The Erosive Potential and Modification of Acidic Foodstuffs

From Laboratory to Clinic

By
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Industrial Ph.D. thesis
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PREFACE AND ACKNOWLEDGEMENTS

This thesis is a part of an Industrial Ph.D. degree that was conducted from November 2002 to November 2005. During these three years I worked in conjunction with Toms Group A/S and the Department of Oral Medicine, Clinical Oral Physiology, Oral Pathology and Anatomy, University of Copenhagen, both in Denmark. Collectively, the work was performed under the supervision of Dr Birgitte Nauntofte (Head supervisor) and Dr Allan Bardow (Project supervisor), University of Copenhagen; Dr Christian Buchwald, University Hospital of Copenhagen (Rigshospitalet); Dr Anni Rasch, Dr Astrid Bork Andersen and the Chief Financial Officer Henrik Frisch at Toms group A/S. I would like to thank all of them for creating an encouraging atmosphere, their constructive guidance and never failing interest and enthusiasm during this study. Further, my sincere thanks are due to Dr Peter Holbrook, University of Iceland, a former supervisor and an invaluable colleague, for many fruitful discussions, support and encouragement throughout the project. Dr Hanne Sand Hansen, University Hospital of Copenhagen is sincerely thanked for the referral of the patients and for useful discussion. I wish to thank all the test persons who participated in this study and thus made this work possible. Mrs Joan Lykkeaa is thanked for the invaluable help in the laboratory. Dr Carsten Thomsen, Mrs Bente Nielsen and Mrs Pia Bast are thanked for their assistance with computer-, accounting- and office issues, respectively. Co-workers at the Department of Oral Medicine are thanked for their help in the daily work and for creating a pleasant atmosphere. I would like to thank my colleagues at Toms Group A/S, for good co-operation, helpfulness, many cozy discussions and for creating a family-like atmosphere. Furthermore, Toms Group A/S steering committee is especially thanked for giving me the opportunity to conduct this study and the freedom to work outside the box.

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During the three-year PhD period, a minimum of 200 hours of obligatory courses at graduate level have been completed, teaching at the Dental School, University of Copenhagen, presentations of the scientific work at eight scientific meetings worldwide, and other oral and written communication have been conducted. Further, obligatory business education has been part of the industrial Ph.D. program and accordingly an obligatory business report has been written. In addition to the more conventional scientific articles and abstracts, this study generated a Patent application, filed July 2004 (Jensdottir T, Bardow A, Nauntofte B, Buchwald C: Acidic solid oral composition without erosive potential in salvia and method for determining the erosive potential in saliva. Patent No: PA 2004 011148). According to the PhD study rules and regulations at the University of Copenhagen: “If the Ph.D. student has written more than one article, these must be incorporated in the thesis. The thesis must be accompanied by an account of the articles’ publishing status. This account should not normally exceed 30 pages.” This present thesis is written as a general overview and the reader is kindly referred to the scientific work presented in I-VI (see below) concerning more detailed information on study design, materials, methods, results and discussions.

This thesis is based on the following six articles, which will be referred to in the text by their Roman numerals (The manuscripts V and VI have been updated by December 2009).


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1. GENERAL INTRODUCTION

1.1 Dental erosion and related diseases
Dental erosion is the chemical wear of the dental hard tissues without the involvement of bacteria, and is therefore unlike dental caries in both etiology and pathogenesis [Eccles, 1979]. Collectively, the tooth-wear diseases comprise, dental erosion, dental attrition (wear of tooth surface against tooth surface) and abrasion (wear of teeth from objects other than teeth). An identical feature of these tooth-wear diseases is that they are chronically destructive to the dental hard tissues, involving a tooth surface destruction, causing irreversible loss of tooth enamel and subsequently dentine. Dental erosion is often misdiagnosed as dental attrition and/or abrasion and is clinically challenging to diagnose [Bartlett, 2005]. Dental erosion may cause increased sensitivity to heat, cold and even provoke pain when severe.

1.2 Etiology and prevalence of dental erosion
Dental erosion can be caused by intrinsic and/or extrinsic factors. Gastro-esophageal reflux [Meurman et al., 1994; Shaw et al., 1998; Barron et al., 2003; Oginni and Olusile, 2005], low salivary flow [Jarvinen et al., 1991; IV; VI], low salivary buffer capacity [Hellstrom 1977; Rytomaa et al., 1998] and frequent vomiting [Bargen and Austin, 1937; Jarvinen et al., 1992] are considered intrinsic factors. Extrinsic factors are dietary, such as frequent and high consumption of acidic foodstuffs including citrus fruits [Grobler et al., 1989], some vegetarian diets [Linkosalo and Markkanen et al., 1985; Al-Dlaigan et al., 2001], infant fruit drinks [Grenby et al., 1990], other acidic beverages [Gedalia et al., 1991a,b; Millward et al., 1994; Johansson et al., 1997; Al-Malik et al., 2001] and acidic candies [Lussi et al., 1997]. Excessive wine consumption or wine tasting [Chaudhry et al., 1997; Gray et al., 1998] and medications such as vitamin supplements [Giunta., 1983; Al-Malik et al., 2001] are also extrinsic factors. Patients with eating disorders may have erosion due to both intrinsic- and extrinsic etiological factors through repeated purging and/or frequent consumption of acidic foodstuffs such as citrus fruits and “diet” soft drinks [Andrews, 1982; Robb et al., 1995; Rytomaa et al., 1998, Bidwell et al., 1999]. Dental erosion of unknown origin is denoted as idiopathic erosion.
Dental erosion is found in all age groups [Lussi et al., 1991; Bartlett et al., 1998; Jensdottir et al., 2004a]. Its prevalence is high and gradually increasing, especially in children and adolescents [Jones and Nunn, 1995; Nunn et al., 1996; Nunn et al., 2003; Downer, 2005]. This can partly be explained with the fact that demineralization of the tooth enamel is more likely to occur in children than in...
adults [Hunter et al., 2000a+b; Anderson et al., 2001] and that soft drink consumption, that is widely recognized as the major risk factor [Jarvinen et al., 1991; Lussi et al., 1991; Milosevic et al., 1997; Jensdottir et al., 2004a], is particularly common among teenagers [Forshee and Storey, 2003; Kassem et al., 2003; Kassem and Lee, 2004].

It has been shown that acidic foodstuffs are very effective salivary stimulants [III-VI], and accordingly, patients who suffer from dry mouth are likely to often consume such products (acidic hard boiled candies) for oral relief. In addition, children are generally known to consume fair amount of acidic foodstuffs in form of juices, carbonated drinks and acidic candies such as lollipops. Consequently, it has become important to know the erosive potential of commercially available acidic foodstuffs such as soft drinks and acidic hard-boiled candies.

1.3 Erosive potential

In this thesis, erosive potential is defined as the potential of any fluid, including saliva, to dissolve tooth substance. However, in the dental literature this term is mostly used in relation to foodstuffs that may cause tooth dissolution. Various methods have been used to determine the erosive potential of soft drinks and other foodstuffs, but no golden method is known. Generally, the existing methods include: determination of the weight loss from tooth pieces to measure the mineral loss after exposure to acidic substances such as soft drinks, softening of tooth surfaces after exposure to soft drinks (measured by using the Knoop hardness test) [Lussi and Hellwig, 2001; Van Eygen et al., 2005], mineral loss from tooth surfaces after exposure to soft drinks; (quantitative microradiography) [Maupome et al., 1998], surface changes of enamel and dentine surfaces after exposure to the foodstuff (electron microscopy) [Azzopardi et al., 2001; Hooper et al., 2004; Venables et al., 2005]; and quantification of released calcium and/or phosphate from teeth exposed to the foodstuff [Grobler et al., 1990]. In addition, determinations of the pH and titratable acid have often been used as indirect measures of the erosive potential of the acidic foodstuffs [Lussi et al., 1995]. The erosive potential of a wide range of soft drinks and acidic foodstuffs has been determined and presented in the literature. Various in-vitro studies have shown fruit juices to have high erosive potential [Lussi et al., 1995; Larsen and Nyvad, 1999] while, several epidemiological studies find carbonated beverages rather than fruit juices to be associated strongly with tooth erosion and, therefore, thought to be the main aetiological factor of extrinsically caused dental erosion [Johansson et al., 1997; Milosevic 1997; Jensdottir et al. 2004a]. Again, other studies have
found both types of drink to be important for dental erosion [Millward et al., 1994; Larsen and Nyvad, 1999].

Surprisingly, to our knowledge, only few experimental [Holloway et al., 1958; Bibby and Mundorff, 1983] and clinical [Lussi et al., 1997] studies have been published on the erosive potential of semi-solid and solid acidic foodstuffs. This can partly be explained by the fact that no acknowledged method has been available to test the erosive potential of solid and semi-solid foodstuffs. Thus, compared to testing acidic soft drinks, the testing of the erosive potential of semi-solid and solid foodstuffs is much more complicated, as it involves collecting the saliva from the mouth upon sucking acidic foodstuffs. Consequently, little is known about the erosive potential of hard-boiled candies in both healthy individuals and dry-mouth patients. This may be concerning as dry-mouth patients are likely to use acidic candies for saliva stimulation and thus for oral relief [Colquhoun and Ferguson, 2004]

1.4 pH, titratable acid and buffer capacity

The pH of any foodstuff refers to its hydrogen concentration. The pH of liquid foodstuffs such as soft drinks can be determined by use of electrodes or by use of colorimetric pH indicators in litmus paper or directly in the fluid. In contrast, the pH of solid foodstuffs is more difficult to obtain, as these have first to be dissolved. Normally, in-vitro dissolution of the solid foodstuff is performed in a 1:1 ratio with Millipore water; the pH is then determined, also denoted as the effective pH [Parker, 2002]. Some solid foodstuffs may need several hours to dissolve and some need to be pulverized or heated to speed up the dissolution process.

Titratable acid corresponds to the normality of an acid, where addition of OH\(^-\) corresponds exactly to the removal of H\(^+\) in the acid being titrated [Siggaard-Andersen, 1963]. In other words the titratable acid is the volume of base needed to obtain a given pH in the fluid. In the erosion literature, a typical titration with 1M NaOH takes place at room temperature, to a predefined pH, most often the average critical pH of human saliva, which is pH 5.5. For a given experiment it is important to know the volume of the fluid that has been titrated and the concentration of base used. Values for the titratable acid are mostly given in ml 1M NaOH if the volume of titrated drink has been around 50-100 ml. This value may, however, be given in other units depending on the volume titrated. The higher the pH to which the soft drink is titrated, the higher the titratable acid becomes (with certain volume and concentration of base). In other words, titratable acid is the expression for the amount of base used to titrate an acidic fluid (or the amount of acid used to titrate alkaline fluid, denoted as titratable base).
to a given pH. Based on these descriptions the term titratable acid will be used individually hereafter. When the titratable acid is known for a given pH interval, the buffer capacity of the drink can also be calculated as:

$$\text{Buffer capacity} = \frac{\Delta \text{base}}{\Delta \text{pH}}$$

Where $\Delta \text{base}$ denotes the molar concentration increase in base (titratable acid) and $\Delta \text{pH}$ denotes the pH change [van Slyke, 1922]. If a large amount of base results in only a minor pH change the buffer capacity is high and vice versa. Accordingly, the titratable acid is strongly related to the buffer capacity. However, the buffer capacity can also be calculated when the concentration of buffers in a fluid is known [van Slyke, 1922], whereas the titratable acid needs to be experimentally determined by titration.

1.5 Role of saliva in dental erosion

Human whole saliva has been shown to have protective effect against mineral loss of tooth substances in-vitro and in-situ [Hall et al., 1999] and is accordingly recognized as an imperative factor in relation to protecting the tooth substance against dental erosion. A previous study has found individuals with reduced salivary flow rate (<0.1 ml/min) to be up to five times more likely to have dental erosion than individuals with higher flow rate, thus salivary flow may be particularly important in protecting against dental erosion [Jarvinen et al., 1991]. Individuals with a low salivary flow rate also often have low salivary buffer capacity [Pedersen et al., 2005]. This may also affect their susceptibility to dental erosion, as individuals with low buffer capacity have been shown to be at risk of developing dental erosion [Meurman et al., 1994]. Additionally, salivary protein which forms the basis of acquired pellicle on the enamel, is known to protect tooth enamel [Meurman and Frank, 1991; Amaechi et al., 1999; Nekrashevych and Stosser, 2003]. Therefore, data on salivary status may help to determine individual susceptibility to developing dental erosion [Jarvinen et al., 1991].

1.5.1 Salivary secretion and flow

Xerostomia is the subjective sensation of dry mouth, whereas hyposalivation refers to an objective measurement of a low salivary flow rate [Nederfors, 2000]. The objective measurement can be obtained by various methods such as the drooling method and the spitting method [Navazesh and Christensen, 1982]. If the salivary flow rate in the unstimulated state is less than 0.1 ml/min and in
the stimulated state less than 0.5 ml/min, the diagnosis of hyposalivation is given. The whole saliva that is collected by such methods is mainly produced from three major salivary glands: the parotid, the submandibular and the sublingual gland. Together they produce about 90% of the whole saliva and the minor salivary glands in the oral mucosa produce the remaining 10% [Ferguson, 1975; Bardow et al., 2004b]. Unstimulated saliva is mainly produced by the submandibular gland. During acid stimulus, the parotid and submandibular glands contribute almost equally and produce most of the saliva, while during mechanical stimulus the saliva is mainly produced by the parotid gland [Schneyer, 1956; Dawes and Wood, 1973; Ferguson, 1975]. Efficient dilution, which is driven by the flow of saliva, is crucial to rinse substances from the oral cavity and protect the oral tissues. The average unstimulated salivary flow is normally 0.2 to 0.5 ml per minute [Heintze et al., 1983]. The average flow of stimulated saliva varies between 1 and 2 ml on average per minute when stimulated by chewing a flavorless substance such as paraffin, parafilm, non-tasting gum etc. The stimulated salivary flow rate can, however, become as high as 5-10 ml per minute when stimulated by a strong acidic taste from acidic foodstuffs or citric acid solutions [Watanabe and Dawes, 1988a,b].

1.5.2 Oral clearance

Oral clearance refers to the elimination of substances from the mouth and thus dependent on the salivary flow. When measuring oral clearance, the disappearance of a substance, normally sugar, from the mouth is determined as a function of time. Two factors, in particular, are important for oral clearance: the salivary flow rate and the frequency of swallowing [Lagerlöf and Dawes, 1985]. Oral clearance after rinsing with citric acid has been shown to be highly dependent on the individual [Bashir et al., 1995]. Slow oral clearance refers to the situation where the substance is present in the mouth for a long period of time (minutes or hours) after ingestion. Such a situation is almost always caused by a low salivary flow rate, and especially a low unstimulated salivary flow rate [Dawes, 1983]. If the oral clearance is slow, acid can accumulate in the mouth and the destructive process of dental erosion is accelerated. The destructive process depends on the type of food- in question and its stimulatory effect on flow and thus how much saliva is produced upon eating or drinking the particular foodstuff. Some foodstuffs have certain physical properties, measured as thermodynamic property of adhesion, which makes them adhere to the tooth substance [Ireland et al., 1995]. Accordingly, the clearance of such foodstuffs will be prolonged. In healthy individuals with normal salivary flow it has been proposed that erosive damage will be limited to the time of ingestion of acidic foodstuffs because of the rapid clearance of acid [Ericsson, 1953]. Thus, the ability to produce
saliva is important for protection against dental erosion, especially with respect to clearing acidic compounds.

1.5.3 Salivary pH and salivary buffer capacity

Salivary pH is one of the most fundamental factors when determining the degree of saturation with respect to hydroxyapatite in saliva. Thus if the salivary pH is not measured precisely as it occurs in the oral cavity the whole determination of the degree of saturation with respect to hydroxyapatite becomes incorrect. Thus, to measure the erosive potential of solid or semi-solid foodstuffs (Specifically in this case: acidic hard-boiled candies) in-vivo, human whole saliva produced upon sucking the acidic candy must be collected without any changes in the salivary pH. However, this is challenging, as the physiology and chemistry behind the salivary pH regulation and buffer capacity is complicated. Bicarbonate, phosphate and protein buffer systems are the three major buffer systems in human whole saliva and their concentrations depend heavily on the secretion rate of saliva [Lilienthal, 1955; Ericsson, 1959; Tenovuo 1997; Bardow et al., 2000a]. Of these three buffer systems, bicarbonate is the primary buffer of human saliva [Izutsu, 1981; Bardow et al. 2000a]. The bicarbonate concentration varies significantly from around 5 mM in unstimulated whole saliva, at a flow rate of 0.5 ml/min [Bardow et al. 2000a] up to 25 mM in stimulated whole saliva, at flow rates above 2 ml/min [Grön and Messer 1965]. The equilibrium for the bicarbonate buffer system is as follows:

\[ CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \]

Where \( CO_2 \) is carbon dioxide, \( H_2CO_3 \) is carbonic acid, and \( HCO_3^- \) is bicarbonate. The average pK of carbonic acid in human whole saliva is 6.15 [Bardow et al., 2000b]. Within the normal pH range of human whole saliva, which is from 6-7.5, the equilibrium is normally shifted to the right due to the pK value of carbonic acid. But the equilibrium will be pushed to the left when the pH of saliva is decreased by the consumption of acidic candies. Thus at pH 4.5, the buffering effect involves a shift from bicarbonate to \( CO_2 \), leaving almost the entire bicarbonate buffer system in the \( CO_2 \) form. Therefore, the \( P_{CO_2} \) will increase as the pH decreases and the equilibrium of the buffer system is pushed to the left. Any loss of the \( CO_2 \) from saliva will cause the pH to rise again and push the buffer system back to the right side of the equilibrium. Therefore, saliva must be collected without loss of \( CO_2 \) in order to avoid pH changes. The amount of \( CO_2 \) loss from saliva is dependent on the partial pressure of carbon dioxide (\( P_{CO_2} \)) in the surroundings and in the saliva. \( P_{CO_2} \) in the
atmosphere is 4 Pa. However, in a completely closed system, the $P_{CO2}$ of stimulated saliva that originally contains 25 mM HCO$_3^-$ and is then acidified to pH 4.0 upon sucking acidic candy becomes 110 kPa [Siggaard-Andersen, 1963], thus more than 25000 times greater than the $P_{CO2}$ of around 4 Pa in the surroundings [III]. Therefore, the greatest challenge when determining the erosive effect of acidic candies in-vivo is the enormous difference in $P_{CO2}$ between the stimulated whole saliva and the surroundings. Any loss of CO$_2$ from the saliva will cause a rapid pH rise that will discard the computations of the degree of saturation with respect to hydroxyapatite and give rise to an underestimation of the erosive potential of the acidic candies. To prevent this, it is important to collect the human whole saliva in a closed system. After the salivary pH has been determined, the titratable base (normally determined by titration with 1 M HCl) can be determined in a similar manner to that of the soft drinks (section 1.4). However, to avoid any loss of CO$_2$, the titration of the saliva has to be performed in a closed, CO$_2$-tight system. After the titration, the buffer capacity can be calculated as the relation between the titration-induced increase in acid concentration and the change in pH as described for the soft drinks (section 1.4) [Lilienthal, 1955; Bardow et al., 2000a]. Human saliva, and especially stimulated human saliva, can have a considerable buffer capacity, which may exceed the buffer capacity of viscous substances as milk [Bardow et al., 2004b]. However, the buffer capacity of human saliva may be considerably less than in some animals [Ericsson, 1962]. Therefore humans may be more susceptible to develop tooth demineralisation such as erosion because their oral pH often balances close to their critical pH.

1.5.4 Degree of saturation with respect to hydroxyapatite
The degree of saturation of saliva with respect to hydroxyapatite (DS$_{HAp}$) is determined from the ionic and the solubility product of hydroxyapatite in human saliva and is decisive in relation to dental erosion. Theoretically, when saliva is undersaturated, erosion is expected to occur, but if saturated or supersaturated, erosion is not expected to occur.

