In-vitro evaluation of erosive potential of vitamin/mineral effervescent tablets commercially available in Switzerland

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1 Abstract

Objective: Aim of the present study was to evaluate the erosive potential of effervescent tablets containing a combination of minerals and vitamins.

Materials and methods: A total of 96 bovine enamel samples were prepared and distributed among eight groups (A-H; n = 12). Samples were immersed (120 s) in the following eight solutions: A: 300 ml water (control); B: tablet with calcium and vitamin D (Qualité & Prix, Coop) dissolved in 300 ml water; C: tablet with calcium and vitamin D3 (well & active, Aldi Suisse AG) dissolved in 250 ml water; D: tablet with calcium and vitamin D3 (Optisana, Lidl) dissolved in 200 ml water; E: tablet with different minerals and vitamins (Actilife All in One, Migros) dissolved in 200 ml water; F: tablet with different minerals and vitamins (Berocca® calcium, magnesium + zinc, orange, Bayer AG) dissolved in 300 ml water; G: tablet with different minerals and vitamins (Isostar® fast hydration powertabs, lemon flavour) dissolved in 250 ml water; H: tablet with magnesium and vitamin C (Qualité & Prix, Coop) dissolved in 300 ml water. Enamel wear was measured using surface profilometry after 10 and 20 cycles of erosion and compared with the baseline profiles. For each solution the following characteristics were determined: average weight of effervescent tablets, pH, concentration of hydrogen ions, citric, malic and ascorbic acid, carbon dioxide, calcium, magnesium, sodium, zinc and phosphate.

Results: Highest mean enamel wear (± SD) after 10 and 20 cycles of erosion was observed for the groups G (1.763 ± 0.368 µm; 5.256 ± 0.755 µm respectively) and H (1.823 ± 0.269 µm; 5.121 ± 0.671 µm respectively). The enamel wear in all other groups (A-F) was significantly lower (p < 0.00179 respectively). Compared with water (control group A), the groups B, C and D after 10 cycles and the groups B and C after 20 cycles showed no significant difference. The other groups, except the group E, presented instead a significantly higher enamel wear.

Conclusions: Depending on their chemical characteristics and more particularly on their calcium-content, effervescent tablets can be regarded as more or less erosive. Effervescent tablets with a low pH, but with high calcium concentration induce less enamel wear than effervescent tablets with the same pH, but with lower calcium concentration or without calcium. Calcium in the effervescent tablets yielded an anti-erosive capacity.
2 List of abbreviations

vol%: Volume percent
EDTA: Ethylenediaminetetraacetic acid
Ip: Ionic activity product
Ksp: Solubility product constant
mat.: Material
H\(^+\): Hydrogen ion
CO\(_2\): Carbon dioxide
Ca\(^{2+}\): Calcium ion
Mg\(^{2+}\): Magnesium ion
Na\(^+\): Sodium ion
Zn\(^{2+}\): Zinc ion
PO\(_4\)\(^{3-}\): Phosphate
SD: Standard deviation
3 Introduction

Both prevalence and incidence of dental erosion, defined as non-carious dental substance loss induced by direct impact of extrinsic or intrinsic acids or chelating agents (GANSS 2006, SCHLUETER et al. 2012), have considerably increased (LUSSI 2006).

The principal extrinsic factor of dental erosion are dietary acids (SCHWEIZER-HIRT et al. 1978), which consumption trends to increase (LUSSI et al. 2012). In the past several decades, many studies investigating the erosive potential of different dietary substances have been performed and a wide range of drinks and foods, such as soft drinks, sports drinks, juices, salad dressings, candies, herbal teas, alcoholic drinks, vinegar, etc., were recognized to be associated with the increase in erosion (LUSSI et al. 2012). Several studies investigated also the erosive potential of some effervescent tablets, especially as effervescent medicament or vitamin C preparations (MEURMAN & MURTOMAA 1986, NUNN et al. 2001, LUSSI et al. 2012).

The acid content of effervescent tablets renders them possibly erosive. Thus effervescent tablets are an interesting element of studies in dental erosion research. In order to form carbon dioxide by contact with water, effervescent tablets consist in fact of a soluble organic acid (e.g. citric, tartaric, malic, fumaric, adipic acid) and an alkali metal carbonate salt (e.g. sodium bicarbonate/carbonate, potassium bicarbonate/carbonate) (STAHL 2003).

At present, there seems to be no studies that analyse in detail the erosive potential of effervescent tablets as dietary supplementation of vitamins and minerals.

Consequently, the aim of this study was to evaluate the erosive enamel wear after contact with effervescent tablets containing a combination of minerals and vitamins (more details about study aim and hypotheses are provided in chapter 3.3).

For a better understanding of the study, some knowledge about the chemical nature of enamel erosion (see chapter 3.1) and about the factors influencing dental erosive potential with respect to diet (see chapter 3.2) must be reviewed.
3.1 Chemistry of enamel erosion

Enamel erosion is a centripetal process characterized by initial softening (hardness loss) of the enamel surface and by followed continuous layer-by-layer dissolution of enamel crystals, which leads to a permanent loss of tooth volume with a softened layer at the surface of the remaining tissue (LUSSI et al. 2011, SCHLUETER et al. 2012). In the initial stage of the enamel erosion process, repair (remineralization) is in theory still possible as the remaining tissue could act as a scaffold, while in the second more advanced stage, in which the mineral of the outer enamel is totally lost, repair is not possible and only the remaining softened enamel beneath the lost hard tissue is remineralizable (LUSSI et al. 2004).

The above-described process of enamel erosion occurs by interaction between enamel surface and hydrogen ions, derived from acid dissociation in water, or chelating agents, i.e. anions that can bind or complex calcium (FEATHERSTONE & LUSSI 2006). However, to interact with enamel, the erosive agents must first diffuse through the plaque, the pellicle, and the protein/lipid coating of the individual enamel crystals themselves (FEATHERSTONE & LUSSI 2006).

