

## INFLUENCE OF APPLE JUICE ON HUMAN ENAMEL SURFACES OF THE FIRST AND SECOND DENTITION - AN IN VITRO STUDY

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### Abstract

Dental erosion caused by acidic beverages is common and occurs with increasing tendency. The aim of this in vitro study was to analyse the erosive potential of apple juice on human enamel samples from the first and second dentition. Apple-juice-containing beverages (n = 23) were selected, and pH and buffering capacity were determined. Enamel samples were prepared from impacted, surgically removed wisdom teeth (20 mm<sup>2</sup>) and from deciduous teeth (16 mm<sup>2</sup>). Prepared enamel slices were incubated with a selected apple juice (pH = 3.5) for up to 24 h; the amounts of released calcium were determined colorimetrically, and mean surface roughness (Ra) of the enamel was measured using an optical profilometric device (perthometer, Mahr, Göttingen, Germany). Controls were incubated with a 0.9 % sodium chloride solution under the same conditions (37 °C, humidified atmosphere of 5 % CO<sub>2</sub> and 95 % air). The surfaces of the enamel samples were visually examined by CLSM (Leica TCS SP2). The pH-values of the apple juices ranged from 3.3 to 4.2. Incubating the enamel slices (from both dentitions) with a selected apple juice caused a time dependent release of calcium. After 24h, the primary dentition showed Ca-release values of  $0.61 \pm 0.035$  mg/ 20mm<sup>2</sup> and the second dentition of  $0.41 \pm 0.085$  mg/ 20mm<sup>2</sup>; the surface roughness for the primary teeth was  $6.8 \pm 1.09$  µm and for the second dentition  $6.2 \pm 0.41$  µm. CLSM show structural changes on all surfaces when compared to the controls. In this in vitro study, the erosive potential of apple juice on teeth of the first and second dentition could be demonstrated. However, it must be considered that numerous modifying factors influence the human enamel surface in vivo; therefore, a direct translation from in-vitro conditions can only be done with caution.

*Key words:* Dental erosion, first and second dentition, apple juice, calcium release, surface roughness, in vitro study

### INTRODUCTION

Over the last ten years dental erosion has become a very common sight and is observed with increasing frequency especially in industrialized countries. One of the consequences of a modern life style is changed eating habits. On the one hand, a healthy diet containing

large amounts of acidic fruit and fruit juices has become vitally important for many people; on the other hand, an ever increasing consumption of soft drinks can be observed [1]. These altered habits have to be taken into account when considering the augmented dental erosion status. The way we consume food, the frequency as well as the time of intake may have a major influence on the severity of loss of the dental hard tissue [30]. Dental erosion is defined as an acidic exposure to the dental surface with loss of minerals from the upper enamel layer. The degree of mineral loss depends upon the intensity of the acid and the exposure time on the dental surface [9]. Various in vitro studies have been conducted in order to determine the erosive potential of acidic food and beverages [5, 22, 27].

One of the first studies aimed at preventing erosion was carried out by Sognnaes et al. (1972). A total of 10,827 extracted human teeth were investigated for possible dental erosion. The authors found that 18 % of all teeth had experienced erosive changes which were primarily located in the mandibular teeth with a high frequency especially in the anterior teeth.

In Switzerland Lussi et al. (1991) found a significantly higher prevalence in a sample of 391 randomly selected subjects. They examined patients of different age groups (26 to 30 and 46 to 50 years) and were able to state that 42.6 % of the older and 29.9 % of the younger age group had erosive damage on at least one occlusal surface.

