

# Calcium phosphate influences erosivity of non alcoholic beverages in vitro

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## Research article

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# Abstract

**Background:** The effect of added calcium phosphate to non alcoholic beverages to reduce erosivity on bovine enamel and dentine was analyzed.

**Methods:** 90 enamel and 90 dentine specimens from freshly extracted bovine incisors were randomly attributed to 9 enamel and 9 dentine groups (n=10 each). Initially, all specimens were weighted using a precision balance. They were suspended in plastic bottles containing 150ml of the following beverages: Cola, orange juice, Red Bull, Bonaqua Fruits. To all beverages 100mg (c1) or 200mg (c2) calcium hydrogen phosphate dihydrate was added. Tap water served as control. After 7 days, median mass loss was calculated in mg. Kruskal-Wallis and Mann-Whitney u-test served for comparison between groups.

**Results:** Cola and Bonaqua Fruits lost erosivity already after addition of 100mg calcium phosphate. Red Bull after 100mg on enamel and 200mg on dentine. Only orange juice remained erosive on dentine and enamel even after the addition of 200mg calcium phosphate.

**Conclusion:** The addition of calcium phosphate may reduce or even prevent the erosivity of some highly erosive non alcoholic beverages on bovine enamel and dentine.

## Background

Dental erosion is a continuous loss of dental hard tissues, i.e. of enamel and dentine, caused by intrinsic (gastric) or extrinsic (dietary) acids. Due to the natural intake of acidic food and beverages, erosion may be regarded as a physiological and age dependent process [1]. It is considered as pathological at the latest when teeth are worn down and functionality is impaired [2], but it may also be detected in earlier stages by patients and dentists when the appearance of the teeth is altered [1]. In contrast to the physiological status of erosion, no age dependence is found for the pathological form [1]. Erosion of teeth is classified as a disease (DA08.12) in the WHO ICD11-classification[3]. Prevalence of tooth erosions is increasing within populations [4]. For Germany, the National Oral Health Survey from 2016 (DMS V) showed a prevalence of 44.8% for 35 to 44-year-olds [5]. The precursor study (DMS IV) which was published ten years before in 2006, showed a prevalence of 16.9% only for the same age group [6]. It must be considered that the diagnostic procedure has changed between the two studies since the BEWE (Basic Erosive Wear Examination) index, which was first published in 2008 by Bartlett et al., was introduced in the DMS V [7]. It can be speculated that the use of the BEWE-index may lead to the documentation of an elevated prevalence since it registers already an "initial loss of surface texture". Another potential bias could be the influence of toothbrushing abrasion since the authors of the DMS V stated, that it could not be differentiated between pure erosions and hybrid forms, e.g. erosions combined with abrasions following oral hygiene procedures [5]. However, the tooth brushing behavior, which was also evaluated in DMS IV and V did not change in the discussed age group between 2006 and 2016 [5]. In summary, there is evidence that the prevalence of dental erosions has considerably increased in Germany within the last decade.

Since pathological forms of erosions are not age depending [1], another independent variable may be the reason for severe levels of erosive breakdown. This could be the increasing consumption of acidic drinks which is observed in developed countries [8] and has been particularly demonstrated for Germany [9]. In several studies, soft drinks have been found to be risk indicators for dental erosions [10–12]. In a former study, we examined erosivity of different common soft drinks on enamel and dentin in vitro. Weight loss of enamel and dentine samples was determined before and after exposure to the acidic drinks [13]. Sprite, Apple Juice, Red Bull, orange juice and fruityfied mineral water showed highly erosive action as opposed to tap water which served as control. To reduce the erosivity of soft drinks and other acidic beverages, it may be reasonable to modify these drinks by adding calcium phosphate.

Therefore, the aim of the present study was to analyze the effect of this added calcium phosphate on the erosivity of modified beverages on bovine dentine and enamel.

## Materials And Methods

The methodological procedure was already described earlier [13]. All animal material (teeth) used in this study originated from the slaughterhouse Schlachthof Bochum GmbH, Freudenbergstr. 45K, 44809 Bochum - Hamme / Germany. All cattle were slaughtered for meat production and not for research purposes.

