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The effect of dilution on the *in vitro* erosive potential of a range of dilutable fruit drinks

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Background. Only one previous study has investigated the effect which dilution has on the erosive potential of dilutable fruit drinks. Based solely on measurement of neutralizable acidity, the authors concluded that the erosive potential of diluting juices may be reduced substantially by the addition of water. This has not been verified in an *in vitro* enamel erosion model.

Objective. The aim of this study was to examine the effect of dilution on the erosive potential of five dilutable fruit drinks.

Methods. For each product, the erosive potential of three dilutions (1 : 3, 1 : 6 and 1 : 15) was assessed by measuring the initial pH and neutralizable acidity; *in vitro* enamel erosion was measured by profilometry following immersion for 1 h.

Results. For the majority of products, increasing dilution from 1 : 3 to 1 : 15 produced a statistically significant rise in initial pH, though they remained markedly acidic. Increasing the dilution factor consistently produced a statistically significant decrease in neutralizable acidity. Increasing the dilution factor from 1 : 3 to 1 : 15 produced a statistically significant reduction in *in vitro* enamel erosion in only three of the five products tested.

Conclusions. Increasing the dilution factor of dilutable fruit drinks within a range likely to be acceptable to the consumer may not effectively reduce their erosive potential.

Introduction

The most recent UK Children's Dental Health Survey found that 53% of 5-year-olds and approximately one-third of 12- and 15-year-olds had one or more teeth that had been affected by non-carious tooth surface loss. Although tooth surface loss has a multifactorial aetiology, erosion is considered to be the principal contributor in these younger age groups[1]. In Western societies, diet is thought to be a major factor in the aetiology of this form of wear and, as such, has received most attention in the dental literature[2]. The dietary components principally involved are citrus fruits and soft drinks[3].

Among young children, consumption of sweetened and acidic drinks continues to increase at the expense of milk and water[4], posing an important public health problem associated with oral health and obesity[5]. In 2005, UK soft drink consumption grew 1% to 13.8 billion litres, trends in consumption indicating a move from carbonated beverages towards diluted and dilutable drinks. In accordance with this trend, it has been observed that many young children drink large quantities of dilutable fruit drinks ('squashes'), researchers having described a 'squash-drinking' syndrome[6]; indeed, one survey of preschool children reported that 15% consumed 50% of their recommended energy intake in 'squash' type of drinks alone[7].

Dilutable concentrates differ from most other soft drinks in that the consumer has control over the composition of what they drink. Because it is not known how many people follow the manufacturer's instructions, it is reasonable to assume that dilutable fruit drinks are consumed in both more concentrated and more dilute forms. At present, there

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are no national guidelines on dilution of drinks, although the Food Standards Agency Wales has recommended that they be 'diluted well with water'.

Despite their popularity, concentrates that require dilution before consumption have received little attention in the literature. It is therefore somewhat concerning that, even among the dental profession, they have had the image of being a 'safer' form of soft drink. One paper went so far as to describe them as being 'diluted and therefore of reduced erosive potential', but provided no evidence to substantiate this[8].

Only one previous study[9] appears to have investigated the effect which dilution has on erosive potential. Based on the measurement of neutralizable acidity, the authors concluded that the erosive potential of the products that they tested could be reduced substantially by the addition of water. They stated, however, that it was not possible to define the degree to which any drink would damage the teeth because this would vary enormously between individuals. Here, we report a study in which measurement of enamel loss (as described by West *et al.*[10]) was used alongside measurement of pH and neutralizable acidity to examine the effect of dilution on the erosive potential of a range of dilutable fruit drinks.

Materials and methods

Selection of products and dilutions

Five dilutable fruit drinks were investigated in this study: Robinsons Whole Orange, Robinsons Apple and Blackcurrant, Ribena Original Blackcurrant, Tesco Apple & Blackcurrant No Added Sugar, and Tesco Value Low Calorie Blackcurrant. This selection was based upon current market and health-related trends, with top-selling brands, less expensive 'supermarket own' products, and 'healthier' options deliberately being included.