In a representative German study Schiffner et al. (2002) investigated age groups from 35 to 44 and 65 to 74 years. Erosive damages in the enamel were found in 6.4 % of the younger and 4.1 % of the older age group; lesions reaching into the dentin were present in 4.3 % of the young and 3.8 % of the old patients. During the last years various epidemiological studies dealt with the occurrence, the severity and the distribution of erosion in children. Millward et al. (1994) were able to state erosive changes in 50 % of 178 investigated children aged four years. The prevalence was significantly greater in children with an elevated socioeconomic status. Al-Malik et al. (2002) described erosive defects in 31 % in a total of 987 children aged two to five years. Sixty percent of those defects were located in the enamel; the remaining 40 % reached into the dentin or even the pulp. An investigation of 42 Swiss children aged 2 to 9 years proved that 100 %

of the primary teeth and 14 % of the permanent teeth had erosive lesions [11]. Al-Dlaigan et al. (2001) were able to determine a significant correlation between erosion and the consumption of soft drinks, alcoholic beverages, fruit, vitamin C supplements and other foodstuff in a study of 14-years-old British adolescents. Citrus fruits, fruit juices, lemonade, fizzy drinks, wine and acidic foods built a food group that is potentially erosive. Järvinen et al. (1991) investigated 106 subjects with erosions and 100 controls that were free of erosion. Numerous studies were able to prove that the risk of developing erosion increases with an intake of citric fruits more than twice a day, a daily consumption of soft drinks or a weekly consumption of apple vinegar [6, 12, 18, 26]. In an in vitro study Lussi et al. (1995) were able to determine the erosive potential of apple juice, Schweppes, oranginas and grapefruit. Dennison (1996) reports a considerable increase in the consumption of apple juice over the years, and that in children younger than 5 years of age apple juice had become the preferred beverage. While fruit juices are generally considered as healthy beverages, there are health concerns regarding their frequent consumption. Apple juices for example contain in addition to several sugars non-volatile organic acids such as quinic, malic, citric and fumaric acids [13, 19].

The aim of this in vitro study was to analyse the erosive potential of apple juice on human enamel samples from the first and second dentition.

#### MATERIAL AND METHODS

From a selection of 23 different apple juice containing beverages, widely available in German supermarkets, the pH was measured by means of a portable pH-Meter with an accuracy of  $\pm$  pH 0.01, equipped with a microelectrode (Novodirect, Kehl, Germany). The total acids (buffering capacities) of four apple juice containing beverages were determined by titration with 0.1 N NaOH and subsequent measurements of the resulting pH values.

For this study, enamel samples were obtained from impacted, surgically removed wisdom teeth from young adults or from deciduous teeth, after exfoliation or extraction for orthodontic reasons from children. The teeth used in this study were collected from the Orthodontics Department of the University Dental Hospital and from oral surgeons in private practices. The adults (permanent dentition) were aged between 19 and 25 years and the children (deciduous teeth) were aged 4 to 7 years. Immediately after tooth extraction, the soft tissue was removed; the teeth were placed into a sodium azide solution (15 mM) for 30 min, and afterwards stored in a 0.9 % sodium chloride solution for up to 7 days. All teeth were microscopically examined for possible irregularities of mineralization, especially for genetically determined diseases of enamel mineralization, for cracks or other defects. The dental crowns of the teeth were evenly divided into 2 to 5 slices, with an enamel window sized for the permanent dentition of about 20 mm<sup>2</sup> and for the deciduous teeth of about 16 mm<sup>2</sup>. The test slices were placed into 12 multi-well plates (Greiner, Labortechnik/Frickenhäuser, Germany), fixed with a dental restorative ma-

