Analysis of the erosive effect of different dietary substances and medications

Adrian Lussi*, Brigitte Megert, Robert Peter Shellis and Xiaojie Wang

Department of Preventive, Restorative and Pediatric Dentistry, School of Dental Medicine, University of Bern, Freiburgstrasse 7, CH-3010 Bern, Switzerland

Abstract

Excessive consumption of acidic drinks and foods contributes to tooth erosion. The aims of the present *in vitro* study were twofold: (1) to assess the erosive potential of different dietary substances and medications; (2) to determine the chemical properties with an impact on the erosive potential. We selected sixty agents: soft drinks, an energy drink, sports drinks, alcoholic drinks, juice, fruit, mineral water, yogurt, tea, coffee, salad dressing and medications. The erosive potential of the tested agents was quantified as the changes in surface hardness (ΔSH) of enamel specimens within the first 2 min ($\Delta SH_{2-0} = SH_{2\,min} - SH_{baseline}$) and the second 2 min exposure ($\Delta SH_{4-2} = SH_{4\,min} - SH_{2\,min}$). To characterise these agents, various chemical properties, e.g. pH, concentrations of Ca, P_i and F, titratable acidity to pH 7·0 and buffering capacity at the original pH value (β), as well as degree of saturation ($pK - pI$) with respect to hydroxyapatite (HAP) and fluorapatite (FAP), were determined. Erosive challenge caused a statistically significant reduction in SH for all agents except for coffee, some medications and alcoholic drinks, and non-flavoured mineral waters, teas and yogurts ($P < 0.01$). By multiple linear regression analysis, 52 % of the variation in ΔSH after 2 min and 61 % after 4 min immersion were explained by pH, β and concentrations of F and Ca ($P < 0.05$). pH was the variable with the highest impact in multiple regression and bivariate correlation analyses. Furthermore, a high bivariate correlation was also obtained between $(pK - pI)_{HAP}$, $(pK - pI)_{FAP}$ and ΔSH.

Key words: Tooth erosion: Erosive potential: Dietary substances: Medications: Chemical properties: Hardness

There is increasing evidence, from *in vitro* and *in situ* studies, that the excessive consumption of acidic drinks and foods poses a risk to dental hard tissues[1–8]. Tooth surface is softened in the early stage, and subsequently bulk material is dissolved, layer by layer, from the tooth surface. This type of tooth wear is defined as tooth erosion and is caused by acids (extrinsic and intrinsic) or chelating agents not involving bacterial action. There is a trend towards the increased consumption of acidic drinks and foods. In 2007, the worldwide annual consumption of soft drinks reached 552 billion litres, the equivalent of just under 83 litres/person per year, and this is projected to increase to 95 litres/person per year by 2012. However, the figure had already reached an average of 212 litres/person per year in the USA in 2009[9]. To decrease extrinsic erosive tooth wear, the emphasis should be on preventive strategies that mainly aim at reducing the exposure of teeth to potentially erosive agents.

As a prerequisite, it is essential for medical personnel and patients to have a thorough knowledge of the erosive potential of popular dietary substances. In the past several decades, studies investigating the erosive potential of different dietary substances and medications have been performed in different countries[3–8,10]. A wide range of drinks, foods and medications, such as soft drinks, sports drinks, juices, salad dressings, candies, herbal teas, alcoholic drinks, vinegar, vitamin C tablets etc., were recognised to be associated with the increase in erosion. Normally, soft drinks are mainly composed of filtered water, artificial additives and refined sugar. Thus, they offer limited nutritional benefit, but energy. Sports drinks, which are designed to replenish fluids lost during activity, typically contain water, electrolytes and sugar. Energy drinks are basically soft drinks that contain some forms of vitamins and other chemicals that boost energy for a very short span.

