

Effects of Calcium on the Erosive Potential of Acidic Candies in Saliva

T. Jensdottir^{a, b} B. Nauntofte^a C. Buchwald^c A. Bardow^a

^aDepartment of Oral Medicine, Faculty of Health Science, University of Copenhagen, ^bToms Group, and

^cDepartment of Otolaryngology, Head and Neck Surgery, Rigshospitalet, Copenhagen, Denmark

Key Words

Critical pH · Dental erosion · Erosive potential · Food modification · Human saliva

Abstract

Theoretical calculations have shown that acidic candies may be potentially erosive upon consumption. However, little is known about the protective effect of adding calcium to potentially erosive candies and about the protective effects of saliva that cannot be fully accounted for by theoretical calculations. Therefore, the aims of this study were to (1) determine the erosive potential of acidic candies with and without calcium and (2) to determine differences between theoretically calculated erosive potential and actual erosive potential in saliva. Twenty healthy test persons sucked acidic candy with and without calcium while their whole saliva was collected into a closed system at different times: baseline, candy-stimulated, and post-stimulated. The erosive potential of the candy was evaluated from candy-induced changes in saliva degree of saturation with respect to hydroxyapatite (HAp) and directly by dissolution of HAp crystals in candy-stimulated saliva. The results showed that similar salivary stimulation was obtained with both candies. The modified candy released more than 13 mmol/l of calcium into saliva, resulting in a lower critical pH, and considerably lower erosive potential than the control ($p < 0.001$). Although a significant correlation was obtained between theoretical calculation of DS_{HAp} and dissolution of HAp crystals ($r_s = 0.65$; $p < 0.001$), many samples obtained by sucking modified candy showed no signs of HAp dissolution in spite of being un-

dersaturated. We conclude that saturation levels and critical pH may not fully reflect when dental erosion is expected to occur in saliva and that calcium addition reduces the erosive potential of acidic candies.

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Dental erosion is the chemical wear of dental hard tissue without involvement of bacteria [Eccles, 1979] and is often caused by extrinsic factors such as frequent consumption of acidic soft drinks [Johansson et al., 1997; Jensdottir et al., 2004]. The degree of soft drink-induced erosion is related to the properties of the drinks consumed [Larsen and Nyvad, 1999; Jensdottir et al., 2005a] as well as drinking frequency and drinking habits [Johansson, 2002; Shellis et al., 2005]. Another extrinsic factor for dental erosion may be acidic foodstuffs and food supplements [Giunta, 1983; Grobler et al., 1989] as the few existing studies on such foodstuffs have shown that they also have erosive potential [Holloway et al., 1958; Bibby and Mundorff, 1975; Lussi et al., 1997; Jensdottir et al., 2005b, 2006b]. However, determining the erosive potential of solid acidic foodstuffs is more difficult than for soft drinks. Thus, solid acidic foodstuffs such as candies and lozenges first have to be dissolved in saliva to release their acidic compounds and thereby become erosive. In this case saliva becomes a matrix for the individual compounds released from the foodstuffs and saliva may thereby play a more important role for the effect of these foodstuffs on teeth than what is the case for soft drinks. The salivary variables that may affect the erosive poten-

tial of solid foodstuffs include the salivary proteins, which via pellicle forming properties can form a diffusion barrier on tooth surfaces that protects against erosion [Meurman and Frank, 1991; Nekrashevych and Stösser, 2003]. But also saliva buffer capacity [Jensdottir et al., 2005b], as well as fluoride, minerals, and metals originating from drinking water, foodstuffs, and oral care products [ten Cate and Duijsters, 1983; Christoffersen et al., 1987] may protect against dental erosion in saliva. In this complex and highly individually determined biological fluid, simple markers for erosion such as pH and saturation level with respect to hydroxyapatite (HAp) may have little explanative power.

