DISSOLUTION OF HUMAN ENAMEL IN STILL FLAVOURED WATER DRINKS IN VITRO

ABSTRACT

AIM: This study aimed to investigate the erosive potential of these drinks using human enamel in vitro. **MATERIAL AND METHODS:** A range of bottled, still flavoured water drinks available in the UK were investigated and their erosive potential was compared by measuring pH and titratable acidity. Six beverages were chosen for the main study and also both a negative (distilled water) and positive control (orange juice). Human enamel specimens were prepared, sectioned and varnished leaving an exposure window visible to have contact with test solutions. Each specimen was randomly allocated in groups of six. Each group was exposed to 20 ml of one of the eight test solutions for 10, 30, 60 and 90 min. Quantitative light-induced fluorescence was used to ensure the teeth selected were free of artefacts and diseased areas. Erosion was measured using non-contact optical profilometry. **RESULTS:** Enamel loss occurred with all test drinks and the positive control ($p<0.05$) and in most cases the still water drinks were just as erosive as the positive control. Only vitamin water at 30 min was not significantly different from the negative control ($p= 0.86$), All drinks tested resulted in significant enamel loss ($p<0.01$). These results may indicate that consumers should think of still water beverages as potentially acidic drinks rather than just flavoured healthy water alternatives. **CONCLUSION:** This study indicates the need for preventive advice to be given by dentists about such beverages and therefore ultimately to make patients and consumers more aware of hidden erosive risks.

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KEYWORDS

Acid. Human enamel. Dental erosion. Flavoured beverages. Non-contact profilometry.

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Received 13 Jul 2014 **Received in revised form** 18 Jul 2014 **Accepted** 24 Jul 2014

INTRODUCTION

Dental erosion is defined as the pathogenic, chemical dissolution of the dental hard tissues by acids, without the involvement of bacteria[1]. Interest in dental erosion has increased in recent years and may be related to an increase in prevalence[2-4].

The diet has been the most extensively studied etiologic variable in erosion[5-8] and is considered to be the most important extrinsic factor[9].

Several studies have shown that consumption of soft drinks can lead to erosion of the dental hard tissues[8,10,11]. It has been proposed that the erosive potential of a soft drink depends on its chemical parameters and models have been developed in which the in vitro erosive potential of a product can be predicted from its titratable acidity, pH, and calcium, phosphate and fluoride concentrations[5].

With erosion becoming more of a concern among dentists and dental researchers[3], there has been increasing attention paid to healthier alternatives. Whilst the consumption of bottled natural mineral water is unlikely to contribute to erosion, the role of flavoured water drinks is still unknown and there have been few studies in this area. Diet is the most extensively researched factor and acidic foods and drinks have been implicated based on clinical and in vitro studies[8-12]. A wide variety of soft drinks are available on the UK market today, the main categories and market share are carbonates 42%, dilutable drinks 23%, bottled waters 16%, fruit juices 10% and non-carbonated (still) and juice drinks 9%[13]. Sales of soft drinks over the last twenty years have increased by 56%. It has been estimated that sales keep rising at about 2-3% a year[13-14]. The data supplied by soft drink manufacturers showed a seven-fold increase in consumption between 1950 and 1990 and that intake is much higher in younger age groups[15].

Consumers are always looking for new tastes and many new breeds of flavoured water products have emerged over the past few years. Many are sold as a healthy alternative to carbonated drinks. In the UK alone sales of flavoured water rose from 247 million litres in 2000 to 435 million litres in 2007 according to the British Soft Drinks Association. Also bottled water becomes a soft drink as soon as you add colours, flavours or sweeteners[13]. This is why flavoured water is sold as a water drink or spring water soft drink. Also the addition of vitamins and other health benefits can make it seem even more attractive to some people.

One recent study looked at the erosive potential of flavoured sparkling water drinks[16]. In this study they used in vitro dissolution assays with hydroxyapatite powder and viewed the results under an electron microscope. All of the waters demonstrated erosive potential. They concluded that sparkling waters should be considered as potentially erosive. However this study did not use relevant erosion times, as 30 min used seems a bit excessive of the actual time spent in the erosive beverage. Also hydroxyapatite powder was used instead of more relevant substrate such as human enamel or dentine.

One study looked at mineral waters and soft drinks in relation to erosion[17]. Using *in vitro* dissolution assays with hydroxyapatite and assays of extracted human teeth they found very low dissolution levels and even undetectable dissolution for several of the drinks studied. They concluded that mineral water appears to be a safe alternative.

