The erosive potential of some alcopops using bovine enamel: an in vitro Study

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Summary

Objectives:

Alcoholic soft drinks have become increasingly popular and have high concentrations of citric acid and alcohol so might have the potential to cause dental erosion. This study aimed to investigate the erosive potential of alcopops on bovine enamel *in vitro*. ***Methods:*** Six bovine upper incisors were prepared and sectioned to give six slabs per tooth, 4x4mm each. Each slab was covered with nail varnish, leaving an exposed window (2x2mm). Samples were immersed in 20ml of each of the test solutions for 20min, 1h, and 24h under gentle agitation (100 rpm). Enamel surface loss was determined using Quantitative Laser Fluorescence (QLF), Non-Contact Profilometry (NCP) and Transverse Microradiography (TMR). ***Results*****:** Enamel loss occurred with all test drinks and the positive control ($p<0.05$), and the depth of lesion correlated with pH and time. No significant difference was observed between 20min and 1h exposure, although both times had significantly ($p<0.05$) greater erosion when compared with baseline. Within each alcopops group significant erosion had occurred at 24h exposure compared with the baseline and previous times. ***Conclusion*****:** All the tested alcopops resulted in significant enamel loss at 24h ($p<0.001$) with direct correlation between degree of enamel loss and both pH and increasing exposure time.

Introduction:

Over the past few years, tooth wear has significantly increased, and the contribution of dental erosion to tooth wear, particularly among children and young adults, has increased in prevalence.[1] Dental erosion is used to describe the 'irreversible loss of dental hard tissues due to acid intake without bacterial involvment'.[2] Intra-oral pH at the enamel surface drops rapidly following an acidic challenge and it comes back to resting pH levels only slowly.[3] As a result, pH remains at low levels over an extended period with frequent acidic drinks intake.[4] The occurrence of dental erosion has been continuously reported in the literature as case report/control studies.[5] The wider availability and the frequent consumption of such acid containing drinks might have led to the increased prevalence of dental erosion.[6] Such acids could be of extrinsic or intrinsic origin. Extrinsic factors mostly include acidic foodstuffs and beverages but the exposure to acidic contaminants can also increase the risk. Intrinsic sources are mainly caused by gastric acid reaching the oral cavity as a result of vomiting or gastro-oesophageal reflux.[7]

Since they were introduced in the market in1995 and the popularity of alcopops has increased and marketed directly at young females aged between 14-16 years. Because these drinks are based on fruits with high citric acid content, alcopops were found to have a highly erosive effect on dental hard tissues ($pH<4.0$).[8-10] More substantial erosion might also be expected because of the risk of vomiting induced by their alcohol content.[11]Therefore, numerous *in vitro* studies were performed in attempts to evaluate the erosive potential of such widely consumed drinks.[12]

The purpose of this study was to investigate the potential demineralisation of four different alcopops on bovine teeth *in vitro*, by assessing their initial pH, buffer capacity, fluoride content, and the amount of enamel lost following exposure to these drinks over a certain period of time.

Materials and methods:

Erosion protocol

Six permanent bovine incisor teeth were cleaned to remove contaminants, polished to create a flat surface, and each incisor was sectioned into six slabs using a diamond disc to give a total of 36 specimens. Each specimen was polished with a clear acid resistant nail varnish (Maxfactor-Infinity®) leaving a 4x 4mm window. Prior to creating lesions, specimens were immersed in freshly made artificial saliva (*Table1)* for two hours then allowed to bench dry for 30min. Specimens were then randomly allocated to each alcopop group. The slabs were attached to lengths of thread and suspended in beakers containing 20ml of each of the test drinks for 20min, 60min, and 24hours, under gentle agitation (100 rpm) at room temperature. Baseline measurements were taken for each sample and after each cycle using Quantitative Laser Fluorescence (QLF, version 2.00c; Inspektor Research Systems, Amsterdam), Non-Contact Profilometry (NCP, Proscan 2000, Scantron, Taunton, UK), and Transverse Microradiography (TMR). Detailed techniques are to be described below.

Test Groups:

Four different brands of alcopops were selected:

*Bacardi Breezer™ Orange Flavour. (BO)

*Smirnoff Ice™. (SI)

*Archers Schnapps Aqua ™ Peach Flavour. (AP)

*Bacardi Breezer™ half sugar Raspberry Flavour. (BHS)

* Orange juice (Tesco value) as a positive control. (OJ)

*De-ionised water as a negative control. (H_2O)

The pH of each drink was measured using a digital pH meter electrode (Jenway 3305, Bibby Scientific Ltd). The electrode was calibrated using a standard buffers pH 4.0 and 7.0. Ten millilitres of each freshly opened drink was placed in a beaker and stirred using magnetic stirrer, at room temperature. Three consecutive readings were taken after which a mean measurement was calculated.