Tooth enamel is composed of hydroxyapatite crystals (Ca$_{10}$(PO$_4$)$_6$(OH)$_$_2$) that are normally surrounded by a moist environment in the mouth. The fluid in the mouth is mostly composed of saliva also comprising a huge number of bacteria as well as desquamated epithelial cells. However, after a meal, the fluid in the mouth may also contain remains of the consumed food and drink. It is crucial for the teeth that the fluid surrounding them is saturated with respect to hydroxyapatite. Theoretically, the degree of saturation with respect to hydroxyapatite depends on the ionic product
for hydroxyapatite ($I_{\text{HAp}}$) in the fluid and the solubility product for hydroxyapatite ($K_{\text{HAp}}$). The solubility product is a constant that is determined experimentally from the ionic product in a solution that has been saturated with respect to HAp. Thus, in the laboratory, powdered tooth substance or pure HAp crystals are allowed to equilibrate with large volumes of water at various pH values and with various background compounds in the solutions. When the HAp is entirely in equilibrium with the fluid mostly determined at a constant pH [Patel and Brown, 1975], a sample of the water is taken and analyzed for its activities of calcium, phosphate, and hydroxy ions. From the activities of these ions the ionic product of HAp can be calculated by the formula:

$$I_{\text{HAp}} = (\text{Ca}^{2+})^{10} \times (\text{PO}_4^{3-})^6 \times (\text{OH}^-)^2$$

If the solution is exactly in equilibrium then this ionic product will represent the solubility product of the compound; here HAp. Numerous determinations of the solubility product of HAp have been made. These include, for example, determinations of the product in human teeth, bovine teeth, bone, experimentally formed HAp crystals, heat pretreated HAp crystals and tooth substance [Patel and Brown, 1975; McDowell et al., 1977]. In this context, the solubility product has been shown to vary between individuals, species, and with heat pretreatment. Apart from varying between species and with pretreatment the solubility product is a thermodynamic constant, meaning that it is affected by temperature [Patel and Brown, 1975; MacDowell et al., 1977]. However, in the dental literature, the solubility product is generally recognized as a fixed value near 117.3 (pK$_{\text{HAp}}$). When the solubility product of HAp is known, it becomes possible to calculate the level of saturation of almost any fluid that could come into contact with teeth.

If the $I_{\text{HAp}}$ of a fluid is equal to or higher than $K_{\text{HAp}}$ the fluid is saturated or supersaturated with respect to hydroxyapatite and no dissolution of hydroxyapatite (tooth substance) will occur. However, if $I_{\text{HAp}}$ is less than $K_{\text{HAp}}$ the fluid is undersaturated with respect to HAp and dissolution will occur. Generally, the relation between the ionic product and the solubility product is expressed as a value for saturation ($D_{\text{HAp}}$):

$$D_{\text{HAp}} = (I_{\text{HAp}}/K_{\text{HAp}})^{1/18}$$

In this equation, the 18 refers to the total number of anions and cations in the salt [Schmidt-Nielsen, 1946]. If $I_{\text{HAp}}$ is smaller than $K_{\text{HAp}}$, $D_{\text{HAp}}$ will be between 1 and 0, mostly expressed as a percentage.
of saturation (i.e. 0.5 equals 50% saturated). If $I_{\text{HAp}}$ is equal to or higher than $K_{\text{HAp}}$, then $DS_{\text{HAp}}$ will be $\geq 1$, which is mostly expressed as how many times the solution is saturated (i.e. 2 equals 2 times saturated). Thereby this way of calculating $DS_{\text{HAp}}$ relates directly to the saturation level in the fluid. However, because this measure for $DS_{\text{HAp}}$ is logarithmic, supersaturation expands over a much wider range of numbers than undersaturation, which is limited within the range of 0.00-0.99. Therefore more linear measures for $DS_{\text{HAp}}$ have also been developed [Larsen and Pearce, 2003] expressing $DS_{\text{HAp}}$ as:

$$DS_{\text{HAp}} = (pK_{\text{HAp}} - pI_{\text{HAp}}) / 18$$

Where the number 18 also refers to the total number of anions and cations in the salt, and $p$ denotes the potency of the solubility and ionic products (i.e. $-\log$). This expression generates a linear measure for $DS_{\text{HAp}}$ with 0 as the level of exact saturation, and this measure has also become widely used in dental research. Regardless of which measure is used, the activity level of free calcium, phosphate, and hydroxy ion activity in the fluid $I_{\text{HAp}}$ is calculated, thus leading to the estimation of the saturation level. However, as calculation of $I_{\text{HAp}}$ is dependent on the activities of phosphate and hydroxy ions, which are especially sensitive to pH (decreasing 10 times per unit below pH 10), pH often becomes the most determining factor for the saturation level. Therefore any changes in pH will disrupt the computations of the degree of saturation with respect to hydroxyapatite. This is why it is necessary to take measures to keep the original pH of the fluid to be analyzed for degree of saturation with respect to HAp.

### 1.5.5 Critical pH

The average critical pH of human saliva is frequently referred to in the literature as a fixed value of pH 5.5 [Schmidt-Nielsen, 1946; Milosevic et al., 1997; Barron et al., 2003] and thus often used as a threshold value for the erosive versus non-erosive potential in the oral cavity. From a chemical point of view critical pH represents the pH at which the ionic product of HAp ($I_{\text{HAp}}$) equals the solubility product of HAp ($K_{\text{HAp}}$). In contrast to calculations of $I_{\text{HAp}}$ and saturation levels with respect to HAp, the pH (expressed as $OH^-$ in the formula for $I_{\text{HAp}}$) becomes the unknown quantity in the calculations of the critical pH. Thus the unknown hydroxyion activity is calculated as a relation between the solubility product of HAp and the calcium and phosphate activities in the saliva. As pH has a major effect on the phosphate activities, the calculation of critical pH cannot be performed in
one step – but has to be iteratively estimated. The average critical pH of human saliva (pH 5.5) is based on mean values for human unstimulated saliva of 0.8 mM total non-protein bound calcium, 4.3 mM phosphate, and a total ionic strength of 0.0275 [Schmidt-Nielsen, 1946]. However, the solubility product with a pK of approximately 116 used for this calculation by Smith-Nielsen [Bjerrum, 1936] was slightly different from the solubility product used today. Had the present solubility product for HAp at 37°C with a pK of 117.3 [MacDowell et al., 1977] been used, the critical pH of this average salivary sample would have been 5.4 instead of 5.5. Nevertheless, the implications of these historical discrepancies are minor as the salivary concentrations of calcium and phosphorus are not fixed, which makes the critical pH a dynamic variable instead of being a constant [Dawes, 2003]. Thus, resting saliva normally has a lower critical pH compared with stimulated saliva due to a higher phosphorus concentration in unstimulated saliva. In extreme cases, calculations have shown the critical pH to vary between 5.1 and 5.7 under normal physiological conditions. Moreover as the salivary calcium and phosphorus concentrations are highly dependent on the individual, the critical pH also varies between individuals. Consequently the critical pH value is a chemical term that can be calculated when the activities of calcium and phosphate as well as the solubility product for HAp are known.

1.5.6 Salivary proteins

Human saliva contains more than 40 different proteins with different biological functions. Many of the proteins have antimicrobial functions and belong to the groups of adaptive (IgA) and innate (Lysozyme, lactoferrin ect.) immune factors [Levine, 1993]. Other salivary proteins have digestive functions such as amylase and others again have lubricating functions such as mucins (Figure 1). A majority of the salivary proteins also have an ability to bind to tooth surfaces. Accordingly, the tooth surfaces are covered with acquired pellicle comprising many of the proteins in saliva [Lendenmann et al., 2000] and the pellicle-forming effect of salivary proteins serves as a protection for tooth enamel [Zahradnik et al., 1976; MEurman and Frank, 1991; Ireland et al., 1995; Amaechi et al., 1999; Nekrashevych and Stosser, 2003]. Thus, recent studies have shown that protein pellicles can both inhibit micro-hardness loss and reduce the increase of enamel surface roughness in-vitro when bovine enamel is exposed to citric acid for 1, 5 and 10 minutes [Nekrashevych and Stosser, 2003]. It is, therefore, likely that salivary proteins under conditions where the saliva is theoretically undersaturated will retard the development of erosion in the mouth.
1.5.7 Other protective factors in saliva

Saliva also contains tooth-protective substances that are not of salivary origin. These substances mainly originate from drinking water, foodstuffs, dentifrice, and food supplements. The most common and important of these is fluoride. In Denmark, the fluoride concentrations in the drinking water vary between 0.01 and up to more than 3.00 ppm. Even on Zealand, which is the relatively small island where Copenhagen is located, the fluoride concentration in the drinking water varies from 0.1 to more than 3.0 ppm [Ekstrand et al., 2005]. Apart from the water source, most dentifrice used in Denmark contains fluoride. Also, the intake of foodstuffs that would not be expected to contain fluoride, but do so, as they might originate from countries with fluoride-rich soil, may be a significant source of fluoride for some individuals (the halo-effect) [Whitford, 1994]. Several studies have shown that the erosive potential of soft drinks and acidic compositions can be reduced in the presence of fluoride [Feagin et al., 1977; Sorvari et al., 1988; Soyman and Stack, 1989; Sorvari, 1989; Lussi and Hellwig, 2001]. These results are in agreement with previous studies showing highly increased remineralization of dental enamel after topical fluoride treatment [Lambrou et al., 1981], even under severe erosive conditions [Ganss et al., 2004]. Similar findings are found in relation to cancer patients following radiotherapy who are recommended to have frequent exposure to low fluoride solutions combined with consumption of hard cheese as it may prevent initial demineralization [Gedalia et al., 1996]. However, as fluoride relatively quickly wears off the tooth
surfaces, a constant saliva concentration of fluoride has to be maintained to obtain protective effects [Lambrou et al., 1981]. In contrast, the literature also presents the opposite view on the effect of fluoride on erosion, with some investigations finding only a limited protective effect of fluoride [Larsen, 2001; Hughes et al., 2004] and others unable to find any effects [Larsen and Richards, 2002]. This conundrum is also discussed by Holbrook and co-workers [2004]. Collectively, most studies point towards a protective effect of fluoride against dental erosion, if present during a moderate acidic challenge. Apart from fluoride, other non-salivary compounds that sometimes present in the oral cavity may also have protective effects against erosion. These include, metals such as, strontium [Christoffersen et al., 1997], aluminum [Christoffersen et al., 1985], chromium, iron [Christoffersen et al., 1987], and copper [Brookes et al., 2003]. The sources of such compounds may also be from dentifrices, food supplements, foodstuffs, fillings, crowns, ceramics, etc. Collectively, these compounds, when present in saliva may protect against dental erosion. As the exposure to such compounds is highly dependent on the individual behavior it is virtually impossible to account for such effects. These would need to be determined by direct testing of the erosive potential.

### 1.5.8 Causes of dry mouth and reduced salivary secretion

The prevalence of xerostomia, the subjective feeling of dry mouth, has been shown to be around 10%, and these findings positively correlate with the objective measurement of reduced salivary flow, hyposalivation [Billings et al., 1996]. The most common cause of xerostomia and hyposalivation is the intake of prescription medication [Handelman et al., 1989; Billings et al., 1996]. Thus, both specific drugs [Sreebny and Schwartz, 1997] as well as the total number of drugs taken per day [Nederfors et al., 1997] are related to xerostomia. When xerostomia and hyposalivation are caused by a single drug, the mechanisms behind the side effect mostly include interactions between the drug and the mechanisms involved in salivary gland activation. However, when xerostomia is caused by the intake of many drugs, the actual mechanisms may be difficult to reveal. In most cases the situation will be normalized when the patient stops taking the drug or shifts to a drug with fewer side effects. Less common causes of xerostomia and hyposalivation are a number of autoimmune diseases such as Sjögren’s syndrome [Pedersen et al., 1999]. These diseases induce irreversible degenerative changes in the salivary glands that in many cases end with a severe reduction in salivary gland function. Xerostomia and hyposalivation can also be caused by the irreversible effects of radiation therapy on the head and neck area [Valdez et al., 1992], where the
reduction in salivary flow relates to the amount of radiation dose given [Eisbruch et al., 2001a]. These patients suffer from xerostomia, however, depending on the radiation dose, they may not suffer from complete lack of fluid in the mouth [Dawes and Odlum, 2004]. In the case of unilateral irradiation it is thought that the non-irradiated salivary glands might partly compensate for the loss of functional gland tissue from the irradiated glands [Eisbruch et al., 2001b]. Although these radiated patients comprise a very small group, their radiation-induced xerostomia and hyposalivation often belong to the most severe end of the spectrum. As it is generally acknowledged that patients with dry mouth seek oral relief through intake of acidic candies, these patients may be at greater risk of developing dental erosion than normal salivating individuals.

1.6 Modification of foodstuffs
Modification is by definition the act of making something different [the free dictionary; 2005]. Modification of acidic beverages to reduce their erosive potential is well known [Grenby et al., 1996; Hughes et al., 2002] and has been found to significantly reduce the erosive potential of the drinks [Attin et al., 2005]. In contrast, modification of solid acidic foodstuffs, such as hard-boiled candies, is less recognized, probably due to the methodological challenges of testing the erosive potential of such products. The available literature on modification of solid foodstuffs is mostly in the form of patents [Parker; 2002], and includes the use of calcium, phosphate, and complex phosphate compounds (polyphosphates) [Baker and Parker, 2003] for reduction of erosive potential of solid foodstuffs such as candies and lozenges. Most of these solid compositions claimed for are, however, only moderately acidic (effective pH > 3.5) compared to commercially available acidic candy with effective pH values ranging from 1.8 to 2.8.
2. THE AIM AND THE HYPOTHESES OF THE THESIS

Evaluation of the erosive potential of acidic food products, including soft drinks, is often done indirectly based on their pH, titratable acid and calculations of the degree of saturation with respect to hydroxyapatite. In contrast to beverages, solid foodstuffs become dissolved in saliva when consumed, and thereby saliva becomes the matrix for all the components contained within the foodstuffs. Therefore, measurements of pH and theoretical calculations of DS_{\text{HAp}} may not fully reflect the erosive potential of acidic foodstuffs, since such calculations do not account for the protective effects of proteins and minerals other than calcium and phosphorus in the saliva. Theoretical calculations may overestimate the erosive potential of an acidic candy, and consequently suggest it is more erosive in the mouth than it actually is. Therefore, actual dissolution of hydroxyapatite crystals in mixtures of acidic foodstuffs and human saliva may give a more reliable measure of the effects of all components in human saliva, protecting as well as potentially erosive (i.e. candy). Further, modification of acidic foodstuffs may reduce their erosive potential in a way that cannot be fully accounted for by theoretical calculations.

The aim of this study was to test the erosive potential of acidic foodstuffs theoretically and experimentally and to assess the possibility of modifying acidic foodstuffs with calcium and/or phosphate addition to reduce their erosive potential. It was hypothesized:

1) That pH is more important than the titratable acid when determining the erosive potential of acidic foodstuffs.
2) That erosive potential cannot be directly predicted from saturation levels and critical pH as salivary proteins have protective effects that can not be assessed.
3) That salivary proteins and minerals, other than calcium and phosphorus, reduce the erosive potential of acidic foodstuffs.
4) That dissolution of HAp crystals in saliva provides a more integrated measure of the erosive potential of acidic foodstuffs than the theoretical determination of the degree of saturation with respect to HAp and thus has greater clinical relevance than theoretical calculations.
5) That modification of acidic beverages with calcium and phosphate can reduce their erosive potential.
6) That modification of acidic candy with calcium can reduce its erosive potential.
3. RESULTS AND DISCUSSION

3.1 Part one: The erosive potential of soft drinks in-vitro (I and II)

The conclusions drawn in the literature are conflicting in their ability to explain the erosive potential of soft drinks. In-vitro studies have often found fruit juices to be a risk factor [Lussi et al., 1995; Larsen and Nyvad, 1999]; while in-vivo studies find carbonated beverages to be the main causative factor of dental erosion [Johansson et al., 1997; Milosevic 1997; Arnadottir et al., 2003; Jensdottir et al., 2004a]. Many different methods in the laboratory are known to determine the erosive potential of soft drinks, but no golden approach is acknowledged. This initiated further investigation and conceivably a development of a new method. Thus, the first part of this study tested different beverages from different categories with various in-vitro methods.

Erosive potential upon prolonged exposure to soft drinks

To begin with, the erosive potential of 16 drinks was determined using a traditional method, including measurements of weight loss and calcium release from tooth pieces [I]. The tooth pieces were immersed in a sample of each one of the 16 drinks for 24 and 72 hours respectively, and calcium release from the tooth pieces to the drink as well as the weight loss of the teeth were measured after immersion. Additionally, pH, calcium, phosphate and titratable acid to pH 5.5, 7.0 and 10.0 were determined in each drink, and from these data the buffer capacity, degree of saturation and the critical pH of the drinks were computed.

High correlation was found between the results after 24 and 72 hours. Therefore data from the 24-hour immersion were used to represent erosion. Although, this study found carbonated cola beverages and sport drinks to have lower pH than pure fruit juices, the juices were found to have higher titratable acid to pH 5.5 and thus higher buffer capacity. Also, the juices were found to have higher erosive potential than cola carbonated drinks when judged from weight loss and calcium release. The weight loss method revealed that the pure fruit juices had the highest erosive potential, with exception of lemon concentrate and milk derived lactic acid. This is in agreement with previous in-vitro methods that found orange juices to have a high erosive potential [Lussi et al., 1995; West et al., 1998; Larsen and Nyvad, 1999]. Nevertheless, this is in contrast to both clinical and epidemiological studies that found strong association between the consumption of cola-type drinks and the prevalence of dental erosion [Johansson et al., 1997; Arnadottir et al., 2003; Jensdottir et al., 2004a]. Except for lemon concentrate and milk-derived lactic acid, the highest
calcium release following immersion of the tooth pieces was found in fruit juices as well as sport and energy drinks. These results are in agreement with previous in-vitro studies showing orange juices to have high erosive potential [Lussi et al., 1995; Larsen and Nyvad, 1999]. Interestingly, this is also in agreement with a previous tooth-piece method that found orange juices to release more calcium from the tooth pieces than cola drinks after 40 minutes of immersion [Grobler et al., 1990]. Nevertheless, our results disagree with in-vivo studies showing cola beverages to be one of the most severe extrinsic factor of dental erosion [Johansson et al., 1997; Milosevic 1997; Arnadottir et al., 2003; Jensdottir et al., 2004a]. This prompted further investigation.

**Erosive potential on immediate exposure to soft drinks**

Subsequently, we tested 20 beverages consisting of carbonated cola drinks and orange juices. Since many of the previous in-vitro studies determined the erosive potential of soft drinks over a longer period of time (minutes and hours), this study investigated the difference in the erosive potential upon immediate or gradual exposure to hydroxyapatite crystals. The erosive potential was determined as the dissolution of hydroxyapatite crystals at a 15-second interval for 3 minutes and after 30 minutes. The study was conducted with and without salivary protein coating on the hydroxyapatite crystals. The experimental set up is demonstrated in figure 2.

The initial erosive potential is the dissolution of HAp crystals for the first seconds and up to three minutes, whereas the dissolution of HAp crystals for the 30-minute period is the end erosive potential [II].
When the time dependency was taken into consideration, it was shown that cola drinks had more than a tenfold higher erosive potential within the first seconds and minutes of exposure to HAp crystals, than the orange juices (Fig 3A). These results agree with the clinical and epidemiological studies [Johansson et al., 1997; Arnadottir et al., 2003; Jensdottir et al., 2004a] that suggest cola beverages to be one of the strongest extrinsic causative factors of dental erosion. These results are concerning, as previous studies have shown a high number of school students to consume cola beverages on a daily basis [Kassem et al., 2003; Kassem and Lee, 2004]. When the erosive potential was determined after 30 minutes, we found that the erosive potential of cola drinks had slowed drastically while the orange juices maintained their low pH and consequently tended to become more erosive than cola drinks (figure 3B).