We therefore propose that estimation of the erosive potential of solid foodstuffs requires a direct quantification of HAp dissolution within the candy-mixed saliva to supplement measurements of pH and saturation levels. We hypothesized that the use of simple measures for erosion such as pH and saturation levels may lead to overestimation of the erosive potential of acidic candies in the mouth. Thus the aims of this study were to determine the erosive potential of acidic candies with and without calcium as well as to determine differences between the theoretically calculated erosive potential and the actual erosive potential. To test the erosive potential under different conditions we used acidic candies with and without calcium. Thus, calcium is known to be effective in reducing dental erosion in acidic solutions [Gray, 1962]. The use of calcium-containing candies also allowed us to test the effect of major variations in saliva critical pH.

Materials and Methods

Study Group and Design

Saliva was collected from 20 healthy non-medicated volunteers, 9 males and 11 females, recruited among students and staff at the School of Dentistry in Copenhagen. The test persons neither ate nor drank at least 1 h before the study. The volunteers were on average 25 years (21–29) of age, weighed 73 kg (54–97) and had a height of 177 cm (155–195). They were fully dentate (28–32 teeth), without active caries and did not suffer from taste or masticatory dysfunctions. Prior to the experiments all volunteers gave informed consent to the protocol, which was approved by the Ethics Committee of Copenhagen, Denmark (No. 03-001/03).

Saliva Collection

The experiments were performed during daytime on 2 separate days by use of non-commercial candies without calcium (control) and with 16.5 g of calcium lactate per kilogram candy (modified) equal to a total calcium concentration of 54 mmol/kg. Both candies, weighing on average 5 g, were based on the same

basic recipe consisting of water, isomalt, tartaric acid, strawberry and rhubarb flavour, and all had the same colour. The candies were given in a randomized order and the test persons were blinded as to which candy they were having. Collections of whole saliva were performed as previously described [Jensdottir et al., 2005a]. Briefly, each collection lasted 19 min and was divided into three periods: 5-min baseline (collected every minute into one syringe), 4-min candy-stimulated (collected every half minute into two syringes), and 10-min post-stimulated (collected every minute into two syringes). Saliva flow rates were determined by gravitation as grams per minute, which is almost equivalent to millilitres per minute. After the saliva collection, the samples were stored on ice in individual closed syringes until pH and P_{CO_2} were determined on a blood gas analyser within a period of 30 min [Bardow et al., 2000]. Hereafter a sample was stored at -80°C for further chemical analyses.

Erosive Potential of Saliva

Candy-stimulated saliva from the two collections was mixed and depleted of its CO_2 content by vacuum, agitation, and acidification [Bardow et al., 2000]. After the saliva was depleted of its CO_2 the pH was adjusted with acid (1 M HCl) or base (1 M NaOH) to the lowest pH obtained in response to sucking the candy. After the saliva pH was adjusted, 2 mg of pure lyophilized HAp crystals (Uni-Crystals, Copenhagen, Denmark) was added to each millilitre of saliva equal to a concentration of 2 mmol/l HAp. The crystals were added to the saliva under constant and standardized stirring speed at room temperature with continuous pH recordings at 15-second intervals for 5 min. If saliva pH was constant after addition of HAp, equilibrium was assumed [Patel and Brown, 1975], and the candy was assessed as non-erosive. In case saliva pH decreased, crystallization was assumed, and the saliva was also assessed as non-erosive. However, in case of a pH rise, dissolution was assumed, and the solution was assessed as erosive. In this case, the process continued to quantify the magnitude of erosion in the saliva solution. Thus, immediately after dissolution of HAp (i.e. 5 min after HAp addition) a back titration with acid (1 M HCl) was performed to estimate how much HAp was dissolved. At the salivary pH values obtained by sucking the candies (pH 4.0–4.5) dissolution of 1 mmol of HAp (MW 1,005) on average requires 14 mmol of H^+ (MW 1) due to the reaction: $Ca_{10}(PO_4)_6(OH)_2 \rightarrow 10Ca^{2+} + 6H_2PO_4^- + 2H_2O$. Therefore the use of 14 μl 1 M HCl (i.e. 14 μg H^+) for back titration resembles the dissolution of 1,005 μg HAp and the use of 1 μl 1 M HCl resembles the dissolution of 72 μg HAp. During the experiment the pH increased on average 0.01 units in modified and 0.08 units in control samples as the results of HAp dissolution. Thereby the pH stayed within the range described. Accordingly the amount of HAp crystals lost per minute in the candy-containing saliva was back calculated from the number of microlitres 1 M HCl needed to reach the pH originally obtained upon sucking the candy. From these data the erosive potential was computed as micrograms of HAp lost during candy stimulation.

