Erosive effect of sports drinks on tooth enamel

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Abbreviations:
EDTA: Ethylene diamine tetra acetic acid, KOH: Potassium hydroxide, NaOH: Sodium hydroxide, TA: Titratable acidity, SD: Sports drinks

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Hydroxyapatite, demineralisation, dental erosion, remineralisation, supersaturation, titratable acidity

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1. Introduction

1.1 Dental erosion
The enamel is the hardest tissue of the body and is about 97% mineralised with calcium hydroxyapatite. On exposure to acids such tissue erosions and this loss is an irreversible process. Eroded tooth surfaces brought about by exposure to the low pH of dietary products is rather common. The prevalence of dental erosion is on the increase and has become a significant clinical problem in many countries. Frequent consumption of soft drinks and carbonated cola drinks showed strong association with erosion of the tooth surface and the low acidity of these drink was recognised as one of the main contributing factors ((Jendottir et al., 2005). Dental erosion is the pathological loss of tooth surface due to a chemical process that has no involvement of microorganisms and is therefore, characterised as non carious (Cairns et al., 2002; Story, 2006). The aetiology of dental erosion is complex and multi-factorial, related to a wide range of extrinsic or intrinsic factors (Coombes, 2005; Milosevic, 1997). Based on

Abstract
SD was introduced for sports performance and endurance. SDs is made fizzy and marketed in various choices for consumers. Strong association between acidic fizzy drinks with erosion of mineralised tissue such as the enamel surface has been reported. Comparative to soft drinks, the erosive effect of SD on tooth enamel was reported of less significant. The study aimed to evaluate the erosive activity of five SDs on tooth enamel. Data on the pH, titratable acidity, and demineralisation rate and weight loss were determined. These, together with data on their calcium content were used to evaluate the erosive potential of the SDs. Although found to be acidic at pH 3.0 to 3.5, all SDs exhibited low erosive activity. This may have been due to the presence of calcium ions in the drinks. Addition of such ions into SD supersaturates the drinks with respect to tooth minerals, thus reducing their erosive effect and enamel loss following periodic exposure to the SDs. An acidic drink may not be erosive if its content of neutralisable acid or TA is low. It is also concluded that SD modified with added ions such as calcium tend to exhibit reduced erosive effect on tooth enamel.

Citation:
earlier studies on enamel erosion, pH or acidity was thought to be an accurate indicator of the erosive potential of a food or drink. Many later studies instead have suggested that total titratable acidity is a more accurate indicator as it gives a measure of the total acid content of a drink, which may therefore be a more realistic means of predicting erosive potential (Cairns et al., 2002; Jendottir et al., 2005).

2. Review of Literature

2.1 Sports drinks
SD or popularly commercialised as isotonic drinks dominates the total volume and value sales of functional drinks in many countries. The demand for SD is on the high scale as they are not only consumed by those who are involved in physical indoor and outdoor activities, but also preferred in official meetings and social events. The change in the life style of the modern urban societies also contributes to the tremendous rise in SD consumption. When isotonic drink first made its name in the market, its role was for better sports performance and endurance for athletes. Various types of SD are commercially manufactured and made available for the choice of consumers with some slight differences in their compositions. This explains for the different erosive potential of different brand of drinks.

2.2 Erosive potential
Many studies have been carried out to study the relationship between acidity and dental erosion (Coombes, 2005; Fathilah and Rahim, 2008; Hara and Zero, 2008). Carbonated drinks, often with acidity in the range of pH 3-5 have been shown to cause great mineral loss from dental hard tissues. However, comparative to acidic fruit juices which are also within similar pH range, the erosive potential of carbonated drinks is often more severe. SD on the other hand is specially formulated carbohydrate-electrolyte products with added ingredients such as calcium, phosphates and fluorides (Shirreffs, 2009). Although sports drinks are different from juices and carbonated beverages when based on its composition, these drinks however are acidic with pH of similar range with the others (Jensdottir et al., 2004).

3. Objective of Research
The aim of this in vitro study was to determine and compare the erosive effect of five types of SD on enamel surfaces of tooth specimens.

