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In vitro Study on Dental Erosion Provoked by Various Beverages Using Electron Probe Microanalysis

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Abstract: Tooth erosion is often based on chemical processes, among others the use of soft drinks or diverse beverages. The aim of this in vitro study was to analyse the erosive potential of different acidic beverages. Over a time span of 6 hours, dental slices (n = 6 slices per tooth) from fully retained wisdom teeth were incubated with different beverages (coca cola, ice tea with lemon, apple juice and white wine). The controls were incubated with a 0.9% sodium chloride solution under the same conditions (37 °C, humidified atmosphere of 5% CO_2 and 95% air). The quantitative elementary analysis for calcium, phosphorus, oxygen and other trace elements in the dental slices in various depths ranging from 5 to 50 µm was carried out using an electron probe micro-analyser (Jeol JXA 8900RL). A beverage-induced loss of minerals, particularly of the 2 main components calcium and phosphorus, especially in the uppermost layers of the enamel down to a depth of 30 µm could be observed. In the depth of 10 µm, the following total mineral loss could be determined: white wine (16%), coca cola (14.5%), apple juice (6.5%) and ice tea with lemon (6.5%). A direct correlation between the loss of minerals and the pH value of the beverages was not observed, because of the buffering effect of the drinks. The conversion of the weight percentages from the chemical analysis of Ca and P to their atomic percentages showed that during erosion the 2 main components were not dissolved in significantly different percentages. In this study the erosive potential of the tested soft drinks and other beverages could be demonstrated. However, it must be considered that numerous modifying factors influence the enamel surface, so an extrapolation from the in vitro study to an in vivo situation can only be applied with caution.

Key words: enamel erosion, soft drinks, in vitro study, microprobe analysis

Introduction

The loss of dental hard tissue as a consequence of frequent wear is increasingly gaining importance. Beside numerous well known etiological factors like increased abrasion, malocclusion or attrition, other parameters which are related to the diet are of special importance. On account of a shift in the eating habits within the last 50 years, the phenomenon of dental erosion of dental hard tissue has gained significant importance with respect to prophylactic measures as well as to restorative techniques. Erosion can be defined as the slow and chronic loss of enamel and dentine and can be compared to the process of demineralisation, which is primarily caused by contact with acids from extrinsic and intrinsic sources without any bacterial activity. Clinically relevant dental erosion only occurs if several unfavourable factors exert an influence on the tooth surface.

Amaechi et al. describe erosion as an acid-induced mechanical wear of the tooth before remineralisation, induced by the minerals in saliva, can occur. As a consequence, all processes that may lead to a demineralisation of enamel and dentine can be regarded as possible factors which can contribute to the development of dental erosion. Diseases which show a tendency to provoke vomiting, and diverse kinds of eating disorders (e.g. bulimia nervosa) as well as hormonal changes (pregnancy) can be listed as possible parameters for intrinsic enamel erosion.

Over the last decades, changes in the dietary habits within the prosperous societies, like unhealthy food intake and a great amount of acidic food, seem to have increased the number of dental erosive changes [3, 19]. The British Society for Paediatric Dentistry quoted in their report from 1993 that an increase of diet-related erosions in children and adolescents could be observed, in which context the dramatic increase in the consumption of acid-containing beverages was emphasised. Similar observations were made by the United States Department of Agriculture (USDA), and it was pointed out that the consumption of fashionable soft drinks has increased dramatically. Carbonated beverages, fruit juices and sports drinks are known to be especially erosive.

These beverages share low pH values as well as a high buffering capacity so that their acidic character is maintained for a considerable time [9, 11]. In a study by Al-Majed et al., a total of 1216 children aged between 5-6 and 12-14 years, was investigated. In 34% and 26% of the cases, they described significant erosions on the front teeth, and they postulated that the amount and time of day of the consumption of soft drinks were important for the severity of the resulting dental damages.

In view of these data, scientific studies of how to prevent dental erosion, caused by unhealthy diets, including acidic foods, seem highly important. In order to determine the erosive potential of different bever-

ages and foods, one can make usage of different examination methods. Besides clinical inspections with a subsequent ranking of the degree of determined severity of the erosion with the help of indices [1], the determination of the Knoop hardness [4], as well as profilometry and the assessment of demineralisation after an acid attack by means of ultrasonic treatment are established techniques [10]. In order to develop appropriate preventive measures, not only the determination of the absolute depth or the width of the changed enamel layer are of great importance, but also the precise processes that take place on the enamel surface during demineralisation. As a consequence, investigation methods have been developed which can be employed to quantitatively and qualitatively determine the mineral loss as well as the distribution of minerals in the eroded enamel layers. [8, 11, 3].

