Influence of Beverage Composition on the Results of Erosive Potential Measurement by Different Measurement Techniques

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Key Words
Acidic beverages · Calcium analysis · Erosion · Inorganic phosphorus analysis · Mineral composition · Optical profilometry

Abstract
The influence of beverage composition on the measurement of erosive potential is unclear. The aim of this study was to evaluate whether beverage composition influences the measurement of erosive potential and to evaluate the influence of exposure in small and large volumes. Eleven beverages were included: water (control), 3 alcopops, 2 beers and 5 soft drinks. For each beverage 15 bovine enamel samples were used: 5 for chemical and 10 for profilometric analysis. After exposure to the beverages (63 min) the resulting solutions were analyzed for Ca and inorganic phosphorus (P_i) content. The samples for optical profilometry were submerged sequentially in 500 ml or in 1 ml of the drinks for 3, 6, 9, 15 and 30 min (total 63 min). For some of the beverages high baseline concentrations of Ca (energy drink) or P_i (cola drink, cola lemon drink, beer, beer lemon) were found. Some of the beverages showed a good correlation between the chemical methods. Profilometry (both for 1 and 500 ml) showed generally lower enamel losses than the chemical methods. Lower enamel losses were found for the profilometry 1 ml compared to the profilometry 500 ml only for the cola drinks. It can be concluded that the composition of the beverages had a significant effect on the determination of the erosive potential with chemical analyses. Drink composition also influenced the effect of small versus large exposure volumes, indicating the need for standardization of exposure parameters.

Dental erosion is defined as an irreversible loss of dental hard tissue due to a chemical process without involvement of microorganisms [Imfeld, 1996]. Dental erosion may be caused by either extrinsic or intrinsic factors. One of the extrinsic causes of dental erosion is excessive consumption of acidic beverages [Dugmore and Rock, 2004]. Different techniques are available to assess the erosive potential of acidic beverages. Frequently used techniques include profilometry and chemical analysis.

Calcium determination and inorganic phosphorus (P_i) determination are used to measure the loss of minerals from the enamel and are well established and accurate [Barbour and Rees, 2004]. Small concentrations of ions released from the enamel can be measured, so it is possible to observe the initial stages of erosion and to use small volumes of the examined solutions. It also allows the use of natural tooth surfaces since polishing is not needed [Barbour and Rees, 2004]. However, chemical methods provide only quantitative and not morphological or mechanical data [Grenby, 1996].

For measuring the loss of surface layers the method of choice is optical or contact profilometry. In optical pro-
filometry there is no physical contact between the probe and the surface, so no damage will occur by scratching of the softened surface [Barbour and Rees, 2004]. A drawback of profilometric techniques is that enamel losses below 2 μm are difficult to measure.

Little is known about the correlation between different methods and the lack of a ‘gold standard’ is a shortcoming in the field of erosion research. Moreover, the influence of the composition of the beverages on the measurements is unclear. In earlier studies comparing methods for measuring the erosive potential of beverages, standard solutions such as citric acid or lactate buffer were used [Ganss et al., 2005; Zero et al., 1990]. As a result, the influence of the composition of beverages on the results obtained by the different methods could not be determined. We hypothesized that the chemical composition of soft drinks and the volume used influence the determination of erosive potential.

The aim of this study was to evaluate whether beverage composition influences the measurement of erosive potential and to evaluate the influence of exposure in small and large volumes.

Materials and Methods

Preparation of Samples

A total of 165 buccal surfaces of extracted bovine incisors, stored in water, were ground flat with water-cooled silicon carbide 220 grit grinding discs (SIA siawat P220, Frauenfeld, Switzerland) and cut into blocks of approximately 5 × 3 mm using a vertical sawing machine with a diamond saw blade (11-4243, Buehler, Düsseldorf, Germany). The blocks were embedded in acrylic resin (Autoplast polymer, Candulor AG, Wangen, Switzerland) and cut into blocks of approximately 5 × 3 mm in the center of the enamel sample.