terial (Venus, Heraeus Kulzer, Dormagen, Germany), and incubated with 2.5 ml of a selected apple juice for up to 24h at 37 °C in a humidified atmosphere of 5 % carbon dioxide and 95 % air in a gas incubator (Heraeus, type Function Line, Hanau, Germany). The control samples were incubated with physiological sodium chloride solution (0.9 % NaCl), pH 6.5 under identical conditions. After incubation of 2 h, 8 h, 16 h and 24 h, the amounts of released calcium were determined colorimetrically (Randox Laboratories, Krefeld, Germany) from the supernatants, and the values were adjusted to an average particle size of 20 mm<sup>2</sup>. Surface roughness (Ra) of the enamel samples was measured after incubation of 0 h (controls), 2 h, 8 h and 24 h using an optical profilometric device (perthometer, Mahr, Göttingen, Germany). Ten measurements, each of a length of 1.75 mm in randomly chosen areas, were performed for each sample and evaluated by means of the MarSurfX20 software. In a separate set of experiments, enamel samples from the first and second dentition were polished to a plane surface, attached to glass slides and incubated for 24 h with apple juice. Then, the surfaces of the dental samples from both dentitions were visually examined by means of a CLSM (Leica TCS SP2, Heidelberg, Germany). A descriptive statistical analysis of the data (non-parametrical test for independent samples, Mann-Whitney test,  $p < 0.05$ ) was performed.

#### RESULTS

In this in vitro study on the determination of the erosive potential of acidic beverages a total of 23 commercially available beverages containing apple juice, were analysed for their pH values. As seen in Table 1 the pH values ranged from 3.3 to 4.2 and the fruit juice concentrations from 30 % to 100 %. Besides on its pH value, the erosive potential of different beverages on dental hard tissue depends upon the buffering capacity. Thus their buffering capacity was determined for four characteristic apple juice containing beverages (Bebivita pH = 4.23; Capri Sonne pH = 3.56, Glan Krone pH = 3.41, Fruxano pH = 3.5) as seen in Fig. 1. As a consequence of this measurement an apple juice (Fruxano, pH = 3.5) with a relatively high buffering capacity was chosen for testing in the subsequent analysis which consisted of determining calcium release, measuring surface roughness and visually analyzing the dental surface via CLSM technique. All studies were carried out for both teeth of the first and second dentition. The prepared dental slices were incubated up to 24 hours with the selected apple juice; the control tooth slices, which were incubated only with physiological sodium chloride solution, served as references for the test samples. The incubation of the dental samples (primary and permanent dentition) with the selected apple juice showed a time dependent release of calcium from the enamel surfaces (Fig. 2). When comparing the primary teeth with the permanent dentition a difference in the time dependent calcium release could be observed. After an incubation time of 24 hours this difference showed a marked statistical significance ( $p = 0.002$ ). After long term incubation the surfaces of the dental samples for both

Table 1. Different beverages containing apple juice which are available in German supermarkets.

<i>Apple juice drinks</i>	% fruit juice	pH
Big Apple (Apollinaris)	60	3.7
Schorle (Odenwald Quelle)	60	3.6
Lift Schorle Apfel (Coca Cola Com.)	55	3.52
Apfel Schorle Alkoholfrei (Merziger)	60	3.43
Apfelschorle (Berg Sport)	60	3.54
Apfeschorle (Gerolsteiner)	60	3.75
Apfeschorle (ALWA)	50	3.55
Fruchtschorle Magic Apple (Punica)	60	3.51
Fruchtschorle Apfel (Bebivita)	60	4.23
Fruchtschorle Apfel (Hipp)	60	3.99
Apfelfruchtsaftgetränk (Tipp)	50	3.32
Capri Sonne Apfel (Capri Sonne)	30	3.56
<i>Apple juices (unfiltered)</i>		
Apfel – Acerola (Hohes C)	100	3.44
Apfel naturtrüb (Merziger)	100	3.36
Apfelsaft naturtrüb (Glan Krone)	100	3.41
Direkt Saft (Kumpf)	100	3.28
<i>Apple juices (filtered)</i>		
Apfelsaft (Albi)	100	3.49
Classic Apfelsaft (Kumpf)	100	3.47
Apfelsaft (Tipp – Goldhand)	100	3.55
Apfelsaft (Fruzano)	100	3.5
Happy Day (Rauch)	100	3.59
Apfelsaft (Milisan)	100	3.64
Milder Apfelsaft (Hipp)	100	3.83

dentitions were visually analysed with the help of CLSM. Fig. 3 demonstrates the loss of minerals induced by apple juice incubation for the primary teeth. When compared to the permanent teeth which showed more superficial lesions (Fig. 4), the defects in the primary dentition reached deeper areas which leads to a greater overall loss of minerals and consequently of tooth structure.

