study design

Various *in situ* designs have been applied to assess the erosive potential of foodstuffs and beverages. Cross-over design studies are usually used as they have the key benefit of using the same subject as its own control. This facilitates the process of subject selection and decreases the number of volunteers required. On the other hand, cross-over design studies last longer and therefore subject's compliance might be compromised. Cross-over designs are also liable to have a 'carryover' effects which can be avoided with a sufficiently wash-out period between treatments or exposures. In the present study, a one week wash-out period was commenced between the two study arms.

5.2.2 Subject selection

Subjects participating in a study should be representative of the population in which the results are intended to be applied to and therefore a randomised selection of the participants is advocated.

All studies looking at the erosive potential of foodstuffs and beverages recruited adults. Adult participants are more likely to comply with clinical protocols and generally have greater availability for appointments. Furthermore, informed consent issues do not pose a problem in adult participants as in children.

Fifteen healthy adults participated in the present *in situ* study. These participants were either dental students or dental nurses, which according to Zero (1995) might be considered as an experimental bias. However, the value of this type of bias is

insignificant to the present study as the results were based on measurements of enamel slabs by surface profilometry and microhardness testing.

All volunteers were screened according to recommendations by Curzon & Hefferen (2001) for intra-oral cariogenicity and erosion studies. The inclusion and exclusion criteria allow for standardisation of the different parameters that could influence the process of dental erosion. Subjects participated in the present study had to have sound dentition with no visual signs of untreated caries or periodontal disease. The presence of periodontal disease has been shown to alter the microbiological consistency of dental plaque (Zero, 1995). Importantly, participant's compliance with a prescribed protocol is crucial when conducting *in situ* studies and poor oral hygiene or dental neglect could be an indicator of poor compliance.

In addition, all subjects had to have a normal salivary flow rate to ensure a normal response to the erosive challenge. The subjects were instructed to remove the upper removable appliances when eating or drinking and during tooth brushing in order to eliminate the potential erosive and abrasive effects of diet and tooth brushing on enamel slabs.

5.2.3 Experimental appliance

An upper removable appliance with U clasps on first permanent molars was used in this study. This appliance was developed by Leeds Dental Institute. The appliance had a similar design to other appliances design used in previous erosive models (West *et al.*, 1998; Magalhães *et al.*, 2008; Hara *et al.*, 2009) and had been used previously in erosive studies without causing any severe problems or discomfort to participants (Abdullah,

2009). Furthermore, the appliance used in this study did not interfere with oral hygiene procedures, allowing participants to remove it during tooth brushing and also during eating and drinking.

The appliance used in this present study contained two enamel slabs secured in the midline of the palatal plate. It has been shown that in palatal appliances, the specimens are well placed for capturing erosion data, although ideally they should be attached to the facial, palatal and occlusal surfaces of teeth where erosion is frequently located. Unfortunately, this is not possible because the location of the specimens in these areas would interfere with the occlusion (West *et al.*, 2011). It has been reported that anteriorly placed specimens show more erosion than posteriorly placed specimens after an *in vivo* challenge, but the differences were not significant (Hooper *et al.*, 2007). For the present study, position of the specimens in the mouth was not important as the immersion of the appliance in the test solutions was carried out *ex-vivo*. The appliance used also had an arched wire placed over the slabs to prevent the abrasive influence of the tongue.

The effect of the appliance used in this study on salivary parameters was assessed previously to identify whether the appliance created conditions different to the *in vivo* situation. No difference in salivary flow rate, either stimulated or un-stimulated, was detected (Abdullah, 1999). This was in agreement with study carried out by Jaeggi & Lussi (1999).

5.2.4 Enamel slabs preparation

Enamel slabs used in this study were prepared from human premolar teeth. It has been recommended that in order to minimise variation between specimens, un-erupted third molars are preferred to be used because of the lack of oral exposure, age of the patient (usually 20-30 years) and a lack of secondary and tertiary age-related changes to the tooth structure (West *et al.*, 2011). This however was very difficult to obtain for the present study due to difficulties in obtaining un-erupted third molars from the Leeds Dental Institute Tissue Bank.

It has been demonstrated *in vitro* that the lingual/palatal surfaces of teeth are more susceptible to erosion compared to labial/buccal surfaces (Tucker *et al.*, 1998). However, West *et al.* (2011) reported that a recent unpublished study by Hooper demonstrated no significant difference in erosion depth between tooth surface or dental arch with regard to wisdom teeth. Enamel slabs used in the present study were prepared from the buccal and palatal surfaces of premolars.

The enamel slabs were stored in distilled water and 0.1% thymol after preparation in order to prevent dehydration and growth of bacteria. Thymol has the ability to perforate cell membranes, so it can destroy the pathogens which may be present on teeth (Shapiro & Guggenheim, 1995). No detrimental effect of thymol on enamel has been reported. There are only few studies that have showed that thymol can affect the permeability of dentine. The amount of mineral dissolution from artificial lesions formed *in vitro* has been found to be reduced in dentine slabs stored in thymol solution (Preston *et al.*, 2007). Enamel slabs were then sterilised by gamma radiation (4080 Gy) prior to their

use. Studies reported that gamma radiation do not alter surface microhardness or the response to demineralisation of enamel (Amaechi *et al.*, 1998; Amaechi *et al.*, 1999b)

Standardisation of enamel slabs was important; all slabs chosen to be included in this study had an indentation length within 60-70 μm . Although the teeth used in the study came from many individuals, the enamel samples used were within a standard hardness and porosity, reducing any errors due to natural biological variation. In addition, all enamel slabs were polished so that they were flat, within 1.0 μm . The process of polishing the enamel slabs to make sure that they were flat was very time consuming.

5.2.5 Study protocol

Many studies have examined 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 *in vitro* studies provided information on the erosive potential of these drinks, it exaggerated the erosive effect due to the absence of modifying influence of saliva.