4. Materials and Methods
The erosive effect of the SD was based on the amount of weight loss and the rate of calcium released from the enamel surface following exposure to the drinks. Characterisation of the drinks which include pH, titratable acidity and calcium content were also carried out to relate and evaluate the erosive potential and buffering capacity of the drinks.

4.1 Preparation of enamel specimens
Non-curious permanent teeth were selected and sterilised in an autoclave prior to use in the experiments. The enamel surface of the teeth were cleaned and then the whole tooth was covered with nail varnish leaving windows of exposed enamel surface with an area of about 30 mm². The sites for the windows were carefully chosen to be at surfaces with the least surface contours (Figure 1.1). Once ready the specimens were divided into six groups, each with three exposed enamel windows. These subgroups were then used in experiments involving each type of SD. The groups were designated as Group I (Revive™), Group II (Excel™), Group III (100plus™), Group IV (Isotonic H2O), Group V (Gatorade™) and Group VI (Mineral water). Calcium and phosphate were listed as amongst the constituents in all the SD. The design of the enamel window was to standardize the surface area of enamel exposed to the SD during the experiments. Mineral water served as a negative control for the study.

Figure 1.1: A tooth specimen showing an exposed enamel window of the size 30 mm²
4.2 Characterisation of sports drinks

4.2.1 Measurement of pH

50 ml of Revive™, Excel™, 100plus™, Isotonic H₂O™, Gatorade™, Mineral water and deionised water were placed into separate clean beakers. The pH of the beverages was then measured using a calibrated pH electrode (Hanna Instruments). The readings were taken three times to ensure reproducibility of results.

4.2.2 Determination of titratable acidity (TA)

50 ml of Revive™ was placed in a clean beaker and in small increments of 20 µl, 0.5 M of sodium hydroxide (NaOH) were added to the drink. Changes in the pH of the drink were monitored and the volume of NaOH required to raise the drink to the pH of 5.5, 7.0 and 10.0 was recorded. The volume of NaOH added was then plotted against pH. Similar steps were also carried out on all the other SD. The TA of each drink was then compared using the Kruskal-Wallis test. The determination of TA was repeated three times on different days to ensure reproducibility of readings.

4.2.3 Determination of calcium content

The content of free calcium ions in the SD was determined using the titration method (Fathilah and Rahim, 2008). 200 µl of the respective drinks was added to a clean beaker containing 1 ml of potassium hydroxide (1.25 N KOH) and 100 µl of calcon, a dye used in the experiment to indicate the end point of the titration. The content was then titrated with ethylene diamine tetra acetic acid (EDTA) (1 %) until the end point which was the appearance of a blue colouration was reached. The volume of EDTA used to produce the end point was recorded. Similar procedure was also carried out on a standard control which contains a known amount of calcium chloride (CaCl₂) (0.1 mg/ml). EDTA is a chelating that complexes with calcium ions. Based on the volume of EDTA used to complex with a known amount of calcium (standard control), the concentration of calcium present in the drinks was calculated.

4.3 Assessment of mineral loss due to sports drinks

4.3.1 Determination of the rate of calcium released

The specimens in Group I through Group VI were immersed in a beaker containing 50 ml of the respective SD. 200 µl of the drinks was pipette out after 5 min and the concentration of calcium in the drinks was determined (Fathilah and Rahim, 2008). The amount of calcium released from the enamel surface was calculated as the amount of calcium determined following exposure to the respective drinks minus the amount of calcium already presence in the drinks.

4.3.2 Determination of weight loss

The method described by Story was employed in this section with slight modification (Story, 2006). The initial weight (w₀) of the tooth specimens in each study groups (Group I-VI) was recorded. The specimens were then immersed in 25 ml of the different type of SD, with constant stirring. After 30 min, the specimens were removed, washed with distilled water and left to dry in an oven at 30° C for 24 hrs. Following that the specimens were re-weighed and the weight loss due to the first exposure to the drinks was calculated. The whole process was repeated everyday over a period of 7-days using fresh sample of SD each time. Graphs of weight loss versus cycles of exposures to each drink were plotted and the erosion potential of the drinks was calculated and analysed.

