Clin Oral Invest
DOI 10.1007/s00784-016-1915-z

ORIGINAL ARTICLE

Erosive effect of different dietary substances on deciduous and permanent teeth

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Received: 29 April 2016 / Accepted: 17 July 2016
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Abstract

Objectives We investigated the effect of different dietary substances on deciduous and permanent enamel.

Materials and methods Enamel specimens were prepared from human teeth ($n = 108$ deciduous molars and $n = 108$ permanent premolars). We measured the chemical parameters (pH, titratable acidity, viscosity, calcium, phosphate, fluoride concentration and degree of saturation) of nine dietary substances. The teeth were immersed in the respective substance (2 × 2 min; 30 °C; shaking), and we measured the baseline surface hardness (SH) in Vickers hardness numbers (VHN), and the changes in SH after 2 min (ΔSH_{2-0}) and the 4 min (ΔSH_{4-0}) immersion. We analysed the differences between deciduous and permanent teeth using the Wilcoxon test and correlated ΔSH to the different chemical parameters.

Results Deciduous teeth were significantly softer (549.53 ± 59.41 VHN) than permanent teeth (590.15 ± 55.31 VHN; $p < 0.001$) at baseline, but they were not more vulnerable to erosive demineralization. Only orange juice, which presented milder erosive potential, caused significantly more demine ralisation in deciduous teeth at ΔSH_{4-0}. Practically all chemical parameters significantly correlated with ΔSH ($p < 0.05$). Substances with lower pH, higher titratable acidity, lower Ca, higher P_i and lower F concentrations, higher viscosity and more undersaturated solutions presented more erosive demine ralisation.

Conclusion Different parameters in dietary substances affect erosive demineralisation in deciduous and permanent teeth, but we generally observed no differences in susceptibility to erosion between both types of teeth; only orange juice (less severe acid conditions) caused perceptible differences.

Clinical relevance We observe that permanent teeth are harder than deciduous teeth, but most substances cause no perceptible difference in erosive demineralisation in both types of teeth.

Keywords Dental erosion · Primary teeth · Permanent teeth · Dietary factors · Chemical properties · Liability

Introduction

The human teeth are constantly subject to a series of physical and chemical attacks that lead to gradual wear of the dental hard tissues during lifetime. However, if the rate of wear increases to clinically relevant levels, it culminates in serious destruction of the teeth and the loss of their function [1]. Such a condition is named erosive tooth wear, and, albeit multifactorial, it has acids as the main cause [2, 3].

When acids come in contact with the dental hard tissues, enamel dissolution begins in a process described as near-surface demineralisation [4], where the acid removes mineral ions from the tooth, leaving a softened surface. These acids are largely present in dietary substances. Nutritional factors play an important role in the development of erosive tooth wear [3], entailing different physical and chemical parameters that modulate dissolution of the tooth mineral [4–11]. These parameters, namely pH, buffering, acid type, as well as calcium, fluoride and phosphate concentrations, will determine the erosive potential of dietary substances. [3]. In general terms, low pH and high titratable acidity are major risk factors, whereas higher calcium concentration is a protective factor in erosive potential of dietary substances.

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Published online: 23 July 2016

Deciduous and permanent teeth are both liable to acid dissolution. In general, the mineral in teeth is calcium-deficient, carbonated hydroxyapatite, a crystalline solid mostly made up of calcium, phosphate and hydroxide ions. However, minerals from deciduous and permanent teeth differ histologically. Deciduous enamel has smaller and more widely spread out prisms, with more complete boundaries [12], suggesting that deciduous enamel is more porous than permanent enamel. Additionally, deciduous enamel has greater variation in organic content [13], it is less mineralised [14], and it has greater carbonate content [15] than permanent enamel. These histological differences are probable explanations why deciduous enamel is significantly less hard than permanent enamel [16–19], and, in turn, why it is more susceptible to cariogenic demineralisation (dental caries) [20].

