Effect on plaque pH of fruit drinks with reduced carbohydrate content

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Objectives To assess the acidogenic response in dental plaque after challenge with four fruit drinks, including two blackcurrant drinks newly formulated, with low levels of carbohydrate.

Methods 24 adult volunteers rinsed, in randomised order, with each of two new formulations of a blackcurrant drink (7% juice with 0.49% and 10% juice with 0.65% carbohydrate concentration respectively), an apple and blackcurrant drink with no added sugar (0.8%), and a mixed citrus fruit drink with a higher carbohydrate concentration (4.5% w/v). Solutions of 10% sucrose and 10% sorbitol were used as controls. Plaque pH was assessed, *in vivo,* before and after the acidogenic challenge using the plaque-harvesting technique.

Results Results showed that the minimum plaque pH after the subjects rinsed with the new blackcurrant drinks was higher as compared with all the other test products and significantly so compared with the mixed citrus drink ($P = 0.0001$). It was also found that with the 7% blackcurrant juice drink none of the subjects and with 10% blackcurrant juice drink only one subject recorded a pH drop below the pH of 5.7. Ten minutes after consumption, both the new formulation blackcurrant drinks produced significantly higher plaque pH than the mixed citrus drink. In addition, overall change in the hydrogen ion concentration over the study period ($\Sigma\Delta cH$) was significantly less with both new blackcurrant drinks compared with the mixed citrus drink.

Conclusions It was concluded that the two new formulations with low levels of carbohydrate had a low acidogenic potential and did not depress the plaque pH below the critical level and their consumption could not be considered to pose a significant risk for enamel demineralisation.

The potential cariogenicity of fruit drinks consumed by infants and young children has been the subject of much study in the past decade.[1–3] Most studies have shown that when drinks that contain fermentable carbohydrate are consumed, there is a drop in the pH of the dental plaque *in vivo*. The production of acid by bacteria in such close proximity to the tooth surface would mean that on consumption enamel demineralisation could occur, hence their cariogenic potential. There has been an attempt by industry to produce drinks that are safer for teeth. In the first instance drinks with 'no added sugar' were marketed. These drinks contained natural sugars such as glucose and fructose. However subsequent research by Duggal et al showed that these drinks had a similar acidogenicity and hence cariogenicity, as drinks that contained sucrose.[1] It has also been shown that glucose, fructose and sucrose produce a similar acidic response in the plaque.[4] Modification of drinks by altering the citrate content has also been studied. It was shown that when small amounts of citrate were added to drinks the acidogenic response in the plaque was reduced.[5]

Another way of making drinks safer for teeth would be to reduce the amount of fermentable carbohydrate to levels that would not produce a significant acidogenic response in the plaque. Imfeld observed an acidogenic response in plaque with very low levels of sugars.[6] However, it would seem obvious that any drink that is aimed at reducing the acid production in the plaque by acidogenic microorganisms must have low amounts of fermentable carbohydrate.

The aim of the present study was to study the acidogenic response in human dental plaque *in vivo* with two fruit drinks modified by the manufacturers and formulated with low levels of carbohydrate compared with two other drinks that were currently available in the market.

Materials and methods

Test products

Four fruit drinks were selected for the study. Two were new formulations of a blackcurrant drink with 7% and 10% juice respectively. These were compared with two conventional drinks widely available in the market; an apple and blackcurrant drink with no added sugar, the second a mixed citrus fruit drink which had a higher carbohydrate concentration than the other products. Details of the drinks, their carbohydrate concentration and the inherent pH are shown in Table 1. Solutions of 10% sucrose and 10% sorbitol were used as positive and negative controls respectively.