![Figure 3A](image1.png)

**Figure 3A** shows the erosive potential of five carbonated cola drinks and five orange juices selected as representatives of these types of drink for the first three minutes upon exposure to HAp crystals.

![Figure 3B](image2.png)

**Figure 3B** shows the erosive potential of the same drinks over the whole 30 min test period. As shown, the sequence of the drinks changed. Thus, some of the orange juices became considerably erosive with time.

The five carbonated cola drinks (1-5) and five orange juices (6-10) in figure 2 are:

1) Coca Cola light
2) Pepsi Max
3) Coca Cola
4) Pepsi Cola
5) Coca Cola light with lemon
6) Capri-Sonne Orange
7) Sun Top
8) Rynkeby with sour oranges
9) Rynkeby with organic oranges
10) Rynkeby with sweet oranges

Although the latter results are in agreement with previous findings [Grobler, 1990; I] it is debatable whether it is the 30-minute time period or the initial acidic exposure that simulates what happens in-vivo when the drink enters the mouth. This study also showed that salivary proteins at low pH values (near pH 2.5) had a considerable protective effect on HAp crystals, while no protective effect
was detected at higher pH values (pH 3.5 and above). Theoretically, the erosive effect when consuming soft drink depends on: (i) the immediate effect of the drink on the surface, (ii) the clearance time [Bashir and Lagerlof, 1996], (iii) the type of drinking method [Johansson et al., 2004]; and (iv) the protective effect of human saliva [Meurman and Frank et al., 1991; III]. Although cola drinks could have been expected to be more erosive than previously reported in laboratory studies, due to their low pH, it was surprising to find them up to 10 times more erosive than orange juices within the first seconds and minutes of exposure. Nevertheless, from a chemical viewpoint these results make sense in the light of the logarithmic nature of the pH scale. Accordingly, the results correlate with the fact that the cola drinks were a whole pH unit more acidic than the juices. However, salivary proteins reduced the erosive potential of the most acidic drinks (cola drinks) by up to fifty percent [II]. We speculate that these findings are due to the relation between the speed of desorption of proteins from the crystals and the speed of the erosive challenge. Thus if the erosive speed (initial erosive potential) exceeded the speed of desorption of proteins from the crystals, a protective effect was obtained, and vice versa. We assume that when the proteins were washed off the crystals, their protective effect ceased, which explains the very limited effects of the proteins on the erosive potential after 30 minutes (end erosive potential). Thus after exhibiting its protective effects the protein coating has to be renewed to withstand a new acidic challenge in the mouth. However, renewal of the protein coating may take considerable time [Zahradnik et al., 1976; Nieuw Amerongen, 1987] and this time may be a critical factor in individuals who tend to sip soft drinks throughout the day, which partly explains their high incidence of erosion [Arnadottir et al., 2003; Johansson et al., 2004]. Another likely explanation for the relation between the protective effects of the proteins and the erosive potential of the drinks may be protein denaturation, which could have occurred in drinks with low pH values, thereby increasing the viscosity of the protein coating on the crystals [Holma and Hegg, 1989] and adding to the protective effects.

### 3.2 Part two: The erosive potential of acidic candies (III -VI)

The literature is sparse when it comes to the erosive potential of hard-boiled candies. Unlike the drinks, the candies are solid and need to dissolve before their erosive potential can be determined. This often occurs in saliva, but measuring the erosive potential of saliva has its limitations. Consequently, the determination of the erosive potential of acidic hard-boiled candies is challenging. The few existing studies on the erosive potential of acidic foodstuffs include
experimental exposure of the foodstuffs to tooth substance with and without saliva [Holloway et al., 1958; Bibby and Mundorff, 1975; Lussi et al., 1997]. The second part of this study determined the erosive potential of acidic candies with two different approaches in two study groups. Firstly, the erosive potential of commercially available acidic hard-boiled candy was determined in healthy test persons [III] and dry-mouth patients [IV] from measurements of salivary pH and with theoretical calculations of the salivary degree of saturation with respect to HAp (DS_{HAp}) upon sucking the candy. Secondly, this study tested the erosive potential of acidic hard-boiled candies upon dissolution of HAp crystals directly in saliva produced upon sucking the candy in both healthy test persons [V] and dry-mouth patients [VI]. The second approach should be interpreted as a more integrated way of determining the erosive potential of acidic candies as it may simultaneously account for harmful as well as protective factors in the human saliva.

In order to prevent any loss of CO₂ and thereby to obtain true oral pH values, the saliva was collected in a closed system. The saliva was collected for a 19-minute period in two groups of healthy test persons [III and V] and one group of dry-mouth patients [IV] (figure 4). All three groups sucked acidic hard-boiled candies [III-V]. Additionally, the patient group [VI] and one group of healthy test persons [V] also sucked the modified candy. Figure 4 shows the study set up and the time interval upon sucking acidic hard-boiled candies.

Figure 4 shows the study set up and time course for collection of saliva in a closed system. The saliva from the healthy test persons was collected into syringes. However, the amount of saliva from the patients was less than from the healthy test persons, and this saliva was also often thicker and thus more difficult to collect. Therefore the saliva from the patients was collected in a smaller system under paraffin oil. The saliva from both groups was collected for the same time interval; firstly 5 minutes unstimulated saliva (baseline), then four minutes stimulated saliva (stimulated) and finally ten minutes unstimulated saliva (post-stimulated).
Effects of the candy on salivary flow

Chewing gum is generally known as an effective salivary stimulant [Dawes and Kubieniec, 2004; Dawes, 2005], while sucking lozenges has been reported to be less stimulating [Dawes, 1992]. This study, however, found healthy test persons to produce five times more saliva when sucking acidic candy than when chewing gum [III]. This was probably due to the acidic components in the candies used and thus in agreement with previous studies showing acidic taste stimulate to a higher salivary flow rate than chewing [Watanabe and Dawes, 1988]. Thus, acidic taste is known to stimulate to the highest salivary flow rates of the four taste modalities known, i.e. salt, sweet, sour, and bitter, [Froehlich et al., 1987] and of the acidic tastes normally used in the food industry (i.e citric, malic and tartaric acid) the tartaric acid is known to have the best salivary stimulating effect [Chauncey et al., 1967]. Additionally, as solid foodstuffs gives a better saliva stimulation than liquid foodstuffs [Guinard et al., 1998] we assume that the salivary flow rates generated in response to sucking the candies were higher than what will be generated in response to drinking acidic soft drinks. The salivary flow peaked already within the first two minutes upon chewing gum and sucking candy, which is in agreement with previous studies also showing the peak salivary flow rate to occur within the first two minutes when chewing and sucking and then to gradually decrease [Dawes and Mcpherson, 1992]. However, with longer periods of stimulation the salivary flow rate will normally be relatively constant, both with acidic stimulation [Chauncey et al, 1967] and chewing stimulation [Shannon, 1958]. Despite lower salivary flow rate and slower recovery in the post-stimulatory period, the same general effects on salivary flow rate upon sucking acidic candy were found in dry-mouth patients and in healthy test persons [IV].

Salivary pH, buffer capacity and \(DS_{\text{HAp}}\)

This study confirmed previous results in finding the stimulated salivary samples to become more supersaturated than the unstimulated saliva when chewing gum [Dawes and Dong, 1995]. However, despite the high salivary flow rates, bicarbonate and protein concentrations, and consequently high salivary buffer capacity upon sucking the acidic candy, the saliva could not compensate entirely for the acidic components in the candy. This caused a serious drop in the salivary pH in both healthy test persons and dry-mouth patients, although it was more pronounced in the patients. Also, the patients failed to reach their baseline pH in the post-stimulatory period [IV]. These findings agree with Distler and co-workers [1993] who found dry-mouth individuals with low buffer capacity to be
less resistant to the acidic attack upon sucking acidic lozenges. As a result, the saliva became
undersaturated with respect to HAp while sucking the candy in both groups, with greater
undersaturation in patients than in healthy test persons (figure 5) [III-V]. However, at least in
healthy individuals these observations might have changed with longer exposure to the candy, as
the salivary bicarbonate concentration, and thus buffer capacity, increases during long times of
acidic stimulation [Dawes, 1969; Dawes, 1974].

Nevertheless, within the four-minute exposure period in this study the results suggests that acidic
hard-boiled candies have erosive potential in both healthy test persons and dry-mouth patients,
although this is more severe in patients than in healthy test persons. This is in agreement with
previous experimental [Holloway et al., 1958; Bibby and Mundorff, 1975] and in-situ studies [Lussi
et al., 1997] that found acidic solid foodstuffs to have erosive potential. It was not surprising to find
that the pH drop was significantly more profound in patients than in healthy test persons, as the dry-
mouth patients had a lower salivary flow rate, which resulted in reduced salivary bicarbonate
concentration. Consequently, this also resulted in a lower buffer capacity and greater
undersaturation with respect to hydroxyapatite among the patients [IV].

*Dissolution of hydroxyapatite crystals in saliva produced upon sucking acidic candies*

This study also determined the erosive potential of acidic hard-boiled candies by dissolution of HAp
crystals in candy-stimulated saliva in healthy test persons [V] and dry-mouth patients [VI]. After

**Figure 5** shows the degree of saturation with respect to hydroxyapatite (DS_{HAp})
upon sucking acid candy in two groups of healthy test persons and one group of
dry-mouth patients [III-V]. Black continuous lines represent the patient group [IV] and broken lines
represent the two groups of healthy test persons, small broken lines [III] and larger broken lines [V].
DS_{HAp} was determined by theoretical calculations based on measurements of saliva pH as well as
calcium and phosphate activities.
The salivary samples were collected in a closed, CO$_2$-tight system upon sucking the acidic candies, hydroxyapatite crystals were immersed in the saliva, in the same way as the hydroxyapatite crystals were added to the drink samples in the first part of this study (figure 2). A pH change was an indicator that dissolution of the HAp crystals had occurred and consequently that erosion was taking place. The actual amount of crystals dissolved is described in a detailed methodological procedure in article V. The results showed that HAp dissolution occurred in 19 of 20 healthy test persons [V] and in 8 of 9 patients in response to sucking acidic hard-boiled candies [VI]. Accordingly, we evaluated the acidic candies as having erosive potential. Thereby it was shown that the theoretical calculations to some degree could predict actual erosive potential of candy. However, the relation between DS$_{\text{HAp}}$ and actual HAp dissolution [V] was not as good as the relation between the pH and HAp dissolution in the drinks [II]. The weaker relationship for the candies was most likely caused by the protective effects of salivary proteins and minerals other than calcium and phosphate.

3.3 Part three: The erosive potential of modified acidic foodstuffs [I, V and VI]

This study modified both solid and non-solid foodstuffs, respectively. Many different substances were potentially interesting, such as calcium and phosphate salts [Gedalia et al., 1991a], fluoride [Amaechi et al., 1998], milk proteins [Bardow et al., 2004; Bardow et al., 2005b] and gum [Hughes et al., 2002; West et al., 2004]. However, as calcium and/or phosphate, in particular, have been shown to be effective in remineralizing the tooth enamel and have consequently been suggested and even used successfully for modification of soft drinks [Gedalia et al., 1991b; Hughes et al., 1999a+b; Hughes et al., 2000] they were chosen here for the modification of pure orange juices.

Modification and the erosive potential of modified beverages

This study modified commercially available orange juice from the Icelandic market with three different ratios of calcium and phosphate. Pure orange juice served as a control. The drinks were named experimental drink A, B and C, respectively and were accordingly modified with 6.67 gram calcium citrate, 4.00 gram calcium tri-phosphate and 8.0 gram calcium tri-phosphate per liter orange juice [I]. The erosive potential of the orange juices was determined as percentage weight loss from tooth piece immersed in the drinks as well as calcium release to the drinks [I]. This study found that modification of orange juices did indeed reduce its erosive potential and the higher the concentration of calcium and/or phosphate in the drinks, the lower the erosive potential became. However, despite the fact that both calcium citrate and calcium tri-phosphate had a considerable
reducing effect on erosion in the tooth pieces, the juices with the calcium tri-phosphate were reported to have slightly metallic taste [I].

**Modification and the erosive potential of modified acidic hard-boiled candy**

Based on the experience with the beverages, only calcium components were chosen to modify the acidic hard-boiled candies [V]. Healthy individuals [V] and irradiated patients [VI] both sucked a modified and a control candy in the setup described in [V]. Acidic hard-boiled candy was hereafter modified with 16.5 grams calcium lactate per kg dough. Both the modified candy with the calcium lactate and the control candy were identical in terms of bulk substances, flavorings and acidification [Jensdottir et al., 2004b]. Despite the considerable amount of calcium in the modified candy, the salivary flow rates obtained in response to sucking the modified and the control candy (candy without calcium) were similar [V and VI]. Additionally, the saliva both increased at the same rate in the presence of the control and the modified candies and correspondingly fell at the same rate during the post-stimulatory period in healthy test persons [V]. Thus, at least for the concentrations used, the calcium addition did not have any noticeable effect on the salivary production upon sucking the hard-boiled candy. The total calcium concentration was significantly 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 [V and VI]. As a consequence of the high calcium concentration in the candy, the estimated critical pH with respect to HAp became significantly lower upon sucking the modified candy [V]. Figure 5 shows the effect of sucking modified and control candy on the salivary pH, the erosive potential upon dissolution of HAp crystals in salivary matrix, and the computed degree of saturation with respect to hydroxyapatite in healthy test persons and dry-mouth patients. In accordance with previous results in this study the salivary pH was lower in patients than healthy test persons upon sucking acidic hard-boiled candy, regardless of control or modified candy. As a result of lower salivary pH in the patient group both candy types were more undersaturated in this group than in the healthy test persons. In fact the only candy that was found supersaturated and thus theoretically non-erosive upon suction was the modified candy among some of the healthy test persons. Interestingly, the data from the dissolution of hydroxyapatite crystals in the saliva revealed that the erosive potential of the modified candy was very low and actually lower among patients than healthy test persons. However, in both groups the erosive potential of the modified candy was significantly reduced compared to the control. As a comparison the control
candy was found over six times more erosive than the modified candy in healthy test persons and over nine times more erosive in dry-mouth patients (figure 6).

These results confirm that modification of acidic solid foodstuffs can reduce their erosive potential similar to that shown for soft drinks. Further, these results also show that salivary protein, minerals and other ingredients of human saliva may have a protective role when consuming acidic foodstuffs. Furthermore this study found a relation between DS$_{HAp}$ and the erosive potential determined by HAp dissolution in saliva while sucking the modified acidic candy similar to that of the non-modified candies. However, the relation between DS$_{HAp}$ and actual HAp dissolution was not as clear for the modified candy as for the drinks. This was most likely related to the protective effect of the salivary proteins and minerals that are not accounted for by calculation. In the modified candies, ten samples did not show any signs of HAp dissolution upon testing, despite being undersaturated with respect to HAp, and thus having pH values lower than their critical pH [V]. Therefore low salivary pH and moderate undersaturation may not necessarily imply that HAp dissolution will occur in the mouth; particularly not when consuming calcium modified acidic foodstuffs.
4. GENERAL DISCUSSION

The importance of determining the erosive potential of various foodstuffs is increasing as several studies indicate that intake of certain acidic foodstuffs is the major causative factor of dental erosion in the industrialized part of the world [Linkosalo et al., 1985; Grobler et al., 1989; Grenby et al., 1990; Gedalia et al., 1991; Chaudhry et al., 1997; Johansen et al., 1997; Lussi et al., 1997; Gray et al., 1998; Al-Malik et al., 2001]. Consequently, the prevention of such an irreversible type of tooth wear is closely linked to an understanding of the effects of acidic foodstuffs on teeth. Judged from the increasing popularity of acidic foodstuffs, a reduction in the intake seems unrealistic. Therefore, modification of acidic foodstuffs may be an important strategic tool in the prevention of dental erosion. The aim of the present study was to determine the erosive potential of acidic foodstuffs, both liquid and solid, and to validate the use of pH and titratable acid as simple indirect measures of erosive potential. The study also aimed to test the possibility of reducing the erosive potential of acidic foodstuffs by modification.

The first part of the study determined the erosive potential of liquid foodstuffs in the form of acidic beverages with both previously known and new in-vitro methods. The known method included determination of the weight loss and calcium release from tooth pieces after exposure to an acidic soft drink. Since the results of this method only reflect the differences in teeth before and after exposure to a soft drink, it does not allow for determination of the time dependency of erosion or for monitoring erosion within the first few seconds upon exposure to the soft drink. Also, this method requires a sudden degree of erosion to have occurred in order to obtain precise results, and thereby it works best in setups with a relatively long exposure time to the soft drink. In contrast to older methods, the new method allowed for continuous monitoring of the erosive potential as a function of time. It also allowed for determination of the erosive potential of soft drinks upon immediate exposure and seemed to be precise even within seconds after exposure of tooth substance to the soft drinks.

The known method found the titratable acid to pH 5.5 to be highly correlated with the erosive potential of soft drinks over a prolonged period upon exposure and was thus in agreement with previous in-vitro studies [Grenby et al., 1990; Lussi et al., 2004]. Consequently, this method found fruit juices to have a higher erosive potential than carbonated cola drinks when judged from weight loss and calcium release from the tooth pieces upon prolonged exposure to soft drinks due to their high titratable acid [Grobler et al., 1990; Lussi et al., 1995; West et al., 1998; Larsen and Nyvad, 1999]. The results after prolonged exposure were also confirmed by the new method used in this
study when the exposure time of HA\textsubscript{p} crystals to soft drink was 30 minutes. However, by immediate exposure of the hydroxyapatite crystals to different drinks it was shown that carbonated cola drinks were considerably more erosive than orange juices. Also the new method showed that the pH is the only relevant measure of the erosive potential within the first seconds and minutes of exposure to soft drinks. Thereby the results of the new method concur with both clinical and epidemiological studies that find strong association between the consumption of cola-type drinks and the prevalence of dental erosion [Grobler et al., 1990; Johansson et al., 1997; Arnadottir et al., 2003; Jensdottir et al., 2004]. Both the new and the known method were performed at room temperature, around 25°C. However, previous studies have investigated the change in the erosive potential of acidic foodstuffs at various temperatures and found the erosive potential to generally increase at higher temperatures [Grobler et al., 1989; West et al., 2000; Barbour et al., 2005]. This suggests that the erosive potential of the soft drinks in our study might have been slightly overestimated by both methods, as soft drinks are normally consumed chilled well below 25°C upon consumption. 

One of the early pioneers in erosion research [Ericsson, 1953] speculated that the erosive effect of foodstuffs was limited to the time of ingestion. Therefore we believe that the titratable acid might not relate to the clinically relevant erosive potential of a soft drink, and most certainly not the erosion that occurs from the time the drink meets the tooth surface until the first swallow occurs. In the worst case, the titratable acid may be directly misleading with respect to erosive potential, as was the case with juices and cola drinks [I]. In contrast, the experiments in this study, conducted with the new method, found the pH to be a reliable predictor of the effects of soft drinks on teeth upon immediate exposure, and thus until the first swallow occurs. During this time the erosive potential of soft drinks was shown to be almost an ideal exponential function of their pH. However, the relation between the erosive potential and pH may account only for a situation where the drinks are present in the mouth for few seconds. Possibly, the titratable acid could become important in dry-mouth patients who may have low salivary flow rate and thereby prolonged exposure to the acid due to reduced low oral clearance such as shown for the acidic candies [IV].

The debate whether the pH or the titratable acid is a better indirect measure of the erosive potential is not new. Holloway and co-workers addressed this issue in 1958 and referred to literature dated back to McClelland in 1926. Holloway et al. discussed the findings that supported the fact that erosion was mainly a function of the pH [Elsbury, 1952] and conversely, findings that suggested that the erosive potential was the function of titratable acid of a solution [Muller and Cortner, 1949].
It was concluded that the titratable acid was more relevant as a predictor of the erosive potential of foodstuffs. Although many recent studies hold the same point of view [Grenby et al., 1990; Lussi et al., 2004], this study has shown that both views may be true, depending on the time of exposure of soft drink to the teeth, and this depends very much on the individual concerned. In general, however, this study has shown pH to be a better indirect predictor of the erosive potential of soft drinks than titratable acid, given the erosion occurs under conditions where the erosive effect is mostly limited to the time of ingestion. This could partly explain the increasing prevalence of dental erosion with high consumption of soft drink from bottles with re-sealable caps which may encourage the habit of sipping (frequent immediate exposure) throughout the day [Arnadottir, 2005].