The aim of the present study was to quantitatively determine the erosive potential of different acidic beverages by means of the microprobe analysis with regard to the possible loss of minerals. These values were compared with untreated enamel surfaces as well as with tooth slices that had been conditioned with an etching gel which is used for adhesive techniques.

Material and Methods

For this study only fully erupted wisdom teeth of young adults were used. These teeth were extracted in the surgery of the university hospital on account of orthodontic or other prophylactic measures. The patients were aged between 21 and 24 years (mean age: 23 years). Immediately after the extraction, the soft tissue was removed, the teeth were placed into a sodium azide solution (15mM) for 30 min and afterwards stored in physiological sodium chloride solution for up to 48 hours. Within the first 48 hours all teeth were microscopically examined for possible irregularities of the mineralisation, especially for genetically induced diseases of enamel mineralisation, cracks or other defects. Each tooth crown was evenly divided into 6 slices, with an enamel window sized about 10-12 mm^2. The test slices were placed into 12 multi-well plates (Greiner, Labortechnik/Frickenhausen, Germany), each well containing a different beverage, and were incubated at 37 °C in a humidified atmosphere of 5% carbon dioxide and 95% air in a gas incubator (Heraeus, type Function Line). The controls (wells no. 1 and 6) were incubated with physiological sodium chloride solution, pH-value 6.5 (0.9% NaCl) under identical conditions.

The following acid-containing beverages were used: well no. 2, Coca Cola, pH-value: 2.3 immediately after opening of the bottle (Lot LSN31711701, 12/2004, Rhein-Main-Sieg Getränke GmbH& Co KG, Liederbach); no.3 ice tea with lemon, pH-value: 2.9 (Lot 100/75/12, 19/09/2004,00:34, Volvic); no. 4 apple juice Apfel/Acerola Hohes C, pH-value: 3.3 (Lot 14.03.04 S2 15:49, Eckes-Granini); no.5 white wine, Meissner Spaargebirge, Müller-Thurgau 1998, pH-value: 3.3, (Lot L/A.P.Nr 010299, Sächsische Winzergenossenschaft Meißen eG). The controls in well no. 6 were treated with an etching gel, containing 37% phosphoric acid (Heraeus Kulzer), for 60 seconds, after the incubation with physiological sodium chloride solution. This treatment was done to see the effects of the etching gel which is used for restoration materials with adhesive techniques. Afterwards all tooth slices were thoroughly cleaned with water spray for about 2 minutes, stored in physiological sodium chloride solution for 24 hours and then dried and stored under dust-free conditions at room temperature, until they were analysed. For the microprobe analysis, a smooth and plane surface, vertical to the enamel surface was required. This was achieved by reducing one side of the tooth slices by at least 1 mm of thickness so that an angle of 90o was formed in relation to the enamel surface, which itself remained untreated..

The quantitative analysis of the elements was carried out with the electron probe microanalyzer (Jeol 8900 RL) in the Institute of Geoscience. This technique is based on the bombardment of a micrometric volume of a specimen with a focussed electron beam. The specimen is polished to a plan surface. The emitted x-rays are measured with detectors.

As the properties of the x-rays are characteristic of the bombarded elements, it is possible to determine the composition of the test materials with wavelength dispersive spectrometers (WDS). With the help of the electron probe microanalyzer, a non-distructive qualitative and quantitative analysis of the elements in a micrometric volume of mineral surfaces is possible.

The detection limits are about 0.01% and the reproducibility of the measurements is in the range of 1%. An accelerating voltage of 15 kV, a beam current of 8 nA and a probe diameter of 2 μm were used.

The high mechanical precision of the sample stage allows the generation of images showing the mapping of element distributions in areas of the μm range. The surfaces of the examined dental slices for the electron probe microanalyzer were polished in the Institute for Geoscience, last polishing step with 0.25 μm diamond powder.

The analyzed elements were calculated in weight % values: CaO, P_2O_5, Cl, F, MgO, SiO_2, Ce_2O_3 and SrO. The analysis of the elements was carried out in depths of 5 μm, 10 μm, 20 μm, 30 μm, 40 μm 50 μm. Each value was established on the basis of 5 analysis points. Each slice received 30 measurements (in reference to the various depths), thus each tooth received a total of 180 analysis points.

