# The **Prostage** potential of thiese smoothiese

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#### Dental erosion

Dental erosion has been defined as the loss of tooth tissue by a chemical process not involving bacteria.<sup>1</sup> In this condition, contact of the tooth structure with acids results in its dissolution. The complications of dental erosion range from minimal tooth surface loss, leading to sensitivity and pain, to excessive loss of clinical crown height, loss

of vitality and poor dental aesthetics. Management of the condition ranges from the application of simple preventive measures and monitoring of their impact, to restoration with composite filling materials or extensive treatment with indirect restorations such as onlays and crowns. This therefore can be a very time consuming and expensive condition to treat.2,3

Acids of intrinsic and extrinsic origin are thought to be the main aetiological factors for dental erosion.<sup>4</sup> For many years exposure of the teeth to extrinsic acids in the diet have been seen to be a major contributory factor to the development of dental erosion. Many studies have identified both fruit-based drinks and carbonated drinks to be potentially erosive.3,5-8

#### Five a day

The UK government's 'five a day' campaign has encouraged the public to consume at least five portions of fruit and vegetables per day<sup>9</sup> to reduce the likelihood of developing serious medical conditions. Many patients see consuming fruit smoothies as a way of achieving this and this is reinforced further by the way such drinks are marketed internationally.

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One serving of fruit smoothie can be counted as up to two of an individual's 'five a day'.<sup>10,11</sup> The consumption of shop-bought smoothies has risen dramatically, from 6.3 million litres in 2001 to 34 million litres in 2006, with consumption projected to treble over the next five years.<sup>12</sup> However, the true level of smoothie consumption is not actually known as these figures exclude consumption of homemade smoothies and those made and purchased in cafés and juice bars.

Historically, fruit smoothies appear to have originated in Brazil as a product of juicing fruit.<sup>13</sup> They first appeared in the UK in 1994 and have steadily increased their share of the soft drinks market.

#### Smoothies and thickies

Legally there is no definition of a fruit smoothie, but it is well accepted in the soft drinks industry that fruit smoothies are made only from pure fruit blended with 100% pure fruit juice (not from concentrate), with no other added ingredients, that is dairy, sugar, sweeteners or water.<sup>14</sup> There are thus many varieties of fruit smoothies, made using different combinations of various fruits and these by their nature contain a variety of organic acids such as citric, malic, phosphoric, oxalic and tartaric. Exposure to such acids has the potential to cause dental erosion.<sup>15</sup> There is nothing to suggest that the increased consumption of smoothies is limited to the UK, thus giving the potential risk of developing dental erosion from their consumption an international dimension.

A variation of the smoothie, though a distinct entity, is the 'thickie'. Thickies contain dairy in some form, usually in the form of yoghurt, with or without fruit. In the production of these drinks yoghurt is deliberately soured or milk curdled by adding bacteria (for example, Lactobacillus acidophilus), which breaks down the milk's lactose forming lactic acid.<sup>16</sup> Although the resultant yoghurt has a low pH, it has no erosive potential due to high levels of calcium and phosphate within it.<sup>17</sup> In the 2008 UK soft drinks report<sup>18</sup> juice and dairy containing drinks were highlighted as being 'set to hit the spotlight' in the future as they were considered 'health targeted products'. They identified that drinks containing dairy cultures conferred 'gut health benefits'<sup>18</sup> as some of the yoghurts used are probiotic and help digestion.

At the time of commencing this work a review of the literature failed to find any research that had investigated the capacity of fruit smoothies to cause dental erosion. This *in vitro* work therefore sought to investigate the pH and titratable acidity of a range of fruit smoothies and observe the effects of exposure to them upon samples of human tooth tissue.