Manufacturers usually recommend that dilutable fruit drinks are diluted 1 : 4 (1 part concentrate: 4 parts water) or 1 : 5. In this pilot study, dilutions of 1 : 3, 1 : 6 and 1 : 15 were selected in recognition of the likelihood that consumers make their drinks either more or less concentrated than recommended. Dilutions of each drink were prepared with tap water as the diluent.

Measurement of initial pH

For each drink, the pH of each dilution was measured using a digital pH meter (Hanna Instruments, Woonsocket, RI, USA). At the start of each session, the electrode was calibrated using test solutions of known pH (Hanna Instruments). Twenty millilitres of each freshly prepared dilution were placed in a glass beaker on a thermostatically controlled electric hotplate at 37 °C. Before reading its pH, each sample was mixed thoroughly using a magnetic stirrer for 1 min. Each dilution was tested three times to give a mean measurement. Between readings, the electrode was rinsed in distilled water to ensure that no cross-contamination occurred.

Measurement of neutralizable acidity

For each drink, neutralizable acidity was measured by placing 20 mL of each dilution in a glass beaker placed on a thermostatically controlled electric hotplate at 37 °C. Then, 0.1 M sodium hydroxide solution was gradually pipetted into the beaker until the pH reached neutrality. Each sample was stirred continuously to ensure thorough mixing. The volume of sodium hydroxide required to raise the pH of each sample to neutrality was recorded. This process was carried out three times for each dilution.

Measurement of enamel loss in vitro

Specimens of enamel were derived from recently extracted, caries-free, permanent teeth. At the time of extraction, donors were resident in areas where the water supplies contained less than 0.3 ppm fluoride. Details of previous residence, however, were not available and it is also likely that fluoride-containing toothpastes were being used. Although the level of fluoride incorporation into enamel was anticipated to be low, this was not further characterized.

Following extraction, each tooth was carefully scraped of any remaining tissue with a scalpel.

It was then rinsed in copious amounts of distilled water. Finally, the crown was sectioned from the root and cut vertically to produce approximately equal sections of enamel. In order to minimize variation in enamel prism orientation, every attempt was made to cut sections from the same coronal area.

Each section was embedded in a vacuum-formed polyurethane mould filled with a low exotherm epoxy resin (Stycast 1266, Emerson & Cuming, Westerlo, Belgium). When the epoxy resin had cured, the specimen was removed and, using an automatic lapping and polishing unit (Kemet International Ltd, Maidstone, UK), ground to fit a stainless steel jig. This had been specifically constructed to hold the specimen precisely during profilometry and to ensure that a stable horizontal platform was maintained. Using abrasive discs of decreasing coarseness (Kemet International Ltd), a smooth, flat area of enamel was exposed, care being taken to remove the minimum amount of tissue. This process was monitored by profilometry, employing a Mitutoyo Surftest SV-2000 (Mitutoyo (UK Ltd), Andover, UK). After its baseline profile had been recorded in duplicate, each specimen was given a unique reference number, which was recorded on its reverse side in indelible ink. It was then stored in isotonic saline at room temperature in an eppendorf tube marked with the same reference number. Immediately before use, an area of enamel was delineated by placing PVC insulating tape (RF Components Ltd, Corby, UK) over the specimen, leaving a 2-mm-wide zone of hard tissue exposed (an approximate enamel area of 10 mm^2).

For each product, five specimens of enamel were randomly allocated to each dilution and placed in a large glass beaker with 250 mL of the drink. The beaker was then placed in a thermostatically controlled water bath at 37 °C, and the mixture was stirred for 1 h. Following exposure, the specimens were removed from the drink and rinsed in copious amounts of distilled water. They were subsequently dried and the tapes were removed. Profilometry was employed to determine surface enamel loss (in μm) in triplicate. Means were calculated from these triplicate measurements. As a control, five specimens were exposed to distilled water for 1 h.

