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Erosive potential of calcium-supplemented citric acid on bovine enamel

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ABSTRACT

Purpose: This in vitro study aimed to evaluate the effect of calcium supplementation on erosive potential of citric acid.

Design/methodology/approach: One houndred bovine enamel specimens were prepared and randomly distributed into the five groups (n=20): Group I (control): 1% pure citric acid solution, Group II: with 1 mM calcium (as calcium lactate pentahydrate), Group III: with 2.5 mM calcium, Group IV: with 5 mM calcium; Group V: with 10 mM calcium. One half of the enamel specimens from each group (n=10) were submitted to a short-term erosion-remineralization (E-R) cycling model (five 1-min erosion challenges in-between six 10-min remineralization periods in artificial saliva) and percentage microhardness change (%SMHC) was evaluated. The other half of the specimens were subjected to long-term erosion-remineralization cycling (six cycles: 5 min in acid solution - 60 min in artificial saliva, repeated for 5 days) and enamel loss was determined by contact profilometry. In chemical analysis of the experimental drinks, the pH value, titratable acidity, and buffer capacity (β) were assessed.

Findings: One percent citric acid supplemented with 2.5 mM, 5.0 mM and 10 mM calcium produced less enamel softening and enamel loss than control group. A trend for less pronounced erosion with an increasing calcium concentration (2.5 mM - 5.0 mM -10 mM) was observed after short-term E-R cycling. Although a similar tendency could be observed after long-term E-R cycling, differences in enamel loss between 2.5 mM, 5.0 mM and 10 mM are not significant. Similar erosive behaviour was observed for 1 mM calcium and control group after both E-R cycling models.

Research limitations/implications: Calcium-containing beverages may be considered as one of the possible approaches to prevention of dental erosion, and may be advised for high-risk individuals who cannot reduce their dietary acidic intake.

Originality/value: This in vitro study imitated erosive challenge in the oral cavity and provide a new approach for prevention of dental erosive wear.

Keywords: Dental Erosion; Tooth; Enamel; Citric acid; Calcium; Microhardness, Profilometry

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PROPERTIES

1. Introduction

There is a large body of evidence, based on the results of growing number of *in vitro* and *in situ* studies, that the excessive consumption of acidic drinks and foodstuffs poses a risk to the dentition. The acid-induced chemical dissolution of mineral from tooth surface is defined as dental erosion, although it could be more appropriately termed 'dental corrosion' or 'biocorrosion' [1], in analogy to metallic corrosion [2]. Unlike in the caries process, where the destruction of hard dental tissues is caused by acids produced by bacteria in dental plaque, dental erosion is due to acids of non-microbiological origin. The eroded dental enamel is softened at the surface and is consequently more prone to removal due to abrasion (process involving foreign objects or substances repeatedly introduced in the mouth and contacting the teeth) and attrition (friction by opposing teeth with no foreign substance intervening) [3]. Erosive wear might lead to the exposure of the dentine, causing a dentine hypersensitivity, and even to pulp exposure in the most severe cases.

Dental erosive potential is a measure of detrimental influence exerted by acidic substances on mineralized dental hard tissues.

A wide range of acids are involved in the process of dental erosion. They may be extrinsic or intrinsic. The intrinsic causes include recurrent vomiting as part of the eating disorders (anorexia or bulimia nervosa) or due to the regurgitation of the gastric contents (gastro-oesophageal reflux) [4]. Extrinsic erosion is caused by low-pH beverages (like fruit juices, carbonated soft drinks), foods (any citrus food, tomato ketchup, salad dressings, pickles), medications (aspirin tablets, effervescent vitamin C) and environmental or occupational exposure to acidic agents (e.g. battery factory workers) [5].

Dietary acids are thought to be the main aetiologic agent, since the consumption of soft drinks has increased considerably over the last few decades, and in the United Kingdom has been reported to have reached 235.3 litres per person per year in 2011 [6].

Organic acids are basic ingredients of many beverages. Dominant acids in fruit drinks are citric acid ($C_6H_8O_7$) [E330] and malic acid ($C_4H_6O_5$) [E296]. Ready-made juices contain ~0.3% of citric acid, fresh orange and apple juice ~1%, and its concentration in lemon juice may reach up to 6%.