Another study looked at the buffering capacities of soft drinks, this included sparkling waters and mineral waters[18]. They measured pH and titratable acidities of many soft drinks and waters and ranked them from most likely to cause a prolonged drop in oral pH as fruit juices, fruit-based carbonated drinks and flavoured mineral waters, non-fruit-based carbonated drinks, sparkling mineral waters and finally the least still mineral waters. Another recent study evaluated the effect of carbonated and non-carbonated beverages, bottled and tap water, on the erosive potential of dental enamel with and without fluoride varnish protection[19]. They used surface roughness (profilometry) as an erosive measurement at baseline and after 14 days of immersion in the beverage.

There have been very few studies in this area considering the current increasing market for flavoured water drinks. Indeed the range of flavours and brands available now show the demand for this type of beverage is growing. The wide range of flavoured waters needs to be investigated and their chemical parameters analysed to check erosive potentials.

With increased awareness among consumers about acid erosion, manufacturers are under pressure to provide research concerning the erosive potential of their products. The aim of this study was to investigate a range of flavoured still waters available on the UK market and measuring their initial pH, titratable acidity (TA) and also the erosive loss of enamel in vitro, following exposure to each test still water drink over a given time.

MATERIAL AND METHODS

PRODUCTS TESTED:

A range of 24 beverages available on the UK market were investigated, shown in Table 1. The pH and titratable acidity (TA) were measured for each beverage. 20 ml of each beverage was used in each titration and pH measurement. This was performed by adding increasing quantities of 0.1 M NaOH sodium hydroxide solution followed by agitation and equilibration (2 min) and measuring using a calibrated pH meter electrode (Jenway 3305, Bibby Scientic Ltd). Titration and pH measurement were repeated three times and a mean measurement taken.

SPECIMEN PREPARATION:

Previously extracted, caries free human adult molars sourced in the UK were used as the enamel substrate within the study. In each case the crown was sectioned from the roots using a dental drill and attached dental diamond disc (Densply International, Australia Pty Ltd). Each tooth was then gently abraded with wet and dry carbide paper and then pumiced. Each was examined under visual and Quantitative Light Induced Fluoresence (QLF, version 2.00c; Inspektor Research Systems, Amsterdam) conditions to ensure that the buccal and lingual surfaces were free from caries, extraction artefacts, restorations and fractures. The crown was then sectioned into smaller specimens using a diamond disc attached to a dental straight hand piece (n = 48). Each specimen was then varnished with a clear acid resistant nail varnish (Maxfactor-Infinity®) and allowed to dry for 24 h. An exposure window of around 5 mm x 5 mm was left. The specimens were then embedded in green-stick wax impression compound (Kerr, CA, USA) with the exposure window facing outwards. Random number tables were used to assign each sample to a category. Specimens were numbered consecutively and then allocated to each group.

TEST GROUPS:

Six different brands of still flavoured water drinks were selected from the original 24 still waters based on their variation in pH and TA: Perfectly Clear - Summer Fruits (PC); Vitamin Water Fruit Punch – Purple (VW); Marks & Spencer Peach and Raspberry (PR); Volvic Water - Touch of Strawberry (VS); Skinny Water (SW); CoolWater - Trim Cranberry (CC).

CONTROL GROUPS:

Orange Juice (Tesco Value) as a positive control (OJ); De-ionised water as a negative control (DW).

EROSION MEASUREMENT:

Eroded wear depths were quantified using a 3D non-contact optical laser profilometry (NCLP) (Proscan 2000, Scantron, Taunton, UK). Baseline measurements were taken for each specimen to evaluate the flatness of the polished enamel surfaces. After each erosion cycle, scans of 5 x 3 mm were obtained. For each specimen the depth of erosion was calculated by subtracting an eroded area from the unexposed area, this was cumulative. A mean value was then calculated.

EROSION OF SPECIMENS:

For each test solution six specimens were attached to lengths of cotton thread and suspended equidistant from the sides in a beaker. Samples were then immersed in 20 ml of test solution for 10 min, 30 min, 60 min, and 90 min, under gentle agitation (100 rpm) at room temperature. After the allotted time, the specimens were removed and rinsed thoroughly with distilled water. The specimens were then allowed to dry in air for 30 min before scanning with the NCLP.

STATISTICAL ANALYSIS:

The results were analysed using SPSS plus 15.0 for Windows. The significance level for all tests was set at 0.05. A one-way ANOVA was undertaken of erosion data after 90 min total erosion time, followed by a Multiple Range Test at a 95% confidence level to test for significant differences in the severity of erosion between test groups.