Results

This in vitro study demonstrated a loss of different amounts of the various minerals in relation to the chosen test solutions. The control tooth slices, which were incubated only with physiological sodium chloride solution, served as references (100 %) for the test samples (Fig. 1). For the quantitative analysis of the concentrations (%wt) of the respective elements, the resulting concentration of each element in the test samples was expressed as per cent of the concentration of the same element in the control samples. The measurements showed a loss of elements of the enamel samples down to a depth of 40 μm, however at a depth of 50 μm a loss of elements could not be observed (Fig 1). Calcium and phosphorus have to be

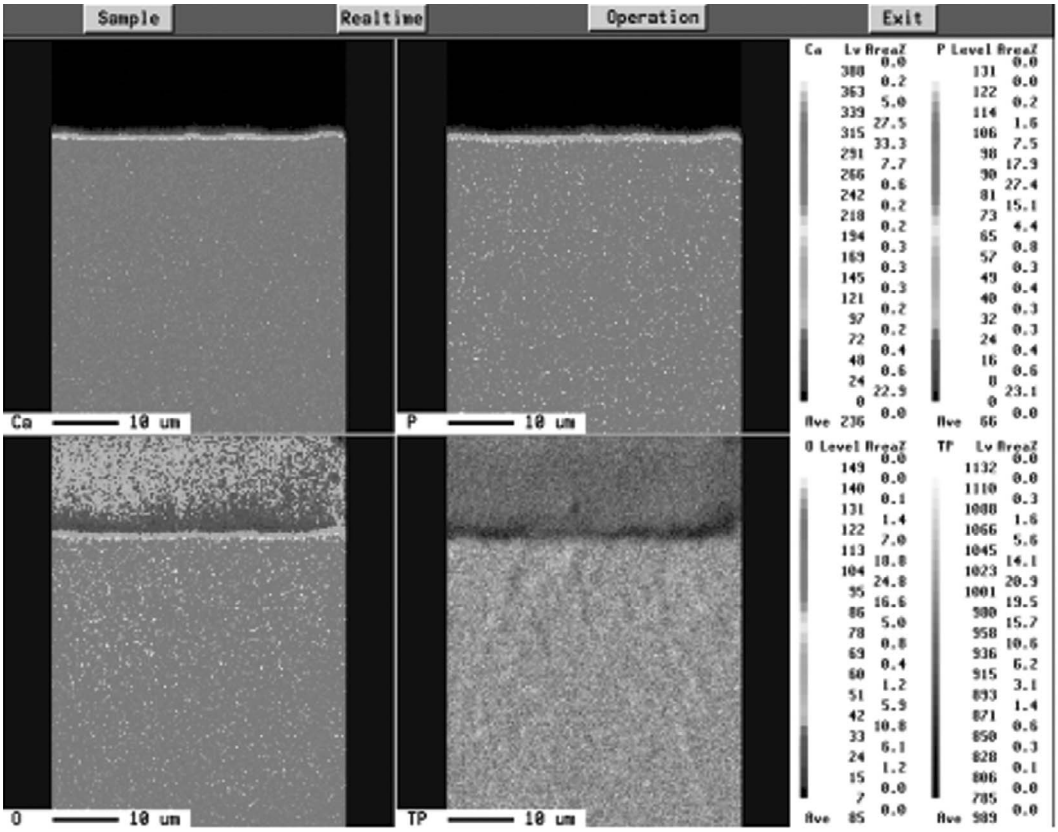

Fig. 1. Enamel sample (tooth slice) incubated with physiological sodium chloride solution, pH = 6.5. The elements calcium, phosphorus and oxygen are unchanged down into a depth of 50 μm. The depth profile (DP) shows no significant change.