After an incubation time of up to 24 hours with the selected apple juice the surface roughness of the enamel samples for both dentitions was also determined. Fig. 5 shows the time dependent increase of surface roughness in the enamel slices for both dentitions.

Fig. 6 a, b show two typical surface roughness profiles of enamel samples from the primary dentition; one was the control, the other shows the result of an incubation with apple juice over 24 hours. However no statistically significant difference of surface roughness after an incubation time of 24 hours ( $p = 0.165$ ) could be determined when comparing both dentitions.

## DISCUSSION

Numerous studies dealing with the influence of food on the development of erosion in the dental hard tissue are described in the literature. Wiegand et al. (2006) investigated a total of 463 German children aged between 2 and 7 years and found erosive defects

in 32 % of all cases, among those 13.2 % reached into the dentin. Erosion of dental hard tissue has to be considered as a multifactorial disease caused primarily by acids [15]. The origin of the acid can be extrinsic and intrinsic [23]. Zero (1996) subdivides the extrinsic factors into environmental influences, nutrition, drugs and also way of life.

Both the adhesion and the displacement of the fluid from the dental surface are factors that might have a further influence upon the erosive process [8]. The capability of bonding to the enamel varies among the various acidic beverages. The stronger the beverage bonds to the enamel the longer its effect on the enamel is going to be and consequently the greater the erosive damage [10]. A large number of techniques have been developed in order to determine both the change in and the loss of tooth surface structure induced by erosion.

Some of the techniques used most frequently are: surface roughness measured with an optical profilometric device, determination of the micro hardness, the scanning surface microscopy, the microradiography, Confocal Laser Scanning Microscopy (CLSM) and the chemical analysis of eluted minerals.

The quantitative light-induced fluorescence (QLF) and the nanoindentation techniques are the most recent and also very promising methods. The surface roughness determination as a physical measurement technique was used in a number of studies to measure erosive effects and was thus used in the present study. Other possibilities to detect erosion caused by food and drink is by measuring surface hardness using the micro hardness test. In vitro investigations are especially suitable to determine early erosive lesions that show a marked softening of surface hardness.

As the surface profilometry is only able to determine defects with a minimum depth of at least 0.5  $\mu\text{m}$  it seems suitable to measure more progressed erosive lesions with this method. In more advanced erosive changes it appears that measuring the dental surface roughness is a very reliable method. This study shows no differences between the first and second dentition after extended incubation with apple juice. However, an advantage of this technique is that it is relatively simple and quick to use especially in small defects [3].

The SEM is a convenient tool to qualitatively determine the most delicate erosive lesions of the tooth surface. However this method is certainly not suitable for a quantitative analysis. [7] SEM is applied as a substitute for light microscopy that was frequently used in the past.

Confocal Laser Scanning Microscopy (CLSM) is a considerably better technique for the qualitative determination of erosion [28]. In this study we could show using CLSM significantly deeper mineral loss in the teeth of the first dentition when compared to those of the second dentition. This visual representation could be confirmed by measurements showing elevated calcium levels in solution from teeth of the first dentition compared to those of the second dentition.