Saliva Degree of Saturation with Respect to HAp

For each sample the degree of saturation with respect to HAp (DS_{HAp}) was determined for conditions at 37°C [Jensdottir et al., 2005b]. The solubility product for HAp [$Ca_{10}(PO_4)_6(OH)_2$] was set at 117.3 (pK) [McDowell et al., 1977], pKw at 13.6, pK for $H_3PO_4/H_2PO_4^-$ at 2.2, for $H_2PO_4^-/HPO_4^{2-}$ at 7.2, and for $HPO_4^{2-}/$

PO_4^{3-} at 12.2 with dissociation constants corrected for ionic strength [Harned and Owen, 1958]. DS_{HAP} was calculated as $(I_{\text{HAP}}/K_{\text{HAP}})^{(1/18)}$. The critical pH was iteratively estimated as the pH at which I_{HAP} equalled K_{HAP} . Iterations were repeated until the pH used for determination of phosphate differed no more than 0.5% from the estimated critical pH. All calculations were processed as a script in a computer program [R Development Core Team, 2004] allowing for process of multiple samples simultaneously [Jensdottir et al., 2005b].

Statistics

Statistical analyses were done with Excel and with the R statistical program [R Development Core Team, 2004]. Differences between candies were determined by the Wilcoxon's signed rank sum test and differences in distribution by the Fisher test. Correlations were analysed by Spearman's rank correlation analysis with correlation coefficients (r_s) and p values given. Straight lines were used to connect points in figure 1 and cubic regression to fit the curve in figure 3. The level of significance was set at $\alpha = 0.05$.

Results

Figure 1A, B show that the saliva flow rates and pH values obtained in response to sucking the modified and the control candy were nearly similar. On average, 4.0 ml of saliva was produced when sucking 1 g of modified candy, and 3.9 ml while sucking the control candy. Figure 1C illustrates that the salivary phosphorus concentration decreased with increasing flow upon stimulation and returned to pre-stimulatory levels in the post-stimulatory period in both candies. As shown in figure 1D the total calcium concentration was nearly 10 times higher upon sucking modified candy compared with the control candy due to the high amounts of calcium released from the modified candy to the saliva ($p < 0.001$). Figure 1E shows a significant drop in estimated critical pH upon sucking the modified candy ($p < 0.001$), which was due to the high calcium content in the modified candy. In contrast the control candy induced a slight increase in the critical pH due to a reduced saliva phosphorus concentration upon stimulation. Figure 1F shows that the saliva became undersaturated with respect to HAp upon sucking the control candy while the modified candy only induced a slight saliva undersaturation during the candy-stimulated period ($p < 0.001$). This suggests that the control candy had erosive potential whereas the modified candy theoretically only would be slightly erosive.

Figure 2 illustrates the DS_{HAP} and the erosive potential as assessed by HAp dissolution. DS_{HAP} was on average 1.5 while sucking the modified candy but only 0.58 while sucking the control candy. Consequently, the HAp dis-

solution experiment showed that the control candy had much higher erosive potential than the modified candy, which was only slightly erosive during the candy-stimulated period ($p < 0.01$). Thus 14 test persons did not experience any dissolution of HAp while sucking the modified candy compared to only 1 while sucking the control candy ($p < 0.001$).