The freshly extracted incisors were ground down to 90 dentine disks and 90 enamel discs of 1 mm height and 5 mm diameter. Specimens were randomly attributed to 9 enamel and 9 dentine groups (n = 10 each). Before weighing by use of a precision balance (Sartorius BP61S, Göttingen, Germany, metering accuracy 0.1 mg), all discs were dried on blotting-paper at room temperature for one hour. The mean initial masses (SD) were 24.5 mg (2.1) for dentine and 34.5 mg (3.2) for enamel discs. Within the dentine and the enamel groups no statistically significant differences were seen for initial masses ( $p > 0.05$ , ANOVA with Bonferroni post hoc test). All specimens of one group were then placed in a plastic basket which was suspended in a plastic box containing 150 ml of the following fluids: Coca-Cola (Coca-Cola Erfrischungsgetränke AG, Germany), Red Bull (Red Bull GmbH, Germany), orange juice Direktsaft (real,- Handels GmbH), Bonaqua Fruits (Mango-Acai) (Coca-Cola Erfrischungsgetränke AG, Germany), tap water (Chlorine free, disinfected with UV-irradiation, public water supply, Witten/Germany, fluoride concentration  $< 0.25$  ppm). None of the commercial products contained any preservatives. Tap water served as a negative control. For every fluid and every material (enamel and dentine), two baskets were set up: 100 mg of calcium hydrogen phosphate dihydrate (C1) was added to the first ones, 200 mg (C2) to the second ones. The fluids were continuously ventilated by an aquarium pump at 37° C for 7 days.

The air inlet was at the bottom of the boxes to ensure an adequate air supply from the ground to the surface of the fluids. The fluids were exchanged daily. After seven days, specimens were removed from the fluids, rinsed for 30 seconds with saline solution and again dried for one hour on blotting-paper at room temperature to ensure the same conditions as at the beginning of the experiment. Finally, specimens were weighed again and mass loss was calculated in mg. PH values of all liquids were

monitored during the whole experiment to ensure stable conditions (Beckmann Coulter GmbH Krefeld, Germany, Serial# 0217107). Figure 1 shows the flow diagram of the study design. Since Kolmogorov Smirnov test did not consistently show normal distribution, median mass losses were calculated. Kruskal-Wallis and Mann-Whitney u-test were used for comparison between groups.

## Results

Table 1 shows the median (min/max) mass losses of enamel and dentine after seven days. As reference, medians from the initial study which was performed using the same methodological procedure are given [13].

Table 1

|   | Hard tissue loss in mg (median, min./max.) |                              |
|---|--|------------------------------|
|   | Enamel <sup>1</sup>                        | Dentine <sup>2</sup>         |
| Coca Cola C1  | 0.25 (-0,10/1.30)                          | 0.55 (0.00/5.50)             |
| Coca Cola C2  | 0.20 (-0,60/1.10)                          | -0.65 (-1.20/0.40)c          |
| Coca Cola R   | 7.45 (6.30/8.50)                           | 6.45 (5.80/7.70)             |
| Orange juice C1   | 2.35 (0.70/3.10)a                          | 12.30 (7.80/17.30)a          |
| Orange juice C2   | 0.75 (0.10/2.00)                           | 5.30 (0.50/10.30)d           |
| Orange juice R  | 23.85 (19.10/34.10)                        | 19.40 (14.70/26.80)          |
| Red Bull C1   | 0.80 (0.20/2.00)                           | 10.55 (7.70/13.50)b          |
| Red Bull C2   | 0.80 (0.20/1.50)                           | 1.35 (-5.20/4.10)e           |
| Red Bull R  | 16.05 (13.40/22.50)                        | 16.85 (14.40/20.50)          |
| Bonaqua Fruits C1   | -0.60 (-1.30/0.20)b                        | 1.20 (-0.90/1.60)            |
| Bonaqua Fruits C2   | -0.05 (-0.80/0.60)c                        | 0.45 (-2.00/2.40)            |
| Bonaqua Fruits R  | 17.95 (15.80/21.30)                        | 16.60 (8.30/21.00)           |
| Tap water   | 0.55 (0.10/0.90)a, b, c                    | 1.65 (1.2/2.40)a, b, c, d, e |
| <sup>1</sup> Enamel: Only values with the same letters are significantly different against water, orange juice_c1 and Bonaqua_c1 at p < 0.001 and Bonaqua_c2 at p < 0.01. |  |                              |
| <sup>2</sup> Dentine: Only values with the same letters are significantly different against water at p < 0.01.  |  |                              |

Initial ph was 2.47 for Coca Cola, 2.59 for orange juice, 2.68 for Red Bull, 3.38 for Bonaqua Fruits, and 7.40 for tap water. On enamel, Bonaqua Fruits\_c1 (p < 0.001) and \_c2 (p < 0.01) were significantly less erosive than tap water whereas orange juice\_c1 was significantly more erosive (p < 0.001) than water. On

dentine, Cola\_c2 was significantly less erosive than tap water, whereas orange juice\_c1 and c2 and Red Bull\_c1 and c2 were significantly more erosive ( $p < 0.01$ ).