Subject selection

Having given informed consent and with the approval of the Ethics Committee, 24 adult subjects aged between 18 and 65 years were selected for the study. The volunteers were chosen on the basis that their

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Table 1 Test products, their carbohydrate content and inherent pH

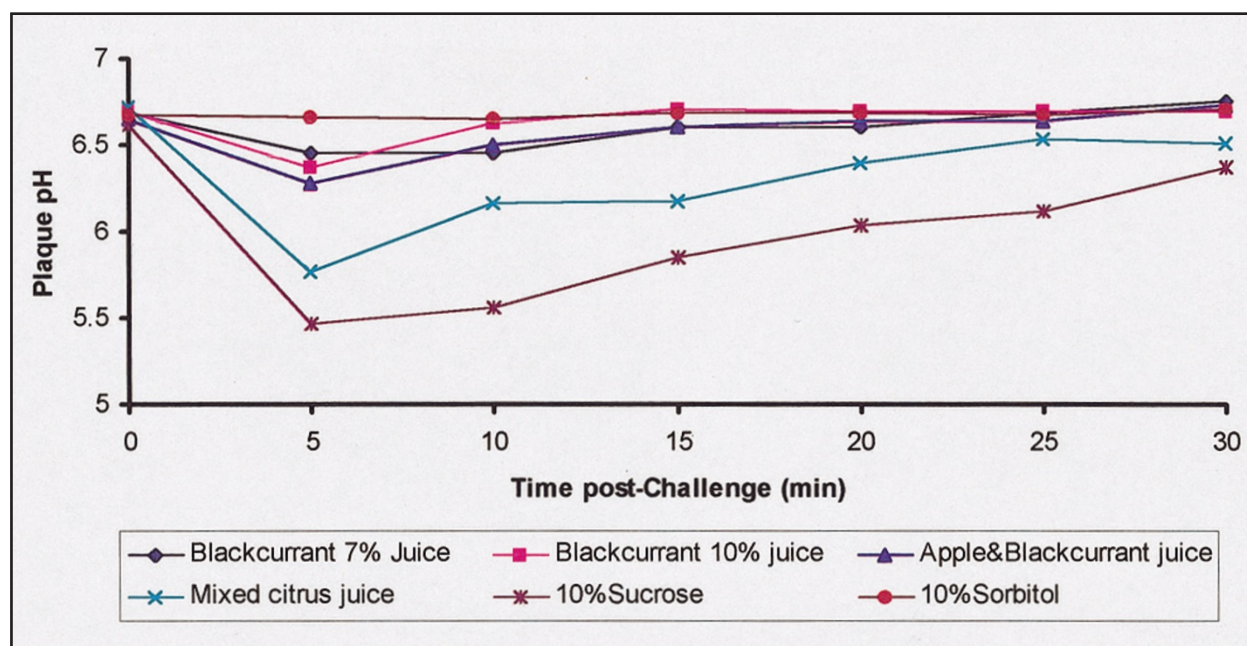| Product	Carbohydrate concentration(%w/v)	Inherent pH
Ribena blackcurrant drink 7% juice (ready to drink formulation)	0.49	3.80
Ribena blackcurrant drink 10% juice (ready to drink formulation)	0.65	3.80
Robinsons Special R (no added sugar) Apple and blackcurrant	0.8	3.24
Waitrose mixed citrus juice drink	4.5	3.57
Sucrose control	10	Neutral
10% Sorbitol control	0	Neutral

Fig. 1 Mean Plaque pH Curves for test and control drinks (*n* = 24)

plaque recorded a pH of less than 5.5, or a fall of at least one pH unit from baseline, on challenge with a 10% sucrose rinse and that they had a minimum DMFS score of 12. These criteria conformed to the guidelines of the Plaque Acidity Working Group of the Food, Nutrition and Dental Health Committee of the American Dental Association.[7]

All subjects were required to refrain from brushing their teeth or from using any oral hygiene aid for 48 hours before the test and to abstain from any food or drink (except water) for 2 hours before the test. Each volunteer was seen at the same time of the day to avoid changes in the circadian rhythm

Plaque harvesting and pH measurement
Plaque pH was measured using the method of Fosdick *et al.*[8] modified by Frostell[9] and Rugg-Gunn *et al.*[10] On each test day, a sample of plaque was taken from the buccal surfaces of four sites of the subject's teeth using a sterile stainless steel straight probe. Subjects were asked to swallow immediately before plaque collection to minimise salivary contamination, and during sample collection care was taken to avoid contamination with blood or saliva. This formed the baseline plaque sample. The collection time for each sample was standardised (30 seconds). The plaque sample was mixed with 20 ml of distilled water and the pH was measured with a

M1-410/M1-415 micro-combination electrode (Microelectrodes Inc. Londonderry, New Hampshire, USA) in conjunction with a Corning 245 pH meter. The pH was read after allowing the reading to stabilise for 30 seconds. Volunteers then rinsed their mouths thoroughly with 15 ml of one of the test drinks for a period of 30 seconds. Plaque samples were harvested from the same areas of teeth 5, 10, 15, 20, 25 and 30 minutes after rinsing, and the pH of each sample was measured as before.