Beverages

Eleven beverages, all available in the Netherlands, were included in this study (table 1). Immediately after opening the bottles, and also after degassing (the drinks were placed on a shaking table set at 200 rpm until no bubbles were visible), the pH was measured 5 times using a calibrated glass pH electrode (Radiometer, PHM 84 Research Meter, G202C, Copenhagen, Denmark) in 100 ml of the beverages. Room temperature in the laboratory was 21°C with a possible variation of ±2°C. Standard buffers, pH 7.01 and 4.00 (20°C), were used (measurement uncertainty for both ±0.015 units) (Merck KGaA, Darmstadt, Germany). Calibration was performed with these buffers every day.

Demineralization Procedures

Before starting the demineralization procedure the samples for chemical analysis were submersed for 3 min in a standard solution of 50 mM citric acid, 0.4 mM KH2PO4, 0.4 mM CaCl2

Table 1. Beverages used in this study, with details of their composition

<table>
<thead>
<tr>
<th>Beverage Type</th>
<th>Producer</th>
<th>pH</th>
<th>Ca, mmol/l</th>
<th>Pi, mmol/l</th>
<th>DS (HA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cola drink</td>
<td>Coca Cola</td>
<td>2.47</td>
<td>0.87 ± 0.04</td>
<td>4.76 ± 0.15</td>
<td>0.005</td>
</tr>
<tr>
<td>Cola lemon drink</td>
<td>Coca Cola</td>
<td>2.73</td>
<td>0.73 ± 0.01</td>
<td>4.90 ± 0.06</td>
<td>0.008</td>
</tr>
<tr>
<td>Orange drink</td>
<td>Fanta orange</td>
<td>3.03</td>
<td>0.06 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Fruit drink</td>
<td>Dubbel friss orange/pink grapefruit</td>
<td>3.35</td>
<td>1.50 ± 0.03</td>
<td>0.51 ± 0.03</td>
<td>0.018</td>
</tr>
<tr>
<td>Vodka alcopop</td>
<td>Smirnoff Ice</td>
<td>3.43</td>
<td>0.15 ± 0.005</td>
<td>0.004 ± 0.001 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Energy drink</td>
<td>Red Bull</td>
<td>3.43</td>
<td>2.40 ± 0.21</td>
<td>0.01 ± 0.02 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Ice tea</td>
<td>Lipton Ice tea</td>
<td>3.80</td>
<td>0.12 ± 0.01</td>
<td>0.25 ± 0.004 0.009</td>
<td></td>
</tr>
<tr>
<td>Beer lemon</td>
<td>Grolsch beer lemon</td>
<td>3.83</td>
<td>0.96 ± 0.02</td>
<td>3.51 ± 0.08 0.068</td>
<td></td>
</tr>
<tr>
<td>Rum lime alcopop</td>
<td>Breezer Lime</td>
<td>3.87</td>
<td>0.17 ± 0.01</td>
<td>0.02 ± 0.001 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Beer</td>
<td>Bavaria beer</td>
<td>4.20</td>
<td>0.72 ± 0.02</td>
<td>5.30 ± 0.14 0.125</td>
<td></td>
</tr>
<tr>
<td>Bottled water</td>
<td>Sourcy bottled water</td>
<td>8.09</td>
<td>1.20 ± 0.03</td>
<td>n.m. -</td>
<td></td>
</tr>
</tbody>
</table>

DS = Degree of saturation; HA = hydroxyapatite; n.m. = not measured.
and 1 mM NaNO₃ (pH = 3) to remove the smear layer from the polished surfaces and subsequently rinsed with tap water. All the beverages were decarbonated.

For the chemical analyses each of the 5 enamel samples was submerged in 1 ml of each beverage in a test tube for 3 min under constant agitation on a shaking table at 100 rpm. After 3 min the samples were lifted from the beverages and the enamel surface was rinsed with 3 ml of demineralized water which was collected in the test tube. From the resulting mixtures 1 ml was used for Ca analysis and 1 ml for P concentration analysis. The same procedure was repeated for exposures of 6, 9, 15 and 30 min (total 63 min). The exposures for the different times were made sequentially on the same specimens.