It was also thought that deciduous teeth would also be more susceptible to erosive demineralisation. However, contradictory results are still found in the literature. While some studies have shown that deciduous teeth presented a greater progression of erosive demineralisation than permanent teeth [21–24], other studies have shown no differences between both kinds of teeth [17, 19, 25, 26]. Given the contradictory results still found in the literature, more comparison studies are still necessary to fully understand the dissolution of both types of enamel in different drinks and foodstuffs. In this regard, the aim of the present study was to analyse the differences in susceptibility to erosion between deciduous and permanent teeth after immersion in different beverages and sour candies, as well as to correlate these findings with the chemical parameters and viscosity of the substances.

Material and methods

Preparation of enamel specimens

A total of 216 human buccal enamel specimens were used in this study, 108 specimens were from (permanent) premolars and 108 specimens were from deciduous molars. Using a diamond saw, we removed the roots from the teeth and embedded them into acrylic resin blocks (Paladur®, Bad Homburg, Germany). For embedding, each deciduous tooth was paired with a permanent tooth, and three pairs of teeth were embedded in each resin block. In order to obtain a flat and highly polished enamel surface, the specimens were serially ground (LabPol 21, Struers, Ballerup, Denmark) with water-cooled silicon carbide paper discs (from grit #500 to #4000) and polished with diamond paste under constant cooling. This procedure removed a standardized layer of 200 μm of the outer enamel. The enamel specimens were then stored in a saturated mineral solution (1.5 mM $CaCl_2$, 1.0 mM KH_2PO_4, 50 mM NaCl, pH 7.0) until the time of experiment [27].

After this procedure we had six enamel slabs in each resin block (three from deciduous and three from permanent teeth). We distributed four blocks per tested substance, totalising 12 deciduous and 12 enamel specimens per dietary substance. Previous studies [7, 11] had already shown that at least 10 specimens would be enough for each dietary substance.

Immediately prior to the experimental procedures, the resin blocks were further polished with 1 μm diamond abrasive paste for 1 min (LaboPol-6, DP-Mol Polishing, DP-Stick HQ; Struers, Copenhagen, Denmark), to ensure the removal of possible remnants from storage.

Tested substances

We tested eight drinks and candies popularly consumed by children and adolescents in Switzerland, as well as one negative control (mineral water) group (see Table 1 for details). For this experiment, all drinks and candies were pre-treated as follows. The carbonated drinks were degassed by stirring at room temperature (10 min). The candy was dissolved in deionized water (5.2 g candy/10 ml water), under constant mixing at 45 °C; the resulting candy solution was then cooled and used at 30 °C for the experiments [11]. As negative control, we used mineral water. All substances were used at 30 °C for the experiment.

Analyses of the chemical parameters and kinematic viscosity of the substances

For the chemical analyses of the drinks and candies, we used the methods previously described in Lussi, Megert, Shellis and Wang [7] and in Lussi and Carvalho [11]. We used 10 g of each solution at 30 °C to measure the initial pH and the titratable acidity to pH 5.5. Titratable acidity was calculated as the amount of base (mmol/L) required to raise the pH to 5.5. For that, an automatic titrator (Toledo DL 53, Mettler Toledo, Electrode DG 101-SC, Software: LabX pro, Schwerzenbach, Switzerland) established the initial pH of the solutions, which were then individually titrated with 0.5 mol/l NaOH in steps of 0.02 ml [4]. Calcium (Ca) concentration was measured with the standard atomic absorption method, using an atomic absorption spectrometer with an air/acetylene flame. Lanthanum was added to all the products and standards (final concentration 0.2 %) to suppress interference from inorganic phosphates (P_i). Total P_i concentration was analysed by the ammonium molybdate method of Chen et al. (1956) [28]. Fluoride (F) concentration was determined using an F ion-specific electrode (Orion 960900, Boston, MA, USA). Before F measurement, we added total ionic strength adjustment buffer (TISAB) to all products and standard solutions (1:1 ratio). The concentrations of Ca and P_i are expressed in mmol/l and those of F in mg/l. The degree of saturation ($pK - pI$) with respect to hydroxyapatite ($_{HAP}$) and fluorapatite ($_{FAP}$) was