Various chemical properties of a potentially erosive agent, such as pH value, titratable acidity, buffering capacity, the concentrations of Ca, P_i and F, have been identified in the literature to be potentially important in determining the erosive potential[4,5,7,11,12]. However, to the best of our knowledge, no thorough analyses of the effects of a wide range of erosive agents have been undertaken. Buffering capacity is associated with the undissociated acid in a solution, and maintains the H^+ concentration and driving force for demineralisation at the site of dissolution[13,14]. The greater the buffering capacity

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of the solution, the longer it will take for saliva to neutralise the acid, and as a result the more tooth mineral may be dissolved before a safe pH value is reached and the dissolution ceases. It is important to distinguish buffering capacity from titratable acidity. The latter measures total available H^+ over a wide range of pH values, whereas the former is defined at a certain pH value.

The aims of the present *in vitro* study were twofold: (1) to evaluate the erosive potential of different drinks, foods and medications; (2) to determine those chemical properties that have an impact on the erosive potential.

Materials and methods

Preparation of enamel specimens

From a pool of extracted teeth, six hundred caries-free human premolars with no cracks on the buccal sites as viewed under a stereomicroscope were selected. After the crowns of all teeth were separated from the roots, the buccal sites were ground flat under water-cooling on a LaboPol-21 rotating polishing machine (Struers, Ballerup, Denmark) as follows: groups of five enamel slabs were embedded into one resin disk (Paladur, Bad Homburg, Germany) in two planar parallel molds. Once the hardening process was complete, the thinner mold (200 μm thick) was removed. The outer 200 μm of enamel were ground away with a silicon carbide paper disc of 18 μm grade. Thereafter, the exposed buccal sides of enamel slabs in the thicker mold (7 mm thick) were serially polished on the polishing machine under constant cooling with silicon carbide paper discs of 8 μm grade for 30 s and with 5 μm grade for 1 min. Then, after being taken out of the molds, the embedded resin disks, each containing five enamel slabs, were polished for 1 min with 3 μm diamond abrasive on DP-Mol polishing cloth (Struers). After each polishing step, the resin disks were rinsed and sonicated for 2 min in tap water. These preparation steps wore away 200 μm enamel substance in the centre of the window. Then all the resin disks with embedded enamel slabs were stored in a saturated mineral solution (1·5 mM-CaCl$_2$, 1·0 mM-KH$_2$PO$_4$, 50 mM-NaCl, pH 7·0)[15].

Tested dietary substances and medications

In the present study, sixty popular drinks, foods and medications in Switzerland were included (Table 1). According to their constituents and applications, these agents were divided into twelve groups: soft drinks, an energy drink, sports drinks, alcoholic drinks, juice, fresh fruit, mineral water, yogurt, tea, coffee, salad dressing and medications. Immediately before the experiment started, the fruits were crushed, and the pulps and seeds were removed by centrifugation; medication tablets and powders were dissolved in tap water according to the suggestions of the manufacturers.

Chemical analysis of tested agents

The pH value and the amount of base needed to raise the pH to 7·0 (titratable acidity) were measured with a titrator (Toledo DL 53, Mettler Toledo, Electrode DG 101-SC, Software: LabX pro, Schwerzenbach, Switzerland). To measure titratable acidity, 10 g of each drink or solution were titrated with 0·5 M-NaOH in steps of 0·02 ml at a temperature of 30°C. The buffering capacity (β) was calculated by using the following equation: $\beta = -\Delta C/\Delta pH$, where ΔC is the amount of base used and ΔpH is the change in pH caused by the addition of the base. In the present study, the buffering capacity at the original pH of the tested products was calculated.

All the tested agents were further analysed for Ca by standard atomic absorption using an atomic absorption spectrometer with an air/acetylene flame. Lanthanum was added to all the products and standards to suppress P$_i$ interference. Total P$_i$ concentration was analysed by the ammonium molybdate method of Chen *et al.*[16]. F concentration was determined using a F ion-specific electrode (Orion 960900, Boston, MA, USA). Before F measurement, all products and standard solutions were mixed with total ionic strength adjustment buffer (TISAB). 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 calculated from the pH and the concentrations of Ca, P$_i$ and F using a computer program[17]. This program assumes a solubility product for HAP of $10^{-58 \cdot 5}$ and for FAP $10^{-59 \cdot 6}$[18,19].