Currently efforts are being made to reduce a chemical damage of dental hard substance by formulating a novel anti-erosive dentifrices. Preventive measures may also involve reduction or elimination of acidic drink from a diet. However, the success of such prophylaxis strategies is difficult to achieve (particularly in children and adolescents), as it largely depends on the patient's compliance. Therefore, a practical approach of reducing the harmful influence exerted by popular acidic drinks on teeth can be modification of their chemical composition [7].

The erosive potential of a drink is influenced by a number of chemical parameters, including: type of acid (pK_A), pH, titratable/neutralizable acidity, buffering capacity, calcium chelating properties, viscosity, adhesiveness, and concentration of calcium, phosphate and fluoride ions.

Modification of beverages by increasing their pH (> 3.8) and reduction of titratable acidity may result in a considerable reduction of erosive potential. However, such a change is unfavourable for technological (shorter shelf life) and sensory reasons (the drink loses its characteristic pungent, refreshing taste). It should be emphasised that increasing a product's pH even to the value of 7.0 does not make the drink completely safe for teeth, as some acid anions (citrate > lactate > phosphate) maintain the ability to bind calcium [8].

According to the Law of Mass Action, the rate of hydroxyapatite dissolution reaction:

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \leftrightarrow 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O \quad (1)$$

could be slowed down if the solvent contains the products of this reaction: the calcium and phosphate ions (PO_4^{3-}, HPO_4^{2-} or $H_2PO_4^-$, depending on pH value). Indirect evidence supporting this phenomenon is the fact that acidic diary products (such as yoghurt and kefir) with its naturally high calcium and phosphate content, have very small, sometimes undetectable, erosive potential despite the presence of lactic acid (pH ~4.0) [9].

Therefore, the degree of saturation (DS) with respect to dental minerals, hydroxyapatite (DS_{HA}) or fluorapatite (DS_{FHA}) is an important chemical parameter related with the rate of erosion exerted by acidic solution [10, 11]. DS is determined by calcium, phosphate, fluoride concentration and pH of the solution. The concentration gradient of those ions is thought to be the basic thermodynamic driving force for dissolution.

Theoretically, solution saturated or supersaturated with respect to hydroxyapatite ($DS_{HA} \geq 1.0$) is not expected to cause enamel dissolution, however, such modification might result in an unpleasant taste and be dangerous and impossible to apply in the food industry. Barbour et al. found an approximately threshold condition for citric acid (pH 3.3) defined by calcium concentration of 120 m*M*, phosphate concentration of 0.57 m*M* [11]. Despite being highly undersaturated (DS_{HA} ~0.104) the solution shows no significant erosive potential with respect to enamel and using greater concentrations of calcium would not provide any additional benefit. However, when consuming more than 520 ml of such citrus beverage there would be a risk of exceeding the tolerable upper intake level of calcium (2.5 g *per* day), resulting in adverse effects (nausea, vomiting, constipation, suppression of intestinal absorption of other mineral nutrients: zinc, magnesium and phosphates, an increased potential risk of kidney stones or prostate cancer) [12]. Obviously, an addition of calcium can play an important role in the prevention of this nutrient deficiency in the organism, but considering the health safety of consumers, it is desirable to establish an optimal anti-erosive and tolerable level of anti-erosive substances added to beverages. One of the commercially available low-erosive products has an effective high calcium content (~1.6 g/dm^3 = 40 m*M*) [13]. A four times lower concentration of calcium (~0.4 g/dm^3 = 10 m*M*) also proved to significantly reduce the erosive potential of orange juice in the *in situ* and *in vitro* investigations [14, 15], though to a lesser extent [15]. A concentration of 0.04 g/dm^3 (= 1 m*M*) of calcium reduces the erosiveness of 1% citrate acid [16] and popular acidic drinks according to some *in vitro* research [17, 18], whereas according to other investigations this very low concentration is ineffective [19].

In view of the above-mentioned conflicting results, this study aimed to investigate the erosive potential of 1% citric acid solution supplemented with low concentrations of calcium. The null hypothesis was that there is no difference among the various solutions.

2. Investigation methodology

2.1. Material

The dental enamel was prepared from freshly extracted, non-damaged bovine permanent mandibular incisors that were obtained from 3-4-year-old cattle, bred locally for human consumption, after negative bovine spongiform encephalopathy (BSE) test. The teeth were stored in 0.5% aqueous thymol solution (pH 7.0) at 4°C when not in use.