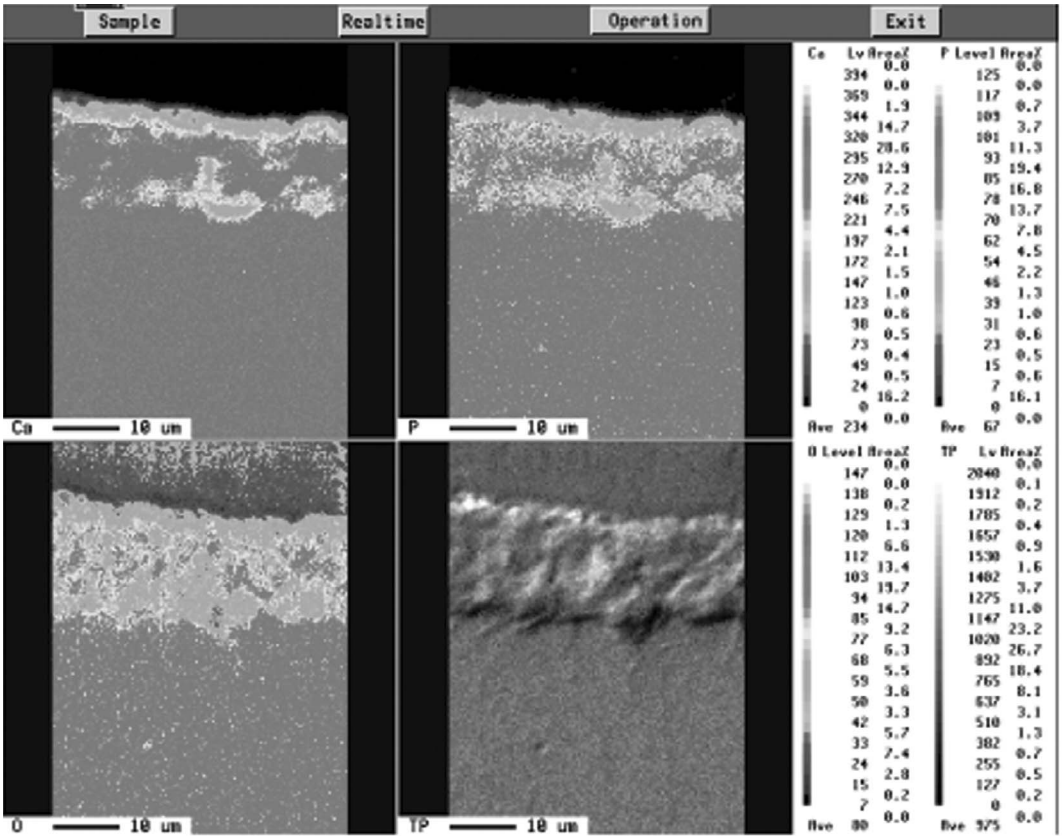

Fig. 2. Enamel sample incubated with Coca Cola, pH = 2.3. The changes in the concentration of calcium, phosphorus and oxygen are displayed into a depth of 20 μm.