Amaechi *et al.* (1999c) introduced a cyclic model to produce dental erosion lesions using simple *in vitro* technique. Lesions were produced by immersing the teeth in aliquots of 20 ml of orange juice at regular intervals six times per day (to simulate 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. Immersion was carried out at room temperature and teeth were stored in artificial saliva at night and between immersions. The experiment was carried out for 24 days, giving a total of

12 hour of exposure to orange juice. The 5 minutes exposure was chosen because it was observed in one study that the pH of saliva and its calcium phosphate saturation returned back to baseline level after 5 minutes rinse with citric acid rinse (Bashir & Lagerlof, 1996). This study concluded that the technique used was suitable to mimic the conditions *in vivo* and can be used to examine the effects of various parameters on dental erosion

The protocol used for the current study was similar to the protocol used in Abdullah's (2009) study. This was developed at the University of Leeds and is a slightly modified version from the method used by Amaechi *et al.* (1999c). 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.

5.2.6 Method of exposure

In situ models involving specimens mounted in appliances have used either extra-oral or *in situ* acid challenge. The extra-oral acid challenge has been used in most studies in order to avoid unnecessary acid exposure of participants' teeth. The extra-oral acid challenge is also standardised. Since all specimens are subjected to exactly the same acidic conditions, tissue loss is less variable than after *in vivo* exposure, which is influenced by differences in the oral environment of the study participants (West *et al.*, 2011).

In the present study, enamel slabs were immersed in 50 ml of test solutions 5 times a day for 2 minutes extra-orally. This amount was chosen as it is believed that most consumers will drink about five soft drinks per day (Mintel, 2008).

5.2.7 Use of surface profilometry

The measurement of tooth surface loss in this study was represented by difference in height, using un-exposed areas as a reference point. The three point height difference was performed by using the laser profilometry software.

Surface profilometry is a quick, simple technique that allows measurements of surface loss of a large area with a high precision provided that the tooth loss exceeds about 0.4 μm (Hooper *et al.*, 2003). This method has been widely used to assess the erosive potential of various products *in vitro* including herbal teas (Phelan & Rees, 2003), various acid solutions (Hughes *et al.*, 2000) toothpastes and CPP-ACP products (Rees *et al.*, 2007) and mouthrinses (Pontefract *et al.*, 2001). The technique has also been adapted for use in clinical trials.

Flattening of the specimen is important when surface profilometry is used to allow reliable detection of minimal loss even below 1 μm (Barbour & Rees, 2004). This, however, can make the enamel surface more susceptible to acid dissolution than it would be under clinical conditions (Meurman & Frank, 1991).

Surface profilometry is a method to use only if there is advanced erosion as it does not account for the subsurface loss of the softened layer in early demineralisation. In the present study, measuring enamel slabs immersed in the smoothie was quite difficult compared to slabs immersed with citric acid where obvious enamel surface loss was noted.

5.2.8 Use of microhardness

Microhardness testing measures the resistance of enamel surface to an indenter penetration. Generally microhardness testing is an easy, non-destructive, reliable and sensitive method of describing changes in mineral density and monitoring early stages of hard tissue dissolution (Featherstone & Zero, 1992). Feagin *et al.* (1969) measured the calcium and phosphate loss or gain during enamel remineralisation or softening and showed that the values correlated well with the enamel microhardness testing values. Similar results have been found for etched enamel (Davidson *et al.*, 1974) and therefore microhardness testing has also been used to assess tooth surface loss due to erosion or erosion/abrasion (Jaeggi & Lussi, 1999).

The key benefit of microhardness technique is that it allows measurements to be undertaken during the study quickly and simply. It provides however, an indirect measurement of enamel loss or gain as opposed to other direct techniques such as transverse microradiography (ten Bosch & Angmar-Mansson, 1991; Featherstone, 1992).

Various loads of the Knoop diamond have been applied in cariogenicity studies ranging from 50g up to 500g and it is recommended that a load between 50-200g be used (Featherstone, 1992). It was found by Graig & Peyton (1958) that a 50g load results in well-defined indentations with a minimum of fractures around the edges. However, Davidson *et al.* (1974) observed that a 100 g load was necessary in order to facilitate optical perceptibility. In this study 100 g load was chosen for the above reason.

5.2.9 Erosive effect from exposure to smoothie and citric acid after 21-days pH cycling protocol

The findings of the *in situ* study were aimed to determine the erosive effect of smoothie and compare it to citric acid after 21-days pH cycling protocol using an *in situ* model.

Citric acid was used as a positive control for this study. It is the acid most commonly used in studies determining the erosive effect of soft drinks because it is the major organic hydroxyl acid found in fruit juices and soft drinks (Ting & Attaway, 1971). 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 (West *et al.*, 2001) therefore this percentage was chosen for the present study.

Comparison of the erosive effect of smoothie with citric acid using surface profilometry and microhardness testing demonstrates that there was a statistical significant difference in tooth surface loss between the two groups at p value < 0.05. Surface profilometry measurements revealed that citric acid caused a greater tooth surface loss compared with smoothie. Measurements with microhardness showed that there was a statistical significant difference (change) in indentation length and enamel microhardness before and after exposure to smoothies and citric acid. Microhardness measurements also revealed that citric acid caused statistically 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=3.67) than citric acid (pH=3.08) and its titratable acidity was three 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 the erosive potential of a drink. Lussi (2006) 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. The research carried out by West *et al.* (1998) showed that enamel erosion was dramatically reduced by the order of 10 times in the *in situ* model. Comparison of the current *in situ* study results with an *in vitro* study is described in the subsequent section.

5.2.10 Comparison of erosive effect from exposure to smoothie and citric acid after 21-days pH cycling protocol with an *in vitro* study

Comparison of the current study results with those obtained from the *in vitro* study conducted by Sukeri (2010) leads to the predictable conclusion that investigating erosion in a laboratory setting grossly overestimated the amount of tooth surface loss that might be expected *in situ*. There was a dramatic difference in tooth surface loss following exposure to smoothies 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 (Meuman *et al.*, 1991). 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* (Hall *et al.*, 1999; Hara *et al.*, 2006). 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 (Birkhed & Heintze, 1989) and protein breakdown (So¨derling, 1989).