Table 1 Basic information of the tested substances and their chemical parameters: pH; titratable acidity to pH 5.5 (mmol OH^-/l to pH 5.5); calcium [Ca], phosphate [P_i] and fluoride [F] concentrations; degree of saturation with respect to hydroxyapatite (($pK - pI)_{HAP}$) and fluorapatite (($pK - pI)_{FAP}$); and viscosity (ν). In parts from Lussi and Carvalho [11]

Tested drinks or sour candies	Brand name/producer	Flavour	Erosion-related ingredients[a]	pH	mmol OH^-/l to pH 5.5	[Ca] (mmol/l)	[P_i] (mmol/l)	[F] (ppm)	$(pK-pI)_{HAP}$	$(pK-pI)_{FAP}$	ν (mm^2/s)
Mineral water	Valser®, Coca-Cola Company	–	–	6.70	–	10.57	<0.005	0.58	−0.35	3.47	0.82
Flavoured mineral water	Valser®, Coca-Cola Company	Lemon and herbs	Lemon, citric acid, ascorbic acid;	3.31	30.00	9.75	0.08	0.63	−14.68	−7.64	0.88
Apple juice	Ramseier Premium, Ramseier Suisse AG	Apple	Apple juice and pear juice;	3.24	70.30	1.17	1.62	<0.05	−15.23	−9.44	1.05
Orange juice	Hohes C, Eckes AG	Orange	Orange juice;	3.56	83.56	1.98	2.57	<0.05	−11.35	−5.81	1.49
Coca-Cola®	Coca-Cola®, Coca-Cola Company	Cola	Phosphoric acid;	2.55	9.32	0.53	5.39	0.05	−20.59	−14.31	1.04
Monster Energy Drink®	Monster Energy Drink®, Vertrieb Spar GmbH, Austria	NA	Citric, sorbic, and benzoic acids, vitamin B, taurine;	3.35	62.39	0.07	0.03	<0.05	−25.05	−19.38	1.08
Red Bull®	Red Bull®, Red Bull GmbH, Austria	NA	Sodium citrate, taurine, vitamin B;	3.35	67.76	1.41	<0.005	0.13	−25.72	−19.38	1.07
Candy spray	Mega Mouth® Candy Spray, Bazooka Candy Brands International Ltd	NA	Citric acid;	2.14	441.75	0.12	0.16	<0.05	−31.68	−26.76	1.58
Sour candy	Haribo® Pommes, Haribo GmbH & Co., Germany	Apple	Citric, malic, and tartaric acids	2.46	88.10	0.07	0.12	<0.05	−30.57	−24.64	7.51

NA not available

[a] Erosion related ingredients are only those presented on the packaging of each substance

calculated from the pH and the concentrations of Ca, P_i and F using a computer programme [29]. This programme assumes a solubility product for $_{HAP}$ of $10^{-58.5}$ and for $_{FAP}$ of $10^{-59.6}$ [30, 31]. The concentrations of Ca, P_i and F, the pH and the titratable acidity were measured in duplicate.

Kinematic viscosity was also measured in duplicate using a viscometer (Micro-Ubbelohde-VisGometer, capillary diameter $\varnothing = 0.63$ mm, constant 0.0095 mm^2/s^2; SI Analytics GmbH, Mainz, Germany), and the viscosity results are expressed in square millimeters per second (mm^2/s).