2.2. Specimen preparation

The pulp tissue was removed from the coronal part of the tooth with endodontic files. Rectangular enamel slabs (5 x 5 mm and x 2.5 mm thick) were prepared from labial surfaces of the teeth using low speed water-cooled diamond saw (Minitom, Struers, Copenhagen, Denmark). The specimens were embedded in acrylic resin (DuroFast, Struers) using hot mounting press machine (CitoPress-20, Struers). Then, the enamel surfaces were subjected to wet-grinding with abrasive paper (500-2000 grit, Water Proof Silicon Carbide Grinding Paper, Struers, Erkrath, Germany) and polishing with felt paper wet by diamond suspension (3 µm, 1 µm Diamont Paste, Struers). This procedure was performed with semi-automatic grinding/polishing device (Tegramin-30, Struers), and resulted in removal of approximately 250 µm of the outermost enamel layer as it was measured with micrometer. Finally, the specimens were ultrasonically cleaned for 15 min with distilled water to remove the smear layer. Specimen with visible surface defects, such as cracks, scratches, white spots, were discarded.

Eighty acceptable specimens having a mean Vickers Hardness Number (VHN) above 330 were selected. Prepared slabs were stored in a saturated mineral solution (1.5 m*M* $CaCl_2$, 0.9 m*M* KH_2PO_4, 150 m*M* KCl, 1 m*M* NaN_3, 20 m*M* TRIS, pH 7.0).

2.3. Experimental design

The enamel specimens were randomly allocated to one of 5 experimental groups (n = 20), described in Table 1.

Table 1.
Experimental groups

Group	Solution	Calcium concentration
I (control)	1% citric acid	0 m*M*
II	1% citric acid	1.0 m*M*
III	1% citric acid	2.5 m*M*
IV	1% citric acid	5.0 m*M*
V	1% citric acid	10.0 m*M*

Calcium was added to 1% citric acid solution (Merck, 1002440500) in the form of calcium lactate pentahydrate (POCH, 877050116). The pH of the solutions was adjusted to 3.82 ± 0.02 with 5 m*M* sodium hydroxide.

Half of the specimens (n = 10) from each group was subjected to short-term erosion-remineralisation cycling model (short E-R). The other half was prepared and submitted to long erosion-remineralisation cycling. A study design is shown in Fig. 1. The erosive potential of the experimental solutions, was evaluated by percentage surface microhardness change (%SMHC) in short-term E-R and by profilometry in long-term E-R cycling.

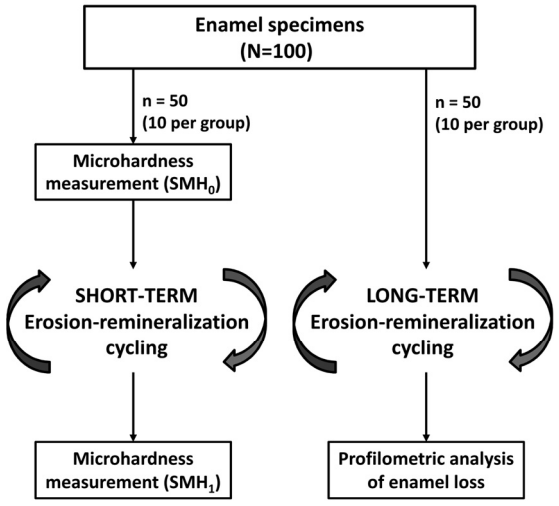

Fig. 1. Experimental design

In short-term E-R model (Fig. 2), enamel specimens, after initial immersion in artificial saliva for 10 min, were subjected to 5 cycles of 1-min exposition in citric acid test solution (10 cm^3/specimen, at 21°C with slow stirring) and 10-min immersion in artificial saliva, which served as remineralizing medium (10 cm^3/specimen, at 35ºC without stirring).