Another extrinsic cause of dental erosion is the intake of acidic solid foodstuffs such as candies and lozenges [Lussi et al., 1997]. It is well known that most people use mouth refreshments for salivary stimulation to obtain the desired freshness and cleanliness in the mouth that often follows increased salivary flow. Others, such as dry-mouth patients, take mouth refreshers for salivary stimulation and for oral relief. Although acidic hard-boiled candies may not be the main causative factor of dental erosion in healthy test persons, they could collectively with other acidic foodstuffs induce the development of dental erosion. This is of particular concern as the candies are in the mouth for a relatively long time during consumption. Furthermore, acidic candies may be a considerably greater causative factor in patients with dry mouth as they both have reduced salivary flow and tend to consume saliva stimulating refreshments frequently. Therefore the second part of this study tested the erosive potential of such foodstuffs in healthy test persons and cancer patients with dry mouth following irradiation to the head and neck area. The second part of this study showed that in healthy individuals sucking acidic hard-boiled candies may result in up to five times more saliva than chewing gum. Saliva is not only considered to have a protective effect in terms of dental erosion, but is also known to remineralize the softened enamel structure after acidic attack [Amaechi and Higham, 2001a,b]. Nevertheless this study found that saliva became significantly undersaturated with respect to hydroxyapatite upon sucking the acidic candies. These findings confirmed the suspicion that hard-boiled candies may have erosive potential in healthy test persons. In view of the fact that the pH drop was significantly greater in patients than in healthy test persons, and both salivary flow and buffer capacity were significantly less, it was expected to find the degree of saturation significantly lower in patients than in healthy test persons. This study also appears to be the first to determine the actual erosive potential of acidic hard-boiled candies in both healthy test
persons and dry-mouth patients when the erosive potential of the candies was determined with dissolution of hydroxyapatite crystals in saliva collected upon sucking acidic candies. Nevertheless, as the acidic hard-boiled candy would normally be close to body temperature (around 37 °C) upon consumption, the erosive potential of the candy in the present study might have been slightly underestimated as the HAp dissolution experiments were performed at room temperature. Interestingly, theoretical computations found a greater undersaturation with respect to hydroxyapatite compared with the actual erosive potential of the acidic hard-boiled candies determined by dissolution of hydroxyapatite crystals. Although this study confirmed that salivary protein pellicles may have a protective effect in the oral cavity, it was also shown that this cannot be accounted for with theoretical calculations. Accordingly, this study also found a similar protective effect from human salivary protein in extremely acidic drinks, such as cola beverages (pH 2.5) upon dissolution of protein-coated hydroxyapatite crystals in soft drinks in-vitro.

An important protective effect against the erosive potential from both soft drinks and solid acidic foodstuffs is the effect of saliva [Hall et al., 1999]. Saliva protects against the acid induced tooth dissolution both by its flow and its composition. We showed that the salivary flow was important for recovery after an acidic challenge from acidic hard-boiled candies [IV], which supports the fact that oral clearance and thus high saliva flow is important in relation to preventing the development of dental erosion [Lagerlöf and Dawes, 1985; Jarvinen et al., 1991]. Additionally, we tested the effect of buffer capacity upon sucking acidic candies and found the salivary buffer capacity to be important [III]. Further, calcium and phosphate in saliva may also have a protective effect in the mouth upon acidic attack and thus are frequently used for modification to reduce the erosive potential of acidic foodstuffs [Grenby, 1996; Hughes et al., 1999a,b; West et al., 1999; Parry et al., 2001; Venables et al., 2005].

The salivary proteins were thought to have the ability to cover tooth surfaces in the mouth in the form of a pellicle. Therefore we tested the effect of a salivary protein coating on both soft drinks and candies. However, out of at least 40 types of protein that have been identified in saliva, we did not test which of them had the most protective effect upon acidic attack. An early study investigated saliva in individuals with erosion and individuals with no erosion. Although no significant difference was found between the amount of saliva and its pH or buffer capacity, the salivary mucins were found to vary between individuals [Mannerberg, 1963]. Consequently, we speculated that the salivary mucins generally due to their tissue coating effects may be one of the more important proteins in terms of protecting the tooth substance upon acidic attack. However, also
other proteins that are known to adhere to the tooth tissues [Bardow et al., 2004b] are for example, amylases, proline-rich proteins, cystatine and statherin and they may also have added to this effect (figure 1).

It has been suggested that modification of acidic foodstuffs and medicine may be an acceptable future strategy for manufactures and consumers to aid to the prevention of dental erosion [Holbrook et al., 2003]. In the mouth, enamel dissolution is known to be a function of the degree of saturation with respect to hydroxyapatite, which is mainly determined by pH, although both calcium and phosphate concentrations are also known to be critical [Barbour et al., 2003]. Accordingly, both calcium and phosphate have been found to be favorable when modifying acidic beverages [Grenby, 1996; Hughes et al., 1999a,b; West et al., 1999; Parry et al., 2001; Venables et al., 2005]. This study modified commercially available pure orange juice and confirmed that both calcium and phosphate reduced its erosive potential. Consequently this study confirmed earlier studies that found development of fruit juices with calcium reduced their erosive potential [Hughes et al., 1999a,b; West et al., 1999; Hughes et al., 2002]. This study did not attempt to modify carbonated drinks, but theoretically we estimated that due to very low pH in cola carbonated drinks the amount of calcium needed to modify such a drink would alter both the taste and the texture of the drink. Furthermore, Larsen and Nyvad [1999] have acknowledged that modification of beverages below pH 3.5 is not realistic. Nevertheless, cola beverages have been modified with calcium salts in earlier studies that successfully reduced their erosive potential [Beiraghi et al., 1989; Hunter et al., 2003; West et al., 2003]. However, some authors suggested that the results should be interpreted with caution and that more research was needed [Hunter et al., 2003]. This might partly explain why modified cola drinks have not been taken up by manufactures. The acidic components of acidic candies have been shown to demineralize the hydroxyapatite crystals in the dental enamel and consequently it has been recommended that modification of candies by reducing the acidity levels might reduce their erosive potential [Grenby, 1995; Grenby and Mistry, 1996]. This study modified acidic candy with calcium lactate without reducing its acidity levels. Reducing the acidity levels would presumably alter some of the characteristics of the candy, but most importantly the taste would be changed. The candy would become less acidic and would thus presumably result in a candy with less saliva stimulating ability [Chauncey et al., 1967]. We found that the acidity level could be unaltered by manipulating other components of the hydroxyapatite compound. Additionally this study found that the salivary proteins, probably due to mucins, amylase, proline-rich proteins, cystatins, statherine, and other tissue-coating salivary proteins do have significant protective effect of the enamel, especially at low
pH levels. Also, other salivary compounds such as fluoride may have added to the protective effects, making some samples non-erosive despite still being theoretically undersaturated. Finally, the hypotheses stated in the beginning of this study have all been concluded upon.
5. GENERAL CONCLUSION

- Cola beverages have a greater erosive potential than fruit juices upon immediate exposure to hydroxyapatite crystals.

- Acidic hard-boiled candy has erosive potential in both healthy test persons and dry-mouth patients.

- The pH is more important than the titratable acid when determining the erosive potential of acidic foodstuffs.

- Modification of acidic beverages with calcium and phosphate reduces their erosive potential.

- Modification of acidic candy with calcium reduces its erosive potential in healthy test persons and dry-mouth patients.

- Salivary proteins reduce the erosive potential of acidic foodstuffs.

- Sucking acidic hard-boiled candy induces a significantly greater salivary flow than chewing gum.

- Undersaturation with respect to hydroxyapatite does not necessarily indicate erosive potential in the oral cavity upon consumption of acidic foodstuffs.

- The erosive potential of acidic foodstuffs cannot be directly predicted from relations between pH and critical pH, as salivary proteins have protective effects that cannot be accounted for by such calculations.

- Dissolution of hydroxyapatite crystals in saliva provides a more integrated measure of the erosive potential of acidic foodstuffs than the theoretical determination of the degree of saturation with respect to hydroxyapatite crystals, hence it has higher clinical relevance than theoretical calculations.
6. FUTURE PERSPECTIVES
With the increasing prevalence of dental erosion and changing dietary habits worldwide pointing to increased consumption of acidic food-stuffs, determining the erosive potential of these foodstuffs is substantial for developing relevant prevention strategies against dental erosion. A future perspective will embrace an extensive epidemiological study where the prevalence of erosion in relation to consumption of acidic foodstuffs, is highlighted. It is hoped that the conclusions drawn from this study may have an impact on future clinical and epidemiological research on dental erosion, caused by intake of acidic foodstuffs. Another obvious future perspective is the further development of mineral and/or protein enriched foodstuffs with no or low erosive potential. As this study did not specifically identify which salivary constituents were protective against dental erosion and how they exert their effects, further effort should be put into identifying these compounds and mechanisms. Accordingly, by use of the model system described in [V] it could also be tested if some individuals are better protected against dental erosion than others due to specific salivary characteristics. Another future benefit of this research is to correlate clinical erosion with actual dissolution of hydroxyapatite in saliva using this model system. Thereby it may be possible to identify salivary variables that are protective against dental erosion among patients that due to their behavior or habits have a high risk of developing dental erosion. Furthermore it could be potentially interesting to identify and determine the protective role of selective salivary protein families with effects against dental erosion upon sucking non-modified acidic candy.
At the same time the consumption of acidic soft drinks and acidic solid foodstuffs is at a historical high level, the prevalence of dental erosion is increasing. This is concerning as these acidic food products are considered to be the main extrinsic cause of dental erosion. Furthermore, certain patient groups suffering from dry mouth may be likely to have a very high intake of these products and at the same time reduced biological protection against dental erosion. Testing the erosive potential of acidic food products, including soft drinks, is often indirectly based on measurements of their pH, titratable acid, and saturation levels with respect to hydroxyapatite. Generally low pH, high titratable acid, and undersaturation with respect to hydroxyapatite of the foodstuff is considered to be indicative of high erosive potential. When testing the erosive potential of solid acidic foodstuff these first have to be dissolved. Thus in the mouth the solid foodstuffs are dissolved in saliva, which thereby forms a matrix for the compounds contained in the foodstuff. Also in relation to soft drinks the protective effects of saliva may be important. As a reduction in the consumption of these products seems unrealistic other measures may be used in the control of dental erosion. Such measures may include modification of the food products by substances that reduce their erosive potential. Consequently the aims of this study were to: 1) determine the erosive potential of acidic solid and non-solid foodstuffs in saliva using various methods; 2) to validate the use of pH, titratable acid; and saturation levels as indicators of erosive potential; 3) to determine protective effects of saliva; 4) to determine the erosive potential of acidic solid foodstuffs in dry mouth patients; and 5) to explore the possibility of food modification to reduce the erosive potential. The results showed that the erosive potential of acidic non-solid foodstuffs in the form of soft drinks is almost solely determined by their pH. However, human salivary proteins may reduce the erosive potential of such drinks by up to 50% within the first minutes of exposure to tooth substance. Furthermore, the results showed that consumption of acidic solid foodstuffs such as acidic candies changes whole mouth-salivary composition so that it may have erosive potential. Also in this relation it was shown that saliva has protective effects as through clearing and buffering, although, strongly reduced in dry mouth patients. Finally modification of both acidic soft drinks as well as acidic candies with calcium and phosphate was shown to significantly reduce the erosive potential of such foodstuffs, and for the candies this was found in both healthy individuals and dry mouth patients.
8. DANISH SUMMARY

9. REFERENCES


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10. APPENDIX

Articles I-VI
ARTICLE I
Properties and modification of soft drinks in relation to their erosive potential in vitro

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KEYWORDS
Dental erosion; Titratable acid; Buffer capacity; pH; Modification

Summary Objectives. The objective was three-fold; (1) to test the erosive potential (EP) of various soft drinks, (2) to determine properties related to the soft drinks that were important for EP, and (3) to test possibilities of reducing the EP of soft drinks by modification.

Methods. Sixteen soft drinks from the Icelandic market including three modified soft drinks were used. The pH, calcium, phosphorus, and titratable acid (TA) to pH 5.5, 7.0, and 10.0 were determined in each drink. From these results the buffer capacity (β) at pH 4.5, 6.3, and 8.5, degree of saturation with respect to hydroxyapatite (DSHAP), and critical pH (DSHAP=1) were calculated. One orange juice was modified by addition of various concentrations of calcium and phosphate. EP was determined as weight loss from tooth pieces after immersion into the soft drinks for 24 and 72 h as well as calcium increase in the soft drink upon immersion.

Results. EP of the drinks varied from 0–10% weight loss and 0–31 mmol calcium increase. The pH in carbonated and sport drinks was lower than in fruit juices, whereas TA and β was considerably higher in fruit juices. Significant correlations were obtained between EP and TA, β, pH, and DSHAP (r = 0.69–0.90). Addition of calcium and phosphate to the experimental drinks considerably decreased their EP.

Conclusion. We conclude that several properties related to soft drinks have an impact on their EP upon long exposure time to teeth and that moderate modification could be a helpful measure to reduce the EP of soft drinks.

Introduction

Dental erosion is the loss of dental hard tissue by a chemical process not involving bacteria. Its prevalence appears to be increasing especially among children1 and adolescents.2 Soft drink consumption
has frequently been reported to be one of the most important risk factors of dental erosion. It is known that soft drink consumption is very high especially among adolescents and may be increasing. Therefore, it is important to investigate those properties of soft drinks that may contribute significantly to their erosive potential. In clinical studies, drinks with low pH such as cola-based carbonated drinks, have often been the drinks most related to dental erosion. Nevertheless in vitro studies have shown fruit juices to have higher titratable acid than cola-based carbonated drinks, which has been used to suggest that fruit juices also may have considerable erosive potential. There is, consequently, a need to clarify this disparity and determine which properties of soft drinks such as pH; titratable acid; and buffer capacity, are the most important with respect to determining their erosive potential. It has also been proposed that the erosive potential of soft drinks may be reduced by modification.

The aim of this in-vitro study was three-fold (1) to test the erosive potential of various soft drinks, (2) to determine properties of soft drinks that were important in causing their erosive potential, and (3) to test the possibility of reducing the erosive potential of soft drinks by modification.

Materials and methods

Study design

This study examined 16 soft drinks from the Icelandic market selected from 70 drinks that have previously been described with regard to pH and the titratable acid. These 16 drinks represented the groups: carbonated drinks, pure fruit juices, juices from concentrate, milk-based drinks, energy drinks, sport drinks, experimental drinks, and distilled water.

Determination of chemical properties of soft drinks

The pH of the drinks was determined at room temperature with an electrode connected to a standard pH meter with a two-digit reading (Radiometer™). Titratable acid (TA) was determined as the volume (in ml) of 1 M NaOH (Titrisol™) required to raise the pH of 50 ml of the drink to pH 5.5, 7.0, and 10.0. For TA a standard titrator and autoburette (Radiometer™) were used. Both pH and TA were determined three times for each drink. Calcium was determined spectrophotometrically by a colorimetric assay based on the Arsenazo III reaction at a wavelength of 680 nm. Phosphorus was also determined spectrophotometrically by a colorimetric assay based on the molybdenum reaction at a wavelength of 670 nm. All samples were measured twice and diluted if necessary to be within the recommended range of the tests.

From the TA results, the buffer capacity was calculated at pH 4.5, 6.3, and 8.5 mmol H⁺/(litre soft drink pH unit) according to the equation: where denotes the amount of strong base that had been added to the drink and denotes the change in the pH of the drink. The degree of saturation with respect to hydroxyapatite (DSHAP) was calculated from the pH, calcium, and phosphate concentrations/activities of the drinks. The critical pH with respect to hydroxyapatite (i.e. the pH value, where DSHAP = 1) was iteratively estimated.

Erosive potential

Tooth pieces were prepared from non-carious molars obtained from the University dental clinic in Iceland. Each tooth piece was approximately one quarter of a tooth crown and weighed approximately 450 mg. Two tooth pieces were used for each drink. One was immersed in the drink for 24 h and the other for 72 h, both in 10 ml volumes of each drink, with constant stirring. The tooth pieces were weighed before being placed directly into the drink and again after immersion and drying at room temperature for a week. The erosive potential was determined as the percentage weight loss upon contact with the drink as well as differences in the calcium concentration of the drinks arising from calcium released from the tooth pieces during immersion.

Experimental drinks

To evaluate the possibility of soft drink modification three experimental drinks with high concentrations of calcium and phosphate were developed from pure orange juice (Trópi, Sól-Viking, Reykjavik, Iceland). Pure orange juice (Trópi) was then used as a control for the experimental drinks (A–C) that was blended as follows:

(A) 6.67 g of calcium citrate dissolved in 1 l of pure orange juice.
(B) 4.00 g of calcium tri-phosphate dissolved in 1 l of pure orange juice.
(C) 8.00 g of calcium tri-phosphate dissolved in 1 l of pure orange juice.
Statistical analysis

Excel and the free statistical software R (www.r-project.org) were used for the analysis. Due to the small sample size all correlations were analysed by Spearman’s rank correlation analysis. All correlation coefficients (r_s) were obtained without the experimental drinks included (n=13). Threshold level of significance was set at p<0.05.

Results

Erosive potential

After 72 h of exposure to the drinks a highly significant correlation was obtained between the weight loss and the calcium release from the tooth pieces (r_s=0.93; p<0.001). However, the corresponding correlation after 24 h of exposure was much weaker (r_s=0.54; p=0.06). We assume that the weaker correlation obtained between the weight loss and the calcium concentration after 24 h could be due to transient precipitation of other calcium phosphate salts in the drinks after dissolution of hydroxyapatite. Such precipitation would most likely occur in drinks with high calcium and phosphate concentrations. Thus, the milk derived lactic acid drink that had the highest concentrations of calcium and phosphorus of the acidic drinks prior to the immersion of the tooth pieces, was also the drink exhibiting most discrepancy between weight loss and calcium release after 24 h (Table 1).

Nonetheless, highly significant correlations were obtained between the weight loss after 24 and 72 h as well as the calcium release after 24 and 72 h (r_s=0.97 and 0.77; p<0.001). For means of simplification, we therefore, chose only to include the results after 24 h for further calculations. Table 1 shows that the loss of tooth substance after 24 h in the drinks varied from 0.0 to 9.9% of the total weight of the tooth pieces and the calcium release varied from 0 to 31 mmol.

Soft drink composition

Table 1 shows that the pH of Coca-Cola and the sport drinks was lower than the pH of the juices. In contrast, the amount of base needed to increase the pH to 5.5 was considerably higher for fruit juices than for Coca-Cola, sport drinks, and mineral water. In fact, of all the drinks in this study, Coca-Cola had one of the lowest titratable acid volumes and buffer capacities in this pH range. By comparing the actual pH of the drinks with their critical pH, all drinks, except for milk, were undersaturated with respect to hydroxyapatite. Not surprisingly, therefore, milk was the only drink, where neither weight loss nor calcium release occurred during the test period.

Determinants of erosive potential

As shown in the second row from the bottom in Table 1 significant correlations were obtained between the calcium release from the tooth pieces and (i) the pH, and (ii) DSHAP of the drinks. As shown in the bottom row of Table 1 significant correlations were obtained between the weight loss from the tooth pieces over 24 h and (i) the pH; (ii) titratable acid to pH 5.5, 7.0, and 10.0; and (iii) the buffer capacity at pH 4.5 of the drinks. Only the pH of the drinks was significantly correlated (negatively) with both the calcium release and weight loss, whereas the most significant correlation (positively) was obtained between the titratable acid to pH 5.5 and the weight loss.