Work to assess salivary film velocity in various parts of the mouth and with intra-oral appliances has shown that salivary exposure is considerable and not necessarily inhibited by the presence of an *in situ* appliance (Dawes *et al.*, 1989; Dawes & MacPherson, 1993; Hall, 1994). Although quantity of saliva is not necessarily the most important factor in reducing acid levels at the tooth surface (Hall, 1994), the chemical composition of freshly secreted saliva *in situ* is likely to offer greater protection than saliva *in vitro*.

Specimens used in this *in situ* study were not exposed directly to tooth brushing and would, therefore, have accumulated a plaque layer which may have modified tooth tissue loss because of erosion (Dibdin, 1981; Tatevossian, 1991). Such modification may be either as a diffusion barrier limiting access by the eroding fluid or as a source of plaque acids generated by plaque metabolism. Additionally, residual intra-oral levels of fluoride (Zero *et al.*, 1988; Jacobsen *et al.*, 1992) would be expected to reduce mineral loss.

5.2.11 Reducing erosion

This current study produced useful information for dental professional and consumers regarding the effect of smoothies on enamel. It is acknowledged that smoothies are considered healthy due to their high content of vitamins, antioxidants and fibres, nevertheless, excessive consumption of smoothies without considering preventive measures can result in detrimental effects on the tooth tissues.

Advice on ways to make consumption of smoothies safer, especially in children, is essential to avoid risk of misuse. Understanding the aetiology of dental erosion means

that reducing the frequency of contact of smoothies with the tooth surface would be the major preventive measure (Imfeld, 1996). Modification of certain habits such as avoiding swishing the drinks in the mouth before swallowing and the use of straw (Tahmassebi & Duggal, 1997) is also useful to reduce the contact and contact time of the smoothies with the tooth surface.

Other advice to consumers can include avoiding immediate tooth brushing after consuming smoothies and postponing it to at least one hour after, rinsing with water or fluoride solution to neutralise and dilute the acid (Lussi *et al.*, 2004), chewing gum to stimulate saliva secretion and finally to drink the smoothie chilled.

5.2.12 Problems encountered

Originally, ion chromatography was planned to be carried out as part of the *in vitro* study to measure calcium and phosphorus ions of the soft drinks. This however, was not carried out due to technical difficulties. The lab was relocated to a new site and some equipment was lost.

The study required a total amount of 60 enamel slabs. To fulfil the strict standardisation criteria many slabs were rejected, mainly because they did not have indentation lengths ranging between 70-80 μm . The process of polishing the enamel slabs to make sure that they were flat was very time consuming. Many slabs were polished to the extent that dentine was exposed; therefore they had to be excluded from the study.

5.2.13 Suggestions for further research

On the basis of the present study it appears that the smoothies have a potential erosive effect on teeth. The current literature does not provide sufficient information on the erosive effect of smoothies, therefore further research is needed to provide more evidence with regard to the properties of smoothies and their effect on teeth.

Further *in vitro* studies looking at the calcium and phosphate chelating properties as well as the mineral contents of smoothies is required. In addition, the need for further well conducted *in situ* studies to assess the effect of smoothies and compare it with other commercially available drinks would be useful. It would be beneficial to look at the effect of brushing following smoothies contact with enamel. Future research should include large-scale randomised control trials, adequately powered to show clinically relevant effects if they exist.

6.0 CONCLUSIONS

From the results of this study on the dental effects of smoothies on enamel erosion, it can be concluded that:

1. Innocent[®] smoothies are acidic and have high titratable acidity.
2. Innocent[®] strawberries and bananas smoothie had an erosive potential to the teeth after 21-days pH cycling protocol using an *in situ* model as demonstrated with surface profilometry and microhardness testing.
3. The erosive effect of Innocent[®] strawberries and bananas smoothie is significantly less as compared with citric acid 0.3% after 21-days pH cycling protocol using an *in situ* model.

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APPENDIX 1: Examples of erosion indices

a) Index according to Eccles (Eccles, 1979)

Class	Surface	Criteria
Class I		Early stages of erosion, absence of developmental ridges, smooth, glazed surface occurring mainly on labial surfaces of maxillary incisors and canines
Class II	Facial	Dentine involved for less than one third surface; two types Type 1 (commonest): ovoid-crescentic in outline, concave in cross section at cervical region of surface. Must differentiate from wedge shaped abrasion lesions Type 2: irregular lesion entirely within crown. Punched out appearance, where enamel is absent from floor
Class IIIa	Facial	More extensive destruction of dentine, affecting anterior teeth particularly. Majority of lesions affect a large part of the surface, but some are localised and hollowed out
Class IIIb	Lingual or palatal	Dentine eroded for more than one third of the surface area. Gingival and proximal enamel margins have white, etched appearance. Incisal edges translucent due to loss of dentine. Dentine is smooth and anteriorly is flat or hollowed out, often extending into secondary dentine
Class IIIc	Incisal or occlusal	Surfaces involved into dentine, appearing flattened or with cupping. Incisal edges appear translucent due to undermined enamel; restorations are raised above surrounding tooth surface
Class IIId	All	Severely affected teeth, where both labial and lingual surfaces are extensively involved. Proximal surfaces may be affected; teeth are shortened

b) Tooth Wear Index According to Smith and Knight (Smith and Knight, 1984)

Score	Surface	Criteria
0	B/L/O/I C	No loss of enamel surface characteristics No loss of contour
1	B/L/O/I C	Loss of enamel surface characteristics Minimal loss of contour
2	B/L/O I C	Loss of enamel exposing dentine for less than one third of surface Loss of enamel just exposing dentine Defect less than 1 mm deep
3	B/L/O I C	Loss of enamel exposing dentine for more than one third of surface Loss of enamel and substantial loss of dentine Defect less than 1–2 mm deep
4	B/L/O I C	Complete enamel loss–pulp exposure–secondary dentine exposure Pulp exposure or exposure of secondary dentine Defect more than 2 mm deep–pulp exposure–secondary dentine exposure

c) Modified Scoring System of Linkosalo and Markkanen (Linkosalo and Markkanen, 1985)