RESULTS

Table 1 shows the mean pH and TA values of all 24 still water drinks tested. Table 2 shows the pH and TA of the 6 test groups and the positive control.

Coolwater Trim Cranberry (CC) had the lowest pH 2.63, whilst Vitamin Water (VW) had the highest at pH 3.60. CC also required the highest amount of NaOH to be neutralised at 14.55 ml. In comparison the positive control Tesco Orange Juice (OJ) needed 27.33ml.

In Figure 1, 10 min erosion cycles as shown over a 90 min period. Significant enamel loss occurred with all test drinks and the positive control ($p<0.05$), only VW at 30 min was not significantly different than the negative control ($p=0.89$). In the first 10 min the negative control distilled water (DW) was significantly less erosive than any of the other drinks ($p=0.001$). VW is the next least erosive being significantly less erosive than OJ, Skinny water (SW), Perfectly Clear (PC) and CC, with the latter having the most enamel loss but it was not significantly different from OJ or PC.

After 30 min of erosion the DW was still significantly lower than the other drinks except VW ($p=0.23$). All the other drinks were just as erosive as OJ and as each other at 30 min. The highest erosion was seen for OJ but this was not significant by more than the others ($p=0.07$).

After 60 min the DW was significantly less erosive than all the drinks. Again CC had the highest loss of enamel but this was not significantly different than OJ, PC or Volvic Strawberry (VS). M & S peach and raspberry (PR) and SW were less erosive then OJ and CC. VW was significantly less erosive than the other drinks, but was significantly more erosive than the distilled water ($p=0.02$).

After 90 min the DW was significantly less erosive than the other drinks ($p=0.01$). OJ and CC had the highest loss of enamel but this was not significantly different than SW, PC or VS.

The amount of enamel loss differed significantly for all six drinks between baseline and 90 min (p=0.02).

Table 1. Mean pH and titratable acidity of soft drinks tested.

Drink Flavour 20ml	pH Mean	SD	TA (ml) Mean	SD
Robinsons Fruit Shoot Blackcurrant	2.97	0.01	10.07	2.10
Cool Water Orange /Peach	3.37	0.02	4.46	2.37
Vitamin Water Fruit Punch Purple	3.60	0.01	5.56	2.54
Robinsons Fruit Shoot H20 Orange	2.92	0.02	9.95	2.05
Drench Orange / Passion Fruit	3.14	0.02	11.4	2.21
This Water Lemon and Lime	2.76	0.01	20.96	1.95
Vitamin Water Raspberry / Apple	3.21	0.01	3.88	2.26
Asda Mango and Passion fruit	3.09	0.01	4.85	2.18
Volvic Touch of Strawberry	2.98	0.00	4.18	2.11
Perfect Clear Red Apple	3.17	0.01	5.98	2.23
Sainsburys Raspberry / Cranberry	2.87	0.01	4.53	2.02
Shapers Boots Raspberry / Mango	3.05	0.01	5.85	2.15
V Water Vitamin Lemon / Lime	2.89	0.01	4.08	2.04
Volvic Touch of Lemon / Lime	2.79	0.02	7.34	1.96
Skinny Water	3.33	0.02	5.81	2.34
M and S Peach / Raspberry	3.46	0.01	5.41	2.44
Volvic Orange / Peach	2.84	0.01	4.91	2.00
M and S Blueberry / Pomegranate*	2.97	0.01	19.13	2.09
Tesco H20 Lemon / Lime	2.74	0.01	7.32	1.93
Shapers Boots Orange Juice Drink	3.11	0.01	11.45	2.20
Tropicana Orange Juice	3.96	0.01	22.46	2.79
Tesco Orange Juice	3.88	0.01	27.34	2.74
White grape and Blackberry *	2.93	0.01	16.9	2.07
Pennine Spring Still Orange / peach	2.71	0.01	7.82	1.91
V Water Green Tea	2.66	0.01	3.38	1.88
Perfect Clear Summer Fruits	3.14	0.02	6.02	2.21
CoolWater - Trim Cranberry	2.63	0.02	14.55	1.85