Soft drink modification

The addition of calcium and phosphate to the experimental drinks had a considerable effect on their critical pH value. Thus the critical pH was lowered from 5.27 in the pure orange juice to 4.24 in experimental drink C. It should, however, be noted that experimental drink C had an unpleasant metallic taste compared to both experimental drinks A and B and pure orange juice, which was the original drink. Nevertheless, lowering the critical pH in experimental drinks B and C considerably reduced the erosive potential of these drinks. In fact the erosive potential was reduced more than six-fold (assessed by weight loss) in experimental drink C compared to the original pure orange juice.

Discussion

Both the factors relating to the properties of the drink itself7,8 as well as associated factors relating to the method of drinking, frequency of consumption, salivary parameters and dental plaque play a role in the development of dental erosion.16,17 The interplay of these factors is complex and clinical studies are often disappointing in their power to explain variations in dental erosion between individuals.2

In this in-vitro study, we focused on the properties of the drink in relation to its erosive potential.
<table>
<thead>
<tr>
<th>Product</th>
<th>pH</th>
<th>C pH</th>
<th>TA 5.5</th>
<th>TA 7.0</th>
<th>TA 10.0</th>
<th>β 4.5</th>
<th>β 6.3</th>
<th>β 8.5</th>
<th>Ca</th>
<th>P</th>
<th>DS_HAP</th>
<th>ΔCa 24</th>
<th>Loss 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure orange juice</td>
<td>3.83</td>
<td>5.27</td>
<td>3.75</td>
<td>5.05</td>
<td>6.21</td>
<td>42.00</td>
<td>15.00</td>
<td>6.00</td>
<td>0.70</td>
<td>3.06</td>
<td>0.08</td>
<td>1.99</td>
<td>1.30</td>
</tr>
<tr>
<td>Pure apple juice</td>
<td>3.59</td>
<td>5.43</td>
<td>2.43</td>
<td>2.79</td>
<td>3.43</td>
<td>24.00</td>
<td>4.00</td>
<td>4.00</td>
<td>0.52</td>
<td>1.94</td>
<td>0.04</td>
<td>3.37</td>
<td>1.20</td>
</tr>
<tr>
<td>Pure grape fruit juice</td>
<td>3.36</td>
<td>5.38</td>
<td>5.92</td>
<td>7.73</td>
<td>9.00</td>
<td>49.00</td>
<td>19.00</td>
<td>6.00</td>
<td>0.51</td>
<td>2.59</td>
<td>0.03</td>
<td>2.89</td>
<td>1.90</td>
</tr>
<tr>
<td>Orange juice from concentrate</td>
<td>3.49</td>
<td>5.92</td>
<td>1.94</td>
<td>2.62</td>
<td>3.11</td>
<td>19.00</td>
<td>8.00</td>
<td>3.00</td>
<td>0.12</td>
<td>1.38</td>
<td>0.01</td>
<td>3.05</td>
<td>1.10</td>
</tr>
<tr>
<td>Malt (malt extract drink)</td>
<td>4.51</td>
<td>5.33</td>
<td>0.59</td>
<td>2.41</td>
<td>5.13</td>
<td>12.00</td>
<td>23.00</td>
<td>16.00</td>
<td>0.32</td>
<td>6.70</td>
<td>0.23</td>
<td>1.18</td>
<td>0.10</td>
</tr>
<tr>
<td>Milk derived lactic acid</td>
<td>3.46</td>
<td>4.27</td>
<td>8.40</td>
<td>10.33</td>
<td>12.15</td>
<td>71.00</td>
<td>18.00</td>
<td>8.00</td>
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<td>Magic-energy drink</td>
<td>2.90</td>
<td>6.36</td>
<td>3.56</td>
<td>5.73</td>
<td>7.66</td>
<td>26.00</td>
<td>24.00</td>
<td>10.00</td>
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<td>0.16</td>
<td>0.00</td>
<td>3.32</td>
<td>1.80</td>
</tr>
<tr>
<td>Gatorade-sport drink</td>
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<td>5.80</td>
<td>1.66</td>
<td>2.38</td>
<td>2.62</td>
<td>14.00</td>
<td>9.00</td>
<td>1.00</td>
<td>0.12</td>
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<td>0.01</td>
<td>3.12</td>
<td>1.50</td>
</tr>
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<td>5.25</td>
<td>6.33</td>
<td>0.16</td>
<td>2.38</td>
<td>2.62</td>
<td>14.00</td>
<td>9.00</td>
<td>1.00</td>
<td>0.12</td>
<td>2.64</td>
<td>0.01</td>
<td>3.12</td>
<td>1.50</td>
</tr>
<tr>
<td>Coca-cola &lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.59</td>
<td>5.79</td>
<td>0.76</td>
<td>2.31</td>
<td>4.99</td>
<td>5.00</td>
<td>19.00</td>
<td>16.00</td>
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<td>0.13</td>
<td>0.02</td>
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<td>Milk</td>
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<td>1.37</td>
<td>27.10</td>
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<td>75.22</td>
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<td>0.00</td>
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<td>4.17</td>
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<td>0.54</td>
<td>1.54</td>
<td>3.77</td>
<td>8.00</td>
<td>13.00</td>
<td>3.00</td>
<td>0.15</td>
<td>0.20</td>
<td>0.03</td>
<td>1.33</td>
<td>0.40</td>
</tr>
<tr>
<td>Lemon concentrate</td>
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<td>5.94</td>
<td>30.91</td>
<td>38.62</td>
<td>40.22</td>
<td>129.00</td>
<td>36.00</td>
<td>13.00</td>
<td>0.25</td>
<td>0.41</td>
<td>0.00</td>
<td>30.95</td>
<td>9.90</td>
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<tr>
<td>Experimental drink A</td>
<td>3.32</td>
<td>4.58</td>
<td>4.91</td>
<td>4.91</td>
<td>3.90</td>
<td>16.00</td>
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<td>0.10</td>
<td>4.40</td>
<td>1.30</td>
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<tr>
<td>Experimental drink B</td>
<td>3.70</td>
<td>4.31</td>
<td>3.81</td>
<td>3.81</td>
<td>16.50</td>
<td>16.20</td>
<td>0.33</td>
<td>5.30</td>
<td>0.50</td>
<td></td>
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</tr>
<tr>
<td>Experimental drink C</td>
<td>4.20</td>
<td>4.24</td>
<td>2.96</td>
<td>2.96</td>
<td>25.20</td>
<td>20.30</td>
<td>0.93</td>
<td>7.90</td>
<td>0.20</td>
<td></td>
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</tr>
<tr>
<td>Correlation with ΔCa 24</td>
<td>—0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>—0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ns.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation with weight loss 24</td>
<td>—0.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ns.</td>
<td>0.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
<td>ns.</td>
</tr>
</tbody>
</table>

Correlation coefficients obtained by Spearman’s rank correlation analysis without the experimental drinks. ns, denotes non-significant.

<sup>a</sup> $p < 0.05$.
<sup>b</sup> $p < 0.001$.
<sup>c</sup> $p < 0.01$. 

**Table 1** The pH, titratable acid (TA), critical pH (C pH), buffer capacity (β), calcium (Ca), phosphorus (P), degree of saturation with respect to hydroxyapatite (DS_HAP), and erosive potential of selected soft drinks and experimentally modified fruit juices.
The erosive potential was determined as weight loss from tooth pieces after immersion into the drinks as well as calcium release from the tooth pieces. Although the weight loss method used for assessment of erosive potential lacks accuracy and precision in relation to actual measures it will give a relative indication of the erosive potential of the drink. By these methods we found that the pH, titratable acid (to pH 5.5, 7.0, and 10.0), buffer capacity (at pH 4.5), and degree of saturation with respect to hydroxyapatite of the drinks all had an effect on the erosive potential. However, only the pH of the drink correlated significantly with both the weight loss and the calcium release from the tooth pieces, whereas the titratable acid to pH 5.5 had a very high correlation with the weight loss. The latter finding is in agreement with many previous studies, showing that the titratable acid of a drink is a good measure of its erosive potential.\(^\text{17,18}\) Moreover, this study showed that fruit juice and especially pure fruit juices had a high titratable acid and accordingly also a high buffer capacity compared to the carbonated soft drink Coca-Cola. These results may explain the high erosive potential of orange juice often shown in the literature.\(^\text{7,17–19}\) Although Coca-Cola did not show a high erosive potential in this in vitro study, many clinical studies have shown strong associations between the consumption of cola-type drinks and the prevalence and severity of erosion.\(^\text{5,9}\) One explanation for this finding may be that cola beverages have a very low pH. Thus, as shown in the results both the pH and titratable acid of the drinks showed strong correlations with the erosive potential.

Only two of the drinks tested, i.e. the milk-derived lactic acid drink and the lemon concentrate, had high titratable acid and at the same time also low pH. Not surprisingly, exposure of the tooth pieces to these drinks resulted in the highest weight losses obtained in this study. Thus according to the results of this study, where teeth were exposed to a limited volume of the drink for a long time, both low pH and high titratable acid (and buffer capacity) will result in erosive potential and both qualities combined will result in the highest erosive potential. Nevertheless, as pointed out by Lussi et al.,\(^\text{20}\) other factors like the degree of saturation with respect to hydroxyapatite also influence the erosive potential of soft drinks as illustrated by the reduced erosive potential in the experimental drinks in this study.

As the calcium concentration increased in the drinks, during the experiment, due to dissolution of hydroxyapatite from the tooth pieces, the composition of the drinks was not constant during the experimental period. Thus, when hydroxyapatite is dissolved calcium, phosphate and hydroxide ions will be released from the tooth substance into the fluid that is causing the erosion. Similarly, the phosphate concentration as well as the pH of the drinks must have increased during the experimental procedure with the tooth pieces. The rise in pH would have been most pronounced in drinks with low titratable acid and buffer capacity like the Coca-Cola and less pronounced in drinks with high titratable acid and buffer capacity like the juices. This explains why the juices had high erosive potential in this study.

Although the composition of the drinks could have been kept constant by using much larger volumes of soft drink (i.e. litres) than the ones used in this study (10 ml) we speculate that the results of this study may better reflect the dynamics that occur in the mouth after a substantial volume of the drink has been swallowed with only small volumes of soft drink still in contact with the teeth. Thus the volume of fluid left in the mouth after swallowing normally only amounts to 0.6-0.8 ml.\(^\text{21}\) The results in this study could also resemble a situation with a slow clearance of acid from the oral cavity due to a low saliva flow rate. In such a situation, the relations described in this study, especially that of the titratable acid, will most certainly become important for the erosive potential of a drink. Thus these relations could be important in patients suffering from dry mouth due to irradiation or Sjogrens syndrome, where the contact between the drink and the teeth will be much longer than in individuals with normal saliva flow rates.

In this context, it is interesting to compare the titratable acid of the drinks with the titratable base of human whole saliva. The titratable base of human resting whole saliva is on average 20 mmol H\(^+\) from pH 7 down to 3.\(^\text{22}\) Calculated in ml 1 M HCl this will be equivalent to 1 ml 1 M HCl in 50 ml of saliva. In comparison the titratable acid of the fruit juices to pH 5.5 was on average 3.5 ml 1 M NaOH (Table 1). Thus, it is unlikely that human saliva can increase the pH of fruit juices up to pH 5.5 unless the volume of saliva is considerably larger than the volume of juice. In contrast, the titratable acid of Coca-Cola was less than the titratable base of human saliva, a finding that at least theoretically would make it possible for human saliva to increase the pH of Coca-Cola in vivo. In support of these
speculations, a previous study has found human saliva ineffective in buffering apple juice, but effective in buffering carbonated water, for the first minutes following intake.23

In agreement with previous studies, this study has shown that calcium and phosphate addition to soft drinks reduces their erosive potential.10,24 Thus the addition of calcium and/or phosphate to a soft drink will increase its degree of saturation with respect to hydroxyapatite and decrease its critical pH. The lower the pH of the drink, the more calcium and phosphate is needed to saturate it. Although it seems tempting to prevent dental erosion by this measure, it is most likely that addition of large amounts of calcium phosphate will give the drink an unpleasant metallic taste as observed in this study. Nevertheless, progress has been made in the production of chemically modified drinks that are less erosive.11

According to the data obtained in this study, we speculate that soft drink modification may be a helpful measure to reduce erosion from drinks with pH values above 4.0. Thus, modification of some fruit juices seems possible, whereas modification of very acidic drinks like Coca-Cola seems unrealistic. Finally, biological factors such as the dental pellicle25 and fluoride originating from dentifrice and diet will also modify the erosive potential by protecting tooth surfaces against erosive effects. It is therefore, a possibility that some drinks, despite having a low pH, high titratable acid, and erosive potential in vitro only have limited erosive potential in vivo.

Conclusion

We found that several properties related to soft drinks such as pH, titratable acid, buffer capacity, and degree of saturation with respect to hydroxyapatite have an impact on the erosive potential of the drink. Furthermore we found that soft drink modification by calcium and phosphate addition to the drinks may be a helpful measure to reduce the erosive potential of some soft drinks.

Acknowledgements

We thank Margrét O Magnúsdóttir for her skilled technical assistance. This study was supported in part by the Icelandic Research Council, the University of Iceland Student Innovation Fund, Sól Viking hf and the Icelandic Dental Association.

References


ARTICLE II
Immediate Erosive Potential of Cola Drinks and Orange Juices

INTRODUCTION

Dental erosion is the chemical wear of the dental hard tissue without the involvement of bacteria (Eccles, 1979). The prevalence of dental erosion is increasing (Arnadottir et al., 2003; Nunn et al., 2003), and soft drink consumption is recognized as one of the main risk factors (Johansson et al., 1997). Clinical studies have found carbonated drinks, especially carbonated cola drinks, to be associated with erosion, most likely due to their low pH (Johansson et al., 1997; Jensdottir et al., 2004). However, in vitro studies have shown that fruit juices may also be potentially erosive, due to their high content of titratable acid (Lussi et al., 1995; Larsen and Nyvad, 1999; Jensdottir et al., 2005a). We speculate that the dynamics of the erosive potential within the first seconds and minutes of exposure may be critical, since the bulk of a soft drink stays in the mouth for only seconds before being swallowed. After swallowing occurs, the residual amount of liquid in the mouth will be reduced to less than 1 mL (Lagerlöf and Dawes, 1984), leaving only a limited amount of drink in contact with the teeth. In healthy individuals, and with reduced rate in dry-mouth patients, the soft drink is mixed with saliva, which then will re-establish its super-saturation level with respect to hydroxyapatite, due to acid clearance (Bashir and Lagerlöf, 1996) and salivary buffering capacity (Jensdottir et al., 2005b).

Equally important in the mouth are the protective effects of the salivary proteins that also may influence the erosive potential of soft drinks (Zahradnik et al., 1976; Meurman and Frank, 1991). The aim of this study was to determine the erosive effects of soft drinks within the first minutes of exposure, and the protective effects of salivary proteins. We hypothesized that the erosive potential of acidic drinks within the first minutes of exposure is closely related to clinical findings showing that soft drink pH is the most important factor for dental erosion, that, due to their low pH, cola drinks are, initially, considerably more erosive than orange juices, and that human salivary proteins may reduce the erosive potential during the acidic challenge.

MATERIALS & METHODS

Ten orange juices and 10 cola drinks were tested. The cola drinks were: Coca Cola, Cola light, Pepsi Cola (all in plastic and glass), Cola light lemon, Pepsi Max, Pepsi Twist, and a local discount Cola drink. Orange juices were: Capri-Sonne Orange, Sun Top, Rynkeby with sweet oranges, sour oranges, and organic oranges, three orange juices made from concentrate, and two made from fresh orange juice, the latter five produced by local supermarket chains. Initially, the pH was recorded (pH0) after 50 mL of the drink was titrated with 1 M NaOH to a pH above 5.5 (Fig. 1A). Then, a 50-mg quantity of freeze-dried hydroxyapatite (HAp) crystals was added to new 50-mL samples of each drink while the pH of the drinks was recorded at 15-second intervals for 3 min (pH1-pH12) and then finally 30 min (pH13) after HAp addition (Fig. 1B). Within the pH range recorded (pH 2.5-4.5), HAp mainly dissolves as: Ca_{10}(PO_4)_6(OH)_2 \rightarrow 10Ca^{2+} + 6H_2PO_4^- +...
2H$_2$O where the released PO$_4^{3-}$ and OH$^-$ ions combine with 14 hydrogen ions, thereby changing the pH in an alkaline direction. The magnitude of this pH rise depends on the amount of titratable acid in the drink. Therefore, the volume of base ($\mu$L 1 M NaOH) needed to reach each pH value obtained by HAp addition (pH$_1$-pH$_{13}$) was determined from the titration curve (Fig. 1C). Thus, titration with 14 $\mu$L of 1 M NaOH represents the loss of 14 $\mu$g of hydrogen ions in the drink, corresponding to dissolution of 1005 $\mu$g of HAp (M$_W$ 1005) crystals due to the stoichiometry of the reaction.

**Initial and End Erosive Potential**

The initial erosive potential was determined as the erosive potential within the first 3 min of exposure of HAp to the drink and was recorded as the slope of a curve obtained by linear regression showing the HAp dissolved (in mg) per liter of drink as a function of time (in sec). When the slope of this curve was non-linear, data points were eliminated successively from pH$_{12}$ and backward. In all cases, data points obtained within the first minute were kept in the analysis. The end erosive potential was recorded as mg HAp dissolved per liter of drink at pH$_{13}$.

**Effects of Human Salivary Proteins**

The combined procedure was repeated with 50 mg of HAp crystals pre-treated with 5 mg of human salivary proteins. The proteins were dialyzed and lyophilized from a pool of 1 liter unstimulated and stimulated clarified whole saliva collected from 100 healthy dental students (upon ethical approval and informed consent). SDS-PAGE revealed that all major salivary proteins were represented in the pool (Schwartz et al., 1995). HAp crystals were coated with the proteins for 24 hrs, resembling the time between daily toothbrushings, at a temperature of 5°C, to prevent denaturation and bacterial growth, in a volume of 2 mL Millipore water at pH 6.5. This pH value allowed for the normal physiological functionality of the proteins. After the crystals were coated, they and the remaining excess protein in the 2-mL solution were lyophilized and added directly to the drink in a manner similar to that for the non-coated crystals. All experiments were carried out at room temperature and were repeated at least three times.

**Statistics**

Statistical analyses were done with Excel and the R statistical program (R Development Core Team, 2004). Differences between juices and cola drinks were analyzed by Wilcoxon’s rank-sum test and correlations with Spearman’s rank correlation analysis ($r_s$). For determination of the initial erosive potential (Fig. 2), the best linear relationships between HAp dissolution and exposure time to the drinks were obtained by linear regression analysis judged from the R-squared values obtained. In Fig. 3, the curves were exponentially fitted. The level of significance was set at $p < 0.05$.

**RESULTS**

The mean pH of the cola drinks was more than one pH unit lower than the mean pH of the orange juices ($p < 0.001$) (Table). Despite the higher pH in the orange juices, their titratable acidity values to reach pH 5.5 were nearly five-fold higher, on average, than in the cola drinks ($p < 0.001$). Nonetheless, the cola drinks clearly had a higher erosive potential within the first minute of exposure to HAp crystals than did the orange juices (Fig. 2A). Thus, the initial erosive potential in the cola drinks was more than ten-fold higher ($p < 0.001$) than in the orange juices (Table), and the initial erosive potential was high in all cola drinks. In contrast, considerable differences were obtained among the orange juices, ranging from an initial erosive potential of around half that of the cola drinks to no sign of erosive potential (Fig. 2A).