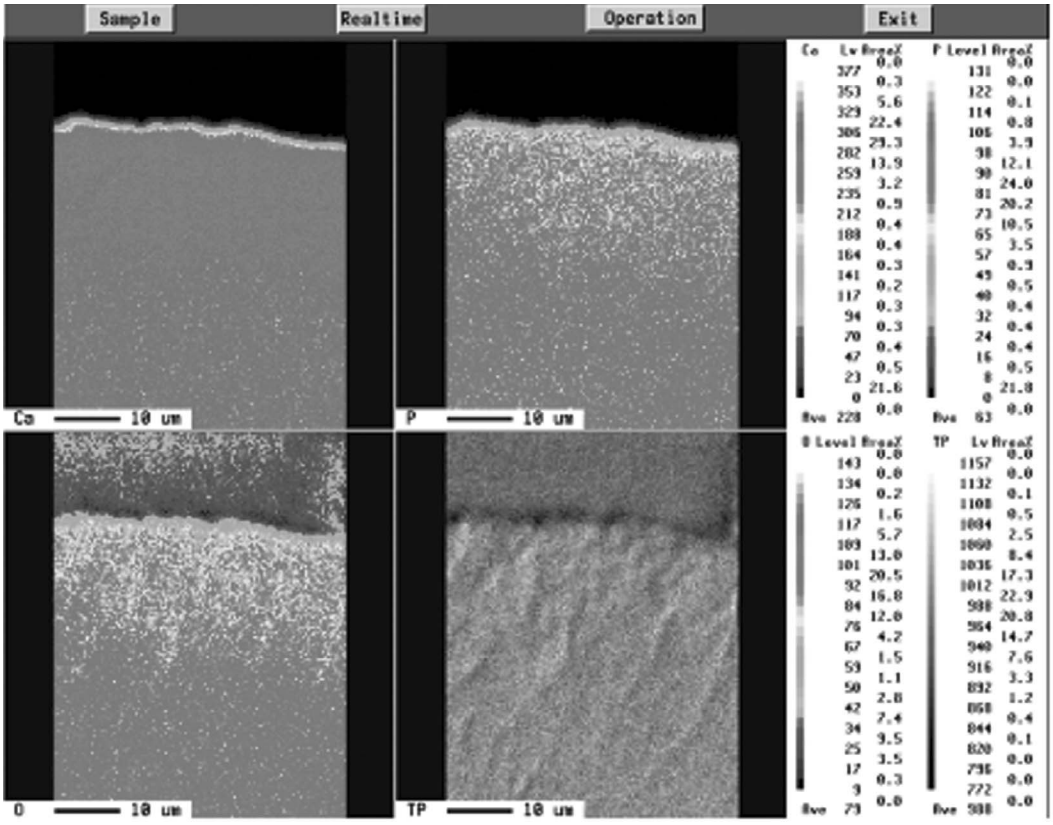

Fig. 3. Enamel sample incubated with ice tea with lemon, pH = 2.9. The significant loss of the elements calcium, phosphorus and oxygen is demonstrated through colour changes.

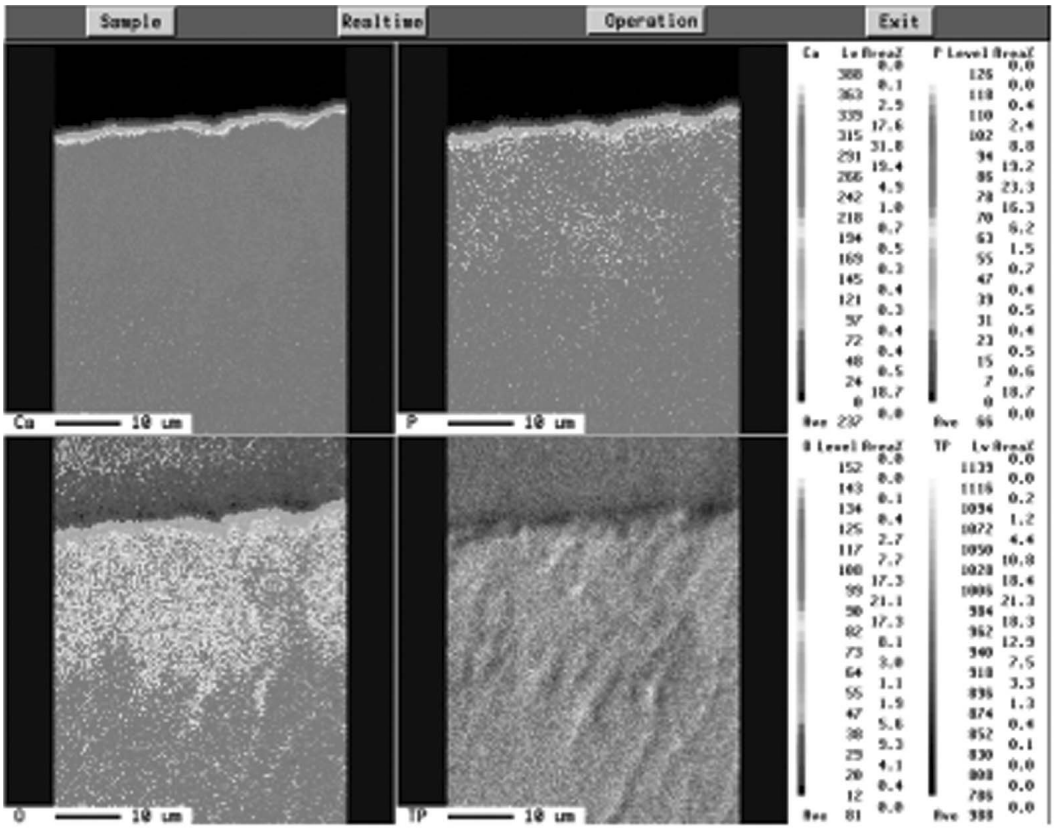

Fig. 4. Enamel sample incubated with apple juice, pH = 3.3 The changes in the concentration of calcium, phosphorus and oxygen are shown.