Grade 0	No erosion
Grade 1 (Incipient)	Loss of surface features of the labial, lingual or occlusal enamel surface, giving a smooth glazed appearance. The dentine is not involved.
Grade 2 (Moderate)	Involvement of the dentine for less than one third of the area of the tooth surface.
Grade 3 (Grave)	Involvement of the dentine for more than one third of the area of the tooth surface.

d) Erosion Index according to Lussi (Lussi, 1996)

Surface	Score	Criteria
Facial	0	No erosion. Surface with a smooth, silky glazed appearance, possible absence of developmental ridges
	1	Loss of surface enamel. Intact enamel cervical to the erosive lesion; concavity on enamel where breadth clearly exceeds depth, thus distinguishing it from toothbrush abrasion. Undulating borders of the lesion are possible and dentine is not involved
	2	Involvement of dentine for less than half of tooth surface
	3	Involvement of dentine for more than half of tooth surface
Occlusal/lingual	0	No erosion. Surface with a smooth, silky glazed appearance, possible absence of developmental ridges
	1	Slight erosion, rounded cusps, edges of restorations rising above the level of adjacent tooth surface, grooves on occlusal aspects. Loss of surface enamel. Dentine is not involved
	2	Severe erosions, more pronounced signs than in grade 1. Dentine is involved

e) **Larsen and Westergaard Index (Larsen and Westergaard, 2000)**

Criteria of facial and oral crown surfaces

Score 0	Original developmental structures, perichymata, are present on part of or on the entire surface
Score 1	Signs of erosion indicated by absence of developmental ridges extending over the entire enamel surface resulting in a smooth, glazed enamel, but without distinct loss of the original morphology of the tooth
Score 2	Signs of erosion and loss of enamel with a change of the original morphology of the tooth surface resulting in a flattening of the surface or a concavity in enamel, the width of which clearly exceeds its depth. Dentine is not involved
Score 3	Signs of erosion and loss of enamel with exposure of dentine in less than one-third of the tooth surface
Score 4	Signs of erosion and loss of enamel with exposure of dentine in more than one-third of the tooth surface.
Score 5	Signs of erosion and loss of tooth substance, changes of the original morphology of the facial or the oral surface as well as of one or both approximal surfaces

Criteria for incisal and occlusal surfaces

Score 0	Original developmental structures are present on the entire surface.
Score 1	Loss of enamel resulting in a smooth, glazed appearance either locally or extending over the entire enamel surface. Areas worn into flat-faceted shapes or rounded cusps are possible. Dentine is not involved.
Score 2	Loss of enamel with exposure of dentine in minor areas.
Score 3	Loss of enamel with exposure of dentine on the entire incisal surface or in larger areas of one or more cusps.
Score 4	Considerable loss of enamel and dentine with lack of one- to two-thirds of the original height of the tooth crown.
Score 5	Excessive loss of enamel and dentine with lack of more than two-thirds of the original height of the tooth crown.

f) O'Sullivan Index (O'Sullivan, 2000)

Site of Erosion on each Tooth	Grade of Severity (worst score for individual tooth recorded)
Code A Labial or buccal only	Code 0 Normal Enamel
Code B Lingual or palatal only	Code 1 Matt appearance of the enamel surface with no loss of contour
Code C Occlusal or incisal only	Code 2 Loss of enamel only (loss of surface contour)
Code D Labial & incisal occlusal	Code 3 Loss of enamel with exposure to dentine
Code E Lingual & incisal/occlusal	Code 4 Loss of enamel and dentine beyond
Code F Multi-Surface	Code 5 Loss of enamel and dentine with exposure of pulp
	Code 9 Unable to assess
	Code - Less than half of surface affected
	Code + More than half of surface affected

g) The UK national Survey of Children's Dental Health Index (Office of National Statistics, 2004)

Score	Criteria
Depth	
0	Normal
1	Loss of surface characterisation, enamel only-on incisor teeth there is loss of developmental ridges resulting in a smooth glazed or ground glass appearance. On occlusal surfaces the cusps appear rounded and there may be depressions producing cupping.
2	Enamel and dentine-loss of enamel exposing dentine. On incisors this may resemble a 'shoulder preparation, parallel to the crest of the gingivae. Particularly on palatal surfaces. The incisors may appear shorter and there may be chipping of the incisal edges. Restorations may be raised above the level adjacent surfaces.
3	
9	Enamel, dentine and pulp-loss of enamel and dentine resulting in pulp exposure. Assessment cannot be made.
Area	
0	Normal
1	Less than one third of surface involved
2	One-third to up to two thirds of surface involved
3	More than two thirds of surface involved
9	Assessment cannot be made

APPENDIX 2: Nutritional information of soft drinks

a) Nutritional information for Innocent® strawberries and bananas smoothie



nutritional information

	per 100ml	per serving (250ml)
energy	228 kJ (54 kcal)	570 kJ (136 kcal)
protein	0.6g	1.5g
carbohydrate	11.9g	29.75g
carbohydrate (of which sugars)	10.5g	26.25g
fat	0.2g	0.5g
fat (of which saturates)	0.1g	0.25g
fibre	1g	2.5g
sodium	trace	trace
vitamin c	8mg	20mg (33% rda)

250ml as a % of an adult's guideline daily amount:

calories	sugars	fat	saturates	sodium	fibre
136	26.25g	0.5g	0.25g	trace	2.5g
7%	29%	1%	1%	trace	10%

b) Nutritional information for Innocent® mangoes and passion fruit smoothie



nutritional information

	per 100ml	per serving (250ml)
energy	229 kJ (55 kcal)	573 kJ (137 kcal)
protein	0.6g	1.5g
carbohydrate	12.2g	30.5g
carbohydrate (of which sugars)	12.2g	30.5g
fat	0.1g	0.25g
fat (of which saturates)	0.1g	0.25g
fibre	1.1g	2.75g
sodium	trace	trace
vitamin c	20mg	50mg (83% rda)