The erosive potential changed over time, more so in the cola drinks (Fig. 2B). Thus, after the first 3 min, the erosive potential in the cola drinks slowed more than 40-fold, whereas the erosive potential slowed only less than three-fold in the orange juices. The slowing of the erosive potential was not due to lack of HAp crystals, since only 36% of the crystals in the cola drinks and only 6% in the juices were lost after 3 min, but due to increased pH. A significant negative correlation was obtained between the slowing of the erosive potential (initial erosive potential/erosive potential from 3-30 min) and the titratable acid values for the drinks ($r_s = 0.83; p < 0.001$). Thus, the juices were able to maintain low pH values throughout the study period, with a pH rise of only 0.13 ± 0.14 unit compared...
with 1.79 ± 0.45 unit in the cola drinks (p < 0.001), resulting in a more constant erosive potential. These dynamics led to the finding that, among the orange juices, which, as a group, were only slightly erosive for the first 3 min, two drinks became the third and fourth most erosive drinks after 30 min (Fig. 2B).

**Effects of pH**

The initial erosive potential was almost an exponential function (R-squared 0.81; p < 0.001) of the pH of the drinks, increasing nearly ten-fold for each time the pH lowered one unit (Fig. 3A). Surprisingly, a negative relationship was obtained between the titratable acidity to pH 5.5 and initial erosive potential (not shown). When the data were reviewed, it became clear that this finding was due to all juices having higher titratable acid values than the cola drinks, and, at the same time, a lower initial erosive potential. The relation between pH and the end erosive potential after 30 min was quite different from that for the initial erosive potential (Fig. 3B). Thus, the end erosive potential increased only around two-fold for every unit the pH was lowered. This shows that, upon prolonged exposure time to a limited volume of soft drink, other factors such as titratable acid also became important for the erosive potential.

**Effects of Salivary Proteins**

Prior to all experiments, the effect of adding 5 mg of proteins to 50 mL of drink was tested. For all drinks, protein addition had no, or only a negligible, effect on the pH and titratable acidity of the drink. Therefore, we were able to test the effect of adding the same amount of proteins now delivered with the HAp crystals to the drinks. The pre-treatment of HAp crystals with salivary proteins reduced the initial erosive potential by 50% at pH values near 2.5 (Fig. 3A). However, at pH values above 3.5, no protective effect was obtained from the proteins. Thus, the protective effect of the proteins on HAp crystals (i.e., the relative reduction in initial erosive potential with salivary proteins) was significantly negatively correlated with pH (r = -0.47; p < 0.05) and significantly positively

**Table.** Characteristics of Cola Drinks and Orange Juices (medians and ranges)

<table>
<thead>
<tr>
<th></th>
<th>Cola Drinks</th>
<th>Orange Juices</th>
<th>P-value</th>
</tr>
</thead>
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<tr>
<td>pH</td>
<td>2.70 (2.39-2.88)</td>
<td>3.73 (3.12-4.08)</td>
<td>&lt; 0.001</td>
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<td>Titratable acidity*</td>
<td>705 (563-1025)</td>
<td>4150 (1600-4450)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Initial erosive potential</td>
<td>3.75 (1.40-5.20)</td>
<td>0.34 (0.00-1.40)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>End erosive potential</td>
<td>480 (377-711)</td>
<td>271 (112-550)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Titratable acidity denotes the amount (μL) of base (1 M NaOH) needed to reach pH 5.5 in the drinks. The initial erosive potential calculated as the amount of HAp (mg) lost per sec per liter soft drink during the first minutes of exposure. The end erosive potential calculated as the amount of HAp (mg) lost per liter soft drink after 30 min of exposure. P-values were obtained by Wilcoxon’s rank-sum test.
correlated with the initial erosive potential ($r_s = 0.65; p < 0.01$). As a consequence of this relationship, the protective effect was higher in cola drinks than in juices on a group basis ($p < 0.05$). Interestingly, only a very limited effect of the proteins was obtained on the end erosive potential after 30 min, and this effect was not dependent on pH (Fig. 3B).

**DISCUSSION**

To predict the erosive potential of a soft drink, the method used should simulate what happens in vivo when the drink enters the mouth. Theoretically, this must be dependent upon the immediate effect of the drink on the tooth surface, the time it takes to clear the drink from the mouth (Bashir and Lagerlöf, 1996), the drinking method (Johansson et al., 2004), the protective effect of saliva (Zahradnik et al., 1976; Meurman and Frank et al., 1991; Jensdottir et al., 2005b), and the amount of residual drink after swallowing. Although the HAp method used in this study is purely experimental, creating a situation with a large crystal surface area in contact with the drink, it offers the possibility for mineral dissolution to be monitored almost instantaneously upon contact with the drink tested.

Cola drinks had more than ten-fold higher erosive potential than orange juices within the first minutes after exposure. This high erosive potential corresponded well to the pH of the cola drinks, which was around one unit lower than that of the juices. Thus, within the first minutes, the erosive potential was nearly an exponential function of pH in both cola drinks and orange juice, as would be expected due to the logarithmic nature of the pH scale. These findings are in agreement with those of Larsen and Nyvad (1999), who found a similar exponential relation between soft drink pH and erosive potential on teeth. However, in their study, the titratable acidity was also found to have an effect on the erosive potential, a finding that has been supported by several other studies (Lussi et al., 1995; Edwards et al., 1999; Jensdottir et al., 2005a). Nevertheless, according to our in vitro findings, we speculate that the titratable acidity is not related to the erosive potential from the time the drink meets the tooth surfaces until the first swallow occurs. The titratable acidity may, however, become important later on, when some of the drink is kept in contact with teeth. Such a situation could occur in dry-mouth patients with low salivary flow rates (Fox et al., 1987) and, consequently, slow oral clearance (Dawes, 1983), with drinks that, due to their physical characteristics, tend to attach to the teeth for a long period of time (Ireland et al., 1995), and in patients with special drinking habits (Ireland et al., 1995; Johansson et al., 2004).

In the mouth, tooth surfaces are covered with the acquired pellicle, comprising many of the proteins present in saliva (Lendenmann et al., 2000), and this pellicle has been shown to protect tooth surfaces against erosion (Meurman and Frank, 1991; Amaechi et al., 1999; Nekrashevych and Stosser, 2003). In this study, we showed that the protective effects of salivary proteins increased with increasing erosive potential within the first minutes of exposure to the drinks. Within this time, the proteins halved the erosive potential of cola drinks with a low pH, while only limited effect was found with the orange juices that had higher pH values and thus lower erosive potential. We speculate that these findings are due to the relationship between the speed of desorption of proteins from the crystals and the speed of the erosive challenge. Thus, if the erosive speed (initial erosive potential) exceeded the speed of desorption of proteins from the crystals, a protective effect was obtained, and vice versa. We assume that when the proteins were washed of the crystals, their protective effect ceased, which explains the very limited effects of the proteins on the erosive potential after 30 min (end erosive potential). Thus, in the mouth, after exhibiting its protective effects, the protein coating must be renewed to withstand a new acidic challenge. However, renewal of the protein coating may take considerable time.

![Figure 3. Erosive potential and soft drink pH. (A) Relationship between the initial erosive potential (i.e., erosive potential during the first minutes of exposure) and the pH (open circles) for all drinks ($N = 20$). As shown, the initial erosive potential was almost a logarithmic function of the pH, increasing ten-fold for each one-unit decrease in pH. The erosive potential of the drinks was reduced, more so in drinks with low pH values and high initial erosive potential, when the HAp crystals were coated with human salivary proteins (bold circles), illustrated by the gray area between the lines ($N = 20$). (B) Corresponding relationship between the end erosive potential (i.e., erosive potential after 30 min) and the pH in the drinks ($N = 20$). Only a very limited effect was seen from the human salivary protein on the end erosive potential of the drinks, as illustrated by the reduction in size of the gray area compared with Fig. 3A ($N = 20$).]
(Zahradnik et al., 1976; Nieuw Amerongen, 1987), and this time may be a critical factor in individuals who tend to sip soft drinks throughout the day, which partly explains their high incidence of erosion (Johansson et al., 2004). Another likely explanation for the relationship between the protective effects of the proteins and the erosive potential of the drinks may be protein denaturation, which could have occurred in drinks with low pH values, thereby increasing the viscosity of the protein coating on the crystals (Holma and Hegg, 1989) and adding to the protective effects.

In conclusion, this study shows that the erosive potential of soft drinks within the first minutes of exposure is solely dependent on the pH of the drinks. Furthermore, it is ten-fold higher in cola drinks when compared with orange juices. However, in cola drinks, human salivary proteins may reduce the erosive potential up to 50%.

ACKNOWLEDGMENTS

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REFERENCES


ARTICLE III
Effects of Sucking Acidic Candy on Whole-Mouth Saliva Composition

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Abstract

Limited information is available on the effects of sucking acidic candies on saliva composition and the protective role of saliva in this relation. Therefore the aim of this study was to determine salivary effects of sucking acidic candies in vivo in relation to individual variations in whole-saliva flow rate (WSFR) and buffer capacity (WS\textbeta{}). Ten healthy young males (24 ± 2 years) sucked a rhubarb-flavoured acidic hard-boiled candy with tartaric acid available on the Danish market. The whole saliva was collected into a closed system, regarding CO$_2$, at different times as follows: firstly, unstimulated saliva for 5 min (baseline), secondly stimulated saliva for 4 min upon sucking the candy, and finally post-stimulated saliva for 10 min. Saliva pH was determined on a blood gas analyser and WS\textbeta{} was estimated from the saliva bicarbonate concentration obtained by the analyser and by ionic balance calculation. The erosive potential of the candy in saliva was estimated from the saliva pH values and degree of saturation with respect to hydroxyapatite (DS$_{\text{HAp}}$). The results showed that saliva pH dropped from 6.5 (baseline) down to 4.5 at the fourth minute of sucking the candy, and returned to pH 6.5 five minutes after stimulation (post-stimulated). DS$_{\text{HAp}}$ decreased upon sucking the candy and saliva from all subjects became undersaturated with respect to HAp. Significant positive correlations were obtained between pH and WSFR ($r_s = 0.47; p < 0.05$) and between pH and WS\textbeta{} ($r_s = 0.65; p < 0.01$).

In relation to WS\textbeta{} we found that 70% of the buffer capacity originating from the bicarbonate buffer system upon sucking the candy was exerted as phase buffering. We conclude that sucking this type of acidic candies changes whole-mouth saliva composition so that it may have erosive potential and that high WSFR and WS\textbeta{} have protective effects against these salivary changes.

Dental erosion is the chemical wear of the dental hard tissue without bacteria and is therefore not associated with dental caries [Eccles, 1979]. The prevalence of dental erosion is gradually increasing, especially among children [Nunn et al., 2003] and young people [Jensdottir et al., 2004]. Aetiological risk factors of dental erosion are categorized into intrinsic and extrinsic factors. Soft drink consumption has been considered to be the major extrinsic risk factor of erosion [Johansson et al., 1997; Jensdottir et al., 2004]. Acidic foodstuffs [Grobler et al., 1989] and food supplements [Giunta, 1983] have also been
shown to be erosive, but only few clinical studies [Lussi et al., 1997] and experimental studies [Holloway et al., 1958; Bibby and Mundorff, 1975] on the erosive potential of acidic candies exist. As dental erosion develops at low pH values, the saliva acid clearance [Bashir and Lagerlof, 1996] and buffer capacity [Leung, 1951; Lilienthal, 1955] become important modifying factors for the erosive potential of acidic candies in vivo. Nevertheless, little is known about the effectiveness of saliva in counteracting the acidic challenge induced by such candies. Furthermore, as patients with reduced saliva flow rates are likely to use such acidic candies to increase saliva flow for relief of oral symptoms [Colquhoun and Ferguson, 2004] there is a need to determine the effect of such candies on saliva composition with respect to their erosive potential.

Thus the aim of the study was to determine saliva composition in response to sucking acidic candy with respect to its erosive potential. The erosive potential of the candy dissolved in saliva was judged by the saliva degree of saturation with respect to hydroxyapatite (HAp) and its pH. We also aimed to test the general belief that saliva by its clearing effect and buffer capacity plays an important role in protecting the teeth against acid-induced dental erosion.

Materials and Methods

Study Group and Study Design

Saliva was collected from 10 healthy non-medicated, male volunteers, 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 24 ± 2 years of age, weighed 75 ± 10 kg, were 184 ± 5 cm tall, fully dentate (28 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 Ethical Committee of Copenhagen, Denmark (No. 03-001/03).

Collection of Whole Saliva

The experiments were performed during daytime on 2 separate days. The saliva collection method used on both days was similar, except for the stimulus used during collection of the stimulated saliva production. On the first day, non-paced chewing on non-acidic sorbitol-sweetened chewing gum weighing on average 1 g/piece was used to stimulate saliva production (taste + chewing), whereas on the second day, a commercially available acidic hard-boiled candy (Sømods-Bolcher, Copenhagen, Denmark), with tartaric acid and rhubarb taste, weighing on average 5 g, was used to stimulate saliva production. For the outside came in contact with the saliva sample. Each collection trial took 19 min and was divided into three periods. The first period consisted of a 5-min collection of unstimulated saliva, collected every minute into a single syringe (baseline). The second period consisted of a 4-min collection of stimulated saliva (chewing gum or sucking acidic candy), collected every 30 s into four syringes (stimulated), after which the stimulus was removed. Finally, the last period consisted of a 10-min collection of unstimulated saliva, collected every minute into two syringes (post-stimulated). All saliva samples were stored on ice in their individual closed syringes and pH and P_{CO_2} was determined within 30 min. The syringes were weighed before and after collection of saliva. In all experiments the flow rates were calculated in grams per minute, which is almost equivalent to millilitres per minute [Navazesh and Christensen, 1982].

Whole-Saliva pH, P_{CO_2}, Bicarbonate and Buffer Capacity

Whole-saliva P_{CO_2} and pH were measured on a standard blood gas analyser (ABL 605, Radiometer, Copenhagen, Denmark) and the Henderson-Hasselbalch equation, HCO_3^- = (P_{CO_2} \times \alpha_{CO_2,saliva}) \times (10^{pH - pK_a}), was used to calculate the bicarbonate concentration, where the absorption coefficient for CO_2 in saliva (\alpha_{CO_2,saliva}) was 0.225 and pK was 6.15 [Bardow et al., 2000a].

Saliva buffer capacity (mmol H^+/litre \times pH) was calculated from total CO_2 concentrations (i.e. the bicarbonate buffer system) and inorganic phosphorus for pH 6.15 as (0.575 \times \text{[total CO}_2\text{]} + 0.340 \times \text{[inorganic phosphorus]}) [van Slyke, 1922]. In samples that were acidified by the candy, total CO_2 (mainly representing bicarbonate at physiological pH) was estimated as the missing anion by ionic balance calculation [Dawes, 1974]. In all other samples total CO_2 was calculated from the saliva bicarbonate concentration.
the following the term buffer capacity refers only to the buffer capacity for pH 6.15 (i.e., $\beta_{6.15}$).

Effect of the Candy in a Non-Buffering Solution
To determine the effect of the candy on pH in a non-buffering solution, the average amount of candy (4.2 g) that was dissolved by sucking was added to 22 ml of Millipore water, equal to the average volume of saliva produced upon sucking over 4 min. The candy was dissolved in the Millipore water by stirring the composition with a magnet rotating in a propeller fashion. The rotating speed of the magnet was adjusted so that 4.2 g of candy was dissolved in 4 min. By this setup ten series of candy dissolution were performed with pH measurements every 0.5 min for 4 min corresponding to the stimulated in vivo measurements.

Inorganic Saliva Composition
Sodium, potassium, magnesium and calcium concentrations were determined by atomic absorption spectroscopy (AAnalyst 400, Perkin-Elmer, Shelton, USA). Prior to determination all samples were diluted (100–1,000 times) in CsCl and thymol-containing Millipore water. When measuring calcium and magnesium, KCl and either SrCl$_2$ or LaCl$_3$ were added to the dilution solution to reduce oxysalts and ionization. Inorganic phosphorus [Goldenberg and Fernandez, 1966] and total protein (Coomassie blue, Bio-Rad Laboratories, Calif., USA) were determined spectrophotometrically. Chloride was determined by coulometric titration (CMT10, Radiometer, Copenhagen, Denmark). All samples were measured at least twice in two separate series.

Degree of Saturation with Respect to HAp in Saliva
All calculations were performed for conditions at 37°C, which is near the temperature of saliva in the mouth. The ionic strength of saliva was calculated from the concentrations of sodium, potassium, magnesium, calcium, chloride, bicarbonate, and phosphate. The $-\log_{10}$ (solubility product), $pK_{sp}$ for HAp [Ca$_{10}$(PO$_4$)$_6$(OH)$_2$] was set at 117.3 [McDowell et al., 1977], $pK_{sp}$ at 13.6, pH for H$_3$PO$_4$/H$_2$PO$_4^-$ at 2.2, for H$_2$PO$_4^-$/HPO$_4^{2-}$ at 7.2, and for HPO$_4^{2-}$/PO$_4^{3-}$ at 12.2 [Bjerrum and Unmack, 1929]. Activity coefficients for calcium and phosphate were calculated by the extended Debye-Hückel equation taking into account the effective diameter of the hydrated ions [Hückel, 1925; Kielland, 1937]. The degree of saturation with respect to HAp ($DS_{HAp}$) was calculated as: $pK - pH/18$ [Larsen and Pearce, 2003]. All salivary data needed for the calculations were imported into the R statistical programme [R Development Core Team, 2004] where the equations for $DS_{HAp}$ were processed allowing for calculation of $DS_{HAp}$ and generation of a text file with the results for multiple samples simultaneously.

Statistics
Statistical analyses were done with MS Excel and with the R statistical programme [R Development Core Team, 2004]. Differences in salivary parameters when sucking the candy and chewing the gum were analysed by Wilcoxon’s signed rank test. Correlations were analysed by Spearman’s rank correlation analysis with correlation coefficients ($r_s$) and p values given. Differences between individuals were determined by the Kruskal-Wallis test. The level of significance was set at $\alpha = 0.05$.

Results
As shown in figure 2, the sodium, chloride and bicarbonate concentrations increased with increasing saliva flow rate both while sucking candy and chewing gum, whereas the calcium concentration changed little and the potassium and phosphate concentrations decreased with flow rate. The increase in sodium, chloride and bicarbonate concentrations significantly increased ($p < 0.001$) the ionic strength of the stimulated saliva, especially during candy stimulation (fig. 3B). Furthermore, as shown in figure 2, the saliva protein concentration was higher both during stimulation by the candy and afterwards at lower flow rates compared to the gum ($p < 0.001$). Although both the candy and the gum had a stimulatory effect on saliva flow rate, the effect of the candy was more than 5 times higher on average (fig. 3A) than that of the gum ($p < 0.001$). Also, the stimulation from the candy lasted longer than the stimulation from the gum, resulting in higher flow rate during the post-stimulatory phase. On average 1 g of candy was sucked per minute stimulating...
Fig. 3. Effects of sucking acidic candy and chewing gum on saliva composition and erosive potential. Black continuous lines represent the acidic candy, dotted lines the chewing gum, vertical bars SEM, and vertical sections the period of stimulation. A Saliva flow rate upon stimulation. B Saliva ionic strength. C Saliva buffer capacity ($\beta_{6.15}$). D Saliva partial pressure of CO$_2$ ($P_{CO_2}$). E Salivary pH and a similar acidic challenge in pure water solution. F Degree of saturation of saliva with respect to HAp. The area below the horizontal line is where the saliva is undersaturated with respect to HAp and erosion is likely to occur.
the production of 5 ml saliva. The test persons that had high saliva flow rates while chewing the gum also had high flow rate while sucking the candy \( (r_s = 0.62, p < 0.01) \).