Measuring the buffering capacity of each drink was carried out by placing 10 ml of each freshly opened drink in a glass beaker and titrating, using the pH electrode, to pH 7.0 by adding increments of 0.1M sodium hydroxide (NaOH).

The fluoride content was measured using a combination fluoride electrode probe (Orion Thermo).

The mean pH, buffer, and fluoride concentration values are all shown in *Table2*.

Erosion measurement:

Lesions were quantified and expressed based on each of the following devices' parameters:

- QLF; this was carried out by placing a patch around the erosion lesion as well as to the sound enamel. The percentage fluorescence loss of demineralised enamel (ΔF) with respect to surrounding sound enamel that relates directly to the amount of mineral lost during demineralisation was then calculated.
- NCP; step-height was systematically measured across the four mid-points of the sides of each slab to give four measures of step height (loss of depth). A mean value for the step height for each slab was then calculated.
- TMR; four sections, 250µm thickness each, were obtained from each slab using a water-cooled diamond wire (Well type 2400, Walter EBNER, Le Locle). The sections were then mounted on brass anvils with nail varnish, allowed to harden overnight, and ground planoparallel to approximately 80-100µm thick sections with a custom- made diamond disc. Sections were mounted on a microradiographic plate-holder (Kodak high-resolution plates), exposed to X-ray source for 35min. The plates were developed following the standard techniques. Lesion variables i.e. mineral loss (ΔZ) and maximum lesion depth (Ld) were quantified using TMR software (TMR 2000, V 2.0.27.16).

All images were stored and analysed by a single examiner and using each equipment software package.

Statistical analysis:

The results were analysed using SPSS 15.0 package. A p-value ($p<0.05$) was considered statistically significant. Continuous variables were expressed as the mean ± SD (Standard deviation of the mean). Levene's Test of Equality of Error Variances was used to identify statistically homogenous groups. One-way analysis of variance and Tamhane *post hoc* tests at a 95% confidence level were performed on the experimental groups to test for statistically significant difference in the severity of erosion between the test groups.

Results:

pH and Buffering capacity

Table 2 shows the mean pH values of the test groups. Archers Peach had the lowest pH 2.95, while Bacardi Orange was the least acidic at pH 3.63. While Smirnoff Ice required the highest amount of NaOH to be neutralised (23.26 ml), only 15.52 ml of NaOH was need for the Archers Peach to reach neutrality.

Fluoride Content

All different flavours of alcopops had more or less similar quantities of fluoride, ranging from 0.01- 0.0143ppm

Figure 1 shows the change in fluorescence (ΔF) occurring for each drink baseline and the 3 time periods. There was an increase in ΔF as the time of immersion in test groups increased. QLF revealed a loss of fluorescence for all specimens from baseline and as the time period extended reflecting an increase in mineral loss. Archers Peach had the greatest change in fluorescence (ΔF=50.65%), Bacardi Orange had the lowest change in fluorescence (ΔF=41.1%), whilst Smirnoff Ice and Orange Juice seemed to have the same effect on the enamel (ΔF=33.33%, 33.96%, respectively). Bacardi Raspberry showed a lesser tendency towards causing enamel erosion (ΔF=29.16%) and water was the least erosive (ΔF=5.26%) among all the test groups.

Mean step height differed significantly for all four drinks between baseline and 24hr ($p<0.001$) as measured by proscan. Other significant differences were observed between baseline/ 1hr, 20min/24hr, baseline/ 20min/ and 1hr/24hr but there was no significant difference between 20min and 1hr specimens (Fig. 2).

Archers Peach resulted in the most dissolution whilst Orange Juice, Bacardi Half Sugar and Smirnoff Ice beverages caused less mineral loss (ΔZ). Bacardi Orange had the least mineral loss (Fig. 3). Similar trends were observed with lesion depth analysis, except that Bacardi Half Sugar expressed more erosive tendency than Orange Juice. None of the trends observed were significant at the 5% level.

Discussion:

Alcopops have become a most appealing drink since their launch in the UK in 1995. Debate around their erosive potential continues.

Buffering capacity and pH remain the most important chemical parameters in determining the erosive potential of an acidic drink. It is well established that enamel dissolution occurs below the critical pH 5.5[13] but it has been reported that erosion could occur slowly at pH levels >8.0[14]. In accordance with results from previous studies[15,16] all examined alcopops caused considerable surface demineralisation. Rees et al (1998, 2000) investigated a wide range of alcopops. [9, 10] They reported greater acidity with a pH that ranged from 2.57-2.86 compared to the current values that ranged from 2.95-3.63 and similar buffering capacity. The erosive potential of some of the drinks (BO, SI, and AP) tested in the current study was previously investigated.[16] Although the respective pH (3.17, 3.2, 3.1) and buffering capacity (23.3, 23.8, 18.17), were similar to the pH and buffering capacity values found in this study, eroded lesions were not quantified.