## Discussion

In an earlier study, common soft drinks were evaluated for erosivity using the same method as in the present study. It was demonstrated that the gravimetric method was appropriate to detect differences in erosivity [13]. Soft drinks, which had shown high erosivity, were included in the present trial. This was done with the purpose to find a possibility to reduce the erosivity of such drinks and consequently to reduce tooth erosion. It was not the aim of the study to generate data that can directly be transferred to the in-vivo-situation, but they should allow a ranking for erosivity of common soft drinks in relation to the non-erosive tap water. To realize this aim, the tooth specimens were subjected to highly erosive conditions.

Calcium hydrogen phosphate dihydrate (DCPD, Brushit,  $\text{Ca}[\text{PO}_3(\text{OH})]\cdot 2\text{H}_2\text{O}$ ) was chosen as an agent because it is soluble at low pH-values of 4 and under and provides calcium- and phosphate-ions which are able to constrain the solubility of hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$ ), the anorganic component of dental hard tissue. Concentrations of 100 or 200 mg/150 ml calcium phosphate were chosen to ensure effectiveness and safety for consumers. According to the Scientific committee of the European Food Safety Authority (EFSA) a group acceptable daily intake (ADI) for phosphates expressed as phosphorus is 40 mg/kg body weight per day: This ADI is regarded as protective for the human population. [14]

That means that for DCPD the ADI is 222 mg/kg bodyweight. In the present study, already 100 mg/150 ml resulted in a considerably reduced erosivity of the soft drinks. If assuming that 1,500 ml soft drink consumption/day can be seen as an upper limit when no abuse is present, this would result in a daily intake of 1,000 mg DCPD/per day. The ADI for DCPD for an adult with 75 kg bodyweight would be 16,650 mg ( $75 \times 222$  mg).

In comparison to the data from our earlier study [13], the erosivity of Cola was reduced by 97% for enamel (from 7.45 mg to 0.25 mg) and by 92% (from 6.45 mg to 0.55 mg) for dentine when 100 mg DCPD/150 ml were added. The respective values for orange juice were 90% (enamel, from 23.85 mg to 2.35 mg) and 37% (dentine, from 19.40 mg to 12.30 mg), for Red Bull 95% (enamel, from 16.05 mg to 0.80 mg) and 38% (dentine, from 16.85 mg to 10.55 mg), and for Bonaqua Fruits 100% (enamel, from 17.95 mg to -0.60 mg) and 93% (dentine, from 16.60 mg to 1.20 mg). For Cola and Bonaqua Fruits, non-erosivity on enamel and dentine could be reached by the addition of 100 mg/150 DCPD when compared to tap water. For Red Bull, this could be reached when 200 mg DCPD/150 ml were added. Even with this addition, orange juice remained erosive on dentine, but not on enamel. However, the erosivity on dentine was reduced by 73% (from 19.4 mg to 5.30 mg) in this case.

DCPD may not be the only calcium phosphate which is able to reduce erosivity when added to soft drinks. Barbosa et al. could abolish the erosivity of Coca-Cola on bovine enamel by adding 5 mM calcium

glycerophosphate (CaGP). This corresponds to 157,60 mg CaGP/150 ml and to 23,24 mg phosphorus/150 ml. In the present study, the addition of 100 mg DCPD including 18 mg phosphorus/150 ml resulted in a non-erosivity for Coca Cola. This seems to show a slight advantage for DCPD. However, the method to detect erosivity was different and therefore the results may not be comparable [15].

Jensdottir et al. added 8 g calcium tri-phosphate to 1 l pure orange juice. As a result, the critical pH for demineralization was reduced from 5.27 to 4.24. The 8 g calcium tri-phosphate corresponds to 315.6 mg phosphorus/150 ml. Coming back to the assumed 1,500 ml soft drink consumption per day, the resulting 3,156 mg phosphorus still seem tolerable.

Another research group tested metallic ions as additives to soft drinks to prevent erosions. [16, 17] They found that ferrous sulfate (human teeth, in situ) as well as combinations of copper, magnesium, manganese, or zinc (bovine tooth powder, in vitro) also show some potential to reduce the erosivity on enamel and dentine. A 7–27% reduction of the dissolution capacity on enamel of two soft drinks was found. [16, 17]

To stop the problem of erosion, several authors looked into application of agents with remineralizing potential after substance loss had already occurred. [18, 19] [20] Bertoldi et al. applied casein phosphopeptide-amorphous calcium phosphate for seven days to eroded extracted teeth and detected partial remineralization. However, the agent was applied for seven days and no further exposure to the erosive agents took place during this time, which is not what happens in real life with its continuous and intermittent exposure to the acidic beverages. [19] Lechner et al. studied remineralization after erosion by beverages with a 90 min exposure to a paste containing calcium and phosphate ions. The results were also an indicator for possible remineralization, however, it might not be practicable on a routine basis, i.e. to alternate drinking and remineralization with every intake of an erosive agent. [20] In summary the attempts to rebuilt enamel or dentine after erosion are inconclusive and it is not clear whether they are of clinical relevance. From a preventive viewpoint, it would be preferable to add a substance to the beverages which avoids erosion in the first place. Therefore, the aim of the present study was to determine whether an addition of calcium phosphate to the erosive beverages would lead to less loss of hard tissue.