For the profilometric analysis each of the 5 enamel samples was submerged in 1 ml of each beverage in a test tube for 3, 6, 9, 15 and 30 min under constant agitation on a shaking table (100 rpm). The pH of these solutions was measured after each exposure. Another set of 5 samples was submerged in 500 ml of each beverage for 63 min under constant agitation on a shaking table (100 rpm) in beakers with a diameter of 9.5 cm. All experiments were performed at room temperature (21 ± 2°C).

Chemical Analysis

P concentration in the beverages was determined using a phosphomolybdate spectrophotometric method [Chen et al., 1956]. The concentration of Ca in the beverages was determined by atomic absorption spectroscopy (PerkinElmer Analytical Instruments, Shelton, Conn., USA) [Vieira et al., 2005]. This was performed in the presence of lanthanum (0.326%) in order to suppress phosphate interference. An air/C₂H₂ flame and a wavelength of 422.7 nm were used.

For the chemical analyses all the beverages had to be diluted with demineralized water. For P analysis most of the beverages were diluted 16 times in total. The beverages with high P concentrations (the colas and the beers) had to be diluted 80 times. For Ca analysis all the beverages were diluted 18.4 times.

The Ca and P losses from the enamel samples were determined by subtracting the Ca or P content of the beverages before the enamel exposure (average of 10 measurements) from the total Ca or P content of the solution after exposure. In addition, the ratio of the Ca dissolved to the P dissolved (ΔCa/ΔP) was calculated for each exposure time.

Lesion depth was calculated from the Ca and P loss using the average Ca and P content per unit volume for bovine enamel and the exposed enamel area [Dijkman et al., 1983]. A Ca concentration in enamel of 25.1%, a P concentration in enamel of 17.61% were assumed and an average enamel density of 2.93 g/cm³ was assumed. This resulted in two depth parameters: d(Ca) and d(P), lesion depth estimated from Ca loss or P loss, respectively. The estimated erosion depth (μm) of the 5 samples was averaged.

Profilometric Analysis

Erosion depth was measured using an optical profilometer (Proscan 2000, Scantron, Taunton, England). Before inclusion of the enamel samples in the experiment, baseline measurements were performed on each sample in order to confirm the flatness of the polished enamel surfaces.

After the demineralization procedure the PVC tape was removed. The samples were scanned over the reference and eroded surfaces. The volume lost due to erosion was calculated with the equipment’s software. Erosion depth (μm) was calculated by dividing the volume loss by the exposed enamel area of the scanned surface. The erosion depths of the 5 samples were averaged. The profilometry resulted in further depth parameters: d(profil1) and d(profil500).

pH Changes and Degree of Saturation

The pH of the solutions after each exposure in the profilometry (1-ml) group was measured. The baseline degree of saturation of the beverage with regard to hydroxyapatite and dicalcium phosphate dihydrate (DCPD) was calculated by means of a computer program [Shellis, 1988], using the baseline Ca and P concentrations of the beverages, together with the pH measured after degassing. To determine the possible influence of saturation of the beverages on the measurement results during the erosion process, the Ca and P concentrations and pH after the 30-min incubation were used to calculate the change in degree of saturation with regard to hydroxyapatite and DCPD after the erosion regime.

Statistical Analysis

For investigation of the relationship between the change in Ca and P concentrations, linear least squares regression was performed. The P concentration was the independent (x) variable. A one-way ANOVA followed by a Bonferroni post-hoc test in SPSS 12.01 (SPSS, Chicago, Ill., USA) was used to test differences between the cumulative erosive depths at 63 min obtained by the chemical methods [average of d(Ca) and d(P): d(CaP), d(prof1) and the d(prof500)]. The significance level for all tests was set at 0.05.

