Quantifying Human Enamel Erosion Caused By Freshly Squeezed Juices

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Aims: The present in vitro study intended to investigate minimal erosive effects of different freshly squeezed fruit juices on human enamel during short time incubation by determination of calcium and phosphate dissolution.
Material and methods: Healthy adult human molars and premolars were cut in 160 blocks (5 mm x 6 mm x 2 mm) and divided randomly in sixteen groups of 10 samples each (n = 10). Each group was assigned for immersion at 37 oC in 5 ml juice, for different spot times 3, 6, 9 and 12 minutes, respectively. The freshly squeezed juices analyzed were orange juice, apple juice, orange mixed with carrot juice (1:1) and apple mixed with carrot juice (1:1). Amount of titratable acid and pH was measured for the tested solutions. Calcium and phosphate release were determined photometrically using VIS-UV/VIS spectrophotometer. ANOVA test compared the data generated ($p < 0.05$).
Results: The apple juice showing the lowest pH and a high value for buffering capacity had the most pronounced erosive effect. The orange juice was less erosive ($p < 0.001$). The least erosive was the orange mixed with carrots (1:1) ($p < 0.001$), which has the highest pH. The enamel demineralization increased when prolonging the immersion time ($p < 0.0001$).
Conclusions: All freshly squeezed juices caused the dissolution of calcium and phosphate in human enamel. Erosive capacity is mainly determined by pH and to a lesser extent by the buffering capacity. The amount of demineralization is directly correlated with the exposure time. Absorption spectroscopy allows detection of very small mineral loss using standardised human enamel samples.

Keywords: dental erosion, human enamel, chemical analysis, in vitro, fresh juice

Introduction

Dental erosion is a non-bacterial process causing loss of dental hard tissues incrementally from the tooth surface with demineralization and changes in tooth morphology. Dietary acids are the most extensively studied aetiological agents and the most important extrinsic factors [1]. In particular, dental erosion by acidic soft drinks appears to be a growing problem and has been the subject of numerous studies [2, 3]. These studies revealed the erosive potential of a wide variety of drinks but few of them have investigated the erosive potential of freshly squeezed juices. "Consumption of one hundred percent fruit juice is closely linked to improved nutrient intake and overall diet quality", highlighted a research presented at the Experimental Biology (EB) meeting in April 2010 in USA [4]. Following these tips, consumption of fresh fruit juices is constantly increasing.

Enamel erosion and enamel softening have been studied by various techniques based on physical [5], chemical [6] or imaging techniques [7]. Most studies described the amount of substance loss after an erosive attack. However, there is little information available on the quantification of mineral loss or the percentage of demineralization within the softened enamel [8].

The aim of the current study was to determine the amount of demineralization of softened enamel with respect to the content of calcium and phosphate after immersion in freshly squeezed juices. The objectives were to get an overview of the differences in erosive potential between juices produced from different fruit types and to describe associations among the chemical parameters of the tested solutions and the degrees of demineralization.

Material and method

Freshly squeezed orange juice (O), freshly squeezed apple juice (A), freshly squeezed orange mixed with carrot juice (1:1) (O+C) and freshly squeezed apple mixed with carrot juice (1:1) (A+C) were investigated for their erosive potential.

The chemical properties of the juices were measured including pH and buffer capacity.

pH

The pH of the drinks was determined at room temperature with pH meter Consort C833.

Buffering capacity

Neutralizable acidity was determined by assessing the amount of 1 N NaOH (mmol/l) needed to raise the pH of 10 ml of solution to pH 7.0.

Preparation of the tooth

Clean and caries-free adult human molars and premolars extracted for orthodontic reasons were collected from patients with their informed consent. The specimens were used to prepare 160 blocks of intact enamel (5 mm × 6

Table I. Mean (SD) calcium and phosphate concentrations, pH and buffering capacity of the fresh juices analyzed

Juice	Calcium mg/dl n=2	Phosphate mg/dl n=2	pH n=2	Buffering capacity mmol/l NaOH
Apple	6.370 (0.024)	5.010 (0.034)	3.535 (0.021)	118
Orange	9.010 (0.034)	15.621 (0.025)	4.230 (0.005)	130
Apple and Carrot (1:1)	13.501 (0.035)	15.620 (0.034)	4.520 (0.028)	80
Orange and Carrot (1:1)	16.361 (0.030)	22.001 (0.039)	4.635 (0.007)	75

mm × 2 mm). The resulting enamel sheets were rinsed with deionised water and divided randomly in sixteen groups of 10 samples each (n = 10). Each group was assigned for immersion in 5 ml freshly squeezed juice in plastic containers at 37 °C. The incubation times in the first tested solution were 3, 6, 9 and 12 minutes, respectively. The same procedures were followed for the rest of juices and groups.

In vitro measurement of erosive potential

The erosive potential of the juices was assessed as a result of the following chemical reaction:

$$Ca_{10}(PO_4)_6(OH)_2 \rightarrow 10Ca^{2+} + 6PO_4^{3-} + 2OH^-$$

The concentration of calcium and phosphate was measured using ultraviolet-visible absorption spectroscopy with VIS-UV/VIS scanning spectrophotometer. The minerals leached out from the enamel in the tested juices were calculated as the difference between the measured values in solution before and after immersion.

Statistical Analysis

Statistical procedures were performed with GraphPadInStat statistics software. One way Analysis of Variance (ANOVA) followed by Tukey's post hoc test compared the data generated. The significance level was set at $p < 0.05$.

Results

Table I shows the concentrations of calcium and phosphate, as well as the pH and buffering capacity of the fresh juices.

For the evaluation of calcium loss (figure 1) and phosphate loss (figure 2) over time, ANOVA test revealed significantly increasing mineral loss from 3 to 12 minutes of erosion time ($p < 0.0001$ for all time spots and all juices).