The first substrate upon which erosive agents have their effect, the dental enamel, consists in large part of mineral (87 vol% assuming that the mineral has the composition of hydroxyapatite) and in small part of water (11 vol%) and organic material like protein and lipid (2 vol%) (FEATHERSTONE & LUSSI 2006, LUSSI et al. 2011). The mineral in dental enamel is composed of a calcium-deficient carbonated hydroxyapatite containing some fluoride (Ca$_{10-x}$Na$_x$(PO$_4$)$_6$$\cdot$$\gamma$(CO$_3$)$_z$(OH)$_{2-y}$$F_y$) and also includes lower concentrations of sodium, magnesium, chloride, potassium and various trace elements (LUSSI et al. 2011). These substitutions in the mineral crystal lattice influence the enamel solubility. Especially carbonate renders tooth mineral more acid soluble than hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$), which in turn is more soluble than fluorapatite (Ca$_{10}$(PO$_4$)$_6$F$_2$) (DAWES 2003, FEATHERSTONE & LUSSI 2006, LUSSI et al. 2011). Because the proportions of the substitutions vary from person to person, and indeed from tooth to tooth, the enamel solubility is not fixed and varies slightly from person to person, and indeed from tooth to tooth (DAWES 2003). In addition, due to the depth dependent change of enamel chemical composition and physical proprieties, the enamel solubility varies even
into the same tooth: with increasing distance from the surface the density and hardness of the tissue tend to decrease and solubility increases (LUSSI et al. 2011).

As previously mentioned, dental erosion is a combination of minerals being dissolved by attack from hydrogen ions and mineral dissolving by calcium being complexed by chelating agents such as citric acid and EDTA (FEATHERSTONE & LUSSI 2006). Hydrogen ions can directly attack the crystal surface of enamel by combining with carbonate and/or phosphate and thus by releasing ions from the involved crystal surface, which leads to direct surface etching (FEATHERSTONE & LUSSI 2006). On the other hand, chelating agents may complex with calcium and, consequently, remove it from the crystal surface (FEATHERSTONE & LUSSI 2006) but also from the surrounding solution, especially saliva (MEURMAN & TEN CATE 1996). In this way, chelating agents decrease the degree of saturation in the surrounding of teeth and favour more demineralization (ZERO & LUSSI 2005).

The degree of saturation in the surrounding solution with respect to tooth mineral is the driving force for mineral dissolution and is defined as the ratio of the mean ionic activity product (Ip) of the solution to the solubility product constant (Ksp) of the tooth mineral (by convention hydroxyapatite); both are based on the calcium, phosphate and hydroxyl concentrations, or more correctly activities (DAWES 2003, LUSSI et al. 2011, WANG & LUSSI 2012). Depending on the ratio of Ip to Ksp, the following degrees of saturation can be defined: 1) if Ip = Ksp, then the solution is just saturated with respect to hydroxyapatite; 2) if Ip > Ksp, the solution is supersaturated and will not dissolve the tooth mineral; 3) if Ip < Ksp, the solution is undersaturated and leads to mineral dissolution until Ip = Ksp (DAWES 2003, WANG & LUSSI 2012).

Another useful measure to understand the mineral dissolution (demineralization) or its opposite, the mineral precipitation (remineralization), is the critical pH, which is defined as the pH at which a solution is just saturated with respect to a particular mineral, such as dental enamel. If the pH of the solution is lower than the critical pH, the solution is undersaturated and can dissolve the tooth mineral. On the other hand, if the pH is above the critical pH, the solution is supersaturated and thus more mineral may precipitate. However, the critical pH is not a constant, since it depends on the activities of the relevant mineral constituents of the solution, especially calcium and phosphate. In general, the more calcium and phosphate are present in a solution, the lower is the critical pH (DAWES 2003, LUSSI et al. 2011). Therefore, it is possible that at a low pH, factors
such calcium and phosphate activities are strong enough to prevent dental erosion and that at higher pH chemicals that complex calcium can cause dental erosion (LUSSI & JAEGGI 2006).

3.2 Diet as extrinsic cause of dental erosion: a multifactorial condition

There is evidence that acidic foodstuffs and beverages play a role in the development of dental erosion. However, the pH of a dietary substance alone is not predictive of its potential to cause erosion as other factors modify the erosive process. These factors are chemical, behavioural and biological (LUSSI et al. 2004). Their interplay is crucial and helps to explain why some individuals exhibit more erosion than others, even if they are exposed to exactly the same acid challenges in their diets (LUSSI 2006).

3.2.1 Chemical factors

The erosive potential of acidic foodstuffs or drinks is not exclusively dependent on their pH, but is also strongly influenced by their mineral content (especially calcium, phosphate and fluoride), their titratable acidity, their buffering capacity and their calcium-chelation properties (LUSSI & JAEGGI 2006). As previously mentioned (see chapter 3.1), the pH and the content of calcium, phosphate and fluoride of a drink or foodstuff determine the degree of saturation with respect to the tooth mineral, which is the driving force for dissolution of the tooth mineral (ZERO & LUSSI 2005, LUSSI & JAEGGI 2006). Apart from the degree of saturation, the ability of an acidic solution to dissolve enamel or dentine depends on its buffering capacity, which is related to the undisassociated acid concentration in drinks and food. The greater the buffering capacity of the drink or food, the longer it will take for saliva to neutralize the acid, and the more mineral may be dissolved before dissolution ceases (LUSSI et al. 2011). Furthermore, the chelating properties of the products can enhance the erosive process by interacting with saliva as well as directly soften and dissolving tooth mineral (LUSSI & JAEGGI 2006). Generally, the titratable acidity of acidic dietary substances is considered more important than their pH, because it will determine the actual hydrogen ions available to interact with the tooth surface (ZERO 1996).
Other more physico-chemical parameters that can influence the erosive potential of foodstuffs or drinks are their adherence to the dental surface, their viscosity and their temperature. In fact, the greater the adherence (LUSSI & JAEGGI 2006), the lower the viscosity (AYKUT-YETKINER et al. 2013) or the higher the temperature of an acidic substance (AMAECHI et al. 1999) is, the higher the likelihood of dental erosion will be.