Results

The pH of the beverages ranged from 2.4 (cola) to 8.1 (bottled water) (table 1). Table 1 also shows the baseline Ca and P concentrations and table 2 shows the changes in Ca and P concentrations for all erosion times and all drinks. P concentration ranged from not detectable (bottled water) to 5.3 mmol/l (beer). Baseline Ca concentration ranged from 0.06 mmol/l (orange soft drink) to 1.3 mmol/l (fruit drink). For most of the drinks the ΔCa/ΔP ratio did not differ significantly from 1.6 except for some of the low exposure times (3 and 6 min), and for the cola drink, orange soft drink, and the ice tea. In table 3, the parameters for the linear least squares regression analysis of the Ca and the P concentrations for all beverages are presented. In most cases a high linear correlation (r² > 0.8) was found, except for the beers (r² = 0.07 and r² = 0.19), cola drink (r² = 0.76), energy drink (r² = 0.63) and cola lemon drink (r² = 0.53). For this reason and because of the problems measuring the P concentration in drinks with a high baseline P concentration, d(Ca) of the beers, cola drink and cola lemon was used for the comparison with profilometry. In table 4 and in figure 1, the cumula-
Table 2. Changes in calcium (Ca) and inorganic phosphorus (Pi) concentrations (mmol/l) of drinks after each exposure, together with the ratio of the changes in Ca and Pi (ΔCa/ΔPi)

<table>
<thead>
<tr>
<th></th>
<th>3 min</th>
<th>6 min</th>
<th>9 min</th>
<th>15 min</th>
<th>30 min</th>
<th>DS (HA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>Pi</td>
<td>Ca/Pi</td>
<td>Ca</td>
<td>Pi</td>
<td>Ca/Pi</td>
</tr>
<tr>
<td>Cola drink</td>
<td>0.15 (0.02)</td>
<td>0.15 (0.05)</td>
<td>1.16 (0.36)</td>
<td>0.26 (0.02)</td>
<td>0.26 (0.09)</td>
<td>1.22 (0.84)</td>
</tr>
<tr>
<td>Cola lemon drink</td>
<td>0.17 (0.03)</td>
<td>0.26 (0.09)</td>
<td>0.71 (0.15)</td>
<td>0.25 (0.10)</td>
<td>0.25 (0.15)</td>
<td>1.81 (0.85)</td>
</tr>
<tr>
<td>Orange</td>
<td>0.13 (0.03)</td>
<td>0.05 (0.15)</td>
<td>2.47 (0.32)</td>
<td>0.14 (0.15)</td>
<td>0.10 (0.15)</td>
<td>1.37 (1.85)</td>
</tr>
<tr>
<td>Soft drink</td>
<td>0.02 (0.01)</td>
<td>0.05 (0.06)</td>
<td>0.16 (0.15)</td>
<td>0.03 (0.02)</td>
<td>0.02 (0.02)</td>
<td>0.15 (0.15)</td>
</tr>
<tr>
<td>Fruit drink</td>
<td>0.17 (0.04)</td>
<td>0.07 (0.03)</td>
<td>2.67 (0.39)</td>
<td>0.29 (0.05)</td>
<td>0.15 (0.03)</td>
<td>2.06 (0.66)</td>
</tr>
<tr>
<td>Vodka alcopop</td>
<td>0.13 (0.05)</td>
<td>0.06 (0.02)</td>
<td>2.14 (0.05)</td>
<td>0.21 (0.01)</td>
<td>0.11 (0.01)</td>
<td>1.97 (0.17)</td>
</tr>
<tr>
<td>Energy drink</td>
<td>n.m. n.m. n.m.</td>
<td>0.05 (0.01)</td>
<td>0.97 (0.01)</td>
<td>0.10 (0.10)</td>
<td>1.25 (1.25)</td>
<td>0.11 (0.23)</td>
</tr>
<tr>
<td>Ice tea</td>
<td>0.11 (0.01)</td>
<td>0.04 (0.01)</td>
<td>2.48 (0.23)</td>
<td>0.15 (0.01)</td>
<td>0.06 (0.01)</td>
<td>2.34 (0.29)</td>
</tr>
<tr>
<td>Beer</td>
<td>0.09 (0.04)</td>
<td>0.02 (0.10)</td>
<td>1.75 (4.12)</td>
<td>0.07 (0.04)</td>
<td>0.14 (0.22)</td>
<td>2.48 (4.23)</td>
</tr>
<tr>
<td>Lemon</td>
<td>0.13 (0.04)</td>
<td>0.05 (0.10)</td>
<td>2.78 (4.12)</td>
<td>0.17 (0.04)</td>
<td>0.07 (0.22)</td>
<td>2.53 (4.23)</td>
</tr>
<tr>
<td>Rum lime alcopop</td>
<td>0.03 (0.02)</td>
<td>0.12 (0.02)</td>
<td>2.42 (0.41)</td>
<td>0.06 (0.02)</td>
<td>0.03 (0.02)</td>
<td>3.33 (0.41)</td>
</tr>
<tr>
<td>Beer</td>
<td>0.10 (0.03)</td>
<td>0.09 (0.07)</td>
<td>4.12 (7.07)</td>
<td>0.09 (0.01)</td>
<td>0.08 (0.13)</td>
<td>1.26 (2.42)</td>
</tr>
<tr>
<td>Bottled water</td>
<td>-0.02 (0.01)</td>
<td>n.m. n.m.</td>
<td>-0.01 n.m.</td>
<td>-0.03 n.m.</td>
<td>-0.01 n.m.</td>
<td>-0.02 n.m.</td>
</tr>
</tbody>
</table>