Before the experiment, carbonated drinks were degassed by stirring at room temperature to avoid the adherence of bubbles to the enamel surface, which will affect the chemical analyses and hardness measurements. The concentrations of Ca, P$_i$ and F, the pH and the titratable acidity were measured in duplicate, and for further calculations of buffering capacity and pK − pI, the mean was determined.

Surface hardness measurement

Surface hardness (SH) of the enamel specimens 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 70 μm. Further indentations next to the previous indentations were made following the experimental procedure. Vickers hardness was calculated from the dimensions of the indentations. 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 WIN-HCU software calculated and illustrated SH.

Study design

After polishing the exposed enamel surface of resin disks (five enamel slabs each) with a 3 μm diamond abrasive, six baseline indentations per specimen were made and measured. The mean SH for each resin disk, i.e. the average SH of five enamel samples, was then calculated. According to the SH distribution, two disks, with a total of ten enamel samples, were assigned to one of sixty groups. Thus, the average SH of each pair of disks was similar. Just before the experimental

Table 1. Basic information and various chemical parameters of the tested agents, e.g. pH value, titratable acidity to pH 7·0, buffering capacity at the pH value, Ca, P_i, and F concentrations, degree of saturation with respect to hydroxyapatite and fluorapatite*

Tested agents	Brand name/producer	Flavour	Erosion-related ingredients	pH	mmol OH⁻/l to pH 7·0	β (mmol/l × pH)	[Ca] (mmol/l)	[P_i] (mmol/l)	[F] (mg/l)	$(pK - pI)_{HAP}$	$(pK - pI)_{FAP}$
Soft drinks											
Carpe Diem Kombucha fresh	Carpe Diem/Carpe Diem GmbH & Co KG	NA	Herbal tea extract and carbonic acid	3·00	39·0	17·6	3·30	0·07	0·39	−19·0	−12·0
Coca-Cola	Coca-Cola/Coca-Cola company	Cola	Phosphoric acid and flavours	2·45	17·5	9·6	1·08	5·04	0·22	−20·0	−13·1
Coca-Cola light	Coca-Cola/Coca-Cola Company	Cola	Phosphoric acid, citric acid and flavours	2·60	19·0	7·3	0·82	4·85	0·22	−19·4	−12·5
Fanta regular orange	Fanta/Coca-Cola Company	Orange	Orange fruit, citric acid, flavours and acidity regulator	2·67	52·5	15·8	0·48	0·08	0·04	−25·2	−19·1
Ice tea classic	n/a /Coop (supermarket in Switzerland)	NA	Black tea extract	2·94	26·5	15·0	0·45	0·04	0·76	−24·2	−16·9
Ice tea lemon	Lipton/Unilever	Lemon	Black tea extract and lemon juice	3·03	24·0	9·4	0·18	0·12	0·58	−24·0	−16·8
Ice tea peach	Lipton/Unilever	Peach	Black tea extract and peach juice	2·94	21·5	8·5	0·12	0·15	0·53	−25·2	−18·0
Pepsi Cola	Pepsi Cola/Pepsi Company, Inc.	Cola	Phosphoric acid, citric acid and flavours	2·39	19·0	11·7	0·33	4·93	0·04	−23·0	−16·9
Pepsi Cola light	Pepsi Cola/Pepsi Company, Inc.	Cola	Phosphoric acid, citric acid and flavours	2·77	15·0	7·4	0·29	4·68	0·04	−20·4	−14·3
Rivella blue	Rivella/Rivella International AG	NA	Milk serum, carbonic acid, citric acid and flavours	3·31	38·0	37·9	4·00	2·17	0·08	−12·0	−5·9
Rivella green	Rivella/Rivella International AG	Green tea	Milk serum, green tea extract, carbonic acid, citric acid and flavours	3·22	44	37·5	3·30	2·41	0·09	−12·9	−6·6
Rivella red	Rivella/Rivella International AG	NA	Milk serum, carbonic acid, citric acid and flavours	3·28	41·5	35·4	3·13	2·28	0·08	−12·6	−6·5
Sinalco	Sinalco/Sinalco International	Orange	Orange juice, carbonic acid, citric acid, mandarin juice, lemon juice, ascorbic acid and flavours	3·12	36·0	10·1	1·14	0·10	0·06	−19·7	−13·5
Sprite	Sprite/Coca-Cola Company	Sour	Carbonic acid, citric acid, acidity regulator and flavours	2·54	39·0	15·0	0·30	0·02	0·02	−28·8	−23·0
Energy drink											
Red Bull	Red Bull/Red Bull GmbH	NA	Taurine and B vitamins	3·30	98·0	45·5	1·94	<0·01	0·11	−26·4	−20·1
Sports drinks											
Gatorade	Gatorade/Pepsi Company, Inc.	Sour	Citric acid and flavours	3·17	46·0	21·8	0·13	2·98	0·05	−19·7	−13·7
Isostar	Isostar/Novartis International AG	NA	Citric acid, flavours and ascorbic acid	3·87	56·5	52·8	8·20	4·49	0·11	−5·9	−0·1
Powerade	Powerade/Coca-Cola Company	Lemon	Malic acid, vitamins B_2 and B_6	3·74	43·0	18·0	0·25	<0·01	0·21	−22·5	−16·2
Juice											
Apple juice	Ramseier/Ramseier Suisse AG	Premium	Apple juice and pear juice	3·41	72·0	43·6	1·96	1·66	0·06	−13·0	−7·0