3.2.2 Biological factors

The biological factors influencing the erosive potential with respect to food and beverages include saliva, acquired dental pellicle, tooth composition and structure, dental anatomy and occlusion, anatomy of oral soft tissues in relationship to the teeth and physiologic soft tissue movement (HARA et al. 2006).

Saliva has been considered the most important biological factor influencing dental erosion prevention due to its ability to act directly on the erosive agent itself by diluting, clearing, neutralizing and buffering acids (HARA et al. 2006). Further, saliva forms a protective membrane, the acquired dental pellicle, and reduces the demineralization rate, respectively enhances the remineralisation, by providing calcium, phosphate and fluoride to eroded enamel and dentin (HARA et al. 2006).

The acquired dental pellicle is a protein-based layer, which is rapidly formed on dental surfaces after its removal. It may protect against erosion by acting as a diffusion barrier or a perm-selective membrane, preventing direct contact between the acids and tooth surface, thus reducing the dissolution rate of dental hard tissue (HARA et al. 2006, LUSSI et al. 2009). Consequently, procedures that remove or reduce the thickness of the acquired dental pellicle, such as tooth brushing with abrasive dentifrice products, professional cleaning with prophylaxis paste, and tooth whitening, may compromise its protective properties and accelerate the erosion process (ZERO & LUSSI 2005).

The type of dental substrate and its composition can also influence the dental erosion, especially if dental erosion is combined with abrasive impact. Dental erosion, in fact, progresses faster in dentin than in enamel, in bovine permanent than in human permanent enamel and in human deciduous than in human permanent enamel (AMAECHI et al. 1999, FEATHERSTONE & LUSSI 2006). Further, tooth mineral is generally more acid soluble than hydroxyapatite, which in turn is more soluble than fluorapatite (as explained in chapter 3.1).
Because dental erosion is highly influenced by the protective factors of saliva, which vary in different sites of the mouth, the different positioning of the teeth in the dental arch may provide different susceptibility of the tooth surface to erosion. This explains why the facial surfaces of upper incisors and lingual surfaces of lower teeth have, respectively, higher and lower susceptibility to erosion (HARA et al. 2006).

Another significant biological aspect is the relationship of the teeth to the surrounding soft tissues and tongue. Tongue seems to be able to remove enamel and dentin softened by erosion and also to have an abrasive effect. Further, unprotected eroded enamel is significant more susceptible to dental erosion when compared to mechanically protected surfaces (HARA et al. 2006).

3.2.3 Behavioural factors

Behavioural factors, which can modify the extent of dental erosion during and after the erosive challenge, include abusive or unusual consumption of foods and beverages, healthier lifestyles involving frequent consumption of acidic fruits and vegetables and regular exercise, unhealthy lifestyles involving chronic alcoholism and frequent consumption of alcopops or illegal designer drugs, overzealous oral hygiene practices with abrasive dentifrices and excessive use of tooth bleaching/whitening products (ZERO & LUSSI 2006).

Behaviours, which increase the contact time of an acidic substance with teeth, are likely to be the main driving force leading to dental erosion in many individuals. Unusual eating and drinking methods, as well as swallowing habits, which increase the direct contact time of acidic foods and beverages with the teeth, such as holding drinks in the mouth before swallowing, are obvious factors that will increase the risk of dental erosion. Particularly destructive can be an exposure to erosive agents at night, such as a night-time baby bottle feeding with acidic beverages, because of the nocturnal absence of salivary flow (ZERO & LUSSI 2006).

The pursuit of a healthier lifestyle, paradoxically, can lead to dental health problems in the form of dental erosion, as it often involves diets rich in acidic food and beverages. Moreover regular exercise, which decreases the salivary flow and requires often low-pH sugar-containing beverages, such as acidic sports drinks, fruit juices and other carbonated and uncarbonated acidic beverages, might also contribute to more pronounced tooth wear (ZERO & LUSSI 2006, LUSSI et al. 2009).
Health conscious individuals also tend to have better than average oral hygiene. However, the frequent tooth brushing with abrasive oral hygiene products or the excessive use of tooth bleaching/whitening products may render teeth more susceptible to dental erosion (ZERO & LUSSI 2006).

At the other end of the spectrum, an unhealthy lifestyle may also be associated with dental erosion. Due to the low pH and the low mineral content of wine and to the increased regurgitation, alcoholics may be at particular risk for dental erosion. But also the frequent consumption of alcoholic soft drinks, so-called alcopops, or of illegal designer drugs, such as ecstasy, is associated with dental erosion (ZERO & LUSSI 2006, LUSSI et al. 2009).

Other factors, such as knowledge, education, occupation, socio-economic status, habits and general health, can influence the whole process of dental erosion development or defence (LUSSI 2006).

Furthermore, for better understanding of the complex entity of dental erosion, it must be considered that the acids involved in dental erosion originate not only from the diet, but also from other extrinsic or intrinsic sources (TEN CATE & IMFELD 1996) and that other forms of tooth wear, especially abrasion and attrition, can interact with dental erosion (ADDY & SHELLIS 2006).

3.3 Aim of the study and hypotheses

The aim of this study was to evaluate the enamel erosive potential of effervescent tablets containing a combination of minerals and vitamins.

The following two hypotheses were taken into consideration:

1) On the basis of the presence of acids, effervescent tablets should have an erosive character.

2) Do to the different composition of the effervescent tablets, different erosive potentials were expected.
Consequently, the null hypotheses of this study were that:

1) effervescent tablets would not have any erosive character;

2) there would be no differences of erosive potential between the different effervescent tablets.
4 Materials and methods

4.1 Experimental design

The accurate description of the materials [mat.] used in this study and their origin are listed in chapter 8.1 as material list.