4.4 Statistical analysis

Data obtained were non-parametric and was descriptively analysed using SPSS Statistical Program version 11.0. pH differences between SD were compared and analysed by Kruskal-Wallis Test. The level of significance was set at p < 0.05.

5. Justification of Research

Sports beverages are often regarded as safe and healthy by consumers as it tends to boost ones energy and replenishes lost electrolytes. Many are however not aware that most of these drinks are carbonated and very acidic. Being a mineralised biological tissue, the enamel begins to demineralise when a critical pH of 5.5 is reached and this gives detrimental effect on the enamel surface. Unlike soft drinks, sports beverages contain added electrolytes that supersaturate oral fluid and at the same time modify the TA of the drinks. The former thus, halt the leaching out of ions from the enamel structure while the later, minimises demineralisation by acids. In other words, an acidic SD may not be erosive if its content of neutralisable acid or TA is low. Results of this study may provide experimental data to support this claim.
6. Results

6.1 Characterisation of sports drinks

6.1.1 pH of sports drinks

Table 1.1 shows the pH of the respective sports drinks and mineral water. All SD were acidic in nature with Gatorade™ being significantly more acidic than the others with pH at 3.02±0.02 (p<0.05). The pH of mineral water was recorded at about neutral.

6.1.2 Titratable acidity

Comparatively all the drinks showed almost similar titratable acidity as the amount of NaOH required to reach pH 5.5 were within the range of (2.45 - 2.80) ml (Figure 1.2). For Excel™, Revive™, Isotonic H₂O™ and 100plus™ the amount of NaOH required to increase the pH of the drinks to pH 7.0 and pH 10.0 were also within the same range of (6.60 - 7.10) ml and (10.40 - 11.35) ml, respectively. Different from the others, the volume of NaOH required by Gatorade™ was significantly lower by about 40 % and 50% to reach pH 7.0 and pH 10.0, respectively (p<0.05). Based on the rapid response of the drinks to the addition of NaOH, it can be inferred that Gatorade™ has the lowest titratable acidity among all the SD under study.

Figure 1.2: Titratable acidity of SD. The amount (ml) of NaOH (0.5 M) required to increase the pH of SD to pH 5.5, pH 7.0 and pH 10. The readings were mean ± standard deviation from nine determinations (n=9)

6.1.3 Calcium content

Calcium was determined presence in all SD. At the lower end, equal concentration was determined for Revive™ and Gatorade™ at 25.00 µg/ml. At the higher end both Isotonic™ and 100Plus™ contains 80.00 and 83.33 µg/ml of calcium, respectively (Figure 1.3).

6.2 Assessment of mineral loss

6.2.1 The rate of calcium released from the enamel surface

Calcium ions were shown released from all SD except Excel™. The demineralisation rate due to 100Plus™ was the highest at 2.00 ± 0.02 µg/ml compared to the others (Table 1.2). Excel™ instead showed deposition of calcium ions. No released of calcium ions was however recorded following exposure of the enamel windows to Excel™ and Mineral water.

Figure 1.3: Calcium content of SD. The readings were the mean ± standard deviation from triplicate samples (n=3)

Table 1.1: The pH of SD recorded using a pH meter. The readings were the mean ± standard deviation (n=3)

<table>
<thead>
<tr>
<th>Beverages</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatorade™</td>
<td>3.02 ± 0.02</td>
</tr>
<tr>
<td>Excel™</td>
<td>3.23 ± 0.01</td>
</tr>
<tr>
<td>Revive™</td>
<td>3.37 ± 0.04</td>
</tr>
<tr>
<td>Isotonic H₂O™</td>
<td>3.43 ± 0.03</td>
</tr>
<tr>
<td>100plus™</td>
<td>3.45 ± 0.02</td>
</tr>
<tr>
<td>Mineral Water</td>
<td>7.33 ± 0.06</td>
</tr>
</tbody>
</table>

6.2.2 The loss of mineral content from enamel surface

Figure 1.4 demonstrated changes in the weight of tooth specimens after repeated immersions in the respective SD over a period of 7 days. An increased in weight from Day-0 was observed in all tooth specimens following the first immersion to the SD. Specimens exposed to 100Plus™ showed the highest weight gained while Gatorade™ showed the least. From Day-2 and Day-3 onwards, the
weight of the specimens decreased back to the initial weight. At the end of Day-7, very minor weight loss was determined only for specimens immersed in Isotonic H$_2$O$^{TM}$ (3.63 ± 0.12) µg, Excel$^{TM}$ (0.75 ± 0.08 µg) and Gatorade$^{TM}$ (0.29 ± 0.14) µg. Mineral water, Revive$^{TM}$ and 100Plus$^{TM}$ exhibited some gain in weight instead (Figure 1.4).