Figure 3 shows the relation between $\log \text{DS}_{\text{HAP}}$ and the erosive potential determined by HAp dissolution in saliva while sucking modified and control candy. As shown the actual dissolution of HAp crystals showed a good correlation with the theoretically determined degree of saturation ($r_s = -0.65$; $p < 0.001$) in all samples and in the control candy only ($r_s = -0.53$; $p < 0.05$). However, no significant correlation was obtained between DS_{HAP} and actual erosive potential in the modified candy only. Furthermore, when sucking modified candy, 10 samples did not show any signs of HAp dissolution upon testing in spite of being undersaturated with respect to HAp, and thus having pH values lower than their critical pH.

Discussion

This study has shown that calcium addition to the degree used in this study may not change the saliva stimulatory effect of candy. The high saliva flow rates obtained with both candies normally suggests a high salivary buffer capacity due to a high bicarbonate concentration [Jensdottir et al., 2005b]. Therefore the physiological protection from saliva flow and its buffer capacity against the acidic challenge from the two candies was assumed similar. This finding allowed us to estimate the isolated effect of adding calcium to candies. Given that each gram of modified candy contained 16.5 mg of calcium lactate the estimated amount of calcium in saliva with a saliva production of 4.0 ml/g became 4.125 mg/ml equal to a total concentration of 13.4 mmol/l. To this value the background contribution from saliva of 1.6 mmol/l (obtained with control) had to be added, giving rise to 15.0 mmol/l of calcium in total. As the actually measured average calcium concentration during the stimulated period was 15.1 mmol/l, the theoretical retrieval of the released calcium from the modified candy in the saliva was around 100%.

Although many different substances were potentially interesting for modification [Grenby, 1996], especially calcium and/or phosphate have been shown to be effective [Hughes et al., 1999; Jensdottir et al., 2005a]. Calcium was chosen because a calcium concentration in the range