For this study 96 bovine enamel samples were prepared and allocated to eight groups (A-H). Each group, containing 12 samples (n = 12), was treated with the following solution:

Group A: 300 ml water (control).

Group B: tablet with calcium and vitamin D (Qualité & Prix, Coop) [mat. 1] dissolved in 300 ml water.

Group C: tablet with calcium and vitamin D3 (well & active, Aldi Suisse AG) [mat. 2] dissolved in 250 ml water.

Group D: tablet with calcium and vitamin D3 (Optisana, Lidl) [mat. 3] dissolved in 200 ml water.

Group E: tablet with different minerals and vitamins (Actilife All in One, Migros) [mat. 4] dissolved in 200 ml water.

Group F: tablet with different minerals and vitamins (Berocca® calcium, magnesium + zinc, orange, Bayer AG) [mat. 5] dissolved in 300 ml water.

Group G: tablet with different minerals and vitamins (Isostar® fast hydration powertabs, lemon flavour) [mat. 6] dissolved in 250 ml water.

Group H: tablet with magnesium and vitamin C (Qualité & Prix, Coop) [mat. 7] dissolved in 300 ml water.
The water volume for the solution preparation is based on the recommendation of the single products. By the description “one glass of water” without a specific volume the effervescent tablet was dissolved in 300 ml water.

The experiment was as follows designed: one part consisted of the chemical characterization of the eight solutions, while the other part (the main part of the experiment) consisted of the erosion procedure and of the profilometric analysis of enamel wear, which data were statistically analysed.

4.2 Experimental procedure

4.2.1 Characterization of the solutions

In order to check the product composition and description (see chapter 8.2) and to study the erosive potential of effervescent tablets, the following properties were determined. For each type of effervescent tablet the average weight was evaluated with an electronic analytical balance [mat. 8]. The hydrogen ions (H$^+$) concentration and the pH of the solutions were then determined with a potentiometer [mat. 9]. By means of enzymatic tests, the concentration of citric [mat. 10], malic [mat. 11] and ascorbic acid [mat. 12] were determined. For evaluation of the carbon dioxide (CO$_2$) concentration, a CO$_2$ meter [mat. 13] was used. The concentration of calcium (Ca$^{2+}$), magnesium (Mg$^{2+}$), sodium (Na$^+$) and zinc (Zn$^{2+}$) ions was measured using atomic absorption spectrometry [mat. 14], while the determination of the phosphate (PO$_4^{3-}$) concentration was done according to the colorimetrically procedure of Fiske and Subbarow (FISKE & SUBBAROW 1925).

For each type of effervescent tablet, two solutions (1-2) were prepared (as described in chapter 4.1). From each solution, two samples were tested, for a total of four test samples (1.1, 1.2, 2.1, 2.2). Table 1 summarizes the means of some relevant solution properties.
Table 1. pH, concentration (mM) of hydrogen ions (H<sup>+</sup>) calcium (Ca<sup>2+</sup>), phosphate (PO<sub>4</sub><sup>3-</sup>) and magnesium (Mg<sup>2+</sup>), citric, ascorbic and malic acid, carbon dioxide (CO<sub>2</sub>).

<table>
<thead>
<tr>
<th>Group</th>
<th>pH</th>
<th>H&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Ca&lt;sup&gt;2+&lt;/sup&gt;</th>
<th>PO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;3-&lt;/sup&gt;</th>
<th>Mg&lt;sup&gt;2+&lt;/sup&gt;</th>
<th>Citric acid</th>
<th>Ascorbic acid</th>
<th>Malic acid</th>
<th>CO&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.20</td>
<td>-</td>
<td>1.2</td>
<td>0.001</td>
<td>0.3</td>
<td>-</td>
<td>0.26</td>
<td>-</td>
<td>0.00</td>
</tr>
<tr>
<td>B</td>
<td>4.10</td>
<td>19.00</td>
<td>33.3</td>
<td>-</td>
<td>0.001</td>
<td>28.94</td>
<td>-</td>
<td>6.71</td>
<td>49.18</td>
</tr>
<tr>
<td>C</td>
<td>4.02</td>
<td>17.40</td>
<td>27.2</td>
<td>-</td>
<td>0.001</td>
<td>46.01</td>
<td>-</td>
<td>-</td>
<td>58.17</td>
</tr>
<tr>
<td>D</td>
<td>3.93</td>
<td>18.04</td>
<td>50.1</td>
<td>-</td>
<td>0.001</td>
<td>42.16</td>
<td>-</td>
<td>14.92</td>
<td>71.57</td>
</tr>
<tr>
<td>E</td>
<td>4.06</td>
<td>16.26</td>
<td>12.4</td>
<td>-</td>
<td>0.001</td>
<td>33.83</td>
<td>1.60</td>
<td>-</td>
<td>52.26</td>
</tr>
<tr>
<td>F</td>
<td>4.14</td>
<td>17.47</td>
<td>8.3</td>
<td>-</td>
<td>0.001</td>
<td>16.0</td>
<td>33.10</td>
<td>6.37</td>
<td>45.40</td>
</tr>
<tr>
<td>G</td>
<td>3.82</td>
<td>17.75</td>
<td>3.4</td>
<td>0.4</td>
<td>0.001</td>
<td>3.8</td>
<td>22.90</td>
<td>0.35</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>4.30</td>
<td>12.25</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>38.7</td>
<td>29.46</td>
<td>1.64</td>
<td>-</td>
</tr>
</tbody>
</table>

4.2.2 Sample preparation and allocation

The 96 enamel samples were prepared from freshly extracted bovine incisors. After removing the organic tissue and cleaning the teeth, the crowns were separated from the roots by sectioning the teeth at the cementum-enamel junction with a water-cooled diamond disc [mat. 15]. The pulp tissue was then removed with endodontic files and the crowns were stored in 0.5% thymol solution until required.