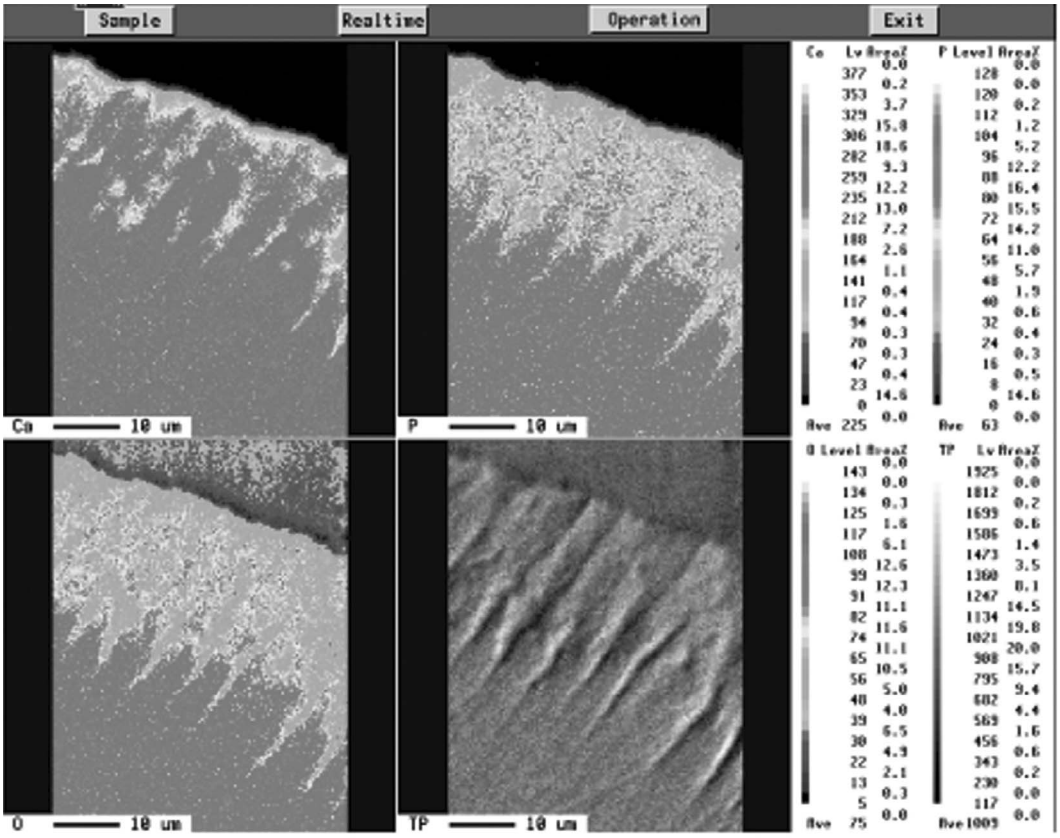

Fig. 5. Enamel sample incubated with white wine, pH = 3.3. The changes in the concentration of the elements calcium, phosphorus and oxygen are displayed.

pointed out as the main components of hydroxyapatite further elements like silicon, cerium, strontium and magnesium play an inferior role. The incubation of the tooth slices with coke (pH = 2.3) lead to a depth-related loss of mineral (Fig. 2). At a depth of 5 μm, the average mineral loss could be established at 15.5%, at a depth of 10 μm the loss of substance was 14.5 % and at a depth of 20 μm the loss could be established at 11%, at a depth of 30 μm a total of 4% were missing, at 40 μm the minerals were reduced by only 1%, and from 50 μm on, no further changes in the enamel were observed. Ice tea with lemon (pH = 2.9) showed a mineral loss in the enamel on a smaller scale (Fig. 3). At a depth of 5 μm, the mean loss of minerals was 6%, at 10 μm 6.5% of the minerals were lacking, at 20 μm only 1.3% were missing, at a depth of 30 μm a small mineral loss of 0.5% can be established, and from 40 μm on, no further loss of minerals is recorded. Similar results were achieved when incubating the enamel samples with apple juice (pH = 3.3). At a depth of 5 μm, the mean mineral loss was 5%, at 10 μm the loss of substance was established at 6.5%, at 20 μm only 2.5% of all minerals were missing, and at depths of 30 and 40 μm the loss was recorded at 0.5%, and from 50 μm on, no further loss of substance were observed (Fig. 4). Significantly higher values were achieved when conditioning the slices with white wine (pH = 3.3). At a depth of 5 μm the average mineral loss was 24%, at 10 μm 16% of all minerals were missing, at 20 μm the value was 7.5%, and at 30 μm 4% were still missing, at 40 μm, 0.5% of loss was recorded, and from 50 μm on no further loss of substance could be measured (Fig. 5). The artificial etching technique lead to a small loss of minerals. At a depth of 5 μm 4.5% of all minerals were missing, at 10 μm an average mineral loss of 4% was recorded, at 20 and 30 μm only 1% was missing, and from 40 μm on no further mineral loss was recorded. The measurements showed no correlation between the pH value of the beverages and the mineral loss (in %) of the enamel.