Figure 1.4: Changes in the weight of tooth specimens following periodic exposures to SD over a 7-day period

Table 1.2: The rate of calcium released from enamel surface following a 5 min exposure of enamel windows to SD. The readings were mean ± standard deviation (n=6). Note: ND denotes non determinable

<table>
<thead>
<tr>
<th>Sports drinks</th>
<th>µg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revive$^{TM}$</td>
<td>0.67 ± 0.01</td>
</tr>
<tr>
<td>Excel$^{TM}$</td>
<td>ND</td>
</tr>
<tr>
<td>100Plus$^{TM}$</td>
<td>2.00 ± 0.02</td>
</tr>
<tr>
<td>Isotonic$^{TM}$</td>
<td>1.33 ± 0.02</td>
</tr>
<tr>
<td>Gatorade$^{TM}$</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>Mineral water</td>
<td>ND</td>
</tr>
</tbody>
</table>

7. Discussion

Exogenous acids coming from acidic food or beverages have been widely implicated in the aetiology of dental erosion (Attins et al., 2005; Lussi et al., 2002; Zero, 1996). Demineralisation of the enamel and tooth wear which softens the tooth surface and the region immediately beneath it were the common effects of frequent contact with these types of beverages (Hara and Zero, 2008; Lussi, 2009). Practically, the effect of acids on dental tissues can be minimised by reducing the intake of acidic foods and drinks, and modifying drinking habits (Moazzez et al., 2000). Studies have shown that acidic drinks modified with the addition of calcium, phosphates or fluoride was able to distinctly reduce the formation of erosive lesions in the enamel (Attins et al., 2003; Larsen and Nyvad, 1999; Lussi et al., 2004). In this in-vitro study the erosive potential of SD was assessed in the laboratory by measuring their pH, neutralisable acidity and their ability to erode the enamel. The calcium content of each SD was also determined as its presence has been suggested to play a role in buffering the erosive effect of acidic soft drinks (Attins et al., 2005).

All five SD tested were acidic with pH in the range of pH 3.0 - 3.5, a range almost comparable to that reported for Coca-Cola$^{TM}$ and orange juice by Jensdottir et al. (2004). In both studies, Gatorade$^{TM}$ was found to be the most acidic (Table 1.1). pH however only measures the initial hydrogen ion concentration in the drinks and do not provide a good indication of the presence of undissociated acid (Cairns et al., 2002; Hara and Zero, 2008). TA is known to give a more realistic measure of the buffering capacity of drinks as the amount of alkali required to bring the pH to a chosen value (pH 5.5, pH 7.0 and pH 10.0) can be quantified. The method used in this study to determine TA has been employed in a number of studies (Cairns et al., 2002; Jensdottir et al., 2006). Gatorade$^{TM}$ showed the lowest TA despite being the most acidic SD (Figure 1.2). Principally, pH only gives an indication of the available H$^+$ in a drink, while TA is a measure of the total amount of neutralisable acids in the drink. The lower pH of Gatorade$^{TM}$ thus mean that the readily available H$^+$ in Gatorade$^{TM}$ is the least among all SD under study while having the lowest TA means that Gatorade$^{TM}$ is the fastest to be neutralised. Based on this argument it can be suggested that Gatorade$^{TM}$ compared to the other four SD would give the least erosive effect on enamel surface.