Statistical analysis of the results for pH, neutralizable acidity, and *in vitro* enamel erosion was carried out using one-way analysis of variance. The threshold for statistical significance was set at $P < 0.05$.

Results

Table 1 presents the initial pH, neutralizable acidity, and *in vitro* enamel erosion data for all five products.

Increasing the dilution factor from 1 : 3 to 1 : 6 produced a statistically significant ($P < 0.05$)

Table 1. Mean initial pH, neutralizable acidity, and *in vitro* enamel erosion by product and dilution.

Product (dilution)	Mean initial pH	Mean neutralizable acidity (mL)	Mean (SD) *in vitro* enamel erosion (μm)
Robinsons Apple and Blackcurrant (1 : 3)	2.93	9.22	8.13 (1.51)
Robinsons Apple and Blackcurrant (1 : 6)	3.00	5.35	7.03 (3.64)
Robinsons Apple and Blackcurrant (1 : 15)	3.20	2.46	6.58 (1.82)
Tesco Value Low Calorie Blackcurrant (1 : 3)	3.20	8.83	8.71 (1.83)
Tesco Value Low Calorie Blackcurrant (1 : 6)	3.27	4.70	4.55 (1.04)
Tesco Value Low Calorie Blackcurrant (1 : 15)	3.43	2.07	3.07 (0.53)
Ribena Original Blackcurrant (1 : 3)	2.87	10.06	7.46 (1.58)
Ribena Original Blackcurrant (1 : 6)	3.03	5.65	3.22 (0.93)
Ribena Original Blackcurrant (1 : 15)	3.20	2.49	2.36 (0.54)
Robinsons Whole Orange (1 : 3)	3.23	8.48	2.72 (0.69)
Robinsons Whole Orange (1 : 6)	3.30	4.87	2.45 (0.35)
Robinsons Whole Orange (1 : 15)	3.40	2.19	2.17 (0.59)
Tesco Apple & Blackcurrant No Added Sugar (1 : 3)	3.50	8.16	3.61 (0.77)
Tesco Apple & Blackcurrant No Added Sugar (1 : 6)	3.53	4.56	3.14 (1.14)
Tesco Apple & Blackcurrant No Added Sugar (1 : 15)	3.60	2.13	1.83 (0.47)
Distilled water	–	–	0.12 (0.04)

rise in the initial pH of only one product (Ribena Original Blackcurrant). Increasing dilution to 1 : 15, however, resulted in statistically significant rises in the initial pH of four of the five products tested [Robinsons Apple and Blackcurrant ($P < 0.001$); Tesco Value Low Calorie Blackcurrant ($P < 0.001$); Ribena Original Blackcurrant ($P < 0.001$); and Robinsons Whole Orange ($P < 0.05$)]. It should be noted that the initial pH of all products remained markedly acidic, even when diluted 1 : 15.

For all products, increasing the dilution factor from 1 : 3 to 1 : 6 and thence to 1 : 15 consistently produced a statistically significant decrease in neutralizable acidity, probability values being, with one exception [Robinsons Whole Orange 1 : 3 v 1 : 6 ($P < 0.05$)], < 0.001.

Increasing the dilution factor from 1 : 3 to 1 : 6 produced statistically significant ($P < 0.001$) reductions in the amount of *in vitro* enamel erosion that resulted from exposure to two of the five products (Tesco Value Low Calorie Blackcurrant and Ribena Original Blackcurrant). Further increasing the dilution to 1 : 15 produced statistically significantly ($P < 0.05$) less *in vitro* erosion resulting from exposure to a third product (Tesco Apple and Blackcurrant). Notably, increasing the dilution of the two Robinsons products failed to reduce *in vitro* erosion by a statistically significant amount.

Discussion

All the drinks tested in this study were highly acidic, even when extremely dilute. Although the values were numerically very similar, it must be remembered that pH is a logarithmic scale. Small changes in pH values therefore equate to larger changes in the hydrogen ion concentration.