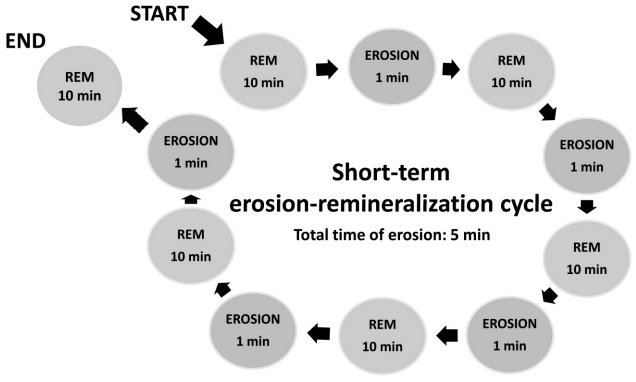

Fig. 2. Short-term erosion-remineralization protocol making a total of 5 min exposure to experimental acid solutions (REM - remineralizing period in artificial saliva)

In long-term E-R model (Fig. 3), enamel specimens, after initial immersion in artificial saliva for 10 min, were subjected to

6 cycles per day, repeated over 5 consecutive days. One cycle consisted of 5-min exposition in citric acid test solution (10 cm^3/specimen, at 21°C with slow stirring) and 60-min immersion in artificial saliva (10 cm^3/specimen, at 35°C without stirring).

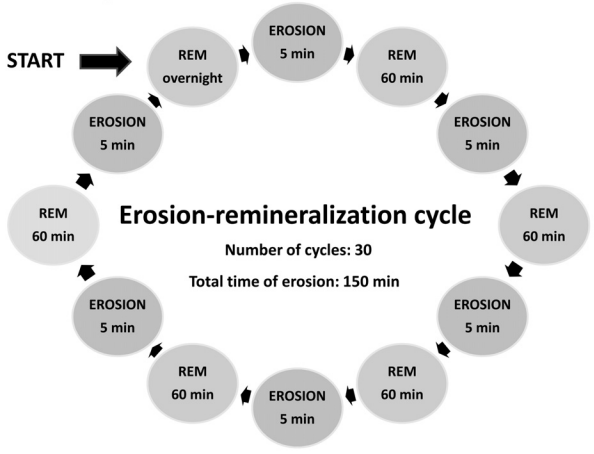

Fig. 3. Long-term erosion-remineralization model, making a total of 150 min exposure to experimental acid solutions

The artificial saliva, used in both cycling protocols, was mixed according to the following formulation: 2.7 g/dm^3 porcine gastric mucin, 0.002 g/dm^3 ascorbic acid, 0.030 g/dm^3 glucose, 0.580 g/dm^3 NaCl, 0.170 g/dm^3 $CaCl_2 \cdot 2H_2O$, 0.160 g/dm^3 NH_4Cl, 1.270 g/dm^3 KCl, 0.160 NaSCN, 0.330 g/dm^3 KH_2PO_4; 0.200 g/dm^3 urea, 0.340 g/dm^3 Na_2HPO_4, pH adjusted to 6.4 by titrating a phosphate buffer to the solution [20]. All solutions were freshly prepared in the morning of each experimental day. During cycling they were not renewed. Before changing solutions, the specimens were washed in deionized water and gently dried with paper towel.

2.4. Surface microhardness measurement

Specimen surface microhardness (SMH) was determined at baseline (SMH_0) and after short E-R cycling (SMH_1) by operator blinded to the group assignment. The indentations were made using a computer-aided FM-700 microhardness tester, coupled to FM-ARS software (Future Tech Corp., Tokyo, Japan). A Vickers diamond was used with a 100-g load and dwell time of 15 s. Five indentations at an interval of 100 μm were made for each specimen and the mean SMH was calculated (Fig. 4). The percentage SMH change (%SMHC) was determined, as follows:

$$\%SMHC = \frac{SMH1 - SMH0}{SMH0} \times 100 \quad (2)$$

2.5. Profilometric measurement

To determine the loss of enamel, prior to the long E-R cycling, one half of enamel specimen was covered by cellulose adhesive tape (Scapa) to obtain a reference area. After cycling, the adhesive tape was carefully removed and speciment were thoroughly washed with acetone solution (pH 7.0) and ultrasonication for 15 min. Enamel loss was quantitatively determined by a contact profilometer (Surftronic 25, Taylor Hobson). The areas protected with the tape during cycling were used as reference areas. Five traces were performed at intervals of about 500 μm on each specimen from the exposed surface to the reference (Fig. 5). The mean depth of the eroded area was calculated in relation to the uneroded surface with specially designed software (TalyProfile Lite, Taylor Hobson).