Many strategies have been proposed to reduce the erosive effect of drinks with low acidity. The addition of various different ions or complexes to erosive drinks showed promising results as the ions were found to supersaturate the drinks with respect to the tooth mineral (Lussi, 2009). In the oral environment, such condition may instead encourage remineralisation rather than demineralisation of the exposed enamel surface. Similar suggestion was also reported in a study involving Sprite Light$^{TM}$ which has added calcium ions. Rinsing the enamel surface with such modified drinks had led to significant reduction in enamel loss (Attins et al., 2005). All the SD under study showed the presence of calcium in the range of 0.75 - 83.33 µg/ml (Figure 3). Saturation of the SD by
these ions may explain for the slow release of calcium ions from the enamel surface following immersion in SD compared to carbonated drinks (Table 2). Calcium released after exposures to carbonated drinks was reported much higher in the scale of microgram per minute. Calcium released from Coke™ for example was at 0.76 µg/min (Fathilah and Rahim, 2008).

The methodology employed by Story (2006) was closely followed to measure the weight loss of tooth specimens due to enamel dissolution caused by the SD. However, comparative to the continuous weight loss measured after a 7-day periodic exposure to the Mountain Dew™ and Red Bull™, the effect observed on the SD in this study was different (Figure 1.4). Very minor weight loss was only observed after Day-1 for all the SD. After Day-2 this lost was instead gradually buffered to about the initial weight. The erosive effect of the SD most probably has been buffered by the added ions in the SD whereby at the end of Day-7, except for Isotonic H₂O which showed very minor weight loss, all the other SD more or less were back to the original weight (Figure 1.4), an indication of remineralisation instead of demineralisation.

Although the erosive potential of various drinks can be compared, it is not possible to define the degree to which any drink will damage teeth especially in the oral cavity where the buffering action of saliva is very effective in neutralising back the acidic pH to neutral since this will vary between individuals. However, buffering potential of saliva between individuals varies as there are many factors that can contribute to dental erosion.

Research Highlights

- Gatorade™, 100Plus™, Isotonic™, Revive™ and Excel™ are very acidic with pH below the critical pH of 5.5 which, at or below this pH demineralisation of the enamel will occur.
- Gatorade™, 100Plus™, Isotonic™, Revive™ and Excel™ contains added calcium ions that help saturates the drinks and buffer the erosive effect of acidic drinks.
- Gatorade™ is the most acidic among all five SD but it has the lowest TA, and thus would be the fastest to be neutralised.

Limitations

In any in vitro study such as this one, many of the variables can be standardised. In the oral cavity however, the presence of oral fluid such as saliva is expected to have significant influence on the erosive potential of SD. This is because the saliva is equipped with buffering components that would also help buffer the acidity of the SD. Hence, observations from an in situ experiment may provide better insight on the consequences of consuming SD on the enamel surface.

Recommendations

Added ions can also be in the form of fluoride and phosphates. The presence of these ions should also be determined as they may have role in buffering the leaching out of ions from the enamel surface.

Funding and Policy

Information on the positive and negative effects of consuming SD should be clearly conveyed to the public users. Oral health educators may play their role by reinforcing important practices such as decreasing the time that the drinks remain in the mouth and to use straws for drinking to potential SD users. It should also be stressed that unless immediate replenishing of electrolytes is required by the body, taking plain water instead would be enough to avoid dehydration.

Conclusion

In conclusion, there is no doubt that the acidic nature of sports drinks has the potential to cause erosion of the enamel surface with in vitro studies supporting this notion. Several properties related to sports drinks such as pH, titratable acidity and calcium content was found to have an impact on the erosive potential. However, added ion such as calcium in SD does have an effect in buffering and reducing the erosive effects of SD. Since SD are widely used by the general population in preference to carbonated beverages, oral health educators should be reinforcing important practices to SD users such as decreasing the time that the drinks remain in the mouth, using straws and avoiding dehydration by drinking plain mineral water.

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Author’s Contribution and Competing Interests

Fathilah AR - supervising the students, designing the research methodology, drafting and writing the manuscript.
Nur Salwa CHR - undergraduate student carrying out laboratory works collection and analysis of data.
Sheril Nur Aida R - undergraduate student carrying out the laboratory works collection and analysis of data.
Sharifah Norul Akmar SZ - Supervision on the analysis of statistical data and proof reading the manuscript.

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