250ml as a % of an adult's guideline daily amount:

calories	sugars	fat	saturates	sodium	fibre
137	30.5g	0.25g	0.25g	trace	2.75g
7%	34%	trace	1%	trace	11%

c) Nutritional information for Innocent® kiwis, apples and limes smoothie



nutritional information

	per 100ml	per serving (250ml)
energy	210 kJ (50 kcal)	525 kJ (125 kcal)
protein	0.5g	1.25g
carbohydrate	11g	27.5g
carbohydrate (of which sugars)	10.3g	25.75g
fat	trace	trace
fat (of which saturates)	trace	trace
fibre	1.8g	4.5g
sodium	trace	trace
vitamin c	21mg	53mg (88% rda)

250ml as a % of an adult's guideline daily amount:

calories	sugars	fat	saturates	sodium	fibre
125	25.75g	trace	trace	trace	4.5g
6%	29%	trace	trace	trace	19%

d) Nutritional information for Innocent® blackberries, strawberries and blackcurrants smoothie



nutritional information

	per 100ml	per serving (250ml)
energy	299 kJ (71 kcal)	748 kJ (179 kcal)
protein	0.6g	1.5g
carbohydrate	11.9g	29.75g
carbohydrate (of which sugars)	11g	27.5g
fat	trace	trace
fat (of which saturates)	trace	trace
fibre	1.5g	3.75g
sodium	trace	trace
vitamin c	25mg	63mg (104% rda)

250ml as a % of an adult's guideline daily amount:

calories	sugars	fat	saturates	sodium	fibre
179	27.5g	trace	trace	trace	3.75g
9%	31%	trace	trace	trace	16%

e) Nutritional values of Diet Coke



Nutritional Information **GDA's** **Ingredients**

Display information per 150ml ▾

Each 150ml serving contains the following of an adult's guideline daily amount:

Calories 0.5 Kcal <1%	Sugar 0.0 g 0%	Fat 0.0 g 0%	Saturates 0.0 g 0%	Salt 0.01 g <1%
---------------------------------------	-----------------------------	---------------------------	---------------------------------	---------------------------------

APPENDIX 3: National Research Ethics Service approval



National Research Ethics Service

NRES Committee Yorkshire & The Humber - South Yorkshire

Millside
Mill Pond Lane
Meanwood
Leeds
LS6 4RA

Telephone: 0113 306 0128

04 August 2011

Miss Hanein Ali
University of Leeds
Child Dental Health Department
Leeds Dental Institute
Worsley Building, Level 6
Clarendon Way
LS2 9LU

Dear Miss Ali

Full title of study: The effects of smoothies on enamel erosion: an in situ pilot study
REC reference number: 11/YH/0228

Thank you for your letter of 21 July 2011. I can confirm the REC has received the documents listed below as evidence of compliance with the approval conditions detailed in our letter dated 30 June 2011. Please note these documents are for information only and have not been reviewed by the committee.

Documents received

The documents received were as follows:

Document	Version	Date
Covering Letter		21 July 2011
Participant Information Sheet	2	21 July 2011
Participant Consent Form	2	21 July 2011

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

11/YH/0228

Please quote this number on all correspondence


Yours sincerely

Ms Sinead Audsley
Committee Co-ordinator

E-mail: sinead.audsley@nhs.net

This Research Ethics Committee is an advisory committee to the Yorkshire and The Humber Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics Committees in England

APPENDIX 4: Leeds Teaching Hospital Trust Research & Development approval

The Leeds Teaching Hospitals 
NHS Trust

Ref: *Amend0304*

04/10/2011

Miss Hanein Ali

Leeds Dental Institute
Child Dental Health Department
Worsley Building
Level 6
LS2 9LU

Research & Development

Leeds Teaching Hospitals NHS Trust

34 Hyde Terrace
Leeds
LS2 9LN

Tel: 0113 392 2878
Fax: 0113 392 6397

r&d@leedsth.nhs.uk
www.leedsth.nhs.uk

Dear Miss Hanein Ali

Re: NHS Permission at LTHT for: The Effects of Smoothies on Enamel Erosion: an in situ pilot study
LTHT R&D Number: DT11/9967
REC: 11/YH/0228

I confirm that *NHS Permission for research* has been granted for this project at The Leeds Teaching Hospitals NHS Trust (LTHT). NHS Permission is granted based on the information provided in the documents listed below. All amendments (including changes to the research team) must be submitted in accordance with guidance in IRAS. Any change to the status of the project must be notified to the R&D Department.

Permission is granted on the understanding that the study is conducted in accordance with the *Research Governance Framework for Health and Social Care*, ICH GCP (if applicable) and NHS Trust policies and procedures available at http://www.leedsth.nhs.uk/sites/research_and_development/.

This permission is granted only on the understanding that you comply with the requirements of the *Framework* as listed in the attached sheet "Conditions of Approval".

If you have any queries about this approval please do not hesitate to contact the R&D Department on telephone 0113 392 2878.

Indemnity Arrangements

Chairman Mike Collier (or Chief Executive Maggie Boyle)

The Leeds Teaching Hospitals incorporating:

Chapel Allerton Hospital Leeds Dental Institute Seacroft Hospital

St James's University Hospital The General Infirmary at Leeds Wharfedale Hospital

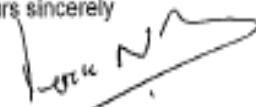


0113 392 2878

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority 'Clinical Negligence Scheme for NHS Trusts' for: (i) medical professional and/or medical malpractice liability; and (ii) general liability. NHS Indemnity for negligent harm is extended to researchers with an employment contract (substantive or honorary) with the Trust. The Trust only accepts liability for research activity that has been managerially approved by the R&D Department.

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm to cover you as investigator and the researchers listed on the Site Specific Information form. Should there be any changes to the research team please ensure that you inform the R&D Department and that s/he obtains an appropriate contract, or letter of access, with the Trust if required.