Enamel cylinders (3 mm in diameter) were drilled out from the labial surface of the crowns using a trephine mill [mat. 16]. The enamel cylinders were then placed centrally in sample moulds (6 mm in diameter) with the labial surface downward and embedded in acrylic resin [mat. 17]. After curing of the acrylic resin by means of a heat-pressure polymerization system [mat. 18], the samples were removed from the moulds and milled [mat. 16] from the dentin side to reach the same height (approx. 2 mm). The enamel surfaces of the samples were then ground flat and polished with water-cooled carbide-rundum discs (1200, 2400, 4000 grit) [mat. 19]. In order to determine reproducibility of profilometric assessment, two parallel scratched line marks (3.5 mm distance) were placed with a custom-made device on the surface of the acrylic resin, near to the enamel border. Following this, the samples were allocated to the eight groups (A-H, n = 12; see chapter 4.1) and stored for a few weeks in water.
4.2.3 Erosion procedure and analysis of enamel wear

Prior to commencing the erosion procedure, the samples were ultrasonically cleaned [mat. 20] from possible impurities caused during the storage. From each sample five baseline profiles were then recorded with a stylus profilometer [mat. 21] with a distance of 250 µm between each profile. The samples were correct positioned, if the scratched line marks were placed perpendicular to the direction of the profilometer traces; to ensure an exact repositioning of the samples, profilometer and samples were equipped with a jig. The stylus profilometer used had a reproducibility of 120 nm and a lowest measurement limit of 0.105 µm, as determined in a previous study (ATTIN et al. 2009)

Once the baseline profiles were recorded, the acrylic resin and a small area of the enamel surface were covered with adhesive tape [mat. 22] following the direction of the scratched line marks, so that only a central area of the samples was exposed. The coverage permitted to avoid any alterations of the sample surfaces under the adhesive tape during the following erosive procedure. In this manner, helpful references for the profilometric analysis of enamel wear could be obtained.

The overall erosion procedure consisted in 20 erosion cycles (40 min). In each cycle the samples were immersed in the respective solution (see chapter 4.1) for 120 s. The sample immersion occurred just after the complete dissolution of the effervescent tablet and the solution was kept in constant motion by means of a magnetic stirrer [mat. 23].

After performing 10 cycles of erosion, the adhesive tape was removed and five new profiles from each sample were recorded. After this determination, the samples were again covered with adhesive tape, as previously described, and another 10 erosion cycles were performed. Afterwards, the tape was removed and five profiles from each sample were additionally recorded.

By exact overlap of the unchanged sample surfaces (non-eroded parts), the enamel wear (eroded area) was calculated with a specially designed software program [mat. 24], comparing the baseline profiles with the respective profiles after 10 and 20 erosion cycles.
4.3 Statistical analysis

The enamel wear data were encoded into a Microsoft Excel [mat. 25] file. For each sample the mean enamel wear of the five profiles after 10 and 20 erosion cycles was calculated. If the mean enamel wear per sample was below the detection limit of the profilometer (0.105 µm), the value for this sample was set as 0 µm. The statistical analysis was then performed using the software program IBM® SPSS® Statistics Version 22 [mat. 26].

Mean, standard deviation (SD), median, interquartile range and 95% confidence interval were calculated.

As the data were not normally distributed according to the Kolmogorov-Smirnov and Shapiro-Wilk tests, the non-parametrical Kruskal-Wallis and Mann-Whitney tests were used to disclose differences between the enamel wear in the different groups at 10 and 20 cycles of erosion. As 28 tests have been applied on the data at the respective time points (10 and 20 cycles), the Bonferroni correction was applied and resulted in a p-value of p < 0.00179 for those tests.

To compare the enamel wear at 10 and 20 cycles within the same group, the Wilcoxon test was used and level of significance set at p < 0.05.
5 Results

Figure 1 shows the enamel wear (mean ± SD) after 10 and 20 cycles of erosion for the eight tested groups (A-H; for more details about the groups see chapter 4).

![Figure 1. Mean enamel wear and SD (µm) in the different groups after 10 and 20 cycles of erosion. Values for 10 cycles that are not statistically significantly different are marked with same capital letters (A-E). Values for 20 cycles that are not statistically significant different are marked with lower case letters (a-e). Groups marked with “ns” show no statistically significant difference between their values after 10 and 20 cycles of erosion.](image-url)
After 10 cycles of erosion, the highest enamel wear was observed for the groups G (1.763 ± 0.368 µm) and H (1.823 ± 0.269 µm). The enamel wear in all other groups (A-F) was significantly lower compared with the enamel wear in these groups (p < 0.00179 respectively). No significantly higher enamel wear after 10 cycles of erosion was observed for the groups B, C and D compared with the water control group A (p = 0.31858, 0.51372 and 0.06882 respectively). The group F, if compared with the water control group A, presented a significantly higher enamel wear after 10 cycles of erosion (p < 0.00179). However, if compared with the groups B and D, the group F showed no significantly higher enamel wear after 10 cycles of erosion (p = 0.00451 and 0.05950 respectively). Significant gain after 10 cycles of erosion was observed for the group E compared with the water control group A (p < 0.00179).

After 20 cycles of erosion, when comparing with the respective values after 10 cycles, the groups D, F, G and H presented a significant increase of enamel wear (p < 0.05 respectively), while the groups C and E presented a significant decrease of enamel wear (p < 0.05 respectively). Only the groups A and B showed no significant difference of enamel wear after 20 cycles of erosion, when comparing with the respective values for 10 cycles (p = 0.317 and 0.080 respectively).