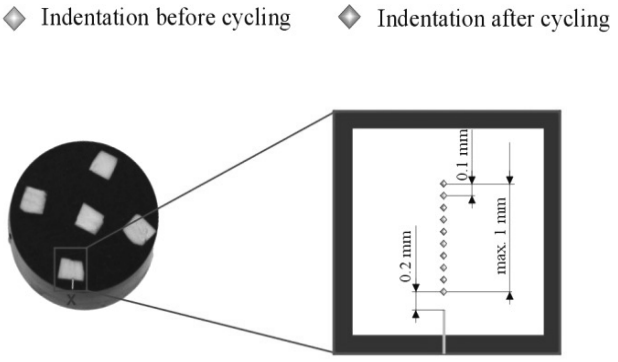

Fig. 4. Diagram of the microhardness indents scheme on the 5 x 5 mm bovine enamel specimens

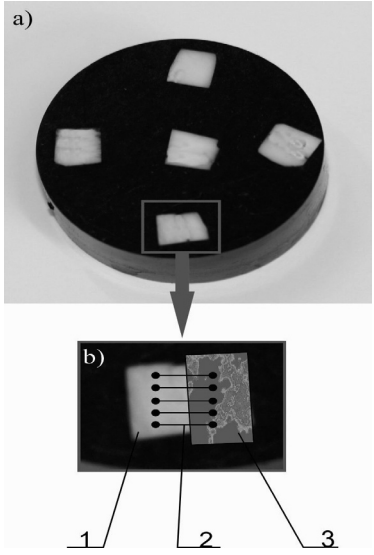

Fig. 5. Scheme of a profilometric tracings (2) on tooth specimen (1). One half of the specimen was covered by adhesive tape (3)

2.6. Chemical analysis of the drinks

Chemical analysis of the solutions were perfomed using a glass electrode (LE409, Mettler Toledo) connected to a standard

pH meter (Five Easy, Mettler Toledo), calibrated with reference buffers of pH 4.00 and pH 7.00, at 21°C. Before measurement, the solutions were mixed thoroughly with a magnetic stirrer for at least 5 min.

The pH of the solutions was adjusted to 3.82 ± 0.02 with 5 m*M* sodium hydroxide. To determine the titratable acidity, 25 ml of each drink were titrated with 0.1 M NaOH in steps of 0.5 mL in 30-s intervals. The amount of base needed to raise the initial pH to 5.5 and 7.00 was measured for each solution under constant stirring at 21°C.

The differential buffer capacity (β) was determined from the slope of a titration curve pH=f(V_{NaOH}) and calculated at the original pH of the tested solutions according to the equation:

$$\beta = \Delta C_B / \Delta(pH) \quad (3)$$

where ΔC_B denotes the amount of base (0.1 M NaOH) added to the drink and ΔpH is the change in the pH of the drink caused by the addition of the base.

2.7. Statistical analysis

The data were tested for normality distribution and homogeneity of variance using the Shapiro-Wilk test and Levene test, respectively. Since the assumptions were satisfied, one-way ANOVA and Tukey's *post hoc* test were performed to check statistically significant differences in %SMHC and enamel loss between the experimental groups. The level of significance was set at $p \leq 0.05$. Statistical analyses were carried out using *Excel 2007* (Microsoft) and *Statistica* software (Statsoft, ver. 8.0).

3. Results

3.1. Chemical analysis

Table 2 compares chemical parameters of experimental solution. They present a comparable $TA_{pH7.0}$. Solutions with calcium show slight higher $TA_{pH5.5}$ and β values than that of pure citric acid solution.

Table 2.
Chemical characteristics of the experimental solutions: mean pH, titratable acidity (TA) to pH 5.5 and 7.0 in mmol OH^-/dm^3, buffer capacity (β)

Group	Calcium	TA pH 5.5	TA pH 7.0	β
I	0 m*M*	35.48	47.39	26.91
II	1.0 m*M*	36.57	47.44	28.96
III	2.5 m*M*	37.20	47.55	30.61
IV	5.0 m*M*	38.74	47.89	31.94
V	10.0 m*M*	39.59	47.95	33.33

3.2. Surface microhardness measurement

A short-term erosion-remineralization cycling resulted in a significant decrease in the enamel surface microhardness in all experimental groups ($p < 0.001$). The %SMHC in the experimental groups after short-term E-R is presented in Table 3. Addition of calcium to the citric acid solution resulted in significantly lower hardness loss compared to the control group with no calcium ($p < 0.05$). There are no significant differences in %SMHC between pure citric acid and solution with 1 mM of calcium ($p = 0.57$).