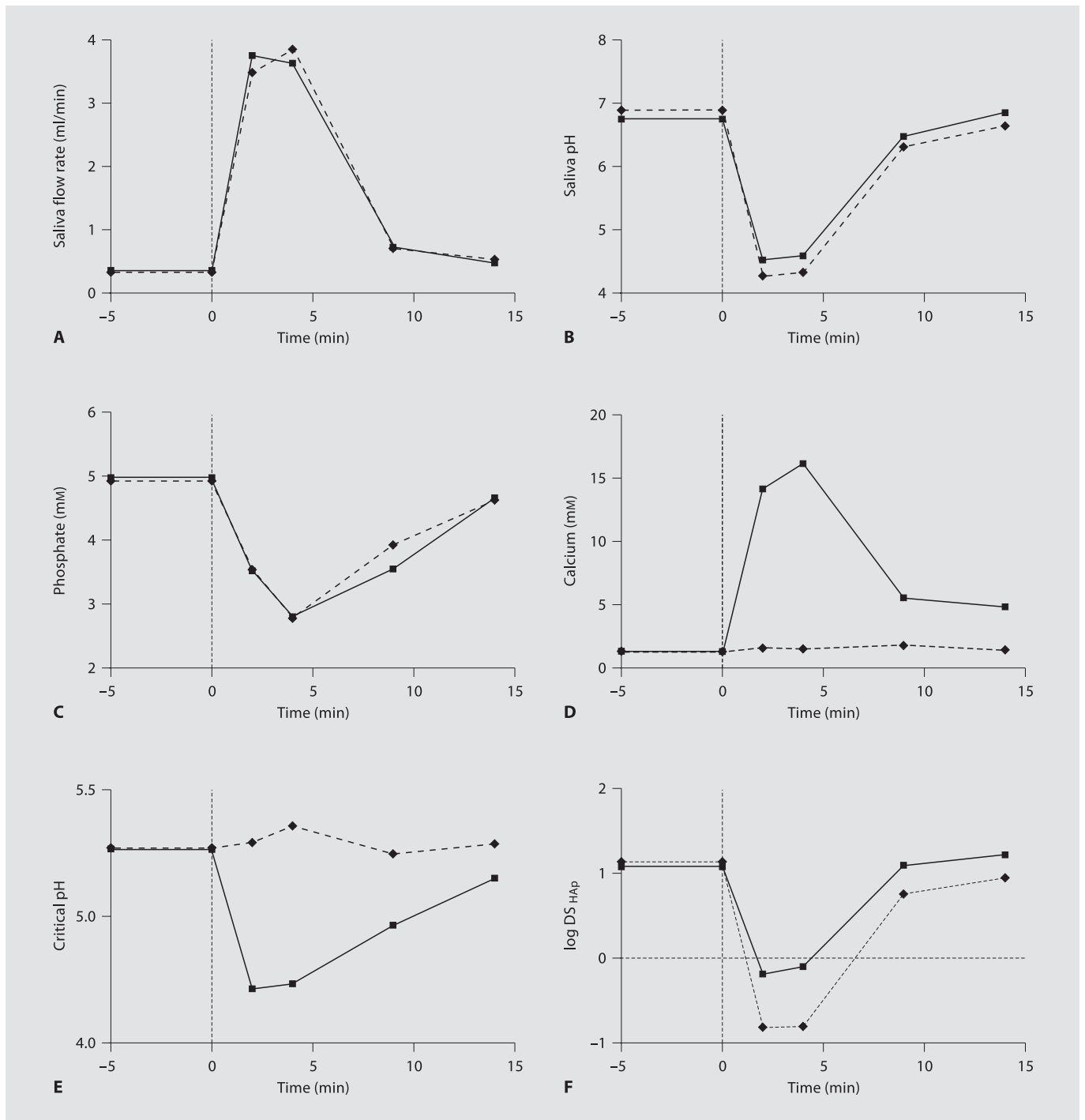


Fig. 1. Effects of sucking acidic candies with (modified) and without (control) calcium on the composition of human saliva. Black continuous lines represent the modified candy and dotted lines the control candy. Vertical grey dotted lines represent the starting of candy stimulation. **A** Saliva flow rate upon stimulation. **B** Salivary pH. **C** Salivary phosphorus concentration. **D** Salivary calcium concentration. **E** Salivary critical pH. **F** Salivary DS_{HAP} (presented as $\log DS_{HAP}$). The area below the horizontal line in **E** is where the saliva is undersaturated with respect to HAP and erosion is likely to occur.

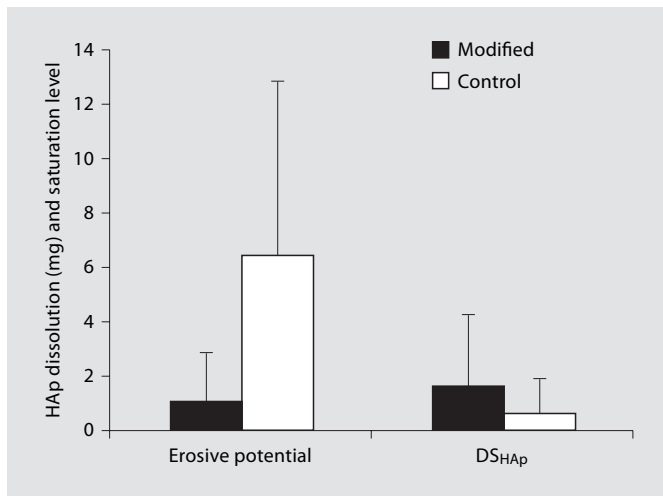


Fig. 2. Effects of sucking acidic candies with (modified) and without (control) calcium on the composition of human saliva. Black columns represent the modified candy and grey columns the control candy during the candy-stimulated period. Left columns show the erosive potential as assessed by HAp dissolution directly in pooled saliva from the two candy-stimulated collections and right columns the DS_{HAp} in stimulated saliva upon sucking modified and control candy. The bars represent the standard deviation.

of 10–25 mmol/l has been shown to be considerably more effective than a similar phosphorus concentration in inhibiting erosion at pH values similar to those in candy-stimulated saliva [Gray, 1962]. In contrast to calcium, which is relatively tasteless, phosphorus may also give a metallic and undesirable taste to foodstuffs [Jensdottir et al., 2005a]. In agreement with the past in vitro results [Gray, 1962] the calcium concentration of around 15 mmol/l obtained in this study significantly reduced the actual erosive potential of the acidic candy, which decreased more than 6 times in response to calcium addition. Thus, in the majority of subjects no sign of erosive potential was observed while sucking the modified candy.