Although baseline acidity has been stated to be a major factor in determining erosive potential[11], it is generally accepted that neutralizable acidity is a better indicator of this capability[12,13]. Based solely on this premise, the results of this study would indicate that erosive potential decreases markedly as the dilution factor increases.

In respect of both initial pH and neutralizable acidity, our results correlate well with those of Cairns *et al.*[9] The latter authors found that dilution had little effect on initial pH, the difference in pH value between the concentrate and a dilution of 1 : 10 being in the order of 0.2 for all drinks tested. Importantly, to attain a pH of 5.5, Robinsons Whole Orange required dilution to a ratio of between 1 : 500 and 1 : 750; Ribena Original Blackcurrant required to be diluted to between 1 : 1000 and 1 : 2000. In order to reach neutrality, Robinsons Whole Orange needed to be diluted to between 1 : 5000 and 1 : 10 000, and Ribena Original Blackcurrant required a ratio of 1 : 10 000.

To put the results of this study in context, a recent study using the same methodology[14] showed Tropicana natural orange juice to have a mean initial pH of 3.68, and a mean neutralizable acidity of 19.68 mL. All five dilutable fruit drinks, even when extremely dilute (1 : 15), had a lower pH than Tropicana. The neutralizable acidity of Tropicana natural orange juice was, however, almost double that of the dilutable fruit drink with the highest neutralizable acidity (Ribena Original Blackcurrant).

Values for *in vitro* enamel loss varied by product. Interestingly, the two Robinsons products showed no statistically significant changes in enamel loss as the dilution factor increased. When the dilution factor was increased from 1 : 3 to 1 : 6, two of the three remaining products (Tesco Value Low Calorie Blackcurrant and Ribena Original Blackcurrant) showed statistically highly significant ($P < 0.001$) decreases in enamel loss; when the dilution factor was increased from 1 : 3 to 1 : 15, all three remaining products showed a reduction in enamel statistically significant (at the $P < 0.05$ level or greater) decreases in enamel loss. The reason for this disparity is not immediately obvious, but may be related to citric acid content. Citric acid is a complex organic acid with three acid dissociation constants (K_a values). At low pH values (around pH 2.0), the acid provides protons which attack the mineral surface and release calcium, whereas at higher pH levels (nearer pH 7.0), the citrate ions draw calcium away from the enamel surface by chelation[15]. Even when diluted, therefore, concentrates with high levels of citric acid will retain a significant erosive potential. To put the results of this study in context, Rees *et al.*[14]

found that immersion in Tropicana resulted in a mean loss of 3.34 μm of enamel.

The results of this study must be interpreted with a certain degree of caution. Consumption of dilutable fruit drinks tends to peak around meal-time, with around 75% of consumption either with main or light meals. *In vivo*, the enamel surface will be covered by a protective salivary pellicle layer and/or plaque layer, and will be protected by the flushing, buffering, and remineralizing effects of saliva[16,17]. Because the consumption of an acidic drink will also stimulate salivary flow[18], it is likely that a more acidic drink will either be cleared from the mouth or neutralized more rapidly. Clearance of a drink from the mouth also depends on the ability of the drink to adhere to enamel[19]. Hence, being more viscous, a more concentrated drink may adhere to the tooth surface and be held in the mouth for longer.

Conclusions

Contrary to popular belief, dilutable fruit drinks are not a 'safe' form of soft drink. Even when extremely dilute, some are capable of causing significant loss of hard tissue. Consumers and healthcare professionals should be made aware of this, and diet advice should be modified accordingly. The public health advice relating to drinks for young children should be clarified to recommend milk and water only as safe drinks for young children. The authors consider that recommending very dilute squash is an inappropriate public health message.

What this paper adds

- Dilutable fruit drinks are widely regarded as a 'safe' form of soft drink. This paper shows that, even when extremely dilute, they are capable of causing significant loss of hard tissue.

Why this paper is important to paediatric dentists

- Dietary advice directed at children and adolescents presenting with erosive tooth wear should be modified accordingly.

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