Table 3.
Mean percent surface microhardness change (%SMHC) with 95% confidence levels after short-term erosion-remineralization cycling

Group	Calcium	%SMHC	95% CI
I	0 m*M*	-13.83 (3.64)^{a}	-11.58 to -16.08
II	1.0 m*M*	-11.79 (3.22)^{a,b}	-8.57 to -15.01
III	2.5 m*M*	-8.49 (2.31)^{c}	-6.18 to -10.80
IV	5.0 m*M*	-6.64 (1.90)^{d}	-5.74 to -8.61
V	10.0 m*M*	-4.20 (1.57)^{e}	-2.83 to -5.57

Figures in parentheses are SD (standard deviation)
Means within columns sharing the same superscript letter do not differ significantly ($p > 0.05$)

3.3. Enamel loss

An 5-day E-R cycling has led to significant enamel loss in all groups ($p < 0.001$). Profilometric measurements of mean enamel loss are presented in Table 4. There are no significant diffences between I (0 m*M*) and II (1.0 m*M*) group ($p = 0.97$). Higer concentration of calcium produce significant less enamel loss ($p < 0.05$). Although a tendency toward lower enamel loss with increasing calcium concentration could be seen, however, differences between 2.5 m*M*, 5.0 m*M* and 10 m*M* are not significant.

Table 4.
Enamel loss (μm) after long-term E-R

Group	Calcium	Enamel loss (μm)	95% CI
I	0 m*M*	8.51 (1.44)^{a}	7.62 to 9.40
II	1.0 m*M*	8.15 (1.63)^{a,b}	8.57 to 9.16
III	2.5 m*M*	6.26 (0.89)^{c}	5.71 to 6.81
IV	5.0 m*M*	5.45 (1.11)^{c,d}	4.76 to 6.14
V	10.0 m*M*	4.24 (1.63)^{d}	3.23 to 5.25

Figures in parentheses are SD (standard deviation)
Means within columns sharing the same superscript letter do not differ significantly ($p > 0.05$)

4. Discussion

The present study intended to evaluate the erosive potential of 1% citric acid solutions with low calcium content on bovine enamel.

Bovine enamel has the advantage that it is easy to obtain in large quantities with good quality (the dental caries in bovine teeth is quite rare). Bovine enamel is thicker and has more uniform chemical composition than that of human teeth, and thus provides a less variable response to erosive agent. Moreover, bovine teeth are easier to prepare due to a large and relatively flat surfaces. On the other hand, bovine enamel is more porous than human enamel and less resistant to acid diffusion, which results in more rapid erosion progression. Therefore, the actual change of surface microhardness might be overestimated and should be interpreted with caution when comparing with human enamel. Nevertheless, in our study bovine enamel specimens were in all experimental groups, thus the above-mentioned phenomenon would affect all the groups. An advantage, however, is that bovine enamel is more susceptible to demineralization than human, and a small erosive changes could be observed between groups.

Citric acid was used because it is dominant acid in fruit beverages and a popular food acidulant. Although citric acid is so-called weak acid, it is very damaging to the tooth. From one molecule of citric acid three hydrogen ions could be obtained. Additionally, the citric anion has the possibility of complexing of calcium cations dissolved from hydroxyapatite [8].

Calcium lactate pentahydrate was chosen, as it is a food-approved substance [E327] and has a good solubility in water. The pH of the examined solutions was adjusted to the same value, so that the results will not depend on hydrogen ions concentration. Specimens were immersed in artificial saliva, because saliva seems to play an important role in reducing the effects of erosive challenges due to its remineralizing and buffering properties as well as the ability to form a protective pellicle layer on dental hard tissues.

Although this *in vitro* study condition excluded many other interactions, e.g. with saliva, it enabled a multigroup comparisons in a highly controlled conditions.

Erosive process occurs in three stages [21]:

1) early demineralization and softening of the tooth tissue without surface loss (nanoscopic changes);
2) microscopic material loss;
3) a clinically visible erosive lesion (macroscopic change).

The erosive potential of modified and unmodified products (as a control group) is most frequently assessed on the basis of enamel erosive lesions in stage 1 and 2. Visual evaluation at stage 3 is very subjective and lacks precision. Hence, the current study employed enamel surface microhardness measurements since this method is appropriate for measurement dental erosion in short-term erosion model [22].