* carbonated drinks

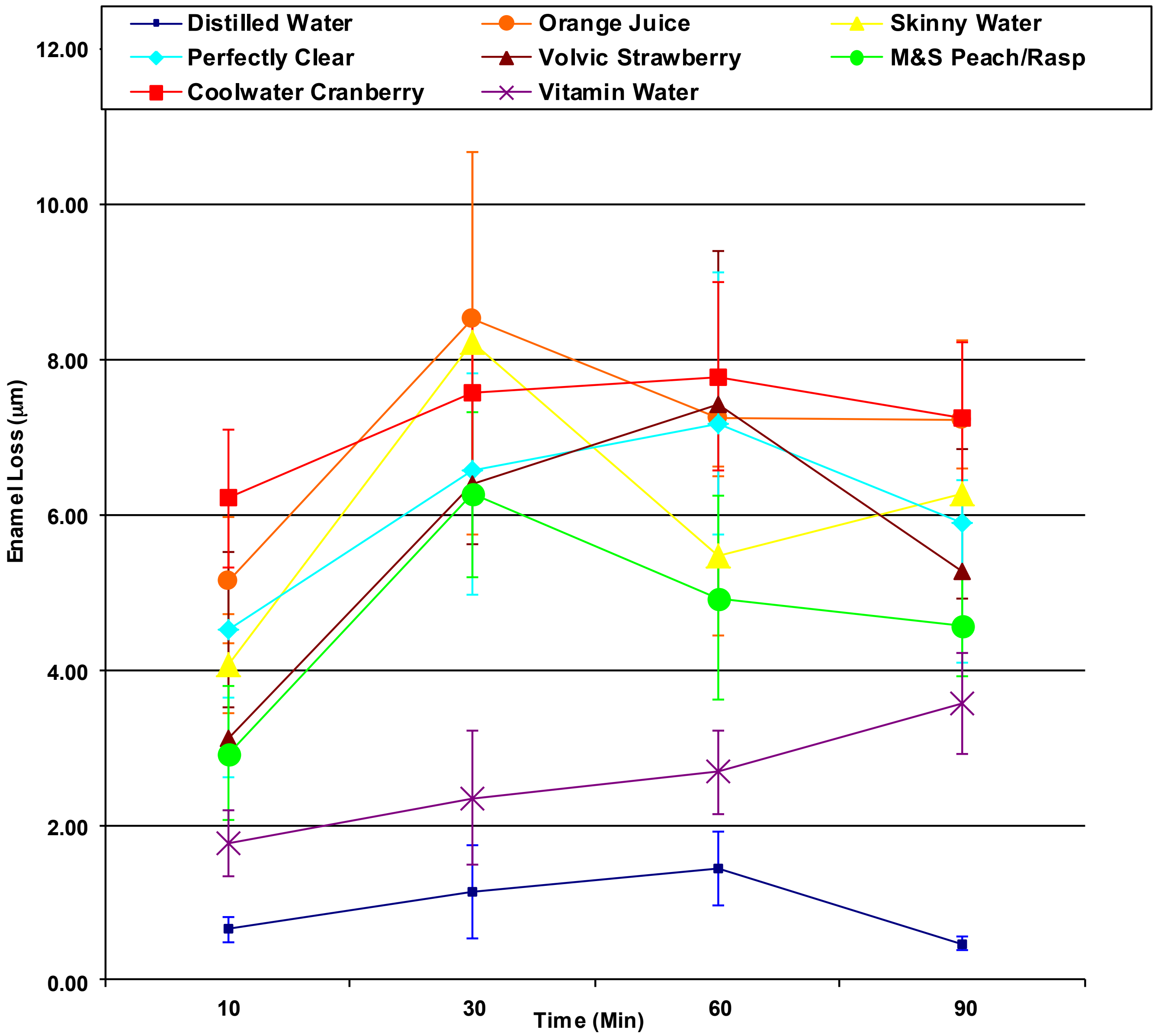
DISCUSSION

New breeds of flavoured water beverages have emerged over the past few years and are being sold as healthy appealing alternatives to carbonated soft drinks. The still, flavoured water drinks selected for this study displayed a wide range of pH value and TA. All of the drinks investigated had a baseline pH below the critical pH of enamel 5.5[20] and therefore required comparable volumes of 0.1 M NaOH to raise their pH to neutrality. CC was the most erosive out of the test drinks having the lowest pH and highest TA, the two intermediate drinks had mid-range pH and TA values, and the two least erosive drinks had the highest pH values and lowest TA. The positive control OJ had the highest pH but also had a high TA. pH and TA are important in determining the severity of erosion. However in this study the degree of erosion could be correlated to the pH, and to a lesser degree, TA.

Table 2: Mean pH and titratable acidity of control and test groups.