Surface hardness measurement

In the present experiment, we measured surface hardness (SH) using nanoindentations. SH was determined with a Vickers diamond under a pressure of 50 mN for 15 s (Fischerscope HM 2000 XYp; Helmut Fischer, Hünenberg, Switzerland). A total of six baseline indentations were made at intervals of 50 μm. Further indentations were made next to the previous indentations after immersion in the substances. SH was automatically calculated from the dimensions of the indentations by the computer programme. The load resolution was ≤0.04 mN and the indentation depth was 600 nm for sound enamel and <1000 nm for most softened specimens. The device allowed fully automatic measurements using a programmable *x*, *y* stage. The SH value for each enamel slab was determined by calculating the average of six indentations. The SH values are presented in Vickers hardness numbers (VHN).

Study design

Before the experiment, acquired pellicle was formed on the enamel surfaces. For that, stimulated saliva was collected from one researcher, by chewing on paraffin, and the whole stimulated saliva was used to incubate the specimens for 2 h at 37 °C. No previous ethical approval was necessary for this procedure. After formation of an acquired pellicle, all enamel specimens were submitted to two consecutive erosive challenges, with respect to the drinks and candies. Each erosive challenge consisted of immersing one resin block (with three pairs of deciduous and permanent enamel) into the respective test substance for 2 min (60 ml/block, 30 °C, under constant agitation (reciprocating movement): frequency of 95 rpm, travel path 50 mm). At the end of the experiment, each pair of enamel specimens had been exposed to the respective test substance for 4 min. A total of 12 pairs of deciduous and permanent enamel specimens were used for each test substance.

Surface hardness was measured at three time-points: before all experimental procedures at baseline ($SH_{Baseline}$), after the first 2 min incubation in the test substance ($SH_{2\ min}$), and at the end of the experiment after a total of 4 min incubation ($SH_{4\ min}$).

Statistical analyses

Since the deciduous and permanent enamel specimens were paired and both kinds of teeth underwent exactly the same treatment, we carried out pairwise analyses. We used Wilcoxon's signed rank sum tests to analyse the erosive effect of each test substance, by analysing the differences in SH val ues before and after incubation in the drinks (analyses made separately for deciduous and permanent teeth). Thereafter, we calculated the changes in SH (ΔSH) within the first 2 min ($\Delta SH_{2\text{–}0} = SH_{2\ min} – SH_{Baseline}$) erosive challenge, the total change in SH after 4 min ($\Delta SH_{4\text{–}0} = SH_{4\ min} – SH_{Baseline}$). Wilcoxon's signed rank sum test was again used to analyse differences in $\Delta SH_{2\text{–}0}$ and $\Delta SH_{4\text{–}0}$ between deciduous and permanent teeth.

We also performed Spearman's correlation coefficient (r_s), to investigate the associations between ΔSH values (dependent variables) and the chemical parameters of the test substances: pH, titratable acidity, and Ca, P_i and F concentrations, degree of saturation with respect to HAP, degree of saturation with respect to FAP and viscosity (independent variables). The significance level for all analyses was set at 0.05.

Results

All chemical parameters and kinematic viscosity of the tested substances are presented in Table 1. All substances, except the negative control mineral water, had $pH < 3.6$. Both kinds of candy (candy spray and sour candy) presented the lowest pH (<2.50) and highest titratable acidity values, where candy spray had the lowest pH and the highest titratable acidity, followed by sour candy. Coca-Cola® also presented $pH < 3.0$, but its titratable acidity was not as low as the other substances.

The highest calcium concentrations were found in mineral water and flavoured mineral water; while the highest P_i concentrations were observed in Coca-Cola®, orange juice and apple juice. The highest viscosity values were observed in both kinds of candy, followed by orange juice (Table 1).