#### Table 1 The drinks investigated in this study

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| Drink  | Manufacturer | Contents (compiled from carton contents labels)  |
| Smooth orange juice<br>(positive control)  | Tropicana    | Juice of fresh oranges   |
| Still mineral water<br>(negative control)  | Volvic       | Still mineral water  |
| Strawberries and<br>bananas fruit<br>smoothie  | Innocent     | 22 crushed strawberries<br>2 pressed apples<br>2 ½ mashed bananas<br>1 ½ squeezed oranges<br>21 pressed red and white grapes<br>A squeeze of fresh lime juice  |
| Kiwis, apples and<br>limes fruit smoothie  | Innocent     | 3 ½ pressed apples<br>1/3 pressed pineapple<br>2 crushed kiwis<br>21 pressed red and white grapes<br>½ fresh lime<br>A dash of spinach and nettle extract  |
| Pomegranates,<br>blueberries and açai<br>fruit smoothie  | Innocent     | 86 pressed red and white grapes<br>2 ½ mashed bananas<br>1 ½ crushed pomegranate<br>1 ½ squeezed oranges<br>153 crushed blueberries<br>102 peeled açai berries   |
| Cranberries,<br>blueberries and<br>cherries fruit smoothie   | Innocent     | 4 pressed apples<br>54 pressed red and white grapes<br>29 crushed cherries<br>160 crushed cranberries<br>90 crushed blueberries<br>A squeeze of fresh lime juice   |
| Yoghurt, vanilla bean<br>and honey thickie   | Innocent     | Fresh low-fat probiotic yoghurt 73%<br>½ pressed apple<br>Honey 9%<br>Vanilla 0.1%   |
| 'Homemade'<br>strawberry and<br>banana fruit smoothie<br>(recipe adapted from<br>Innocent strawberries<br>and bananas fruit<br>smoothie) | Innocent     | 450g strawberries<br>200g mashed banana<br>315g pressed apples (juice and pulp)<br>215g squeezed oranges (juice and<br>pulp)<br>125g pressed green and black grapes<br>(juice and pulp)<br>15 ml freshly squeezed lime juice |

Details derived from manufacturers' data.

Source: Innocent website at www.innocentdrinks.co.uk/things\_we\_make/smoothies/ (accessed 24 January 2011)

In addition, the effects of the removal of fruit smoothie constituents upon erosive potential were also investigated.

#### THE STUDY

This was an *in vitro* investigation in which five varieties of shop-bought fruit smoothies including a thickie were investigated, with respect to their initial pH, titratable acidity and effect upon exposure to the surface microhardness and profile of extracted human teeth.

The effect of a 60 minute exposure to each drink was investigated using specially prepared samples of extracted,

# human buccal/palatal enamel.'

Table 1 gives details of the drinks studied that also included positive (Tropicana smooth orange juice) and negative (Volvic still mineral water) control drinks, as well as a homemade fruit smoothie made using the Innocent strawberries and bananas fruit recipe as a guide, sourced from the carton label of the commercially available drink with the quantities of fruit used converted to grammes to ensure reproducibility. Five versions of this homemade strawberry and banana smoothie were made for testing that omitted certain key ingredients: strawberry omitted; orange and lime omitted; banana omitted; apple omitted; grape omitted.

For each homemade smoothie all ingredients were placed within the jug of a commercially available smoothie maker and blended for 120 seconds.

#### Titratable acidity and initial pH measurement

For each fruit smoothie and the positive control drink, five 100 ml samples were titrated to a pH of 7.0 using 0.1M sodium hydroxide (NaOH) while being stirred constantly with a magnetic stirrer set at a uniform rate. The initial pH and the change in pH by adding increments of 0.1M NaOH were recorded using a calibrated temperature compensated pH electrode. The mean initial pH reading and volume of 0.1M NaOH required to raise this to pH 7.0 was recorded. The mean and standard deviation of these values were calculated for all the drinks. The mean titratable acidity values were also expressed as the standardised titratable acidity (STA). This is the mean volume of 0.1M NaOH required to neutralise one litre of drink.