Similar to previous studies, the relation between buffering capacity and mineral loss was weak. [13,17] The most and least erosive drinks (Archers Peach, Bacardi Orange), in terms of pH, had similar buffering capacity. On the other hand, Smirnoff Ice and Bacardi Raspberry showed approximately similar pH and buffering capacity, although the former had slightly lower pH (3.18) as opposed to 3.26.

It has been documented that high fluoride concentration is capable of reducing erosive wear.[18] In this study, the F^- content in the alcopops ranged from <0.0125 – 0.011ppm. Even at this low concentration a 10% protection against erosion is possible.[19]

However, it has been reported that this protective effect only occurs at a pH greater than 3 with a reduction in erosion by up to 28%. [20] Therefore, increasing the concentration of fluoride in drinks with a pH below 3 has no effect. This study supports this observation, as AP had the lowest pH (2.95) yet the highest F concentration resulting in the greatest mineral loss of all the alcopops tested in this study. We found very low concentrations of fluoride at 0.01ppm compared to greater fluoride content in their drinks (0.36 to 0.45ppm). Despite the greater acidity, a one-hour immersion in their chosen alcopops resulted in a surface loss that ranged from 1.5-13.5µm.[9]

Conflicting results have been found after profilometry to measure surface loss. Despite similar pH values for different alcopop flavours tested by Rees et al (1998), the surface loss was low at 1.80-3.28µm [10] compared to more significant surface loss after one-hour immersion reported here (24.2-44.4µm). The relatively higher fluoride content cannot account for the low lesion depth because, and as was mentioned earlier, only at pH above 3 does fluoride play a role in decreasing the erosive effect of such drinks. With the reported pH between 2.57 and 2.86 the fluoride concentration would not have a significant protective effect.

Erosive potential is dictated by the total acid content and/or the type of acid that mostly constitutes the drinks.[9] Bacardi Breezer contains citric, lactic, and malic acids and this combination was found to be responsible for its erosive potential. A noteworthy finding is that, in bovine enamel, malic acid had less erosive effect than citric acid, but this is valid for the first 15-30min, after which, both acids tend to have similar erosive tendancy.[21] In this study, the effect of individual acids within each drink was not investigated.

Salivary pellicle is another factor to be considered when studying the progression of dental erosion. It acts as a barrier preventing the diffusion of acids towards the tooth surface. In order to achieve maximum protection, salivary pellicle should be allowed to mature for at least 24 hours to reach maturation and provide protection against erosion.[22] Pellicle grown for 7 days *in vivo* or longer periods (18 hours) *in vitro* did not offer greater protection.[23] Consequently, in the present study; the two-hour pellicle may be insufficient to resist the erosive capability of the acidic drinks thus accounting for the high degree of erosion/demineralisation.

Structural differences between bovine and human enamel play a major role in the spread of erosive lesions. Bovine enamel is more porous than its counterpart in human teeth, hence, less resistant to acid diffusion and lesions tend to progress rapidly.[24]Solubility rates increase as temperature rises, and thawed or even room temperature drinks were found more erosive than ice-cold drinks.[25] This study was conducted at room temperature, which although not controlled did not fluctuate greatly. Removing the smear layer, through polishing and grinding enamel surfaces, renders the enamel surface more susceptible to acid attacks.[26] This would expose the more porous subsurface, and as a result destruction is more progressive.

All the above-mentioned factors: the use of bovine teeth, temperature, and smear layer removal would constitute a worse case scenario in order to magnify erosive changes.

Certain oral parameters were impossible to replicate using this *in vitro* model system; of these are salivary flow rate, clearance, remineralising and neutralising capacity. It has been reported that the ingestion of acidic drinks stimulates salivary flow, which in turn either wash the drink away or neutralises it.[27] On the other hand, the clearing effect of saliva is dependent on the viscosity of the drink and its ability to adhere to the enamel surface. According to the same study, viscous drinks are more likely to adhere and therefore, have longer contact to enamel surfaces.[28] This parameter along with the type of acid content of each drink has not been discussed in this study. These obvious differences can make the *in* *vitro* results somewhat exaggerated compared to the *in vivo* situation. The effects of exposure of the dentition to these alcopops beverages *in vivo* would be expected to be dependent on both the amount of drink consumed and its frequency, together with the biological modifying factors and the drinking habits.[29]

Different flavours of the same drink were also tested in a similar study and found to cause a less damage that ranged from 1.80-3.28.[10] In contrast, a slightly higher pH levels of the drinks currently tested resulted in a more significant surface loss after one-hour immersion that ranged from 24.2-44.4μm. Their relatively higher fluoride content did not justify the less lesion depth because, and as was mentioned earlier, only at pH levels above 3 fluorides play a role in decreasing the erosive effect of such drinks. With pH levels that ranged from 2.57-2.86 the amount of fluoride contained in those drinks cannot be considered to have the expected protective effect against acidic attacks.

Conclusion:

The results of this *in vitro* study demonstrated that alcopops have a significant erosive effect on bovine enamel, similar to that of Orange juice.

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