To analyze the erosivity, specimens from bovine teeth were used in the present study. The reason, why bovine teeth were used instead of human once, was discussed earlier [13]. It has been shown for erosion studies, that bovine teeth are an equivalent substitute to human teeth [21]. The specimens of the present study had the same diameter of 5 mm and thickness of 1 mm and therefore the same surface (55.0 mm<sup>2</sup>) resulting in the same exposure to the tested liquids. This makes the results comparable to the results from the earlier study [13].

This study wanted to examine, whether an addition of calcium phosphate to erosive drinks would prohibit erosion in teeth. This could be confirmed under the conditions of this experiment.

# Conclusions

Erosivity of common non-alcoholic drinks as determined in vitro by substance loss of bovine enamel and dentine varies widely. In conclusion, the addition of calcium phosphate may reduce or even prevent the erosivity of some highly erosive non alcoholic beverages on bovine enamel and dentine.

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## Declarations

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## Data availability statement

The dataset supporting the conclusions of this article is included within the article and its additional file.

## **Authors' Contributions**

SZ was developing the study protocol and wrote the main manuscript text

NB was performing the laboratory tests (dentine specimens)

BT was performing the laboratory tests (enamel specimens)

MB was supervising the laboratory work and was responsible for quality assurance

CBZ was responsible for statistical analysis and wrote the main manuscript text

All authors reviewed the manuscript

## **Competing interests**

None of the authors declares any conflict of interest.

## **Figures**

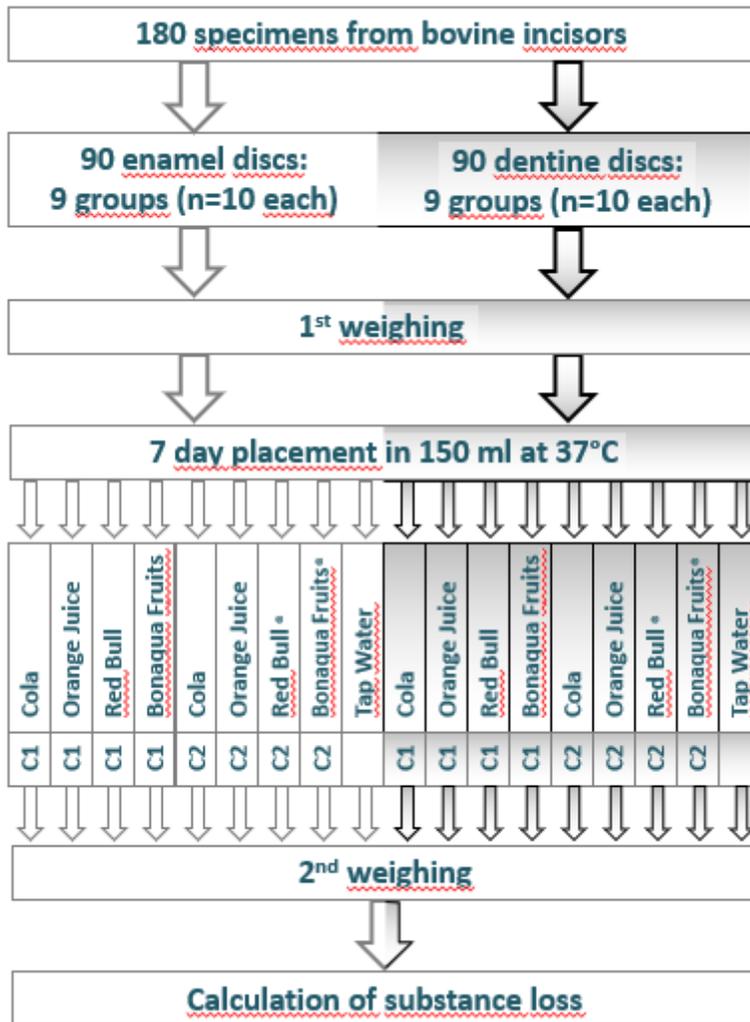


Figure 1

Flow diagram of the study design

## Supplementary Files

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