Table 2 shows $SH_{Baseline}$ values, as well as mean change in surface hardness after the first ($\Delta SH_{2\text{–}0}$) and second ($\Delta SH_{4\text{–}0}$) erosive challenges, for both deciduous and permanent teeth, according to the different tested substances. Overall, deciduous teeth presented significantly lower surface hardness at baseline (mean ± SD 549.53 ± 59.41) than permanent teeth (590.15 ± 55.31; $p < 0.001$). All substances, except mineral water, caused erosive demineralisation in both kinds of teeth. The most erosive substance for both deciduous and permanent teeth was candy spray, which, already after the first erosive challenge ($\Delta SH_{2\text{–}0}$), caused a loss in hardness more than six times greater than that observed with orange juice. Comparing the erosive effects of each substance on both kinds of teeth, we generally observed no differences in surface hardness loss between deciduous and permanent enamel ($p > 0.05$). The only significant differences between deciduous and permanent teeth were seen after 4 min incubation in orange juice ($p = 0.023$). Looking at the $\Delta SH_{4\text{–}0}$ values (Table 2), we see that orange juice caused more erosive demineralization on deciduous teeth than on permanent teeth (mean ± SD −165.92 ± 41.41 and −138.67 ± 34.87 for deciduous and permanent teeth, respectively), but (besides mineral water) orange juice caused the least demineralisation in comparison to the other substances.

The association between change in surface hardness and the different chemical parameters and kinematic viscosity are presented in Table 3. In general, we observed a moderate to strong, significant correlation of pH, Ca concentration, $(pK - pI)_{HAP}$, $(pK - pI)_{FAP}$, with ΔSH in both deciduous and permanent teeth. Substances with lower pH values (less acidic), with lower Ca concentrations and that had greater degrees of undersaturation (more negative $pK - pI$ values) presented more erosive demineralisation. Weak, albeit significant, correlations were also found for titratable acidity, P_i and F concentration as well as viscosity (Table 3), where substances with higher titratable acidity, higher viscosity, higher P_i and lower F concentrations caused more erosive demineralisation.

Discussion

The excessive consumption of acidic substances is widely associated to erosive demineralisation in both deciduous and permanent teeth [22, 32]. In the present study, we have tested erosive substances that are commonly consumed by children and young adolescents in Switzerland. These substances have considerably low pH values and high titratable acidities, which are suggestive of their high erosive potential. Mineral ion concentrations, namely Ca, P_i and F concentrations, also play a role in enamel dissolution [7, 11, 33–35]. Interestingly, analyses for Ca, P_i and F concentrations showed only moderate to weak correlations

Table 2 Mean and standard deviation (SD) for surface hardness at baseline (SH_{Baseline}; in HV), and the difference in surface hardness between baseline and the first erosive challenge ($\Delta SH_{2\text{–}0}$), as well as between baseline and the second erosive challenges ($\Delta SH_{4\text{–}0}$; in HV), for deciduous and permanent teeth, respectively

Test substance	Deciduous teeth								Permanent teeth							
	SH_{Baseline}		$\Delta SH_{2\text{–}0}$			$\Delta SH_{4\text{–}0}$			SH_{Baseline}		$\Delta SH_{2\text{–}0}$			$\Delta SH_{4\text{–}0}$		
	Mean	SD	Mean	SD	*p* value	Mean	SD	*p* value	Mean	SD	Mean	SD	*p* value	Mean	SD	*p* value
Mineral water	547.97	68.87	−8.43	16.07	0.117	−14.82	24.26	0.084	591.43	47.63	−3.60	28.58	0.239	−10.98	30.87	0.117
Flavoured mineral water	524.81	49.75	−144.97	28.61	0.002	−276.69	48.97	0.002	598.26	44.29	−134.94	15.53	0.002	−280.35	22.73	0.002
Apple juice	544.27	40.08	−154.71	58.98	0.002	−283.23	44.44	0.002	564.61	45.69	−124.16	64.62	0.002	−267.81	50.81	0.002
Orange juice	581.62	53.16	−64.55	25.81	0.002	−165.92	41.41	0.002	602.70	53.57	−60.75	27.48	0.002	−138.67	34.87	0.002
Coca-Cola®	518.36	53.15	−165.79	43.71	0.002	−279.98	53.90	0.002	544.86	50.23	−156.42	56.36	0.002	−310.18	58.07	0.002
Monster Energy Drink®	551.54	66.91	−156.58	37.38	0.002	−264.00	51.73	0.002	592.57	51.16	−144.62	34.18	0.002	−255.84	44.17	0.002
Red Bull®	570.50	68.13	−169.52	62.97	0.002	−307.11	60.64	0.002	602.53	58.31	−154.01	65.44	0.002	−280.84	80.94	0.002
Candy spray	574.82	43.43	−406.37	47.81	0.002	−487.11	46.28	0.002	621.00	55.51	−396.69	66.54	0.002	−508.42	56.16	0.002
Sour candy	531.88	66.75	−189.87	68.82	0.002	−313.48	80.84	0.002	593.40	67.26	−180.42	49.78	0.002	−322.84	62.38	0.002