Means with SD in parentheses. The degree of saturation (DS) with respect to hydroxyapatite (HA) after the final 30-min exposure is also given. n.m. = Not measured.

tive results of Ca, P, and the profilometric analyses are presented. The highest enamel loss was found for cola lemon drink in the d(prof500) group (13.54 μm). The drinks concentrating in the middle part of the graph (dashed lines) showed lower erosive potential for profilometry compared to the chemical analysis. The two colas showed lower erosive potential in the d(prof1) group compared to the d(prof500) group and higher erosive potential for the d(Pi) compared to the d(Ca). In figure 1, also the rank order in which the different methods placed the drinks can be assessed. For the beverages the influence of the measurement method on its rank order in erosiveness (1 is lowest, 11 is highest erosion) is marked, e.g., the orange soft drink is the 7th most erosive drink in d(Ca) but the 4th most erosive with d(Pi); similarly, ice tea is 8th with d(Ca) and 4th with d(prof500). One-way ANOVA showed a significant effect of measuring technique (p < 0.05) for all beverages except ice tea and the fruit drink. d(CaP) showed an enamel loss that was significantly higher (p < 0.05) than that of d(prof1) for the rum lime alcopop (p < 0.0001), energy drink (p = 0.007), vodka alcopop (p = 0.034), beer (p < 0.0001, d(Ca) only) and orange soft drink (p = 0.0001). When compared to

Table 3. Parameters for the linear least squares regressions of Ca concentration on Pi concentration for all beverages

<table>
<thead>
<tr>
<th></th>
<th>Slope</th>
<th>y intercept</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cola drink</td>
<td>0.47</td>
<td>0.14</td>
<td>0.75</td>
</tr>
<tr>
<td>Cola drink lemon</td>
<td>0.75</td>
<td>0.17</td>
<td>0.53</td>
</tr>
<tr>
<td>Orange soft drink</td>
<td>0.76</td>
<td>0.07</td>
<td>0.98</td>
</tr>
<tr>
<td>Fruit drink</td>
<td>1.69</td>
<td>0.06</td>
<td>0.91</td>
</tr>
<tr>
<td>Vodka alcopop</td>
<td>1.45</td>
<td>0.05</td>
<td>0.99</td>
</tr>
<tr>
<td>Energy drink</td>
<td>2.17</td>
<td>0.14</td>
<td>0.86</td>
</tr>
<tr>
<td>Ice tea</td>
<td>1.07</td>
<td>0.08</td>
<td>0.80</td>
</tr>
<tr>
<td>Beer lemon</td>
<td>0.10</td>
<td>0.44</td>
<td>0.19</td>
</tr>
<tr>
<td>Rum lime alcopop</td>
<td>1.49</td>
<td>0.06</td>
<td>0.97</td>
</tr>
<tr>
<td>Beer</td>
<td>0.05</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>Bottled water</td>
<td>6.94</td>
<td>0.09</td>
<td>0.18</td>
</tr>
</tbody>
</table>

The Pi concentration was the independent (x) variable.
0.001) and orange soft drink (p = 0.001). The d(prof1)
showed a significantly lower enamel loss than the
d(prof500) only for the cola drink (p = 0.002) and the
cola lemon drink (p = 0.003).