Calibration of the system was carried out using standard solutions of pH 7.0 and 4.0, and tests of standards run randomly between the plaque pH readings. In between each reading the electrode was cleaned with a stream of distilled water and placed in a standard solution of pH 7.0. This ensured stable readings and a constant check on drift.

Each volunteer received one of the test drinks in an order determined by a randomisation schedule, with a 7-day interval between each test day to avoid any carry over effect. The study was single blind; blind to the person performing the plaque sampling and pH measurement.

Analysis of results
pH curves were constructed using the mean plaque pH readings of each test drink.[11] For each drink the results were initially analysed

Table 2 Mean minimum pH, pH at 10 minutes after rinsing, number of subjects with pH < 5.7 and sum of changes in hydrogen ion concentration after rinsing with test drinks and the controls (*n* = 24)

Drink	Mean (SD) pH at 10 min	Mean (SD) minimum pH	No. (%) of subjects with pH <5.7	Median ΔcH
Blackcurrant 7% juice	6.45 (0.36)*†	6.29 (0.35)**	0 (0%)*	5.959×10^{-7}**
Blackcurrant 10% juice	6.63 (0.35)**	6.28 (0.27)**	1 (4%)*	3.724×10^{-7}**
Apple and blackcurrant juice	6.50 (0.41)	6.17 (0.47)	3 (13%)	1.845×10^{-7}
Mixed citrus fruit juice	6.16 (0.49)	5.72 (0.44)	12 (50%)	4.315×10^{-6}
Sucrose 10%	5.56 (0.35)††	5.34 (0.32)††	22 (92%)††	9.865×10^{-6}††
Sorbitol 10%	6.65 (0.37)	6.47 (0.45)	0 (0%)	5.359×10^{-8}

*$P < 0.005$, **$P = 0.0001$ (compared with mixed citrus juice drink)
†$P = 0.034$ (compared with blackcurrant 10% juice); ††$P = 0.0001$ (compared with 10% sorbitol)

for three parameters:
1. Minimum pH,
2. pH recorded 10 minutes after rinsing,
3. Number of subjects where a pH drop below 5.7 was recorded.

In order to quantify the shape of the Stephan curves a further variable, $\Sigma\Delta cH$, was evaluated as described by Edgar.[12] For this, the hydrogen ion concentration, cH (the negative of the antilogarithm of the pH), was generated and then the difference between the cH value at each time point and the cH value at baseline was calculated to give ΔcH. The sum of these differences gives a numerical value for the depth and duration of the curve. Statistical evaluation was performed using an analysis of variance (ANOVA) model appropriate for a 6-way crossover design and statistical significance was assessed at the conventional level of 5%. Following ANOVA, the method of Least Significant Differences was used to make pre-specified comparisons between pairs of drinks for all of the parameters assessed. Each blackcurrant drink was compared with all other test drinks and the two control solutions were compared.

Results

The Stephan curves[11] for the pH over time of each test drink and the two control solutions are shown in figure 1.

Minimum pH, and pH at 10 minutes

The mean minimum pH reached in the plaque is shown in Table 2. The minimum pH reached with the two new formulation blackcurrant drinks was 6.29 ± 0.35 and 6.28 ± 0.27 with blackcurrant 7% and 10% juice drinks respectively. It should be noted that both resulted in a slightly higher plaque pH than the apple and blackcurrant drink (6.17 ± 0.47) and significantly higher ($P = 0.0001$) compared with mixed citrus fruit drink (5.72 ± 0.44), and 10% sucrose (5.34 ± 0.32). There were no statistically significant differences between the two new blackcurrant drink formulations.