#### Effect of exposure to the drinks upon surface microhardness and contour of tooth samples

The effect of a 60 minute exposure to each drink was investigated using specially prepared samples of extracted, human buccal/palatal enamel. Their fluoride history was unknown. In total 40 teeth were used in this study. In preparation their roots were removed and the resultant crown sectioned longitudinally to leave buccal and palatal halves. Each half was mounted with their buccal/palatal face outermost in epoxy resin mixed according to the manufacturer's instructions. Once the resin was set this surface was finished flush with the surrounding mounting epoxy resin using a PM5 precision lapping and polishing machine and a slurry of calcined aluminium oxide powder with a particle size of 9  $\mu$ m, for subsequent exposure to the test drinks (five teeth per drink).

Before the commencement of any experimental work, the surface microhardness and baseline surface profiles of all specimens were determined, following marking the specimens so that a 2 mm field of measurement was reproducibly identifiable. To achieve this, four indentations were made in the mounting epoxy resin. When joined by two parallel lines 2 mm apart a zone of measurement was defined. Both surface hardness and profile measurements were made at the centre of this and across it.

Following baseline measurement adhesive masking tape was applied to the tooth specimens to mask out their surfaces other



than a 2 mm strip as detailed by the reference indentations. Following immersion in the drinks, the tape was removed and measurement of the surface microhardness and profile was again undertaken. Before and following immersion all specimens were stored at 37 °C in distilled water to prevent desiccation of the tooth specimens.

#### RESULTS

The majority of the drinks investigated had a baseline pH below the critical pH of enamel (5.5) and required comparable volumes of 0.1 M NaOH to raise their pH to neutrality as the positive control. Only two drinks (Volvic still mineral water, the negative control, and the yoghurt, vanilla bean and honey 'thickie') displayed a higher pH, though to neutralise the thickie, a lesser quantity of alkali addition was required. The immersion of the tooth samples in the drinks brought about reductions in their surface hardness but these were only significant (p <0.001) for the cranberry, blueberry and cherry fruit smoothie and homemade strawberry and banana fruit smoothie. There was no reduction in surface hardness in the case of the teeth immersed in the thickie. Omission of certain ingredients from the homemade smoothie affected the magnitude of surface hardness reductions seen. With regard to the loss of surface contour of the tooth samples following immersion in the drinks, as assessed by depth loss, there were significant differences between the drinks (p = 0.0064) with the thickie and negative control not causing depth loss and the kiwi, apple and lime smoothie producing most depth loss (28.26 [5.45] µm).

#### DISCUSSION

In this work three commonly used laboratory tests were used to investigate the potential of the drinks to bring about dental erosion in vitro. All the methods used in this study have the limitation that they cannot replicate exactly the conditions encountered in the oral environment but they do enable one variable at a time to be studied under carefully controlled conditions and their performance compared to control drinks. As no human subjects are directly involved in drink consumption there is minimal risk and so longer exposure times can be used than would be encountered in vivo. In this context it should noted that tooth substance loss in such tests is considered to be ten fold greater than would occur intra-orally.8

The commercial smoothies investigated in this study were selected as they represented world brand leaders whose constituents were readily declared. Their inclusion did not imply that they were considered by the researchers to be any better/worse than competitor beverages.

# Baseline pH and titratable acidity (STA)

The methods used to measure baseline pH and the titratable acidity were similar to that used by others.14-21 In the present study, however, 100 ml of drink was used due to the thickness of both the fruit smoothies and the yoghurt 'thickie' investigated. On a practical basis such a volume promoted the efficient mixing of drink and chemical reagents. In contrast, other beverage studies only used 20 ml of drink, perhaps due to the lower viscosities of the drinks under investigation.21 In the present work a non-heating magnetic stirrer, set at the highest stirring rate, was used to provide sufficient momentum to permit mixing due to the drinks' thick consistencies. As Shellis et al. found, stirring rate influences both the rate of erosion and rate of dissolution of tooth substance so this was kept constant in the work reported here.22

It is recommended that smoothies are stored in a fridge and so, when determining pH and titratable acidity, these tests were conducted as soon as practicable, upon removal of the drink from the fridge, which was at a temperature of 4 °C. Although the quantity of 0.1M NaOH required to bring about neutrality of the drinks is reported in this study, the standardised titratable acidity (STA) is also given, as advocated by Syed and Chadwick to permit ready inter study comparisons.<sup>23</sup>