Yours sincerely



Dr D R Norfolk
Associate Director of R&D

Approved documents

The documents reviewed and approved are listed as follows

<i>Document</i>	<i>Version</i>	<i>Date of document</i>
NHS R&D Form	3.1	28/07/2011
SSI Form	3.1	28/07/2011
Directorate Approval		28/09/2011
Protocol	1	08/05/2011
REC Letter confirming favourable opinion		11/07/2011
Insurance/ Indemnity		10/09/2011
Poster		Not Dated
Patient information sheet (REC Approved)	2	21/07/2011
Consent form (REC Approved)	2	21/07/2011

APPENDIX 5: Patient Information Sheet



UNIVERSITY OF LEEDS

Department of Paediatric Dentistry

Level 6, Worsley Building

Clarendon Way, Leeds, LS2 9LU

Tel: Direct Line +44 (0)113 343 6177

Tel: Enquiries +44 (0)113 343 6138

Fax: +44 (0)113 343 6140

Leeds Dental Institute

Patient Information Sheet

Study title:

The effects of smoothies on enamel erosion: an in situ study

“ You are being invited to take part in the above research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part”

What is the purpose of the study?

We are about to undertake a research looking at the effect of smoothies on dental erosion (tooth wear). We will use two different solutions in our study and will compare their effects on dental erosion. The solutions are strawberries and banana smoothie and citric acid.

Why have I been chosen?

We hope to recruit volunteers for this study. All we ask of volunteers is that they are willing to take part in the study; that they are at least 18 years old; in general good health and are free from visual signs of dental decay or gum diseases. Volunteers should have sufficient natural teeth to retain a removable appliance. Before you are enrolled on the study, you will need to be ‘screened’. This will involve a short dental examination, to enable us to establish whether you meet all our criteria. We will ask you a few simple questions about your general health. You will also have the opportunity to ask us any questions you may have about the study.

Do I have to take part?

Participation in this study is entirely voluntary. If you decide you would like to take part, you will be given a copy of this information sheet to keep, and we will ask you to complete and sign a form which gives your written consent to take part. If after reading this and thinking about the information given, you decide you would not like to take part that is fine. Even if you decide you would like to take part in the study, but you later decide you no longer wish to continue, you can withdraw at any time, and you do not have to give us a reason unless you want to. If you do decide to withdraw from the study at any point, please let us reassure you that your future dental care at the Dental Hospital will in no way be compromised. If you are a member of staff of the Trust or University, you are under no obligation whatsoever to take part in this study, but if you decide you would like to take part, you can still withdraw at any point without having to give a reason.

What will happen to me if I take part?

If you decide you would like to take part, and our screening procedures identify you as a suitable volunteer, an upper removable appliance similar to an orthodontic plate will be constructed for you. You will have to wear this removable appliance which contains two sterilised enamel sections. These enamel sections are the parts that we will do our tests on. The appliance should be worn for 21 days at all times, except at mealtimes, while drinking and teeth brushing, and overnight to collect dental plaque on enamel sections. You will then be asked to dip the appliance for 2 minutes each time, 5 times daily in fresh 50 ml of test solutions. The solutions are strawberries and banana smoothie and citric acid.

We will show you how to remove and re-insert the appliance, and you will have the opportunity to ask any questions, and to make sure you are happy and confident in using the appliance, before starting the study.

After 21 days, the appliances will be collected by the study investigator. Enamel sections will be removed in the laboratory and will be tested for enamel loss.

What do I have to do?

You will need to agree to wear the appliance we will construct for you, and to agree to remove the appliance during mealtimes and during teeth brushing. You will need to use the fluoridated toothpaste we will give you. You will need to immerse the appliance for 2 minutes each time, 5 times daily in fresh 50 ml of test solutions. You will also need to agree to come into the test centre at the dates and times agreed. In recognition of any inconvenience and out of pocket expenses you will incur, you will be paid a fee of £150 for taking part in the study. This may affect any benefits you currently receive. This money will be paid to you at the end of the study. In order for us to pay you, you will need to complete a bank details form, and provide us with your National Insurance Number. This information will be held confidentially.

What are the side effects of taking part?

The only disadvantage to you as a volunteer will be that you might find it slightly inconvenient wearing the appliance initially however, this will disappear in the following day as you will get used to the appliance. If there is any pain or discomfort you can contact us on telephone numbers provided below and we will arrange an appointment to adjust the appliances if needed.

What are the possible benefits of taking part?

There are no direct immediate benefits to you for participating in our study. However, you will be helping us understanding the effect of consuming smoothies on dental erosion.

Will my taking part in this study be kept confidential?

Yes, any information we gather will be kept confidential. You will not be identified by name in any reports or publications.

What will happen to the results of the research study?

We hope our research will be well received by the dental community. You will not be identified by name in any reports we write.

Who is funding the research?

I am a full time professional doctorate student in paediatric dentistry at the University of Leeds. As part of the professional doctorate programme I am doing this research. The research is funded by the Leeds Dental Institute.

Contact Information

Name	Telephone	e-mail
Miss Hanein Ali	07861071352	efy9ha@leeds.ac.uk
Dr Jinous Tahmassebi	(0113)3433955	j.tahmassabi@leeds.ac.uk

Thank you for reading this information sheet.

APPENDIX 6: Consent Form



UNIVERSITY OF LEEDS

Department of Paediatric Dentistry
Level 6, Worsley Building
Clarendon Way, Leeds, LS2 9LU
Tel: Direct Line +44 (0)113 343 6177
Tel: Enquiries +44 (0)113 343 6138
Fax: +44 (0)113 343 6140

Title of Research Project: The effects of smoothies on enamel erosion: an in situ study

Name of Researcher: Hanein Ali

Initial the box if you agree with the statement to the left

- 1 I confirm that I have read and understand the information sheet dated explaining the above research project and I have had the opportunity to ask questions about the project.
- 2 I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without there being any negative consequences. In addition, should I not wish to answer any particular question or questions, I am free to decline.
- 3 I understand that my responses will be kept strictly confidential. I give permission for members of the research team to have access to my anonymised responses. I understand that my name will not be linked with the research materials, and I will not be identified or identifiable in the report or reports that result from the research.
- 4 I agree for the data collected from me to be used in future research
- 5 I understand that relevant sections of data collected during the study may be looked at by individual forms (University of Leeds), from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

APPENDIX 7: Poster advertisement of the study



Are you 18 years old or older?