After 20 cycles of erosion, the highest enamel wear was again detected in the groups G (5.256 ± 0.755 µm) and H (5.121 ± 0.671 µm). All other groups (A-F) presented significantly lower enamel wear (p < 0.00179 respectively). The second highest enamel wear was observed in the groups D (0.234 ± 0.062 µm) and F (0.627 ± 0.703 µm). The enamel wear in all other groups, except G and H, was significantly lower compared with the enamel wear in these groups (p < 0.00179 respectively). No significantly higher enamel wear after 20 cycles of erosion was observed for the groups B and C compared with the water control group A (p = 0.11350 and p = 0.01727 respectively). Significant gain after 20 cycles of erosion was again observed for the group E compared with the water control group A (p < 0.00179).
6 Discussion

6.1 Materials and methods

In the present study, the samples were prepared from bovine enamel. Because of different genetic, environment and diet, bovine and human enamel are not identical (LAURANCE-YOUNG et al. 2011). However, the studies using bovine enamel for dental erosion experiments are numerous and the reasons are various. First of all, bovine teeth compared with human teeth are easier to obtain in large quantities, in better condition and with a more uniform composition (YASSEN et al. 2011). Bovine teeth often stem from cattle from the same region with similar environmental and nutrition factors (WEGEHAUPT & ATTIN 2010). Furthermore, they do not have caries lesions, other defects or a history of fluoridation measures that might influence the outcomes of dental erosion (WEGEHAUPT & ATTIN 2010, YASSEN et al. 2011). Another reason for the use of bovine teeth is their larger size, which on one side makes the handling easier and on the other allows the preparation of more samples from the same tooth, resulting in a reduction of differences between the teeth and also in more comparable samples and groups (WEGEHAUPT & ATTIN 2010, LAURANCE-YOUNG et al. 2011).

As in several other studies evaluating the erosive potential of acidic substances, the enamel wear was measured by use of surface or, more precisely, contact profilometry. This method is simple and fast to perform, has been thoroughly validated and is therefore addressed as “the gold standard” (HALL et al. 1997, BARBOUR & REES 2004, GANSS et al. 2005, SCHLUETER et al. 2005). However, contact profilometry has the disadvantage that the stylus can penetrate the eroded enamel surface and, consequently, can cause surface damages and lead to an overestimation of early erosion depth (SCHLUETER et al. 2011).

In order to standardize the samples by providing the test surfaces with uniform composition and erosion pattern, the samples were flattened and polished (MELLBERG 1992). Therefore it has to be considered that polished enamel surfaces are more susceptible to acid dissolution than natural enamel surfaces (GANSS et al. 2000).
The exposure time of the samples in the solutions during a single erosive cycle has been set to 120 s, as in a previous study (WEGHAUPT et al. 2011) and as recommended by Wiegand and Attin (WIEGAND & ATTIN 2011). This duration seems to be representative for a rapid consumption of an acidic beverage (MEURMAN et al. 1987).

Last but not least, it has to be kept in mind that the chosen in-vitro-model did not completely reflect the intra-oral situation, where numerous other factors, such as saliva, acquired pellicle, abrasive attacks, etc., influence the erosive enamel wear (for more details about the multifactorial condition of dental erosion see chapter 3.2).

6.2 Results

Both the null hypotheses of this study have to be rejected. The results of this study suggest that effervescent tablets, depending on their chemical composition, can cause more, less or no enamel wear. Within the tested solutions there were in fact groups that showed high enamel wear (groups G and H), low enamel wear (groups D and F), no significant enamel wear (groups A-C) and even “enamel increase” (group E).

Comparing the results with the chemical characteristics of the solutions (see Table 1 in chapter 4.2.1), the pH seems to be not the only reason for the different erosive potentials of the tested effervescent tablets. All the solutions of the tested effervescent tablets had a similar pH (in the range from 3.82 to 4.30), which was much lower than the pH of the water control group (8.20). However, only the groups G and H after 10 and 20 cycles of erosion and the groups D and F after 20 cycles of erosion showed significantly higher enamel wear compared with water. The groups B and C presented instead no significant difference respect to water.

The factor that seems to influence the erosive potential of the investigated effervescent tablets mostly is their calcium concentration. It could be observed that effervescent tablets with a low pH, but with high calcium concentration (groups B-F) induced less enamel wear than effervescent tablets with the same pH, but with lower calcium concentration (group G) or without calcium (group H). This was particularly evident by comparing the group B with the group H. While the group B, that had a low pH (4.10) and a high calcium concentration (33.3 mM), showed no significant difference if compared with water, the group H, which had a higher pH (4.30) but no calcium, presented one of the highest enamel wears. In other words, calcium seems to have an anti-erosive potential.
These findings correlate well with the literature. Various studies demonstrate in fact that the addition of calcium to acidic beverages can decrease their erosive potential (ATTIN et al. 2003, ATTIN et al. 2005, HARA & ZERO 2008, WEGEHAUPT et al. 2011). Furthermore, foods with a natural high calcium content, such yoghurt, show a low or no erosive potential despite their low pH (LUSSI et al. 2004, LUSSI et al. 2012).

Based on the results of this study, especially individuals at high risk for dental erosion and those with active erosion should prefer vitamin and mineral supplements in a non-effervescent tablet form or at least avoid effervescent tablets without or with less calcium. If not possible, by the intake of effervescent tablets (as well as other foods and drinks) with a high erosive potential, some preventive measures should be taken into consideration. It is for example advisable, not to hold and swish the erosive solution in the mouth and to avoid tooth brushing immediately after the intake. Neutralization of the low intra-oral pH with fluoride-/calcium-/phosphate-containing products, sugar-free chewing gums or water might also be recommended (WIEGAND et al. 2008, LUSSI et al. 2009).

However, further studies with conditions that better represent the intra-oral and clinical situation are necessary to determine which role effervescent tablets really play in the dental erosion.