	Test Drinks and Positive Control	pH Mean	SD	TA (ml) Mean	SD
1	Perfect Clear Summer Fruits	3.14	0.02	6.02	2.21
2	Vitamin Water Fruit Punch – Purple	3.60	0.01	5.56	2.54
3	Marks & Spencer Peach /Raspberry	3.46	0.01	5.41	2.44
4	Volvic Water - Touch of Strawberry	2.98	0.00	4.18	2.11
5	Skinny Water	3.33	0.02	5.81	2.34
6	CoolWater - Trim Cranberry	2.63	0.02	14.55	1.85
Control	Tesco Orange Juice – Positive Control	3.88	0.01	27.34	2.74

Figure 1. The loss of enamel for specimens exposed to the selected soft water drinks for 10, 30, 60, and 90 min.



It was found that all the still water drinks caused significantly more erosion than the negative control. Only VW at 30 min was not significantly different than the negative control (DW). In most cases the amount of enamel loss was the same as the positive control.

The CC, SW, PC and VS were the most erosive drinks, being just as erosive as the positive control OJ. PR was the least erosive but in most cases. VW was significantly less erosive in all cases and was still as erosive as most of the other drinks.

The results of this study are in agreement with other studies investigating flavoured waters[16,18], where enamel surface changes consistent with erosive dissolution are demonstrated when exposed to such products. However studies in this area are few, most concentrate of soft drink beverages such as fruit juices, carbonated products and sports drinks[19, 21, 22].

The erosive measurements were cumulative and eventually there was a levelling off before being sectioned teeth were checked under QLF for any diseased or irregular areas that could cause variation in the results. This method allows for the early detection of demineralisation of enamel. It measures a loss in auto-fluorescence of enamel in the presence of demineralisation. QLF may have an important role to play in erosion due to its sensitivity to mineral changes and being non-invasive[23].

There is some biological variation of fluoride content of the enamel between teeth of different individuals and even areas of the same tooth. Fluoride incorporation into the apatite lattice has been shown to be protective against erosion[24]. For our sampling this was controlled to some degree by randomly allocating specimens to different test solutions. The polished enamel specimens used in this study present the worst cause scenario as the natural surface layer has larger crystallites, higher carbonate and fluoride concentration and therefore more resistant to attack. Once this amorphous layer is removed by polishing it exposes the more readily demineralised subsurface layers[25].

Saliva is known to have many properties that can serve a protective function against dental erosion such as dilution, clearance, neutralisation and buffering of dietary acids. The formation of the acquired pellicle by the adsorption of salivary proteins and glycoproteins, protects the enamel surface[26]. The fact that no salivary pellicle layer was present would alter the erosive effects, as in the natural oral environment this offers protective effects and therefore would be more rapid than erosion *in vivo.*

All the solutions used in this study were all at room temperature when substrates were exposed to them. Erosion of the teeth by soft drinks is likely to be influenced by the temperature of the beverages; therefore it would be better to control the temperature to ensure more accurate dissolution measurements. Eisenburger and Addy[27] (2003) concluded that erosion depth increase significantly with acid temperature. Therefore given the higher temperature of the oral cavity there may be expected to be more dissolution occurring *in vivo.*

One of the limitations of the model presented in this study is the fact that *in vivo* the erosive drinks are normally only briefly in contact with the teeth and saliva is continuously present. The opportunity for any remineralisation or continuous buffering would have been limited. Also oral clearance was also not simulated and thus these studies would have lead to higher erosion than in the *in vivo* situation.

The use of non-contact light profilometry was an advantage over other methods as there was no alteration of the samples surface. It is non-invasive and suitable for the measurement of fragile softened layers of enamel[28].

Conditions such as relevant exposure times, temperature, human enamel substrate and stirring rate allowed the model used to represent the best possible parameters that the time for this study allowed.

This study offers a good screening method as there are many erosive still waters are on the UK market. This study offered a wide range from well known water companies, to new designer waters to supermarket brands. Whilst providing useful information, future studies should now be conducted using an *in situ* model, where effects of saliva, pellicle and other conditions found in the mouth could be incorporated.

CONCLUSION

It was found that *in vitro* exposure of human enamel specimens to flavoured still water drink beverages resulted in surface loss of enamel consistent with erosive dissolution. These types of beverages should be considered to have an erosive challenge similar to orange juice.

These investigations may lead to a better understanding of the erosive potential and also make the public more aware about them being potentially acidic rather than simply flavoured waters.

Preventive advice on their consumption should be provided by dentists who can advise patients on the relative erosive potential of such drinks. Useful information from such studies could be given to manufacturers for improvement of their products to provide less erosive beverages.

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