Also both blackcurrant drink formulations and the apple and blackcurrant drink resulted in higher plaque pH values 10 minutes after rinsing than did the mixed citrus fruit drink. All test drinks resulted in a higher mean pH at 10 minutes as compared with the 10% sucrose control. The plaque pH with the blackcurrant 10% juice drink was only minimally lower than that with the 10% sorbitol control (6.63 ± 0.35 and 6.65 ± 0.37 respectively). The plaque pH with both new blackcurrant drink formulations was significantly higher as compared with the mixed citrus fruit drink ($P < 0.005$). When the two new low carbohydrate formulations were compared with each other, it was seen that the pH after rinsing with blackcurrant 10% juice drink was also significantly higher than that with blackcurrant 7% juice drink ($P = 0.034$).

Number of subjects with plaque pH below 'critical pH' (5.7)

Both the new formulation blackcurrant drinks were similar to the 10% sorbitol solution in the proportion of subjects with a plaque pH below 5.7. In contrast, plaque pH below 5.7 was recorded in 50% of the subjects who rinsed with the mixed citrus fruit drink and in 92% of the subjects with 10% sucrose control, significantly more than either of the new blackcurrant drinks ($P < 0.005$).

Change in hydrogen ion concentration

The curves formed following challenge with the blackcurrant drinks gave $\Sigma\Delta cH$ values of 5.959×10^{-7} and 3.724×10^{-7} with blackcurrant 7% and 10% respectively. These values represent a considerably smaller overall change in pH than the $\Sigma\Delta cH$ value of 4.315×10^{-6} for the mixed citrus juice drink ($P = 0.0001$) and 9.865×10^{-6} for 10% sucrose control.

Discussion

Though there is no single test which can unambiguously determine the cariogenicity of any food or drink *in vivo*, studies on their ability to depress plaque pH may give an insight into their potential to cause demineralisation. Measurement of plaque acidity, principally as changes in the plaque pH over a period of time, form an important group of tests for assessing potential cariogenicity of foods and drinks.[13] The Human Plaque Acidity Model Working Group agreed that methods for measuring plaque pH would satisfactorily identify non-acidogenic foods when compared with positive and negative controls such as 10% sucrose and 10% sorbitol, respectively.[14] There is however continuing debate in the dental literature on the most appropriate way to measure the plaque pH. Both indwelling electrode systems[6] and the harvesting technique, as used in our study, have been widely used and are both generally accepted as scientifically sound techniques. An important feature of the plaque pH model is their capacity to provide evidence of the acidogenic potential of foods under normal conditions of use. The fall in plaque pH after rinsing with sucrose has been correlated with caries increment.[15]

All the drinks investigated in the current study contained fruit juices, with the two new formulations containing only blackcurrant juice. Of the two drinks against which these were compared one contained a combination of blackcurrant and apple and the other mixed citrus fruits. The two new formulations contained lower levels of fermentable carbohydrate (0.49% and 0.65% w/v) compared with the blackcurrant and apple drink (0.8%) and mixed citrus juice drink which had the highest carbohydrate content (4.5%). The plaque pH after rinsing with the drinks seemed to reflect the carbohydrate concentration as is evident in the minimum pH values for the drinks. The mean minimum pH was significantly higher when the subjects used the two new blackcurrant drink formulations as compared with the mixed citrus drink. The minimum pH values for the two new drinks was higher, though not significantly, as compared with the apple and blackcurrant drink whose carbohydrate content was also under 1%. It should be noted that the inherent pH of the two new formulations was slightly higher as compared with the other test drinks (Table 1), though this is unlikely to affect the plaque pH which is a measure of the organic acid production by the plaque microorganisms. Also, in our study the plaque was sampled for pH measurement at baseline and then 5 minutes post-challenge. Some investigators have opted for sampling at 2 minutes[16] or 3 minutes post-challenge.[17] There is a concern that sampling at 5 minutes might not always record the minimum pH and the authors acknowledge this is a possibility. However, numerous studies have been published in the literature that have used the time intervals as used in the present study.[1,2,5]

It was decided that in order to make an in-depth study of the acidogenic profile of the drinks it was important to study the individual variations in the acidogenic response within the study group. The analysis of the mean minimum pH values alone does not reflect possible variations between subjects. To do this the number of subjects who recorded a minimum pH below the critical pH (5.7 used in this study) with the tests and controls was analysed. A fall of plaque pH below the critical value is usually associated with the start of demineralisation of the enamel.