Figure 3C shows an increased buffer capacity \( (\beta_{6.15}) \) in saliva upon stimulation and more so with the acidic candy than the chewing gum \( (p < 0.001) \). Thus the saliva buffer capacity was, on average, nearly twice as high while sucking the candy than while chewing the gum (fig. 3C). Figure 3D shows that the partial pressure of carbon dioxide while sucking the acidic candy was significantly higher than when chewing gum \( (p < 0.001) \). In contrast, no major changes in saliva \( P_{CO_2} \) were obtained in response to chewing gum. Figure 3E shows that sucking on the candy resulted in a serious pH drop below pH 5.5, the average critical pH of human saliva with respect to HAp [Schmidt-Nielsen, 1946]. The pH dropped instantly upon sucking and was below the critical pH during the last 3 min of sucking. For comparison the pH change in a non-buffering water solution is superimposed onto figure 3E. As shown the pH drop in the non-buffering solution occurred more rapidly and was considerably more profound than the pH drop in the saliva. Figure 3F shows that saliva became undersaturated with respect to HAp while sucking the candy. In contrast the degree of saturation increased upon chewing the gum.

**Individual Correlations**

There was a significant positive correlation between saliva flow rate and pH values while sucking on the candy \( (r_s = 0.47; p < 0.05) \). Furthermore, analysis revealed that the 10 persons tested in this study differed significantly with regard to saliva flow rate while sucking on the candy \( (p < 0.001) \). Thus some persons with high saliva flow rates did not experience as low pH values as other persons with low flow rates. There was also a significant positive correlation between the saliva buffer capacity and pH values produced upon sucking the candy \( (r_s = 0.65; p < 0.01) \). As for the flow rates, some persons generally had a high buffer capacity \( (\beta_{6.15}) \) upon sucking the candy while others had a low buffer capacity \( (p < 0.01) \). Finally the bicarbonate buffer system alone \( (i.e. \text{the concentration of total } CO_2) \) showed the best correlations with the pH values induced by the acidic candy \( (r_s = 0.67; p < 0.01) \).

While sucking the candy \( \Delta S_{HAP} \) was significantly correlated with flow rate \( (r_s = 0.43; p = 0.05) \), buffer capacity \( (r_s = 0.61; p < 0.01) \), and concentration of total \( CO_2 \) \( (r_s = 0.62; p < 0.01) \). In contrast the candy-induced changes in saliva calcium and phosphate concentrations did not have any effect on \( \Delta S_{HAP} \) while sucking.

**Discussion**

This study tested the effect of sucking acidic candy and chewing non-acidic gum on saliva composition. In agreement with previous studies we found that the acidic taste stimulation from the candy stimulated much higher saliva flow rates than chewing stimulation [Watanabe and Dawes, 1988]. However, the same trend was obtained between saliva ionic composition and flow rate for both stimulants (fig. 2). This finding indicates that the relative contribution from various salivary gland types, mainly the submandibular and the parotid, to the whole saliva was the same at comparable flow rates when chewing the gum and when sucking the candy. The fact that the saliva protein concentration was higher both during and after stimulation by the candy might indicate that sucking the candy resulted in more sympathetic stimulation than chewing gum [Turner and Sugiyama, 2002].

The high secretion rate produced by sucking the candy resulted in production of more than twice as high saliva bicarbonate concentrations upon saliva secretion to the mouth than while chewing the gum (fig. 2). The high saliva bicarbonate concentration in concert with acidification from the candy, taking place in the mouth, increased the saliva \( P_{CO_2} \) nearly 5-fold compared to chewing gum (fig. 3D). These \( P_{CO_2} \) values induced by the candy were much higher than what is normally reported for whole saliva [Gron and Messer, 1965] and possessed the greatest methodological challenge in this study. Thus in order to measure the erosive potential of the candy, whole saliva had to be collected without any changes in its \( P_{CO_2} \) and pH to resemble the condition in the mouth. Of the three buffer systems in human saliva, i.e. bicarbonate, phosphate and protein [Lilienthal, 1955], the bicarbonate is the most important [Bardow et al., 2000b], but also has the ability to change pH if \( CO_2 \) is allowed to escape from saliva. Thus the buffering effect of bicarbonate involves a shift to the \( CO_2 \) form:

\[
CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+
\]

Within the normal pH range of human whole saliva, which is from 6.5 to 7.5, the equilibrium is shifted to the right, but it will be pushed to the left when the pH of saliva is decreased. Therefore, in a closed system, the \( P_{CO_2} \) increases proportionally to decreases in pH induced by acidification. In this study we assumed that the mouth mostly is a closed system because the test persons kept their mouths closed throughout the testing period. Nonetheless, with an estimated mean total \( CO_2 \) concentration of 18 mM upon sucking the candy (fig. 2), we ex-
pected the mean saliva P\textsubscript{CO\textsubscript{2}} to be around 80 kPa (i.e. 18/\alpha\textsubscript{CO\textsubscript{2}\textsubscript{saliva}}). However, the measured mean value in this study was only around 22 kPa (fig. 3D). Thus, in agreement with previous theoretical suggestions [Izutsu, 1981], a major part of the bicarbonate buffer system (70\%) is lost during buffering in the mouth. These findings illustrate that the mouth is not a completely closed system, but rather a semi-closed system where phase buffering easily can occur, meaning that the buffer system changes from a dissolved phase to a gas phase during buffering. These abilities of the bicarbonate buffer system in combination with its high concentration in response to sucking the candy provided considerable protection against the acidic candy. Thus individuals with a high buffer capacity were better at maintaining moderate pH values than individuals with a low buffer capacity. In addition the acid clearing effect of saliva also had a protecting effect, but considerably less than the buffer capacity. These findings are different from the effect of saliva on dental caries where the saliva flow rate seems to be much more important than the saliva buffer capacity [Bardow et al., 2003].

Nonetheless, in spite of the protective effects from the saliva buffer capacity and acid clearance, this study also indicates that this type of candy still has the potential to lower saliva pH considerably. This is in agreement with previous experimental [Holloway et al., 1958; Bibby and Mundorff, 1975] and in situ studies [Lussi et al., 1997] showing erosive potential of acidic candies. Thus, given that the candy is acidic and the exposure time is long enough, saliva is not able to overcome the acidic challenge, which will result in low salivary pH and a theoretical erosive potential. The theoretical erosive potential as judged from saliva pH and DS\textsubscript{HAp} may, however, not completely reflect what will happen in the mouth. Thus in this study the saliva pH dropped to 4.5, which is near the critical pH with respect to fluorapatite in human whole saliva [Larsen and Pearce, 2003], meaning that fluoride most likely would have protected the teeth against demineralization in spite of saliva being undersaturated with respect to HAp. Also the pellicle forming and tooth protecting effects [Nekrashevych and Stösser, 2003] of the high saliva protein concentration during candy stimulation were not accounted for in the DS\textsubscript{HAp} calculations. Thus, because of both salivary fluoride and proteins the pH and DS\textsubscript{HAp} estimate for the erosive potential may be somewhat overestimated. In contrast the saliva concentration of tartrate arising from the candy was not determined. Therefore no correction was made for the formation of ion-pairs, meaning that the erosive potential in this perspective could have been underestimated. Accordingly, the true erosive potential of these types of candies in human saliva still needs to be tested directly on HAp crystals or on tooth substances.

The present clinical study addressed only healthy persons. Although the consumption rate of acidic candies is unknown, it is thought that children and young adults are the largest consumption groups. It is, however, generally known that acidic sugar-free candies are recommended as stimulants for patients with reduced saliva flow due to radiotherapy, Sjögren’s syndrome or because of dry mouth of any other origin. As this and other studies [Lussi et al., 1997] have shown that acidic candies may be potentially erosive even in healthy persons, patients suffering from dry mouth, slow oral clearance, low buffer capacity and bicarbonate concentration may be at even higher risk for dental erosion in relation to sucking acidic candies. It is therefore recommended that future research also should aim to test the erosive potential of acidic candies among patients with reduced saliva flow who are likely to use such stimulants for relief of oral symptoms [Colquhoun and Ferguson, 2004].

In conclusion, this study has shown that sucking the acidic candy that was tested in this study, induces changes in whole-mouth saliva composition that may have erosive potential and that high saliva flow rates and buffer capacities have some protective effect against these changes.

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ARTICLE IV
Effects of sucking acidic candies on saliva in unilaterally irradiated pharyngeal cancer patients

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Summary Patients who have received radiation therapy on the head and neck area often use acidic candies to relieve symptoms of dry mouth. Therefore, the aim of this study was to determine the erosive potential in relation to teeth of an acidic candy in 10 such patients. The patients sucked the candy 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). Previously published normative values were used for comparison. The results showed that saliva became significantly more undersaturated with respect to HAp in irradiated patients, and failed to return to baseline values during the post-stimulatory period, which it normally does in healthy individuals. Thus, prevention of dental breakdown in these patients should involve counseling regarding choice of stimulant for dry mouth relief.

KEYWORDS Radiation therapy; Dry mouth; Erosive potential; Dental erosion; Saliva flow rate; Saliva buffer capacity

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Introduction

It is well known that patients who have received radiation therapy on the head and neck area often suffer from dry mouth,1 undergo a oral microbial shift towards a more cariogenic microflora2 and higher plaque levels, are at risk of developing rampant caries,3 and due to the feeling of dry mouth4 may experience dietary changes towards a higher intake of acidic saliva stimulating food products. In concert these conditions may lead to severe dental breakdown and impaired oral health for the patients. In spite of a possible intake of acidic food products like candies and lozenges in such patients, little is known about the erosive potential of these products, which in this context is the potential of the food product when dissolved in saliva to erode dental hard tissue.

In contrast to dental caries that is of bacterial origin, dental erosion is defined as the chemical wear of dental hard tissue without bacteria5 and can be caused by both sugar-containing and sugar-free acidic foodstuffs.6 Some early studies showed that acidic candies have the potential to erode human tooth enamel under experimental conditions.7,8 More recent studies have shown that such candies may also be potentially erosive in humans.9,10 As dry mouth patients often have low saliva flow rates11 and buffer capacity,12 they may be at greater risk of developing dental erosion when consuming acidic lozenges and candies than healthy individuals.13 Among dry mouth patients, the ones who have received radiation therapy on the head and neck belong to the more severe end of the spectrum with considerable reductions in saliva flow rate, and therefore these patients may be at even higher risk for developing dental erosion when consuming acidic candies.

We hypothesized that irradiated dry mouth patients will experience more pronounced changes in saliva composition while sucking acidic candies than healthy controls due to lower saliva flow and buffer capacity. To test this hypothesis we determined the erosive potential of acidic candies when dissolved in whole saliva in vivo in a group of patients irradiated on the head and neck against pharyngeal cancer in the tonsil area. Six patients were irradiated on the right side and four were irradiated on the left side. The patients were treated with a 4 MV photon linear accelerator by angled wedge fields. The field covered the diseased tonsillar fossae and the homolateral neck lymph node regions. All patients had the homolateral glandula submandibularis and the homolateral glandula parotis included in the target field where they received 66 Gy. The study was conducted at least three months after the radiotherapy. Six of the ten patients were smokers at the time of the study and additionally two had a history of smoking. Four patients received prescription medication on a daily basis. Nine of the irradiated patients had complaints of dry mouth11 that had occurred in relation to the radiation treatment. Prior to the experiments all volunteers gave informed consent to the protocol, which was approved by the Ethical Committee of Copenhagen, Denmark (No. 03-001/03).

Collection of whole saliva

The participants had nothing by their mouth at least one hour before the experiment was conducted during daytime (10 a.m. to 2 p.m.). The saliva was stimulated with a commercially available acidic hard-boiled candy (Soemods-bolcher, Copenhagen, Denmark) with tartaric acid and rhubarb flavor, weighing on average 5 g. To avoid loss of CO₂ and thus changes in saliva pH, saliva was collected in a closed system under paraffin oil as previously described.14 All participants were instructed not to swallow any saliva and to keep their mouths closed whenever they were not delivering saliva into the collection system. Each collection lasted 19 min, and was divided into three periods.10 The first period consisted of a 5-min collection of unstimulated saliva (−5 to 0 min), collected every minute into one paraffin oil filled glass vial (baseline). The second period consisted of a 4-min collection of stimulated saliva (sucking acidic candy, 0–4 min), collected every minute into two paraffin oil filled glass vials (stimulated saliva). The third period consisted of a 5-min collection of unstimulated saliva (−5 to 0 min), collected every minute into one paraffin oil filled glass vial (baseline). The second period consisted of a 4-min collection of stimulated saliva (sucking acidic candy, 0–4 min), collected every minute into two paraffin oil filled glass vials (post-stimulated, 5 min for each). The paraffin oil filled glass vials were stored on ice and weighed before and after collection of saliva enabling calculation of saliva flow rates by gravitation (g/min), which is almost equivalent to ml/min.15 Within less than 30 min, the saliva sam-

Materials and methods

Study group and study design

The study group consisted of seven men and three women with a median age of 51 years (26–67), a height of 173 cm (145–182), a weight of 70 kg (46–90), and unilaterally irradiated on the head and neck against pharyngeal cancer in the tonsil area. Six patients were irradiated on the right side and four were irradiated on the left side. The patients were treated with a 4 MV photon linear accelerator by angled wedge fields. The field covered the diseased tonsillar fossae and the homolateral neck lymph node regions. All patients had the homolateral glandula submandibularis and the homolateral glandula parotis included in the target field where they received 66 Gy. The study was conducted at least three months after the radiotherapy. Six of the ten patients were smokers at the time of the study and additionally two had a history of smoking. Four patients received prescription medication on a daily basis. Nine of the irradiated patients had complaints of dry mouth11 that had occurred in relation to the radiation treatment. Prior to the experiments all volunteers gave informed consent to the protocol, which was approved by the Ethical Committee of Copenhagen, Denmark (No. 03-001/03).
ple was extracted from underneath the paraffin oil into individual CO₂ impermeable syringes for further analyses.

Saliva composition

Whole saliva P CO₂ and pH were measured on a standard blood gas analyzer and the saliva bicarbonate concentration was calculated. Sodium, potassium, calcium, chloride, bicarbonate, and phosphate concentrations were determined by atomic absorption spectroscopy as previously described. Chloride was determined by the mercury-chloride/iron-TPTZ reaction and total phosphate was determined by the molybdenum reaction. All samples were measured at least twice in two separate series. The saliva buffer capacity was estimated for pH 6.15 from total CO₂ concentrations (i.e., the bicarbonate buffer system) and inorganic phosphorus. In samples that were acidified by the candy, total CO₂ (mainly represented as bicarbonate at physiological pH) was estimated as the missing anion by ionic balance calculation as previously described.

Degree of saturation with respect to hydroxyapatite in saliva

All calculations were performed for conditions at 37 °C, which is near the temperature of saliva in the mouth. The ionic strength of saliva was calculated from the concentrations of sodium, potassium, calcium, chloride, bicarbonate, and phosphate. The solubility product for HAp (Ca₁₀⁻₅(PO₄)₆(OH)₂) was set at 117.3 (pKₘ₄). pKₘ₄ at 13.6, pK for H₂PO₄⁻/H₃PO₄ at 2.2, for H₂PO₄⁻/HPO₄²⁻ at 7.2, and for HPO₄²⁻/PO₄³⁻ at 12.2. All dissociation constants were corrected for the ionic strength of the saliva. Activity coefficients were calculated by the extended Debye–Hückel equation taking into account the effective diameter of the hydrated ions. The ionic product for HAp (pH₄) was calculated from the activities of calcium, phosphate, and hydroxyl ions in saliva. The degree of saturation with respect to HAp (DSH₄) was calculated as: (pKₘ₄−pH₄)/18. All salivary data needed for the calculations were imported into the R statistical program (www.r-project.org) where the equations for DSH₄ were processed allowing for simultaneous calculation of DSH₄ in multiple samples.

Statistics

Statistical analyses were done with Excel and with the R statistical program (www.r-project.org). For reference, values previously obtained on healthy test persons in a similar experimental setup were used. In the figures, these reference values are shown as dotted lines. Differences between patients and reference values were determined by the Wilcoxon’s rank-sum test and differences in distribution by the Fishers exact test. Correlations were analyzed by Spearman’s rank correlation analysis with correlation coefficients (rₙ) and p-values given. The level of significance was set at p < 0.05.

Results

As shown in Figure 1A, the irradiated patients had slightly lower baseline saliva flow rates than the healthy individuals. Although the acidic candy had a clear stimulatory effect in both groups, the patients exhibited less increase in saliva flow rate upon stimulation by the acidic candy than that we have previously found in healthy individuals (p < 0.001). During the post-stimulation period, saliva flow rates were also lower in the patients (p < 0.001). Figure 1B shows that due to low whole saliva pH while sucking the candy the bicarbonate buffer system was shifted to the CO₂ form, resulting in increased partial pressure of carbon dioxide (P CO₂). When P CO₂ is interpreted to reflect a measure of the bicarbonate buffer system this figure also indicate that the concentration of this buffer system was considerably reduced in the patient group compared to that of the healthy individuals (p < 0.001). As a consequence of the reduced bicarbonate concentration the patients also had a significantly lower estimated buffer capacity than that found in healthy individuals (p < 0.05). As a high buffer capacity prevents the candy-induced salivary pH drop, the patients clearly had reduced protection against the acidic load released from the candy.

On average, one gram of acidic candy was dissolved per minute in healthy individuals, giving an average whole saliva flow rate of 5 ml per minute. However, in the patients only 0.7 g of acidic candy dissolved per minute, giving an average flow rate of 2.4 ml saliva per minute. In terms of dilution this means that the candy was diluted five times its weight in saliva from healthy individuals and only 3.4 times its weight in saliva from the patients. Consequently, the concentration of acid in saliva was higher in the patients than in the healthy individuals. Thus Figure 1C shows that sucking acidic candy resulted in a considerably greater pH drop in unilaterally irradiated cancer patients than seen before in healthy individuals (p < 0.001). In addition, saliva
pH values failed to reach baseline in the patients after the removal of the acidic candy, which normally occurs in healthy test persons during the 10-min post-stimulatory period. Figure 1D shows that, in the patients, the saliva became considerably undersaturated with respect to HAp while sucking the candy. Although we have previously observed undersaturation with respect to HAp upon sucking acidic candies in healthy individuals, we found here that undersaturation among patients was considerably greater ($p < 0.001$). Furthermore, even at the end of the 10-min post-stimulatory period, half the patients had saliva that was still undersaturated with respect to HAp, whereas all healthy individuals had saliva that was supersaturated ($p < 0.05$).

Correlation analyses showed that the degree of saturation with respect to HAp in saliva was highly dependent on the saliva flow rate becoming more saturated with higher flow rates ($r_s = 0.66; p < 0.001$). Also, the degree of saturation was dependent on the saliva $P_{CO_2}$ and buffer capacity, becoming more saturated with higher $P_{CO_2}$ ($r_s = 0.57; p < 0.001$) and with higher buffer capacity ($r_s = 0.41; p < 0.001$).

**Discussion**

It has been shown that sucking acidic candies reduces pH values and saturation levels with respect to HAp in human saliva, and that acidic candies therefore may have erosive potential even in healthy test persons. As dry mouth patients are known to have low saliva flow rates and thus slow oral clearance and low saliva buffer capacity, we speculated that the erosive potential of acidic candies would be even higher in such patients. However, limited information is available on this topic, although one study reports higher erosive potential of acidic candy in one patient irradiated on the head and neck. Therefore the aim of this study was to determine the effect of...
sucking acidic candy in a group of irradiated patients with a well-known history of dry mouth.

The present study has shown that the candy-induced drop in pH and thereby undersaturation of saliva with respect to HAp was significantly greater in the irradiated dry mouth patients than that we have previously shown for healthy individuals. Although low saliva pH and undersaturation with respect to HAp does not necessarily imply that dental erosion occurs, these findings indicate that the erosive potential of the acidic candy was considerably higher among the unilaterally irradiated cancer patients than among healthy persons. Additionally, even 10 min after removing the candy from the mouth half the patients had saliva that was undersaturated with respect to HAp, whereas no healthy individuals had undersaturated saliva. Undersaturation of saliva is normally limited to the time of exposure to the acidic foodstuff, however, in irradiated dry mouth patients much longer periods of undersaturation should be expected. Although, this is what could be intuitively expected, a number of complex variables are involved in this process, such as the composition of the candy, the amount of the acidic candy dissolved per ml of saliva, the saliva flow rate, and the saliva buffer capacity. This study has shown that the concentration of acid in saliva becomes higher in irradiated patients than in healthy persons. This inevitably will lead to lower pH values in patients and more pronounced acid induced effects on the dentition must be expected. Along this line the patients also had compositional differences in their saliva compared to the healthy, which may accelerate such processes even further.