6.3 Conclusions

In conclusion, this study has shown that, depending on their chemical characteristics and more particularly on their calcium-content, effervescent tablets can be more or less erosive with respect to dental enamel.
7 References


LUSSI A, SCHLUETER N, RAKHMATULLINA E & GANSS C: Dental erosion--an overview with emphasis on chemical and histopathological aspects. Caries Res 45(suppl 1): 2–12 (2011)


8 Appendix

8.1 Material list

mat. 1: Calcium + Vitamin D, Qualité & Prix, orange flavour, made in Germany for Coop, Basel, Switzerland, L41
mat. 2: Calcium + D3, well & active, lemon/lime flavour, made in Germany for Aldi Suisse AG, Schwarzenbach, Switzerland, L2305/5
mat. 3: Calcium + Vitamin D3, Optisana, orange flavour, Krüger GmbH & Co. KG, Bergisch Gladbach for Lidl Schweiz, Weinfelden, Switzerland, L2300638
mat. 4: Actilife All in One, orange flavour, made in Switzerland for Migros-Genossenschafts-Bund, Zurich, Switzerland, CH.04710463
mat. 5: Berocca® calcium, magnesium + zinc, orange flavour, Bayer AG, Zürich, Switzerland, L7N687
mat. 6: Isostar® fast hydration powertabs, lemon flavour, distributed under the authority of nutrition et santé S.A.S., Revel, France by Wander AG, Neuenegg, Switzerland, L2300535
mat. 7: Magnesium + Vitamin, Qualité & Prix, lemon flavour, made in Germany for Coop, Basel, Switzerland, L26
mat. 8: Mettler AT261 Delta Range, Mettler-Toledo GmbH, Greifensee, Switzerland
mat. 9: Titroprocessor 686, Metrohm swiss made, Herisau, Switzerland
mat. 10: Citric acid Test kit, cat. no. 10139076035, Roche Diagnostics GmbH, Mannheim, Germany
mat. 11: L-Malic acid Test kit, cat. no. 10139068035, Roche Diagnostics GmbH, Mannheim, Germany
mat. 12: L-Ascorbic acid Test kit, cat. no. 10409677035, Roche Diagnostics GmbH, Mannheim, Germany
mat. 13: CarboQC, Anton Paar® GmbH, Graz, Austria
mat. 14: ContrAA® 300, Analytik Jena AG, Jena, Germany
mat. 15: IsoMet® Low Speed Saw with diamond wafering blade, Buehler GmbH, Düsseldorf, Germany
mat. 16: Mill/drill system, PROXXON GmbH, Föhren Germany
mat. 17: Paladur, Heraeus Kulzer, Hanau, Germany
mat. 18: Ivomat IP3, Ivoclar Vivadent AG, Schaan, Lichtenstein
mat. 19: Water Proof Silicon Carbide Paper, Struers, Erkrath, Germany
mat. 20: Ultrasonic cleaner, Bransonic® 220, Branson Ultrasonics SA, Geneve, Switzerland
mat. 21: Perhometer Concept, Mahr, Göttingen, Germany
mat. 22: Tesa®, Beiersdorf, Hamburg, Germany
mat. 23: IKA-Combimag RCT, IKA®-Werke GmbH & Co. KG, Staufen, Germany
mat. 24: 4D Client, custom designed software, University Zurich, Zurich, Switzerland
mat. 25: Microsoft Corp., Redmond, United States
mat. 26: International Business Machines Corp., Armonk, New York, United States
### 8.2 Effervescent tablets – Composition and recommendations of manufactures

<table>
<thead>
<tr>
<th>Product</th>
<th>Composition</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium + Vitamin D Qualité &amp; Prix Orange flavour Made in Germany for Coop, Basel, Switzerland</td>
<td>Acidifiers: citric acid, malic acid; calcium carbonate (500 mg Ca/tablet); sodium bicarbonate (0.10 g Na/tablet); corn starch; beetroot juice concentrate; sweeteners: sodium cyclamate, saccharin; flavourings; colourings: riboflavin; vitamin D (5 µg/tablet); maltodextrin.</td>
<td>1 tablet/day, to dissolve in one glass of water.</td>
</tr>
<tr>
<td>Calcium + D3 well &amp; active Lemon/lime flavour Made in Germany for Aldi Suisse AG, Schwarzenbach, Switzerland</td>
<td>Acidifiers: citric acid; calcium carbonate (400 mg Ca/tablet); acidity regulator: sodium bicarbonate; inulin; sweeteners: sodium cyclamate; flavourings: lemon, lime; sweeteners: saccharin-sodium; vitamin D3 (2.5 µg/tablet); colourings: riboflavin.</td>
<td>1 tablet/day, to dissolve in one glass of water (250 ml).</td>
</tr>
<tr>
<td>Calcium + Vitamin D3 Optisana Orange flavour Krüger GmbH &amp; Co. KG, Bergisch Gladbach for Lidl Schweiz, Weinfelden, Switzerland</td>
<td>Acidifiers: citric acid, malic acid; calcium carbonate (500 mg Ca/tablet); acidity regulator: sodium bicarbonate; starch; colouring beetroot juice concentrate (maltodextrin, beetroot juice concentrate); sweeteners: sodium cyclamate; natural flavourings; sweeteners: saccharin-sodium; colourings: riboflavin-5'-phosphate; vitamin D3 (5 µg/tablet)</td>
<td>1 tablet/day, to dissolve in one glass of water (200 ml).</td>
</tr>
<tr>
<td>Actilife All in One Orange flavour Made in Switzerland for Migros-Genossenschafts-Bund, Zurich, Switzerland</td>
<td>Acidifiers: citric acid; 9 minerals: calcium carbonate (240 mg Ca/tablet), magnesium oxide (115 mg Mg/tablet), iron gluconate (4.2 mg Fe/tablet), sodium selenite (16.5 µg Se/tablet), zinc sulphate (3 mg Zn/tablet), sodium molybdate (15 µg Mo/tablet), manganese-II-sulfate (0.6 mg Mn/tablet), cupric sulphate (0.3 mg Cu/tablet), chromium-III-chloride (12 µg Cr/tablet); acidity regulator: sodium carbonate; sorbitol; 12 vitamins: C (80 mg/tablet), niacin (16 mg/tablet), E (12 mg/tablet), pantothenic acid (6 mg/tablet), B6 (1.4 mg/tablet), B2 (1.4 mg/tablet), B1 (1.1 mg/tablet), A (400 µg/tablet), folic acid (200 µg/tablet), biotin (50 µg/tablet), D (2.5 µg/tablet), B12 (2.5 µg/tablet); flavourings; defoamers: E 1521, E 473; sweeteners: aspartame, acesulfame K, mannitol; release agents: E 551; colourings: beta-carotene.</td>
<td>1 tablet/day, to dissolve in one glass of water (200 ml). Children: ½ tablet.</td>
</tr>
<tr>
<td>Product</td>
<td>Active Ingredients</td>
<td>Dosage and Administration</td>
</tr>
<tr>
<td>---------</td>
<td>-------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Berocca® calcium, magnesium + zinc Orange flavour Bayer AG, Zürich, Switzerland</td>
<td>Active ingredients: vitamin B1 (15 mg/tablet), vitamin B2 (15 mg/tablet), vitamin B6 (10 mg/tablet), vitamin B12 (10 µg/tablet), vitamin C (500 mg/tablet), biotin (150 µg/tablet), folic acid (400 µg/tablet), nicotinamide (50 mg/tablet), pantothenic acid (23 mg/tablet), calcium (100 mg/tablet), magnesium (100 mg/tablet), zinc (10 mg/tablet). Excipients: flavourings; sweeteners: aspartame; sodium chloride (0.04 g/tablet) and other excipients.</td>
<td>Adults and children older than 12 years: 1 tablet/day, to dissolve in one glass of water.</td>
</tr>
<tr>
<td>Isostar® fast hydration powertabs Lemon flavour Distributed under the authority of nutrition et santé S.A.S., Revel, France by Wander AG, Neuenegg, Switzerland</td>
<td>Dextrose; Acidifiers: citric acid; sodium bicarbonate; starch; maltodextrin; natural lemon flavouring and other natural flavourings; potassium carbonate (48 mg K/tablet); magnesium carbonate (17 mg Mg/tablet); calcium carbonate; sodium chloride (for a total of 0.17 g Na/tablet); calcium phosphate (for a total of 44.4 mg Ca/tablet; sweeteners: aspartame; vitamin C (12 mg/tablet); vitamin E (1.4 mg/tablet); vitamin B1 (0.12 mg/tablet); colourings: beta-carotene.</td>
<td>Dissolve 2 tablets in 500 ml of water for a portion. To eat while warming up (150 ml every 15 min) and regularly during and after exercise (up to 2 hours after exercise) (150 ml every 15 min).</td>
</tr>
<tr>
<td>Magnesium + Vitamin C Qualité &amp; Prix Lemon flavour Made in Germany for Coop, Basel, Switzerland</td>
<td>Acidifiers: citric acid; magnesium carbonate (300 mg Ca/tablet); sodium bicarbonate (0.20 g Na/tablet); maltodextrin; vitamin C (80 mg/tablet); corn starch; flavourings; sweeteners: sodium cyclamate, saccharin; colourings: riboflavin.</td>
<td>1 tablet/day, to dissolve in one glass of water.</td>
</tr>
</tbody>
</table>
9 Curriculum Vitae