The results obtained for the pH measurements after
each exposure in the 1-ml profilometry showed very little
change in pH (~0.02 to +0.03) after the erosion process
for most beverages. Only the cola and the cola lemon
showed a small rise in pH (0.1) after 30 min. None of the
beverages was supersaturated with respect to hydroxy-
apatite or DCPD after a 30-min erosive exposure in 1 ml
of the samples (table 2). The highest degree of saturation
for hydroxyapatite was found for the beers. The highest
rise in the degree of saturation after 30 min was found for
the energy drink.

Discussion

In this study bovine enamel was used. Most in vitro
studies use bovine enamel since it is considered a suitable
substitute for human enamel [Zero, 1996]. Although
Meurman and Frank [1991] did not observe any differ-
ence in the progression of erosion or the surface ultra-
structure of erosive lesions between bovine and human
prismatic enamel, another study showed that morpholog-
ical differences such as a higher porosity exist when com-
pared to human enamel, which results in higher rates of
artificial caries lesion formation [Featherstone and Mell-
berg, 1981]. It should be considered that in this study a
comparison between the methods was performed and not
an extrapolation of the results to the clinical situation.

The P_i analysis of the beers and the Ca analysis of the
energy drink yielded negative values. For these beverages
the differences between the Ca analysis and the P_i analy-
sis and the negative values may possibly be explained by
their chemical composition. Because of the high baseline
concentrations of Ca and P_i it was sometimes necessary
to use high dilutions which may have increased the mea-
surement error. A high concentration of P_i in the colas
and the beers could also have interfered with the Ca mea-
surements by calcium binding, but this was prevented by
adding a high concentration of lanthanum. However, the
amount of Ca and P_i released from the enamel into solu-
tion by erosion was for some beverages relatively small
compared to the baseline concentration of Ca or P_i in
the beverages, especially for the short exposure times. This
resulted in small changes in mineral concentration, which
did not always exceed the measurement error and made
it difficult to obtain reliable measurements.

Previous studies, using standard erosive solutions
such as citric acid or lactate buffer reported almost per-
fect agreement between the Ca and P_i analyses [Ganss et
al., 2005; Zero et al., 1990]. Because of the use of standard
solutions in these studies the influence of the composi-
tion of the erosive solution on the results could not be
determined. In the present study, for three of the drinks
a Ca/P ratio (table 2) with significant deviations from 1.6
(the Ca/P ratio of bovine enamel) was found. This sug-
gests that reprecipitation occurred during the erosive
exposure. However, in none of the drinks could a super-
saturation with respect to other calcium phosphates be
calculated. No explanation could be found for the phe-
nomenon which, especially for the orange drink, does

| Table 4. Cumulative loss of enamel after 63-min total exposure to the beverages |
|---------------------------------|----------------|----------------|----------------|----------------|
|  | d(Ca) | d(P_i) | d(prof1) | d(prof500) |
| Cola drink | 4.44 ± 0.22 | 9.22 ± 1.25 | 2.08 ± 0.58 | 8.04 ± 3.62 |
| Cola lemon drink | 6.72 ± 0.36 | 8.97 ± 1.75 | 6.42 ± 1.15 | 13.54 ± 4.31 |
| Orange soft drink | 3.64 ± 0.22 | 5.50 ± 0.38 | 2.29 ± 0.88 | 2.37 ± 0.51 |
| Fruit drink | 6.55 ± 0.53 | 5.67 ± 0.72 | 4.24 ± 2.53 | 3.27 ± 1.17 |
| Vodka alcopop | 5.00 ± 0.85 | 4.94 ± 0.88 | 2.98 ± 1.06 | 2.69 ± 1.35 |
| Energy drink | 4.25 ± 0.96 | 4.84 ± 0.18 | 2.34 ± 0.85 | 2.69 ± 0.96 |
| Ice tea | 3.23 ± 0.48 | 3.11 ± 0.33 | 1.80 ± 1.34 | 3.08 ± 0.63 |
| Beer lemon | 1.99 ± 0.32 | –1.79 ± 2.02 | 1.12 ± 0.98 | 0.00 |
| Rum lime alcopop | 4.09 ± 0.31 | 3.43 ± 0.29 | 0.84 ± 0.70 | 1.47 ± 0.72 |
| Beer | 1.30 ± 0.23 | –0.38 ± 1.16 | 0.00 | 0.00 |
| Bottled water | –0.33 ± 0.92 | 0.01 ± 0.01 | 0.00 | 0.00 |