Would you be interested in participating in a research study?

We are investigating the effect of smoothies on enamel erosion

What we will need from you is to wear an appliance, which will allow us to collect samples of your plaque & Investigate whether smoothies have an effect on enamel erosion.

For further information please contact Hanein Ali

on 07861071352 or efy9ha@leeds.ac.uk



Leeds Dental Institute



University of Leeds School of Dentistry

with the

Dental Hospital at Leeds

Subject code:	Randomisation no.:	Screening no.:
---------------	--------------------	----------------

Case Record Form

The effects of smoothies on enamel erosion An in situ study

Ethics Committee Ref No:11/YH/0228

Supervisors

Dr Jinous Tahmassebi

Prof Monty Duggal

Division of Child Dental Health

Research Investigator

Dr Hanein Ali

Division of Child Dental Health

Volunteer Personnel Sheet

Personnel Information

First name:		Family name:	
Date of birth: __/__/_____		Marital status (Optional):	
Gender: M <input type="checkbox"/> F <input type="checkbox"/>			
Contact address:			
		Post code:	
Tel:	Mobile:	e-mail:	
Emergency contact			
Name			
Relationship			
Address:			
		Post code:	
Tel:	Mobile:	e-mail:	

Researcher's name:	Date:	Signature:
--------------------	-------	------------

Subject code:	Randomisation no.:
----------------------	---------------------------

MEDICAL HISTORY

Any medical conditions to report?	YES	NO
--	------------	-----------

Please list any relevant previous and current medical conditions (including allergies) and surgery that the subject has experienced in the table below*.

MEDICAL CONDITION	START DATE <small>(DD / MM / YYYY)</small>	ONGOING	STOP DATE <small>(IF APPLICABLE)</small>
/...../.....	YES / NO/...../.....
/...../.....	YES / NO/...../.....
/...../.....	YES / NO/...../.....

*Note that if any treatment (s) is/are currently being taken for any of the above conditions this (these) must be recorded on the CURRENT / CONCOMITANT MEDICATIONS page.

<p>Signed by (Investigator).....</p> <p>Print Name (Investigator).....</p>

Dated (DD/MM/YY)

--	--	--	--	--	--	--	--

Subject code:	Randomisation no.:
---------------	--------------------

Current/ Concomitant medication

Drug's name	Cause of medication	Dosage	Date started	Date stopped (if applicable)

Investigator's signature:..... Date __/__/____

Subject code:	Randomisation no.:
---------------	--------------------

Dental Examination

DMFT score

Right

Left

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Total DMFT:

Number of natural teeth:

Note: Subject must have at least 18 natural teeth to be eligible for inclusion.

Oral Cavity Examination

Soft Tissues:

Normal

Abnormal

Describe abnormality

Salivary Flow Rate:

1- Unstimulated: __. __ __ ml/min. (Must be ≥ 0.25 ml/min)

2-Stimulated: __. __ __ ml/min. (Must be ≥ 0.8 ml/min)

Researcher's signature:..... Date __ / __ / ____

Subject code:	Randomisation no.:
---------------	--------------------

Inclusion Criteria Sheet*

	Yes	No
1. Age: Aged between 18-65 years.	<input type="checkbox"/>	<input type="checkbox"/>
2. General Health: Satisfactory medical history with no clinically significant and relevant abnormalities of medical history.	<input type="checkbox"/>	<input type="checkbox"/>
3. Dental Examination: <ul style="list-style-type: none"> i. In possession of at least 18 natural teeth. ii. Free from visual signs of untreated caries or periodontal disease. iii. Not taking any drugs or on special diet that could affect the salivary flow rate or oral pH. iv. Unstimulated Salivary flow rate \geq 0.2 ml/min and Stimulated Salivary flow rate \geq 0.8 ml/min. 	<input type="checkbox"/>	<input type="checkbox"/>
4. Compliance: Understand and is willing, able and likely to comply with all study procedures and restrictions.	<input type="checkbox"/>	<input type="checkbox"/>
5. Consent: Demonstrate understanding of the study and willingness to participate as evidenced by voluntary written informed consent.	<input type="checkbox"/>	<input type="checkbox"/>

***Note:** If any of the above questions are answered “No”, the subject should be discontinued from the study as a “Screen failure” on the study conclusion page.

Researcher’s signature:..... Date__ / __ / ____

Subject code:	Randomisation no.:
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Exclusion Criteria Sheet*

	Yes	No
<p>1. Disease:</p> <p>a. Current or previous history of serious, severe or unstable physical or psychiatric illness, any medical disorder that may require treatment or make or make the subject unlikely to fully complete the study, or any condition that present undue risk from the study product or procedure (e.g. diabetes, history of aphthous ulcer).</p>	<input type="checkbox"/>	<input type="checkbox"/>
<p>b. A condition or medical history that requires prophylactic antibiotic therapy for dental treatment likely to cause bacteraemia.</p>	<input type="checkbox"/>	<input type="checkbox"/>
<p>2. Medication:</p> <p>c. Antimicrobial therapy within 14 days prior to screening.</p> <p>d. Treatment with antibiotics within 28 days prior to screening.</p> <p>e. The use of any medication or erosive products that might result in reduced salivary flow rate.</p>	<input type="checkbox"/>	<input type="checkbox"/>
<p>3. Dental Details:</p> <p>v. Dental disease that require immediate treatment</p> <p>vi. Oral surgery or extraction within 6 weeks prior to study initiation.</p> <p>vii. The wearing of removable prostheses or fixed or removable orthodontic appliances that could affect the conduct of the study.</p>	<input type="checkbox"/>	<input type="checkbox"/>
<p>4. Allergy/Intolerance: Known or suspected intolerance or hypersensitivity to any of the agents used in the study.</p>	<input type="checkbox"/>	<input type="checkbox"/>

***Note:** If any of the above questions are answered “Yes”, the subject should be discontinued from the study as a “Screen failure” on the study conclusion page.