with enamel demineralisation, whereas the combination of these variables (together with pH), represented by the degree of saturation, showed strong correlations with erosive demineralisation [7].

In practical terms, the degree of saturation of a specific substance with respect to enamel mineral ($(\mathrm{p}K-\mathrm{p}I)_{\mathrm{HAP}}$ and $(\mathrm{p}K-\mathrm{p}I)_{\mathrm{FAP}}$) is a good predictor of the driving force of enamel dissolution. Positive values, $(\mathrm{p}K-\mathrm{p}I)_{\mathrm{HAP}}>0$ and $(\mathrm{p}K-\mathrm{p}I)_{\mathrm{FAP}}>0$, indicate supersaturated solutions, which tend to cause mineral precipitation; whereas negative values, $(\mathrm{p}K-\mathrm{p}I)_{\mathrm{HAP}}<0$ and $(\mathrm{p}K-\mathrm{p}I)_{\mathrm{FAP}}<0$, denote undersaturated solutions, which tend to induce erosive demineralisation of the enamel [6, 7]. All the substances in Table 1, besides mineral water, presented negative $(\mathrm{p}K-\mathrm{p}I)_{\mathrm{HAP}}$ and $(\mathrm{p}K-\mathrm{p}I)_{\mathrm{FAP}}$ values, so it was not surprising that both deciduous and permanent enamel presented a significant loss in surface hardness after 2- and 4-min incubation in the solutions.

Viscosity has also been associated with erosive demineralisation, where higher viscosities favours less demineralisation [5, 36, 37]. It is theorised that viscosity can affect the Nernst layer [5], which is the semi-static layer of solution adjacent to the surface of the tooth. In more viscous

Table 3 Spearman's correlation coefficients (r_s) and the respective *p* values for correlations between the chemical properties and the changes in surface hardness (ΔSH) in deciduous and permanent teeth

Property	Deciduous teeth				Permanent teeth			
	$\Delta SH_{2\text{–}0}$*		$\Delta SH_{4\text{–}0}$†		$\Delta SH_{2\text{–}0}$*		$\Delta SH_{4\text{–}0}$†	
	r_s	*p* value	r_s	*p* value	r_s	*p* value	r_s	*p* value
pH	0.732	<0.001	0.717	<0.001	0.733	<0.001	0.807	<0.001
Titratable acidity	−0.458	<0.001	−0.459	<0.001	−0.464	<0.001	−0.439	<0.001
[Ca]	0.589	<0.001	0.517	<0.001	0.608	<0.001	0.560	<0.001
$[P_i]$	−0.213	0.027	−0.192	0.047	−0.198	0.040	−0.261	0.006
[F]	0.463	<0.001	0.398	<0.001	0.479	<0.001	0.410	<0.001
$(\mathrm{p}K-\mathrm{p}I)_{\mathrm{HAP}}$[a]	0.741	<0.001	0.715	<0.001	0.756	<0.001	0.736	<0.001
$(\mathrm{p}K-\mathrm{p}I)_{\mathrm{FAP}}$[a]	0.740	<0.001	0.698	<0.001	0.758	<0.001	0.722	<0.001
Viscosity (ν)[b]	−0.426	<0.001	−0.410	<0.001	−0.423	<0.001	−0.342	0.001