Enamel consists of 34 to 39 percent calcium by weight (dry weight) and 16 to 18 percent phosphorus by weight. That is why a quantitative determination of

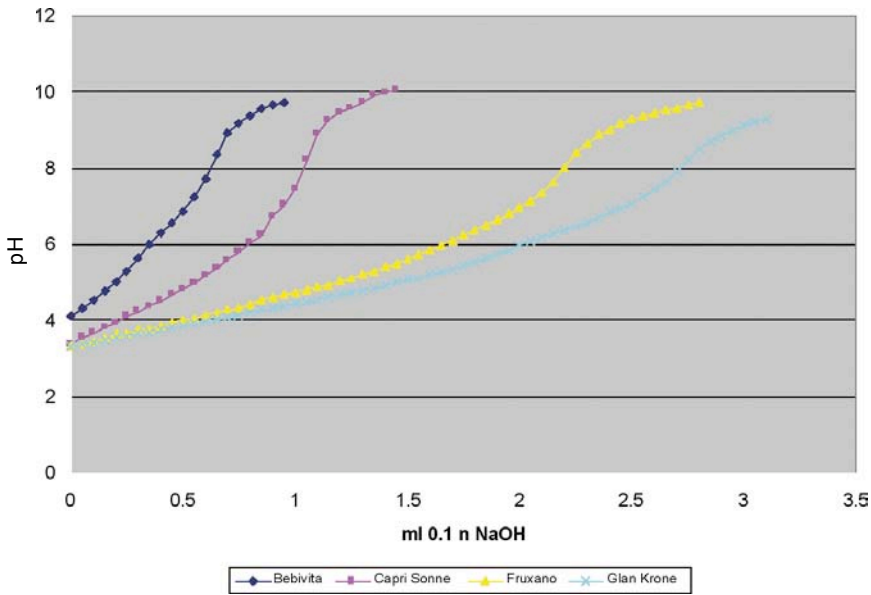


Fig. 1. Buffering capacities of four selected apple juice containing beverages.

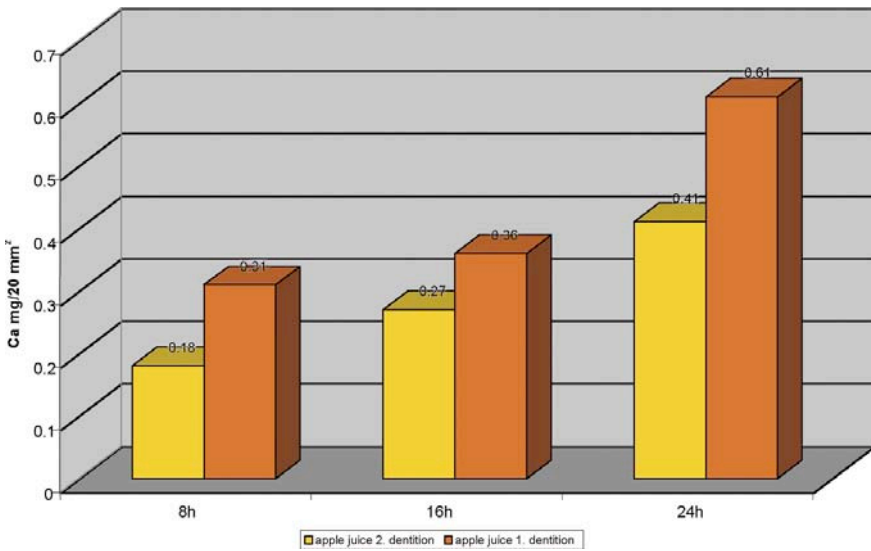


Fig. 2. Mean calcium release (mg/20 mm<sup>2</sup>) from human dental enamel samples (first and second dentition) after an incubation time of 8-24 hours.