Researcher’s signature:..... Date__ / __ / ____

Leeds Dental Institute



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with the

Dental Hospital at Leeds

Subject code:	Randomisation no.:
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Fitness and Eligibility to Participate in the Study

In the investigator's opinion, on the basis of the screening assessments and Inclusion and Exclusion criteria, is the subject eligible to participate in the next part of the study?

Yes No

Researcher's signature:..... Date__/__/__

Subject code:	Randomisation no.:
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Screening Visit Check List

Personnel sheet completed	Yes	No
Medical history checked	Yes	No
Dental Examination completed	Yes	No
Inclusion criteria sheet completed	Yes	No
Exclusion criteria sheet completed	Yes	No
Eligibility sheet completed	Yes	No
Participant's Information Sheet	Yes	No
Participant's Consent Form	Yes	No

Researcher's signature:..... Date__ / __ / ____

Subject code:	Randomisation no.:
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First visit

Date --/--/----

Upper and lower alginate impression	
Dental scaling and polishing	
Any problems	Yes No
If yes please give details	

Second visit

Date --/--/----

Fitting of appliances	
Any problems?	Yes No
If yes please give details	
Instructions and restrictions	
Appointment arranged for next visit	Yes No

Third visit**Date --/--/----**

Removal of appliance	
Any problems	Yes No
If yes please give details	

Fourth visit**Date --/--/----**

Fitting of appliances	
Any problems	Yes No
If yes please give details	
Appointment arranged for next visit?	Yes No

Fifth visit**Date --/--/----**

Removal of appliance	
Any problems	Yes No
If yes please give details	

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Division of Child Dental Health

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Adverse Events

Adverse Event		Onset Date	End Date	Duration	Outcome	Pattern	Intensity	Relation to study	Action taken	Serious*
		___/___/___	___/___/___							
		___/___/___	___/___/___							
		___/___/___	___/___/___							
		___/___/___	___/___/___							
<u>Duration(Units)</u>	<u>Outcome</u>	<u>Pattern</u>	<u>Intensity</u>	<u>Relationship to study</u>	<u>Action Taken (regarding the study)</u>			<u>Serious</u>		
1.S-Seconds 2.M-Minutes 3.H-Hours 4.D-Days	1.Resolved 2.Ongoing	1.Continuous 2.Intermittent	1. Mild 2. Moderate 3. Severe	1.Not relate 2.Unlikely 3.Possible 4.Highly possible	1. None 2. Interrupted 3. Discontinued			1.No 2.Yes*		
* All serious adverse events must be reported to the study monitor within 24 hours and require special action										

Subject code:	Randomisation no.:
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Study Conclusion

Did the subject complete the entire study?	Yes	<input type="checkbox"/>	No*	<input type="checkbox"/>
<i>If "No" is checked, please complete the following (please check as an appropriate):</i>				
Screen Failure		<input type="checkbox"/>		
Adverse Event		<input type="checkbox"/>		
Lost of Follow-up		<input type="checkbox"/>		
Protocol Deviation		<input type="checkbox"/>		
Withdrawal of Volunteer		<input type="checkbox"/>		
Other		<input type="checkbox"/>		

Researcher's Signature

I confirm that I have reviewed all the data collected in this Case Report Form and take responsibility that the information is accurate and complete.	
Researcher's Name	
Researcher's Signature	Date ___ / ___ / _____

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Case Record Form

Study no:

The Effects of smoothies on enamel erosion An in situ study

DIPPING DIARY LOG & APPOINTMENT CARD

SUBJECT CODE

--	--	--	--	--

RANDOMISATION NUMBER

--	--	--	--	--

Supervisors

Dr Jinous Tahmassebi

Prof Monty Duggal

Division of Child Dental Health

Research Investigator

Dr Hanein Ali

BRIEF INFORMATION FOR COMPLETION OF
THE FOLLOWING DIARY CARDS

- Brushing should occur initially in the morning and before bed.
- Guidelines for the suggested frequency of dipping in citric acid:

Frequency	Suggested Dipping Times					Interval
5 x per day	08.00	11.00	1.00	3.00	6.00	2-3 hrs

- Each dipping should last for 2 minutes; however the exact length of dipping and the time it takes place should be recorded in the relevant diary log.

Thank you for your co-operation.

STUDY CONCLUSION

Did the subject complete the entire log? Yes No*	
<i>If "No" is checked, please comment on missing dips/ logs and why:</i>	
Missed individual dips	<input type="text"/>
Missed entire legs	<input type="text"/>
Other comments	<input type="text"/>
	<input type="text"/>

I confirm that I have reviewed all the data collected in this Dipping Diary Log and take responsibility that the information provided by the subject complete.

Signed by (Investigator).....

Print Name (Investigator).....

Dated (DD/MM/YY)

APPENDIX 9: Leeds Dental Institute Tissue Bank approval

Leeds Dental Institute

Department of Oral Biology
The University of Leeds
Clarendon Way
Leeds LS2 9LU

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UNIVERSITY OF LEEDS

Miss Hanein Ali
Department of Paediatric Dentistry
Leeds Dental Institute

1st August 2011

Dear Hanein,

Re: Tissue Bank Application no: 120711/HA/68
Title: The effects of smoothies on enamel erosion: an in situ pilot study

I am pleased to inform you that your above application has been accepted by the Dental Research Ethics Committee.

Attached is a copy of your protocol, version 1.

With best wishes for the success of your project.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'Julie McDermott'.

Julie McDermott
Research Secretary
Leeds Dental Institute

Professor Jennifer Kirkham BSc PhD
Head of Department

The Leeds Teaching Hospitals NHS Trust