One important compositional difference between the two groups was the difference in the bicarbonate buffer system, which is the main buffer in human saliva under normal conditions. Bicarbonate is most likely secreted from the salivary glands in response to the increased metabolic activity and is therefore dependent on the salivary flow rate and increases with increasing flow. Thus the lower $P_{CO_2}$ in the saliva of the patient group can be explained by their lower saliva flow, which is characterized by a low bicarbonate concentration. When saliva is mixed with the acidic candy the salivary pH drops which pushes the bicarbonate buffer system almost entirely to the CO2 form resulting in an increased salivary $P_{CO_2}$. Therefore the lower $P_{CO_2}$ in the patients was indicative of a lower bicarbonate concentration than in healthy and thus a lower overall buffer capacity. The low pH values in the patients in response to the candy were therefore most likely attributed to their low buffer capacity in combination with low saliva flow rates, slower acid clearance, and higher concentration of acid (released from the candy) in their saliva. Collectively, these findings may help to explain why individuals with low saliva flow rates have been shown to be at increased risk for developing dental erosion. As our study group consisted of patients unilaterally irradiated on the head and neck, which are known to suffer from dry mouth while still having some saliva secretion intact, it is important to emphasize that the effects shown in this study must be even more pronounced in bilaterally irradiated patients.

All ten patients reported increased saliva secretion and the majority mentioned relief of oral dryness upon sucking the candy. This is in accordance with the objective findings in this study as the patients produced on average ten times more saliva upon sucking the acidic candy compared to the flow rate in the pre-stimulatory state. Interestingly, although the reference group produced nearly twenty times more saliva upon sucking the acidic candy, both groups, healthy individuals and patients, had twice as high flow rates of whole saliva after the 10-min post-stimulatory period compared to the pre-stimulatory state, in spite of the relatively high baseline flow in the patients. This suggests that the acidic stimulus lasted longer than 10 min and supports the patient reports of having more saliva in the mouth after sucking acidic candy. The relatively high baseline flow in patients could most likely be explained by a compensatory increase in saliva from the glands, mostly the parotid gland, in the non-irradiated side.

Despite the positive stimulatory effects of sucking acidic candy on saliva flow, and the relief of oral dryness, the patients should be concerned about the effect that acidic candy could have on their oral health if consumed on daily basis. Thus prevention of dental breakdown in irradiated dry mouth patients should involve counseling regarding choice of stimulant for relief of dry mouth.

**Acknowledgements**

We thank all the test persons who participated in the study. Special thanks go to Dr Anni Rasch and Dr Astrid Bork Andersen for useful discussions and co-operation. Ms Joan Lykkeaa is thanked for skilful laboratory assistance throughout this study. Financial support from the Danish Ministry of Science, Technology and Innovation, Toms Group A/S, Denmark and the University of Copenhagen is gratefully acknowledged.
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ARTICLE V
Effects of Calcium on the Erosive Potential of Acidic Candies in Saliva

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Key Words
Critical pH \cdot Dental erosion \cdot Erosive potential \cdot Food modification \cdot 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 undersaturated. 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.

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 barri

er on tooth surfaces that protects against erosion [Meur-

man 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 ex

planative 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 ero

sion such as pH and saturation levels may lead to overes-

timation 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 theo-

retically calculated erosive potential and the actual ero-

sive 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 volun-

tees, 9 males and 11 females, recruited among students and staff at the School of Dentistry in Copenhagen. The test persons nei

ther 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 volun-

tees 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 sepa-

rate 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 blind-
ed as to which candy they were having. Collections of whole sa-

liva 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 milliliters per minute. After the saliva collection, the samples were stored on ice in individual closed syringes until pH and P CO2

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 CO2 content by vacuum, agitation, and acidifica-

tion [Bardow et al., 2000]. After the saliva was depleted of its CO2 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 milli-

litre of saliva equal to a concentration of 2 mmol/l HAp. The crys-
tals 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 dis-

solved. 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 aver-

age requires 14 mmol of H+ (MW 1) due to the reaction:

\[
Ca_{10}(PO_4)_{6}(OH)_2 \rightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O
\]

Therefore the use of 14 µl 1 M HCl (i.e. 14 µg H+) for back titration simulates 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 in-

creased 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 crys-
tals lost per minute in the candy-containing saliva was back cal-

culated 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_2PO_4/H_2PO_4 at 2.2, for H_3PO_4/HPO_4^{2-} at 7.2, and for HPO_4^{2-}/

Acidic Candies with Calcium
PO_{4}^{3-} at 12.2 with dissociation constants corrected for ionic strength [Harned and Owen, 1958]. DS_{HAp} was calculated as \((I_{HAp}/K_{HAp})^{(1/18)}\). 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 dissolution 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 DS_{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.
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$_{\text{HAp}}$, 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 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$_{\text{HAp}}$ 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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References


ARTICLE VI
Title

Erosive potential of calcium modified acidic candies in irradiated dry mouth patients

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Short title

Acidic candies with calcium in dry mouth patients

Key words

Saliva stimulation, erosive potential, food modification, dry mouth, irradiation treatment

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Summary

Purpose: Patients who have received irradiation therapy on head and neck are known to suffer from reduced saliva flow and may therefore use acidic candies to relieve symptoms of dry mouth. However, acidic candies have erosive potential even in healthy individuals. Therefore the aim of this study was to determine if calcium-modified acidic candies have reduced erosive potential in irradiated cancer patients.

Materials and methods: Nineteen cancer patients (26-70 years) ipsilaterally irradiated on head and neck sucked control and calcium-modified acidic candies while their whole saliva was collected into a closed system. The erosive potential of both candies was evaluated from saliva degree of saturation with respect to hydroxyapatite (HAp) and by dissolution of HAp directly in candy-stimulated saliva. The results were compared to normative data previously obtained on twenty healthy test persons (21-29 years).

Results: No significant difference was obtained in the saliva flow between control and calcium-modified candy. However, the saliva became significantly less undersaturated with respect to HAp when sucking calcium-modified compared to control candy (p<0.001) and more undersaturated for both candies in ipsilaterally irradiated cancer patients compared to normative data (p<0.001). HAp dissolution was significantly lower sucking the modified candy compared to the control candy (p<0.01) and, surprisingly, slightly lower in patients compared to normative data.

Conclusion: Modified acidic candy with calcium has reduced erosive potential in patients irradiated on head and neck area and could therefore be a favourable stimulant for dry mouth relief.
**Introduction**

Patients who have received radiation therapy on the head and neck area have markedly diminished saliva flow rates even after only small doses of radiation (Cooper et al., 1995). Such low saliva flow rates may lead to dry mouth and changed swallowing abilities (Logemann et al., 2003) that again may result in dietary changes such as higher intake of acidic saliva stimulating foodstuffs (Colquhoun and Ferguson, 2004). Early studies have shown that acidic candies have the potential to erode human tooth enamel under experimental conditions (Holloway et al., 1958; Bibby and Mundorff, 1975). More recent studies have shown that acidic candies may also be potentially erosive when dissolved in saliva from healthy test persons with normal salivary flow (Lussi et al., 1997; Jensdottir et al., 2005) and that acidic candies have even higher erosive potential in irradiated dry mouth patients (Jensdottir et al., 2006). We have previously shown that the erosive potential of saliva stimulating acidic candies can be reduced in healthy test persons by addition of calcium to the candy composition. Such calcium-modified candies can still be effective in stimulating saliva (Jensdottir et al., 2007) and could therefore be used for saliva stimulation in dry mouth patients. Thus the aim of this study was to determine the erosive potential of acidic candies with calcium (modified) and without calcium (control) in dry mouth patients.

**Materials and methods**

**Study group and design**

Saliva was collected from nineteen cancer patients all ipsilaterally irradiated on the head and neck area against pharyngeal cancer in the tonsil area. The patients comprised fourteen men and five women with a mean age of 51 years (26-70), mean weight of 76 kg (47-101) and a mean height of 175 cm (145-186). Ten patients were irradiated on the right side and nine on the left side. The patients were treated with a 4MV photon linear accelerator by angled wedge fields. The field covered the diseased tonsillar fossae and the homolateral neck lymph node
regions. All patients had the homolateral glandula submandibularis and the homolateral glandula parotis included in the target field where they received 66 Gy. The study was conducted at least three months after the radiotherapy and all patients were without recurrence of their former cancer. All experiments were conducted at the dental clinic at the Department of Oral Medicine, University of Copenhagen, Denmark. The test persons neither ate nor drank at least one hour before the study. Prior to the experiments all participants gave informed consent to the protocol, which was approved by the Ethical Committee of Copenhagen, Denmark (No: 03-001/03). Normative data were obtained from 9 males and 11 females who were healthy and unmedicated having a mean age of 25 years (21-29) and previously presented in Jensdottir et al. (2007).

**Saliva collection**

The experiments were performed during daytime between 10 am and 4 pm by use of non-commercial acidic candies without calcium (control) and with 16.5 grams of calcium lactate per kilo candy (modified). Both candies, weighing on average 5 g, were based on the same basic recipe consisting of water, isomaltose, 1% tartaric acid w/w, strawberry and rhubarb flavour, and all had the same light red colour. Collection of whole saliva from all patients was performed under paraffin oil as previously described (Bardow et al., 2000). Unstimulated saliva was collected for 5 minutes at baseline where after the candies were given in a random order and sucked for 10 min each with a one-hour pause between the two collections. The patients were blinded as to which candy they were having. Saliva flow rates were determined in g/min, which is almost equivalent to mL/min (Navazesh and Christensen, 1982). After saliva collection, samples were stored on ice in individual closed syringes until pH and $P_{CO2}$ was determined on a blood gas analyser within 30 min (Bardow et al., 2000). Hereafter aliquots were made and either analysed immediately or stored at -80°C for chemical analyses.
Saliva analyses

Salivary concentrations of sodium and potassium were determined by atomic absorption spectroscopy (AAS) in the emission mode and total calcium by AAS in the absorption mode. Salivary chloride and total phosphate were determined by colorimetric methods: chloride after the mercury–iron–TPTZ reaction and total phosphate after the molybdenenum reaction. The erosive potential of saliva was determined directly by dissolution of HAp crystals in saliva and indirectly by calculations of the degree of saturation with respect to HAp. Briefly, candy-stimulated saliva was depleted of its CO$_2$ content by acidification, vacuum and agitation where after the pH was adjusted to the pH obtained in response to sucking the candy. HAp crystals were then added to the saliva at a concentration of 2 mg/mL with continuous pH recordings for 5 minutes. If the saliva pH was constant or decreased the candy was assessed as non-erosive. In case the pH increased, dissolution was assumed, and then the magnitude of erosion was quantified by back titration (Jensdottir et al., 2006$^a$ and 2007). The degree of saturation with respect to HAp ($DS_{\text{HAp}}$) was determined from measurements of saliva pH, calcium, phosphorus, and ionic strength (calculated from all major ions in saliva) and the critical pH was iteratively estimated from the same compositional values used for calculating $DS_{\text{HAp}}$ (Jensdottir et al., 2007).

Statistics

Statistical analyses were done with Excel and with the R statistical software (R Development Core Team, 2008) and results were given as mean ± standard deviations. As the estimate of $DS_{\text{HAp}}$ is highly non-linear, because all undersaturated samples will have saturation levels between 0 and 1 and all supersaturated samples will have saturation levels above 1, the degree of saturation for each sample was transformed to a linear estimate, i.e. log$_{10}$ ($DS_{\text{HAp}}$), before analyses. Also saliva pH and critical pH were linearised prior to analyses, i.e. 10^-pH or critical pH. Results on HAp dissolution could not be linearised, due to multiple zero values,
and therefore differences in HAp dissolution were analysed by the Fisher’s exact test for count data. All remaining results, including the linearised pH, critical pH and DS_{HAp} were analysed by a two-sample t-test. For text and figures the linearised values were averaged and then transformed to standard mean values of pH and critical pH, i.e. \([-\log_{10}(\sum\,10^{-\text{pH}_X}/n)\], as well as for DS_{HAp}, i.e. \([10^{\big((\sum\log_{10}\text{DS}_{HAp})/n)\}]. The level of significance was set at p<0.05.

Results

Figure 1 shows the saliva flow rate, pH, and calcium concentration in response to sucking control and modified candy in patients as well as corresponding normative data for these variables. As shown, no difference was obtained in saliva flow rate between the two candies within patients and normative data, whereas the saliva flow rate was significantly lower in patients than normative data with both candies (p<0.001). Candy stimulated saliva pH values were lower in patients than normative data, both with respect to the modified candy (p<0.01) and the control candy (p<0.01). Within the patients saliva pH became higher when they sucked the modified candy (p<0.01). The calcium concentration in saliva in both groups became significantly higher when sucking calcium-modified candy compared to control candy (p<0.001) and slightly higher in patients than in healthy individuals.

Figure 2 shows saliva degree of saturation with respect to hydroxyapatite, critical pH of saturation with respect to hydroxyapatite and erosive potential determined by dissolution of hydroxyapatite crystals directly in saliva from patients compared to normative data. As shown, the saliva was on average undersaturated in all samples, although, the degree of saturation was significantly higher for both groups (p<0.001) when sucking the modified candy (30% saturated in patients and 70% for normative data) compared to the control candy (3% saturated in patients and 20% for normative data). The degree of saturation was lower in patients when sucking both control (p<0.001) and modified candies (p<0.05). The critical pH
became significantly lower (p<0.001) when the modified candy was sucked in both groups and the critical pH was similar between groups.

All patients experienced HAp dissolution with the control candy compared to only three patients who experienced HAp dissolution with the modified candy (p<0.001). For normative data nineteen subjects experienced HAp dissolution with the control candy compared to only six subjects with the modified candy (p<0.001). No significant difference was obtained between the numbers of patients and healthy who experienced dissolution of HAp with the two candies. Nonetheless, comparison of the mean results for HAp dissolution in Figure 2 shows that the erosive potential tended to be higher with the control candy among patients compared to normative data and slightly lower with the modified candy among patients compared to normative data.

Discussion

This study aimed to determine the erosive potential of acidic candies with calcium (modified), and without calcium (control) in cancer patients ipsilaterally irradiated on head and neck area. This patient group was chosen because radiation therapy on head and neck clearly causes a severe reduction in saliva flow rates even after only small doses of radiation (Cooper et al., 1995). In agreement with a recent study on healthy individuals (Jensdottir et al., 2007) represented as normative data in the present study, calcium-modified acidic hard-boiled candy also had markedly reduced erosive potential in dry mouth patients. The challenges of the present study on dry mouth patients were mainly that irradiated patients only produce small amounts of saliva upon sucking the acidic candies. Limited volume of sample made analyses of erosive potential more difficult than the analyses originally used for obtaining the normative data. Also the collection of the saliva occurred slightly differently since the texture of the saliva was thicker and stickier among the patients. Therefore their saliva could not be
collected into syringes as used for the normative data but the saliva was instead collected under paraffin oil as previously described (Bardow et al., 2000). When candies are sucked and dissolved in saliva, saliva becomes the matrix for soluble constituents of the candy including the acid, which then can make the saliva potentially erosive. Using techniques requiring only minute volumes of saliva for analyses, the saliva pH showed to be lower in patients than in healthy. This was presumably due to a diminished diluting effect of acid in the patients who had low saliva flow rates compared to the healthy test persons. Furthermore, a low saliva flow rate also results in a low saliva buffer capacity (Bardow et al., 2000) and therefore a reduced ability of the saliva to raise its pH upon sucking the acidic modified candy. Analyses of the saliva composition showed that both candies on average resulted in saliva that was undersaturated with respect to HAp. Nonetheless, direct quantification of HAp dissolution in the saliva matrix showed that the calcium-modified candy had significantly reduced erosive potential in both patients and for normative data. In fact, in many patients no dissolution of HAp occurred in modified candy saliva during the five-minute test period. Also, the modified candy seemed to have slightly lower average erosive potential in irradiated patients than in healthy. A reason for the slightly lower erosive potential among the patients could be that they, due to a reduced saliva volume for dilution, gained higher calcium concentrations in saliva sucking the modified candy. The increased calcium could explain the lower erosive potential as calcium along with phosphate and pH are essential variables for the saturation level with respect to hydroxyapatite (Jensdottir et al., 2006b).

The critical pH, which is the pH value where saliva is exactly saturated with respect to hydroxyapatite, was similar in patients and normative data. Because the patients had much lower saliva pH values, but similar critical pH values, they consequently became more undersaturated with respect to HAp than the normative data. The critical pH is like the degree of saturation with respect to HAp, a compound measure including both the salivary calcium
and phosphate activities as well as the hydroxyl ion activity, which has to be iteratively estimated because the critical pH itself is a specific pH value. High calcium and phosphate activities results in a low critical pH value and vice versa.

The reason why the critical pH was similar in patients and normative data, in spite of higher calcium concentrations in the patients, was because the patients were lower in phosphate than the normative values (data not shown). Accordingly, the explanation for the slightly lower erosive potential, in spite of much lower degree of saturation, cannot be found among inorganic parameters for calcium and phosphate. However, differences in saliva fluoride concentrations between patients and normative data may have influenced the results. Thus, even low concentrations of fluoride may reduce demineralisation of tooth substance at pH values comparable to ones obtained in this study (Attin et al., 2005). Another explanation for the discrepancy between saturation levels and actual erosion may be related to the organic components in saliva. We speculate that the sticky and protein rich saliva in the patients offered a relatively increased protection compared to the less protein rich and more aqueous saliva normally found in healthy subjects. Indeed, salivary proteins are very effective in protecting tooth substance against acid demineralisation (Bruvo et al., 2009).

In conclusion, modified acidic candy with calcium was shown to have reduced erosive potential compared to non-modified candy in irradiated head and neck cancer patients. Therefore modification of acidic candies with calcium could be used as an effective saliva-stimulant for relief of dry mouth.

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Figure Legends

Figure 1
Saliva flow rates, pH and total calcium in ipsilaterally irradiated patients on head and neck area in response to sucking acidic hard boiled candy without calcium (light grey columns) and with calcium (dark grey columns) compared to normative values (Jensdottir et al., 2007). P-values above columns show the level of significance obtained between the control and calcium modified candy. P-values between columns show the level of significance obtained between patients and normative data. Numbers shown within columns represent the mean value for each column.

Figure 2
Saliva degree of saturation with respect to hydroxyapatite (DS$_{\text{HAp}}$), critical pH of saturation with respect to hydroxyapatite and erosive potential determined by dissolution of hydroxyapatite crystals directly in saliva from ipsilaterally irradiated patients on head and neck area compared to normative values (Jensdottir et al., 2007). The control candy (without calcium) is represented in the light grey columns and the modified candy (with calcium) is represented in the dark grey columns, respectively. P-values above columns show the level of significance obtained between the control and calcium modified candy. P-values between columns show the level of significance obtained between patients and normative data. Numbers that are shown within columns or in labels above columns represent the mean value for each column.
Figure 1

![Bar chart showing saliva flow, pH, and calcium levels in healthy and patients groups.](image1)

**Healthy Saliva Flow**: 3.7 mL/min, **Patients Saliva Flow**: 1.6 mL/min, **Healthy Saliva pH**: 4.0, **Patients Saliva pH**: 3.3, **Healthy Saliva Calcium**: 15.1 mg/mL, **Patients Saliva Calcium**: 17.4 mg/mL.

Figure 2

![Bar chart showing saturation ratio and critical pH in healthy and patients groups.](image2)

**Healthy Degree of Saturation**: 0.2, **Patients Degree of Saturation**: 0.7, **Healthy Critical pH**: 5.3, **Patients Critical pH**: 4.7, **Healthy Erosive Potential**: 1.0, **Patients Erosive Potential**: 0.7.