Means with SD.
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 seem to be systematic, looking at the decline of the Ca/P ratio with increasing exposure time. The short exposures and the drinks with low erosive potential were found to be irrelevant for the calculation of the Ca/P ratio because of the low enamel losses.

Profilometric analysis showed a trend for lower enamel loss compared to Ca and P_i analysis. These results are in agreement with the findings of a previous study where Ca/P_i analysis and contact profilometry were compared [Ganss et al., 2005]. The difference found between the chemical methods and the profilometry may be explained by the fact that the erosion process does not only remove enamel layers but also causes a 'softening'. Profilometry does not account for the subsurface loss of the softened layer. The depth of the softened layer is unknown but may be more than 10 μm [Eisenburger et al., 2004].

Removal of reaction products and the supply of fresh acid are important for the continued formation of erosion lesions [Eisenburger and Addy, 2003]. In a study investigating the relationship between enamel erosion and liquid flow rate, it was concluded that the rate of erosion is dependent on liquid velocity, exposure time and the total volume of the acidic solution [Shellis et al., 2005]. In this study agitation of 1 ml probably results in a different replacement of liquid at the enamel surface compared to agitation of a 500-ml reservoir, thus influencing the erosion rate. However, regarding the exposure volume of the beverage, our study found significant differences between the measured erosive potential only for the two cola drinks.

Some authors have observed that a small change in the degree of saturation resulted in a difference in the dissolution of enamel and that it is an important parameter that defines the ability of a solution to demineralize enamel [Barbour et al., 2003; Finke et al., 2000; Margolis et al., 1999; Tanaka and Kadoma, 2000]. Only in a small volume of the beverages would a significant rise in saturation be expected. To ascertain whether this was the case in the present study, the saturation with respect to hydroxyapatite was determined for all 1-ml volumes before and after exposure. It was found that the degree of saturation rose with increasing exposure time. However, this was seen in all beverages and the highest rise was found for the energy drink. This did not explain our findings as only the cola drinks showed a lower erosion in the 1-ml exposure.

Although pH is a parameter in the calculation of the degree of saturation, it has been reported as a separate factor in erosive potential [Larsen and Nyvad, 1999; Margolis et al., 1999]. A rise in pH would result in a slowing

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**Fig. 1.** Cumulative loss of enamel as measured by the four techniques, showing both the quantitative loss and the rank order for each beverage. To facilitate comparison between the techniques for each beverage, the points have been connected. The plot area is divided into three areas: little or no erosion (–2 to 2 μm), moderate erosion (2–6 μm), and severe erosion (6–14 μm).
down of the erosion process. Only for the cola drink and the cola lemon drink a measurable, if low (0.1), rise in pH was found. As this corresponds to the observed reduced erosion for the two cola drinks in the 1-ml exposure model, we assume that pH was a determining factor.

In conclusion, the present study has shown that the composition of the beverages had a significant effect on the determination of the erosive potential with chemical analyses. This should be considered when choosing an appropriate measurement method. Optical profilometry is suggested as a beverage-independent alternative. Beverage composition also influences the effect of small versus large exposure volumes, indicating the need for standardization of exposure parameters such as exposure times, volumes and flow rate of the drinks during exposure to prevent differences in erosion rate due to differences in liquid velocities.

References