In long-term E-R protocol (lasting for 5 days), irreversible loss of dental hard tissue occurs, hence other methods for assessment should be applied, such as surface profilometry or confocal laser scanning microscopy. On extensively demineralized enamel surface it is not possible to accurately measure microhardness from the indentations.

It has to be emphasised that the most reliable research into the effect of food on teeth are *in situ* investigations, i.e. extraorally assessment of tooth specimens worn in the oral cavity by volunteers on custom-made intra-oral appliances, orthodontic brackets or bands [23].

Sometimes erosive potential is determined solely on the basis of chemical parameters of a product, most frequently: pH, titratable acidity and buffer capacity. According to many suggestions the best determinant of erosiveness is titratable acidity (expressed by the amount of base needed to raise the initial pH to 7.0) [24]. However, some authors indicate that both pH and titratable acidity or buffer capacity are reliable prognostics of the erosive process, but for the sake of accuracy it should be said that pH characterises erosive potential better when a beverage is consumed in large quantities and its time of contact with dental tissue is short, whereas titratable acidity is a better determinant if a small amount of a beverage is mixed with saliva and stays in the oral cavity longer. [25,26] However, it should be pointed out that both pH and titratable acidity or buffer capacity give merely an approximate indication of the erosive potential of a product, as it depends on many other factors. Among them, the degree of saturation with respect to hydroxyapatite seems to be most important. On the other hand, it has been shown that, two acidic solutions with similar DS may have completely different erosive potentials [11]. This imply that other variables exist and interfere with each other, such as calcium:phosphate molar ratio (Ca:P ratio), type of acid and its calcium chelating properties, undissociated acid concentration, adhesiveness, and they should be also taken into consideration when formulating beverages with a reduced erosive potential.

It can be assumed that the erosive potential of a product decreases as the concentration of calcium increases, as was shown in %SMHC after short-term erosion, and in enamel loss after long-term acidic challenge. However significant differences between 0 m*M* and 1 m*M* calcium solutions were not detected in this study.

On the market there are or there were some original products with high calcium content (sometimes with vitamin D_3), e.g. *Calci-Cola* (20 m*M* calcium), *Calci-Sport* (24.5 m*M* calcium), *Calci-Orange* (24 m*M* calcium) by Calcium Beverage Company (USA), *7-Up plus* (11 m*M* calcium), *Minute Maid Ca* orange juice (44 m*M* calcium, Coca Cola Comp.) [25]. Although *in vitro* research showed a less detrimental effect of these beverages on teeth compared to their unmodified versions, on the labels only their „bone reinforcement" and „osteoporosis preventive" effect is highlighted. An exception was *Ribena Toothkind* blackcurrant juice (23.75 m*M* of calcium) produced by SmithKline Beecham (currently GlaxoSmithKline), which was withdrawn from sale, though, due to the controversial advertising campaign. A series of *in vitro* and *in situ* investigations has demonstrated a significantly lower erosiveness of blackcurrant juices and concentrates modified with different concentrations of calcium compared to non-modified juices [15,27]. Also a prototype carbohydrate-electrolyte sports orange drink with 355 ppm of calcium (~8.75 m*M*) ingested during exercise was less harmful to the teeth [28]. Of the sports drinks, *Sukkie* and *Endura* have been shown to have a very low erosive potential due to high calcium content: calcium amino acid chelate in Endura (3 m*M* calcium) and calcium lactate in Sukkie (11 m*M* calcium) and high pH (~4.9). However, the taste of those drinks is less acceptable than that of more acidic sports beverages [29].

Bearing in mind the health safety of consumers, it is necessary to establish maximum amounts of substances added to drinks on the basis of tolerable upper levels, taking into consideration a scientific evaluation of risk, various degrees of sensitivity of particular consumer groups as well as consumption of these nutrients with food and from other sources, e.g. with water. More research is needed to evaluate the possible interactions between calcium and other food ingredients [30].

5. Conclusions

Within the limitations of this *in vitro* study it might be concluded that:

- erosiveness of citric acid could be reduced without changing the pH of the solution
- 2.5 m*M*; 5.0 m*M*; 10 m*M* concentrations of calcium reduce erosive potential of 1% citric acid solution in short-term and long-term erosion-remineralization model
- difference between erosive behaviour of pure 1% citric acid solution without and with 1 m*M* of calcium is small and insignificant.

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