Name, Vorname (n): Lunghi, Nancy

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I would like to express my deep gratitude to my supervisors, Prof. Dr. Thomas Attin and Dr. Florian Wegehaupt, for their professional guidance, support, encouragement and useful critiques of this research work.

I would also like to thank Ms Beatrice Sener, Ms Claudia Cucuzza-Honti and Ms Priska Irenen-Weber, who helped me determinate the chemical characteristics of the effervescent tablets, and Mr Klaus Becker and Ms My-Lien Lai for their help and support during the sample preparation and the profilometric analysis. My grateful thanks are also extended to Dr. Malgorzata Roos for her advice and assistance in doing the statistical analysis of the data.

Last but not least, I wish to thank my family and my friends for their support and encouragement throughout my study and my life.


11 Declaration

Masterarbeit

Ich erkläre ausdrücklich, dass es sich bei der von mir im Rahmen des Studiengangs Master of Dental Medicine eingereichten schriftlichen Arbeit mit dem Titel

In-vitro evaluation of erosive potential of vitamin/mineral effervescent tablets commercially available in Switzerland

um eine von mir selbst und ohne unerlaubte Beihilfe sowie in eigenen Worten verfasste Masterarbeit* handelt.

Ich bestätige überdies, dass die Arbeit als Ganzes oder in Teilen weder bereits einmal zur Abgeltung anderer Studienleistungen an der Universität Zürich oder an einer anderen Universität oder Ausbildungseinrichtung eingereicht worden ist.

Verwendung von Quellen

Ich erkläre ausdrücklich, dass ich sämtliche in der oben genannten Arbeit enthaltenen Bezüge auf fremde Quellen (einschliesslich Tabellen, Grafiken u. Ä.) als solche kenntlich gemacht habe. Insbesondere bestätige ich, dass ich ausnahmslos und nach bestem Wissen sowohl bei wörtlich übernommenen Aussagen (Zitaten) als auch bei in eigenen Worten wiedergegebenen Aussagen anderer Autorinnen oder Autoren (Paraphrasen) die Urheberschaft angegeben habe.

Sanktionen

Ich nehme zur Kenntnis, dass Arbeiten, welche die Grundsätze der Selbstständigkeitserklärung verletzen – insbesondere solche, die Zitate oder Paraphrasen ohne Herkunftsangaben enthalten –, als Plagiat betrachtet werden und die entsprechenden rechtlichen und disziplinarischen Konsequenzen nach sich ziehen können (gemäß §§ 7ff der Disziplinarordnung der Universität Zürich sowie §§ 51ff der Rahmenverordnung für das Studium in den Bachelor- und Master-Studiengängen an der Medizinischen Fakultät der Universität Zürich

Ich bestätige mit meiner Unterschrift die Richtigkeit dieser Angaben.

Datum: 10.10.2014

Name: Lunghi Vorname: Nancy

Unterschrift:.................................

* Falls die Masterarbeit eine Publikation enthält, bei der ich Koautor/-in bin, wird meine eigene Arbeitsleistung im Begleittext detailliert und strukturiert beschrieben.