A good correlation was obtained between the actual dissolution of HAp crystals and DS_{HAp}, indicating that predictive calculations of erosive potential to some extent can also be performed for saliva. However, the difference between the calculated degree of saturation with respect to HAp and the measured erosive potential upon sucking acidic candy with and without calcium were considerable. These findings support the fact that salivary proteins have a significant protective effect against acid-induced dental erosion and that this protective effect cannot be accounted for theoretically. Along this line

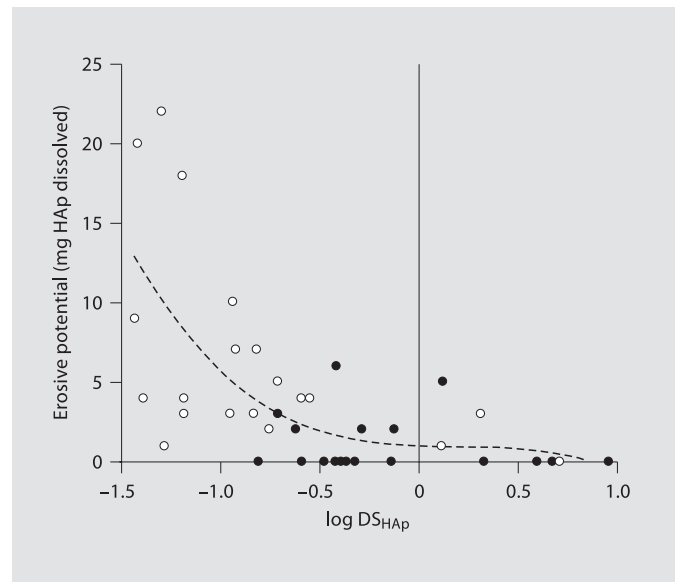


Fig. 3. Relation between DS_{HAp} (log DS_{HAp}) and actual dissolution of HAp crystals within the candy-stimulated saliva. Open circles represent control candy and filled circles modified candy. The vertical grey line shows where the saliva is saturated with respect to HAp.

considerable protective effects from salivary proteins against soft drink-induced dental erosion have previously been shown [Meurman and Frank, 1991; Jensdottir et al., 2006a]. In the case of acidic solid foodstuffs the protective effects of saliva may be even greater as not only the saliva proteins in the form of a pellicle on tooth surfaces, but also fluoride, minerals and metals from the mucosa and food debris may come in contact with tooth substance and protect against dissolution while sucking the candy. Consequently, the theoretical erosive potential as judged from saliva pH and DS_{HAp} may not fully reflect when erosion is likely to occur in the mouth. Thus in saliva, undersaturation may show that erosion can happen, but not necessarily that erosion will happen. Therefore, the critical pH, which is often referred to as a fixed value of pH 5.5 [Schmidt-Nielsen, 1946], may not fully reflect when erosion is likely to occur in saliva.

The variable concept of critical pH discussed by Dawes [2003] was also very much proven in this study. Thus, calcium addition to the candy made it possible to decrease the critical pH by more than half a pH unit. This decrease in critical pH happened while the subjects were maintaining nearly the same saliva flow rates as with the control candy and without any self-reported difference in

taste. Therefore, under extreme conditions the critical pH in human whole saliva may range from well above pH 6 [Dawes, 2003] to well below pH 5 without affecting normal physiological functions such as taste and the rate of saliva secretion.

In conclusion, this study has shown that calcium addition can be used to give a major reduction in erosive potential of hard-boiled candies in healthy test persons. Furthermore, this study has shown that actual erosion in the form of HAp dissolution may not necessarily occur when saliva drops below the critical pH of 5.5 for a short period of time.

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