[Ca], $[P_i]$, [F]: calcium, phosphate and fluoride concentrations, respectively

[a] Degree of saturation with respect to hydroxyapatite ($_{\mathrm{HAP}}$) and fluorapatite ($_{\mathrm{FAP}}$)

[b] Correlation coefficients for viscosity calculated excluding values for sour candy

*$\Delta SH_{2\text{–}0} = SH_{2\mathrm{min}} – SH_{\mathrm{Baseline}}$

† $\Delta SH_{4\text{–}0} = SH_{4\mathrm{min}} – SH_{\mathrm{Baseline}}$

substances, the Nernst layer tends to be more "static," leading to a generally lower clearance rate and, in turn, slower ionic exchange rates between the solution and the underlying tooth mineral [36]. Consequently, the Nernst layer will tend to have a higher concentration of ions, and eventually become less undersaturated with respect to enamel, leading to less demineralisation [3]. Furthermore, because substances with higher viscosity have a lower penetration coefficient [5], viscosity probably plays a role on the "near-surface demine ralisation" process described by Shellis, Barbour, Jesani and Lussi [4]. Our results, by contrast, seem to contradict these arguments at first. We observed only a weak, albeit significant, correlation between viscosity and enamel dissolution, suggesting in principle that greater viscosities cause more dissolution. Looking at the other parameters, however, we observe that these substances also presented lower pH values and higher buffer capacities. These latter two variables play a much more important role in erosive demineralisation than viscosity. So we suggest that, in the presence of other chemical properties, viscosity plays only a minor role in erosive demine ralisation. Moreover, if one disregards the viscosity values of candy spray and sour candy, one can observe that orange juice had a noticeably higher viscosity in comparison to the remaining substances, which, in turn, presented relatively less demineralisation.

Our results also showed that deciduous teeth presented significantly lower baseline hardness than permanent teeth. This is understandable, given the differences between deciduous and permanent enamel described in the "Introduction" section, as well as previous findings [16–19]. Since deciduous teeth were less hard than permanent teeth, it was expected that they would be more liable to dissolution. Remarkably, we generally observed no difference in demineralisation between both deciduous and permanent enamel after 2- or 4-min incubation in the different substances. The only difference observed was after a 4-min incubation in orange juice, where deciduous teeth presented greater demineralization than permanent teeth.

Many studies have analysed whether deciduous teeth are more liable to erosive demineralization than permanent teeth, but publications on this topic still present contradictory data (for a review see [38]). In one study, Amaechi, Higham and Edgar [24] used orange juice, with total exposure time ranging from 6 to 12 h, and they showed that erosion progressed faster on deciduous enamel than permanent enamel. Other studies have also observed differences between deciduous and permanent teeth, where deciduous teeth also presented more demineralization than permanent teeth [21, 23]. However, the vast majority of other *in vitro* studies report no significant differences between both kinds of teeth [16, 17, 19, 25, 26, 39]. In our case, we tested eight different erosive substances, each with different erosive potentials, yet only orange juice caused a significant difference between deciduous and permanent teeth. These contradictory results could, therefore, be explained by a combination of two main reasons. On the one hand, it can be due to the different chemical properties of the erosive substances [6, 7, 11, 33–35], and on the other hand, it can be related to the severity of the erosive challenge.

Regarding the first point, we observed that orange juice presented a relatively high P_i concentration, high calcium concentration and the highest pH value (besides mineral water), and, albeit undersaturated, it presented the least negative degree of saturation value (Table 1). First, it has been previously argued that there are four different species of inorganic P_i (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}). The concentrations of each of these species present in solution are closely dependant on the pH [40]. In acidic substances, most P_i species are in the form of $H_2PO_4^-$, whereas the most important species in the ion activity of enamel is PO_4^{3-} present in higher pH [7, 11]. Therefore, the protective effect of P_i in acidic substances is very small [7], which explains why P_i had a negative correlation to enamel demineralisation (Table 3). Secondly, the major factors affecting enamel demineralization are pH, calcium concentration and the degree of saturation with respect to enamel [4, 6, 8, 41]. In regard to orange juice, the combination of these factors led to a much milder demineralisation on both kinds of enamel (Table 2).