Although much literature exists upon the pH and titratable acidity of single fruit juices no published work to date has examined combinations of fruit juices, such as those found in fruit smoothies, with one exception.21 Most work hitherto has focused on orange, apple, grapefruit and lemon juice and more exotic fruit juices such as blackcurrant, guava, apricot and grape.24-28 Although Blacker et al.20 investigated the pH and titratable acidity of smoothies it is difficult to compare the results with the present work as only one fruit smoothie is common to both studies: the shop-bought strawberry and banana fruit smoothie. In addition, testing was carried out in the previous study at room temperature. Therefore, comparison between the studies is not practicable. Increases in temperature have been demonstrated to increase acid dissociation with an erosive drink.27,29

In this work there were significant differences between the drinks tested in the present study in terms of baseline pH reading and titratable acidity. The variations observed are probably a reflection of the complex interplay of different acid constituents and certainly worthy of detailed future chemical analysis. It is interesting to note that all the smoothies examined had pH values less than 5.5 and titratable acidity values approaching that of the positive control drink. They thus had the capacity, on this basis,

to bring about dental erosion. In terms of pH the exception to the statement was the Innocent yoghurt, vanilla bean and honey 'thickie', which had a mean baseline pH reading of 5.70. On first examination this appears to be at odds with the work of Bamise and Bamise who investigated the acidic content of commercially available yoghurt drinks in Nigeria.<sup>30</sup> The yoghurt drinks which they investigated, however, were fruitbased and displayed baseline pHs that ranged from 3.51 to 4.12. Such differences could be accounted for by the fact that the Nigerian yoghurt drinks investigated contained fruit concentrate and stabilisers whereas the Innocent yoghurt, vanilla bean and honey 'thickie' investigated in the present study did not contain any fruit. Touyz found that products from fruits were always acidic whether they were fresh, juiced or dried.<sup>31</sup> The presence of fruit would therefore lower the pH and account for the apparent difference seen.

In terms of titratable acidity the yoghurt, vanilla bean and honey 'thickie' required the least amount of 0.1M NaOH to neutralise it compared with the other drinks. This could be accounted for by the fact that this drink did not contain any source of fruit and contained dairy products unlike the other drinks investigated. Other *in vitro* studies have demonstrated that the addition of calcium to both orange juice<sup>32</sup> and in the form of UHT milk to carbonated beverages<sup>23</sup> reduces titratable acidity, thus reducing the potential to bring about dental erosion.

Despite being of a similar recipe to the shopbought variety the homemade version of the strawberry and banana fruit smoothie required more than 0.1M NaOH to neutralise it. Various reasons could account for this finding that include the use of different fruit varieties, fruit at different stages of ripening and also possible heightened acid activity due to the relative freshness of the homemade smoothie. Grobler et al.25 found that the amount or proportion of acids found in fruit varied from fruit to fruit, between different varieties of the same fruit and fruit in different stages of ripeness. It should be pointed out that Innocent fruit smoothies do not contain any preservatives or stabilisers but are gently pasteurised, during which degradation of acids may occur.33

#### Surface hardness and profilometry

This work sought to assess the effects of immersion of prepared human tooth samples upon these parameters. In discussing this it is important to note that the fluoride history of the teeth used, by virtue of the anonymous nature of donation, was unknown. Fluoride incorporation into the apatite lattice has been shown to be protective against erosion.<sup>34</sup>

In relation to tooth sample preparation a precision lapping machine was used to flatten and polish the tooth samples tested in preparation for pre- and post-exposure profiling. In this process it is inevitable that the enamel tested by both profilometry and hardness determination was subsurface enamel and may also have included zones of dentine. Subsequent exposure of these samples to the drink may therefore have resulted in an artificially elevated measurement of tooth surface loss, as dentine is softer than enamel and more susceptible to softening upon acid exposure.