Although this pH value has been given different estimates, a conservative value of 5.7 was proposed by Mühlemann.[18] It was found that with the 7% blackcurrant juice drink none of the subjects, and with the 10% blackcurrant juice drink only one subject (4% of the sample) recorded a pH drop below 5.7. With the apple and blackcurrant drink 13%, mixed citrus fruit juice 50% and with the 10% sucrose control 91% of the subjects had a pH drop to below the critical pH. The differences were statistically significant when the two new formulation blackcurrant drinks were compared with the mixed citrus drink.

Another parameter that was assessed was the pH of the plaque 10 minutes after the acidogenic challenge. It was thought that a comparison of the plaque pH value 10 minutes after challenge would reflect the speed with which the acid was cleared from the plaque. At

10 minutes after use, plaque pH following consumption of either of the two new formulation blackcurrant drinks was higher than plaque pH following consumption of the citrus drink and the 10% sucrose control but similar to the apple and blackcurrant drink. Some authors have reported that a true measure of plaque acidity is its hydrogen ion concentration. A comparison of this parameter gives a better idea of the acid production in the dental plaque than expressing the pH value which represents a logarithmic scale and makes it difficult to envisage the true acidity of the environment. The change in hydrogen ion concentration is expressed as $\Sigma\Delta$cH . In our study the overall change in pH throughout the test period as measured by $\Sigma\Delta$cH was also highly significantly lower with both new formulation blackcurrant drinks as compared with the mixed citrus juice drink, and lower but not significantly as compared with the apple and blackcurrant drink. The overall change was 6.0% and 3.8% of the change with 10% sucrose for the 7% blackcurrant juice and 10% blackcurrant juice drinks respectively

It was also interesting to note that the plaque pH after 10 minutes of using the drinks was significantly higher when the subjects used the blackcurrant formulation with 10% juice as compared with the drink with 7%, in spite of its slightly higher carbohydrate concentration. This suggests a quicker clearance of acid from the plaque with the 10% juice drink. This may be due to the composition of the 10% formulation which also contained a slightly higher level of citrate (17.7 mM compared with 10.9 mM in 7% formulation). Previous work has shown a reduction in the drop of plaque pH when citrate was added to a blackcurrant drink[19] or to a sucrose solution.[5] Although the mechanism has not been established unambiguously, it is believed to arise from inhibition of phosphofructokinase and enolase which is known to occur with citrates at low concentrations.[20] This inhibition of glycolytic enzymes could reduce the concentration of the acid formed by bacterial fermentation of carbohydrate. One of the drinks tested, however, was a mixed citrus juice, which would be expected to have higher levels of citrate than the blackcurrant 10% juice formulation. In this case, any benefit from added citrate at low concentration is outweighed by the increased titrateable acidity due to higher concentrations of citric acid which tend to reduce pH in the vicinity.[20]

Conclusion

These results discussed above would suggest that reducing the amount of fermentable carbohydrate in a fruit drink to very low levels would lead to a significantly lower acid production in the dental plaque *in vivo*. It is difficult to imagine and would be naive to believe that the use of fruit based drinks can ever be stopped completely. However, given the concerns of the dental profession and the known possible detrimental effects of such drinks, the emphasis should be on less frequent usage and the development of drinks that are safer for teeth. The use of such drinks would limit the potential damage to teeth if they are misused, as they sometimes are, in spite of the advice of the dental profession. The two new formulations of blackcurrant drinks with very low levels of carbohydrate tested in the study had a low acidogenic potential and did not depress plaque pH below the critical level and their consumption could, therefore, not be considered to pose a significant risk for enamel demineralisation.

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