The most erosive was the apple juice ($p < 0.001$) followed by orange, apple mixed with carrot (1:1) and orange mixed with carrot juice (1:1) ($p < 0.001$).

Discussions

In the present study, a photometric method for specific determination of calcium and phosphate loss during erosive attacks was applied. This allowed the assessment of erosive potential of freshly squeezed fruit juices during short time incubation. The solutions investigated displayed low values for pH and a wide range of values for buffering capacity. The buffering effect of the fresh fruit juice can be explained as the ability to keep pH unaffected by the dissolution of enamel and dilution with saliva. The greater the buffer capacity of the drink, the longer it will take for saliva to neutralize the acid and dissolution brought to an end. The freshly squeezed apple juice showing a high buffering capacity and the lowest pH had significantly the most pronounced erosive effect on human enamel in vitro. The least erosive loss of minerals was observed for orange mixed with carrots (1:1), which has the highest pH.

The dissolution of calcium and phosphate in permanent teeth enamel was caused by all of the tested fresh juices. The enamel demineralization was increased when prolonging the immersion time.

Dental enamel forms the top layer of our teeth and consists of 34–39% m/m (g per 100 g) calcium and 16–18% m/m phosphorus [9]. During erosion, calcium and phosphorus from the enamel are dissolved, which eventually leads to a collapse of the enamel surface structure [10]. Therefore, determination of dental enamel dissolution by assessing the amount of calcium or phosphate dissolved

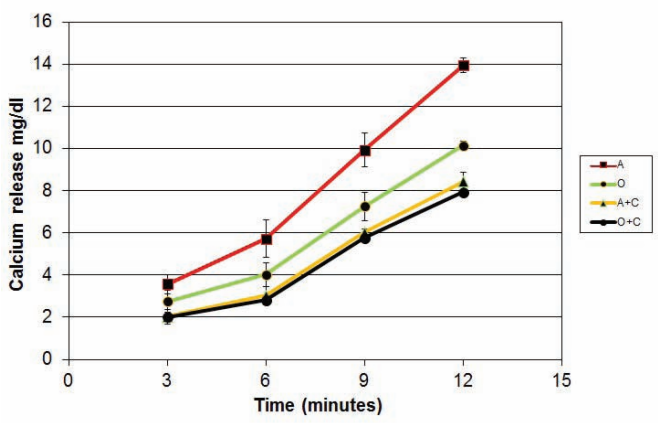

Fig. 1. Mean ±SD calcium release (mg/dl) from human enamel specimens stored for 3, 6, 9, 12 minutes in different fresh juices, n = 10 for each group

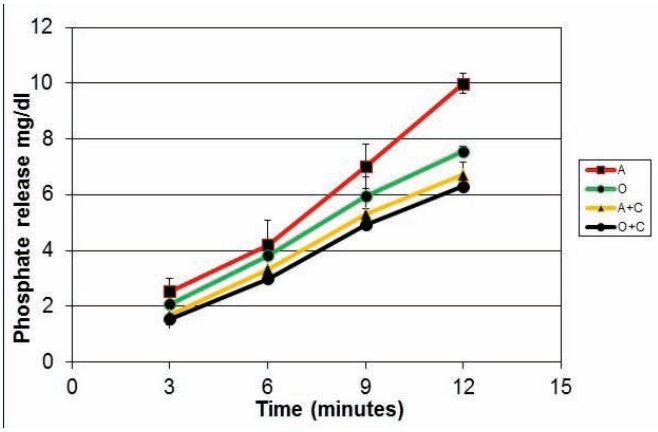

Fig. 2. Mean ±SD phosphate release (mg/dl) from human enamel specimens stored for 3, 6, 9, 12 minutes in different fresh juices, n = 10 for each group

from the apatite crystals is important for assessing dental erosions. In previous studies, amount of mineral dissolved by erosive drinks, acids, and foodstuffs was assessed [11, 12]. To our knowledge, the present research is one of the few systematic studies attempting to elucidate the erosive character of different pure fresh squeezed fruit juices via photometric determination of mineral loss during short time incubation. Under the given conditions, there was a clear influence of pH on erosive effects. Correlation of pH and erosive potential has already been proven in other studies by roughness measuring or other surface measuring methods [11, 13].

The data for enamel demineralisation found in this in vitro study have to be carefully transferred to the clinical situation. The pellicle formation on the enamel surface resulting in a diffusion barrier might have an effect on mineral loss within the softened enamel [14, 15]. A series of studies have demonstrated in situ the efficacy of the pellicle in reducing erosion [16]. Thus, it can be speculated that demineralisation of softened enamel in vivo is smaller than found in the current in vitro study. Buffering capacity of saliva also might decrease the erosive potential of acidic drinks [15]. Besides the variables tested, other variables must be taken into account in future studies for developing strategies to minimize dental erosion.

Conclusions

The high sensitive approach used in the present study allows investigation of minimal erosive effects during short time exposition of enamel to erosive fresh fruit juices through photometric determination of calcium and phosphate loss.

Assessment of erosive dissolution has the advantage that allows detection of very small mineral loss using unpolished, native tooth samples.

Increasing immersion time of human enamel in erosive solutions may cause increasing demineralization.

Erosive capacity is mainly determined by pH and to a lesser extent by the amount of titratable acid.

A low pH fresh juice (i.e. apple) mixed with a higher pH juice (i.e. carrot) might not only improve the taste but it might reduce the erosive potential of the solution.

Demineralization caused by freshly squeezed fruit juices suggests the need for a rational protocol to encourage the consumption of healthy fruit juices but to render them less harmful with minimizing dental erosion.

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