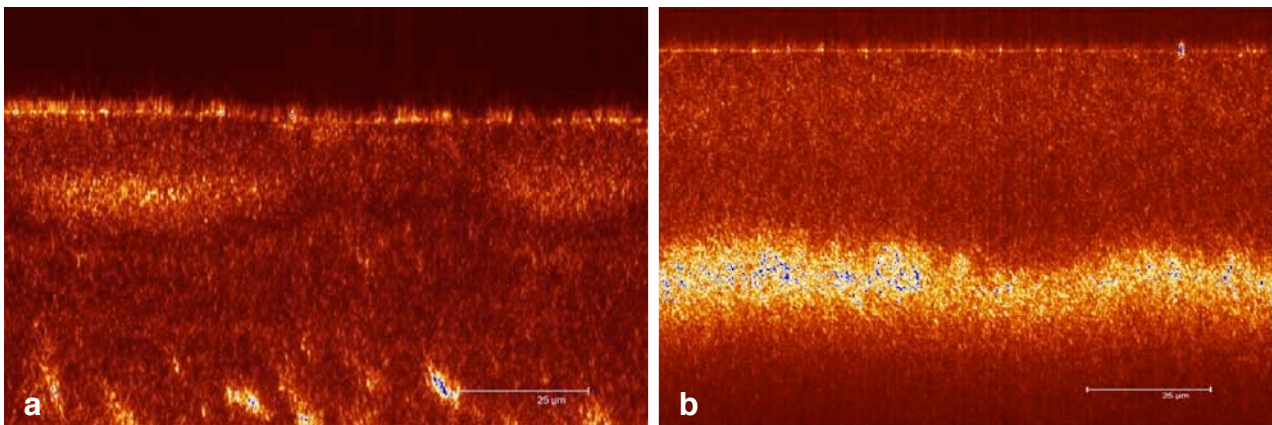


Fig. 3. Confocal laser scanning microscopy images (xz-cut) of enamel surfaces of primary teeth. a) Control dental sample, b) enamel sample after long-term incubation with apple juice.

the calcium and phosphorus dissolved through the erosive procedure is an appropriate method in order to

measure erosion [3]. All these methods measure tiny amounts of mineral loss and can therefore be used for

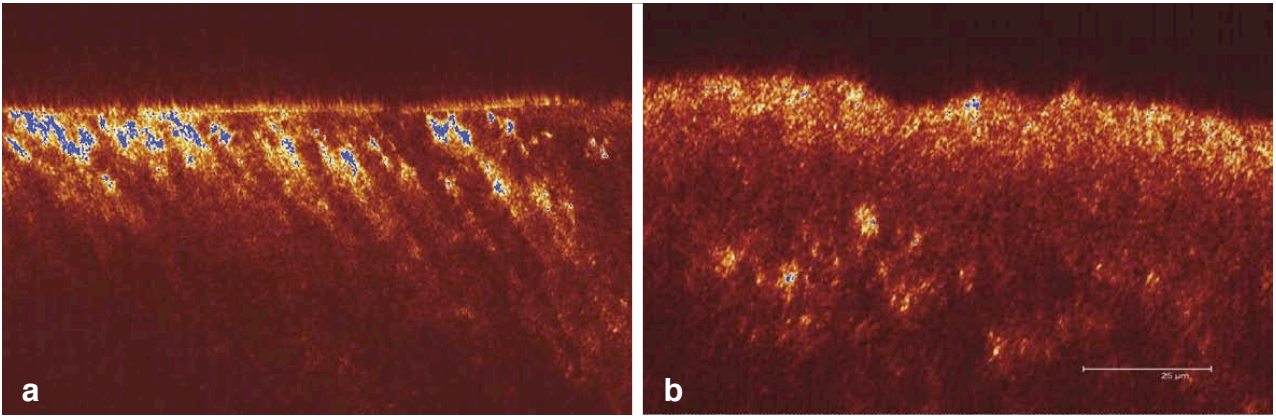


Fig. 4. Confocal laser scanning microscopic images (xz-cut) of enamel surfaces of permanent teeth. a) Control dental sample, b) enamel sample after long-term incubation with apple juice.