Discussion

In the present in vitro study the erosive potential of various acidic beverages with different pH-values on the enamel surface into depths of down to 50 mm was evaluated. In numerous clinical studies the erosive capacity of various foods and beverages has been observed. Linkosalo and Markkanen compared a group of adult subjects, adhering to a special lacto-vegetarian diet, with a corresponding control group. The persons from the diet group showed erosive changes of their teeth in 77% of the cases. Frequent consumption of acid-containing salad dressings, citrus fruit and acidic berries was reported to be a possible factor that fosters damages on the enamel surfaces. Many fruit juices, soft drinks as well as confectionery contain citric acid in typical concentrations of 15 to 45mmol/1 [18, 13].

In a study with schoolchildren, who were supposed to follow a special diet plan, Millward was able to establish a correlation between the frequency of consuming carbonated beverages and the severity of the

erosive changes. Furthermore, a relation to the consumption of acidic fresh fruit and mixed pickles could be confirmed. Over the last 10 years, an enormous increase in the turnover of carbonated beverages, such as soft drinks and corresponding diet drinks, has occurred. In their in vitro study Parry et al. incubated different mineral waters and soft drinks with powdered hydroxyapatite, and they measured the loss of calcium and phosphate. They were able to prove that sparkling mineral waters had a smaller erosive potential, whereas the tested soft drinks lead to a significant release of calcium and phosphate.

Bartlett and Coward examined the influence of gastric acid and of carbonated beverages on extracted molars with regard to the loss of calcium ions. Both liquids lead to a significant release of calcium ions; however, gastric acid caused a higher release. In our study, the quantitative loss of the main elements like calcium and phosphorus as well as other elements that are present in smaller amounts, such as magnesium, silicon, strontium and cerium was determined in different depths via electron probe micro-analysis. A beverage-related loss of all minerals down into depths of 40 µm could be shown. In contrast to the study of Bartlett and Coward, only fully retained wisdom teeth, which showed no changes of the enamel due to extrinsic or intrinsic influences, were used in this in vitro study.

In their in vitro study Vanuspong et al. also used fully retained wisdom teeth in order to determine the erosive potential of different beverages on the dentine surface: however, the influence of the beverages on the dentine was referred to as a possible change of the surface profile as measured with a profilometer. In a different in vitro study [7], the hardness and elasticity of the enamel surface of extracted erupted molars was tested, after they had been incubated with solutions containing citric acid (pH-value between 2.3 and 6.3). The study showed a pH-dependant loss of hardness and a corresponding increase in the modulus of elasticity. In our study, we were not able to establish a correlation between the mineral loss of the enamel samples and the pH-value of the beverages: besides the pH-value, several other parameters like the buffering capacity and additives to the beverage, like chelating agents or the concentration of minerals, have to be taken into consideration when explaining the erosive capacity [14]. Barbour was able to show that an increase of calcium ions is able to diminish the erosive effect.

The decline in enamel hardness after incubation with an acidic beverage indicates a possible damage of dental hard tissues, induced by acidic beverages, which is in accordance with our quantitative study on the determination of the total mineral loss of the enamel.

The conversion from the weight percentage, obtained from the chemical analysis of Ca and P, to their atomic percentages documents furthermore that these main components are not dissolved in significantly different proportions during erosion. The ratio of the elements Ca to P stayed nearly stable.

The direct application of the results from the analysis of this in vitro study to a possible threat to the dental tissue under in vitro conditions cannot be fully made, due to the fact that too many individual factors influence the environment of the oral cavity. Besides the anatomic shape and composition of the dental hard tissues, the conditions of the saliva and the buffering capacity, the frequency and duration of the beverage intake as well as the other dietary habits play a crucial role; therefore, the considerable individual properties have to be taken into account. The present data on the mineral loss in the upper enamel layers confirm the results from countless in vivo and in vitro studies that have proved the erosive characteristics of acid-containing beverages.

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