Of all the drinks investigated, the thickie showed the least reduction in tooth substance surface hardness. This finding could be explained by the presence of high calcium and phosphate ions in the yoghurt that prevent the dissolution of dental enamel by the law of mass action, therefore making it more resistant to indentation.<sup>23</sup> Similarly Gedalia *et al.* found acid softened enamel samples were rehardened after exposure to milk.<sup>36</sup>

Upon removing the acidic constituent of orange/lime juice from the homemade strawberry and banana fruit smoothie there was less reduction in surface microhardness than when these constituents remained. This, however, is at odds with the greater depth loss seen when orange/lime juice was removed from the drink.

With reference to the changes in surface contour seen of all the fruit smoothies investigated, the kiwi, apple and lime smoothie produced the most tooth substance loss at 28.26 µm after immersion for 60 minutes. This was nearly double the amount of tooth loss produced by the next erosive drink, which was fresh orange juice (positive control) at 15.39 µm. It is generally considered that in laboratory tests orange juice removes 4 µm per hour.24 The elimination of orange/lime juice from the homemade smoothie markedly reduced the depth loss seen. Although apples contain citric acid (3%), their major acid constituent is malic acid (95%)<sup>25</sup> and this, coupled with presence of citric acid from the lime and kiwi fruit, may account for the considerable reduction in surface hardness seen for the kiwi, apple and lime fruit smoothie. It should also be borne in mind that assessments of erosive potential should use a variety of tests to gain an overall assessment, for no single test has proven to be a reliable predictor of tooth tissue loss.35

Plain yoghurt, as used in the dairy-based smoothie tested in this work, is a low pH food<sup>34</sup> recognised as being non-erosive due to its calcium and phosphate constituents and buffer capacity. It was therefore not surprising to see that the Innocent yoghurt, vanilla bean and honey 'thickie' produced no tooth surface loss

# 'The ''thickie'' showed the least reduction in tooth

# substance surface hardness.'

or deterioration in surface microhardness. The increases in the amount of tooth structure and hardness seen could be the result of deposition of organic and mineral material upon the surface of the affected tooth samples.<sup>36</sup>

#### Conclusions

Although the 'thickie' drink was not dentally erosive in this study, it contains 29.89 g of fermentable carbohydrate per 250 ml bottle according to the manufacturers' data. Such a level of carbohydrate is classed as high (15 g per 100 g) and therefore regular consumption of such beverages, due to the risk of developing caries, is not recommended by some.<sup>37</sup> It may therefore not be wise to advocate this as an alternative type of 'safer' smoothie in a patient with dental erosion.

Various organisations and government bodies have advocated the consumption of fruit on a daily basis, citing fruit smoothies as a valid source.38,39 A recent survey showed 60% of parents gave their children fruit smoothies as they felt it was an easy way for them to consume their fruit portions.40 Recently the World Health Organisation (WHO) developed nutritional education guidelines to encourage the development of nutrition education in health promoting schools in Europe.<sup>41</sup> In the spirit of this the Scottish Government have issued guidelines on what can be consumed as a drink in school premises as part of their policy document Healthy eating in schools - a guide to implementing the nutritional requirements for food and drink in schools (Scotland) Regulations 2008.42 Permitted drinks include plain water (still or carbonated), milk drinks and drinking yoghurts, fruit juices and blends of these. In these regulations lunchtime consumption of fruit juice is limited to a portion size of no more than 200 ml. It is, however, known that other drinks are consumed in school hours and these are either brought in lunchboxes from home (71% of drinks consumed within school) or purchased at school (26% of drinks consumed within school)11 and their quantity and consumption is therefore outside of controlled mealtimes. It is therefore likely that some children may snack on fruit smoothies as they are perceived to be nutritionally healthy. If this is frequently carried out the results of this in vitro study, with its limitations, suggests they may be heightening their risk of developing dental erosion. Such a practice should therefore be discouraged and any claimed nutritional

benefits of smoothie consumption be reaped at mealtimes only.

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