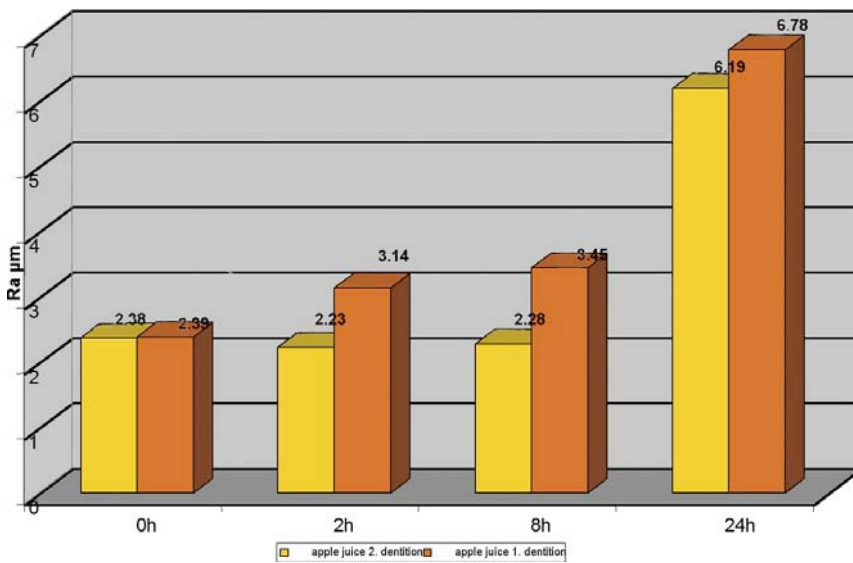


Fig. 5. Mean surface roughness (Ra µm) of human dental enamel samples (first and second dentition) after an incubation time of 2-24 hours.

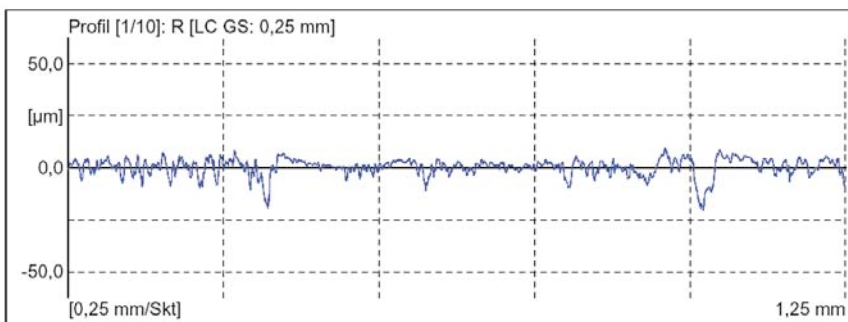


Fig. 6a. Profile of a dental enamel surface (first dentition); control sample.

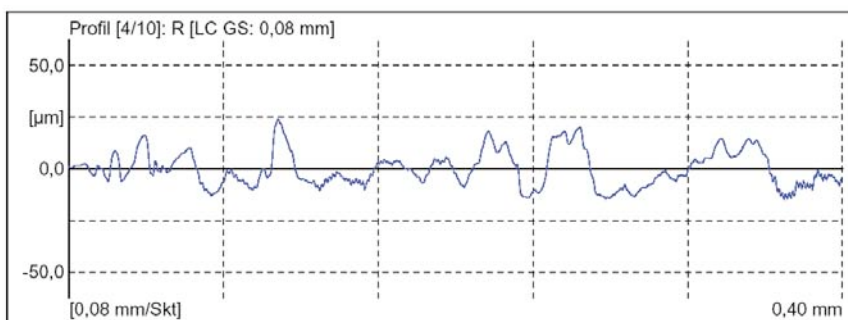


Fig. 6b. Profile of a dental enamel surface (first dentition) after long-term incubation with apple juice (24 h).

both early and progressed erosive defects. Modifying parameters such as the use of fluoride dentifrice and the composition and the flow rate of the saliva have an additional influence upon the severity of the erosion [8, 17].

Besides the well known factors which must be considered, the aetiology of erosion is very complex and not yet fully clarified.

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