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A Formulation to Mimic the Human Salivary Protein Buffering System

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One of the many biological functions of human saliva is to keep the intra-oral pH at a constant level. This salivary feature is of utmost importance to oral health and the integrity of teeth. Saliva contains three buffer systems providing pH stabilization: the bicarbonate, phosphate and protein systems. Unlike for the first two, the constituent(s) and mode of action of the latter are unknown, mainly because of the complexity and interference with the other two buffering systems. This study was thus designed to simulate and quantify the action of the protein buffer system by a simple synthetic and straightforward strategy. The synthetic approach had the advantage that all buffering components were assessable individually for comparison with human saliva. A solution containing 5 m*M* di-hydrogen phosphate and 10 m*M* hydrogen carbonate, 10 μ*M* amyloglucosidase from the fungus *Aspergillus niger* and 340 μ*M* lysozyme from hen egg white was used to simulate the salivary protein buffering system. Lysozyme exhibited 57% and amyloglucosidase 35% sequence homology with their salivary counterparts. Monotonic acid/base titration showed two almost congruent titration curves of saliva and the protein formulation with a slight difference in acidic buffering power of 10 μ*M* H^+. In human resting saliva the protein buffering system contributed 35% of the total acidic and 48% of the basic buffering power compared to 31 and 43% in the protein formulation. Our investigation showed that the salivary protein system has 3 buffering optima at pH 4.4, 5 and 6.5 whereas the synthetic protein buffer formulation has two at pH 4.4 and 6.5. It is concluded that the protein formulation under study is able to mimic the performance of the acidic and a part of the basic salivary protein buffering system.

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Validation of a Colorimetric Calcium Assay for Short Time Erosion Analysis

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Aim of the study was the quantification of enamel erosion by colorimetric determination of calcium and the comparison of this method with current physical procedures. Plano-parallel polished enamel surfaces were used for all experiments. Areas of 4.5 mm^2 were isolated with tape. Erosion of those areas was induced by treating with HCl (100 μl, pH 2.0, 2.3 or 2.6).The acid was continuously stirred using a pipette tip. (1) *Short erosion* (5–60 s): Every 5 s a sample of 5 μl was taken to analyse the dissolved Ca with Arsenazo-III-reaction (colorimetric assay, 650 nm). (2) *Long erosion* (240 s): Every 15 s the acid was changed and analysed. The mineral loss was calculated by measuring impression depth changes of a Knoop diamond and by stylus profilometry. These calculated values were compared with the dissolved Ca measured by the Arsenazo-III-method. Enamel dissolution could be calculated by the Ca contents determined in the acid samples. Dissolution of Ca showed a linear correlation with time ($r > 0.995$) and pH of the acid used. The Ca contents correlated with the impression depths of a Knoop diamond during the first 30 s ($r = 0.82$), which showed high coefficients of variation of about 80% after 15 s acid contact. Profilometrically measured enamel loss during 60–240 s erosion time correlated with the dissolved mineral calculated by Ca determination ($r > 0.94$). Reproducibility, linearity and accuracy of the Ca determination were better than the results obtained with Knoop diamond indentation and profilometry. In conclusion, quantification of dissolved enamel in 5 μl fractions by Ca detection with the colorimetric assay was linear in the range of 5–30 s acid contact. For short time erosion this method is superior to diamond indentation and profilometry.

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Erosive Effect of Acidic Drinks and Acids on Bovine Enamel in vitro

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High consumption of soft drinks and fruit juices, eating disorders and gastric reflux are known to be important factors in the etiology of dental erosions. The aim of this study was to investigate the erosive effect of three acid beverages, two acidic components of these drinks and HCl (gastric juice). Bovine anterior teeth were sectioned to obtain four pieces (specimens) large enough to locate a circular enamel window (4 mm diameter). Nail varnish was used to block out the surrounding tooth substance. Seventy two specimens were randomly divided into six groups: cola drink (pH 2.6), orange juice (pH 3.9), grapefruit juice (pH 3.4), phosphoric acid (adjusted to same pH as cola), citric acid (adjusted to same pH as grapefruit juice) and 0.01 *M* HCl (pH 2.1). Each specimen was subsequently subjected to 3 ml of the different drinks/acids stepwise for 4 + 4 + 4 min. Then the drinks/acids were analysed for calcium by atomic absorption spectroscopy. Another 12 specimens were ground flat before exposure to HCl to compare with the unground, natural enamel surfaces. Ca release ($\mu g/mm^2$) was highest for orange juice (mean 64.8 ± 2.20 SD) and lowest for citric acid group (4.37 ± 1.93). Ca release for cola, grapefruit juice, phosphoric acid and HCl was 9.24 ± 1.43, 30.3 ± 4.08, 25.0 ± 1.60 and 49.4 ± 8.29 respectively. There were significant differences ($p < 0.05$) between all the groups. There was no difference in Ca release for ground and unground enamel surfaces. Although the pH was nearly the same for citric acid and the 2 juices, the Ca release varied greatly. When comparing a cola drink with phosphoric acid with the same pH, the Ca release was highest in the pure acid. These results support the concept that pH alone is an unreliable factor in classifying the erosive potential of acidic beverages.