Regarding the second point, our results, as well as those from Amaechi, Higham and Edgar [24], show significant differences in susceptibility between deciduous and permanent teeth after incubation in orange juice. Since orange juice caused a less severe demineralisation on both kinds of enamel, this "milder" erosion actually led to a more perceptible difference in mineral dissolution between deciduous and permanent enamel. Contrarily, when substances present greater degrees of undersaturation (more negative $pK - pI$ values), the rate of mineral dissolution tends to be at a maximum [6]. Conse quently, the demineralisation tended to be stronger in such solutions, and, in turn, it caused similar degrees of mineral loss in both deciduous and permanent enamel. In other words, differences in the susceptibility to erosion between deciduous and permanent enamel is probably only observable in less acidic conditions. Given the "correct" circumstances ("mil der" acidic environments), differences between the two enamel types will be more distinguishable, whereas these differences will tend to be more imperceptible in more severe acid conditions. This could also explain, in parts, why deciduous teeth are more susceptible to caries [20] (milder acid conditions). In any case, further studies are still necessary to systematically investigate this effect, and verify how different acid properties modulate mineral dissolution in both types of enamel.

It is also important to mention the strong erosive potential of some acidic candies. The taste preference of children has gradually changed for more sour flavours [42], and a high consumption of acidic drinks and candies are consistently found among both children and adolescents [3, 38, 43]. These new candies contain different types of organic acids, and they cause considerable drop in the salivary pH and are more acidic than orange juice [44, 45]. Our results alert to the high erosive potential of these sour candies and sprays [43, 46–48]. Additionally, it is also important to bear in mind that a frequent consumption of acidic substances during childhood and adolescence tends to persist on to adulthood [49], and that erosion on deciduous teeth should be regarded as a predicting factor of erosion on permanent teeth [38, 50, 51]. Our results also point to the fact that these acidic substances causes erosive demineralisation on both deciduous and permanent teeth, which will probably lead to deleterious effects in both children and adolescents/young adults.

In conclusion, different chemical parameters and viscosity of substances are associated with different erosive potentials. In general, we observed no differences in susceptibility to erosion between deciduous and permanent enamel. This is probably due to the generally more severe acidic conditions present in most of the substances tested. However, less severe acid environments (i.e. with orange juice) may produce perceptible differences between the two types of teeth.

Acknowledgments The author thank G. Fischer and Prof. Häusler, Institute of Mathematical Statistics, University of Bern, for their support with the statistical analyses, and also Brigitte Megert for her valuable help in the laboratory. We are particularly grateful to the Department of Preventive, Restorative and Pediatric Dentistry, University of Bern, Switzerland, for providing the chemical analysis data from the data pool of the Department.

Compliance with ethical standards

Ethics The present experiment complied with the ethical standards of the 1964 Helsinki Declaration and its later amendments. This study was also in accordance with the approved guidelines and regulations of the local ethical committee (Kantonale Ethikkommission: KEK). The teeth were extracted by dental practitioners in Switzerland, pooled into two groups of deciduous and permanent teeth, and stored in chloramine until the time of the experiment. Before the donation, the patients (and parents, in case of children) were informed about the use of their teeth for research purposes. All patients (and parents) gave their oral consent. Because we selected teeth from a pool of permanent or deciduous teeth, the local ethics committee categorizes these specimens as “irreversibly anonymised”, so no previous approval from the committee was necessary.

Conflict of Interest The authors declare that they have no conflict of interest.

Funding The work was supported by the Department of Preventive, Restorative and Pediatric Dentistry, University of Bern, Switzerland.

Informed consent For this type of study, formal consent was not required (KEK: Req-2016-00332).

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