Table 1. *Continued*

Tested agents	Brand name/producer	Flavour	Erosion-related ingredients	pH	mmol OH⁻/l to pH 7·0	β (mmol/l × pH)	[Ca] (mmol/l)	[P_i] (mmol/l)	[F] (mg/l)	$(pK - pI)_{HAP}$	$(pK - pI)_{FAP}$
Carrot juice	Biotta/Biotta AG	Carrot	Carrot juice, orange juice, agave juice, lemon juice and ascorbic acid	4·16	70·5	55·7	4·40	1·20	0·04	−6·6	−1·4
Grapefruit juice	n/a /Coop	Grape fruit	Grapefruit juice	3·15	168·5	71·4	2·30	2·17	0·03	−14·2	−8·4
Orange juice, Del Monte	n/a /Migros (supermarket in Switzerland)	Orange	Orange juice	3·74	108·0	66·7	2·38	2·36	0·03	−9·8	−4·5
Orange juice, Hohes C	Hohes C/Eckes AG	Orange	Orange juice	3·56	121·0	62·5	1·98	2·57	0·03	−11·4	−5·8
Fruit											
Apricot	NA	Apricot	NA	3·25	317·0	125·0	1·20	5·95	0·02	−13·6	−8·0
Kiwi	NA	Kiwi	NA	3·25	206·5	142·9	3·35	4·47	0·02	−11·9	−6·3
Orange	NA	Orange	NA	3·60	113·0	47·6	2·18	1·27	0·03	−11·8	−6·3
Alcoholic drinks											
Bacardi Breezer orange	Bacardi/Bacardi & Company Limited	Orange	4·4 % alcohol and orange juice	3·16	60·0	26·1	0·19	0·14	0·03	−22·5	−16·7
Cynar	Cynar/Campari Gruppo	NA	16·5 % alcohol	4·00	6·0	5·8	2·01	0·13	0·07	−12·0	−6·5
Beer, Carlsberg	Carlsberg/ Carlsberg Group	NA	5·0 % alcohol	4·20	17·5	8·3	0·74	5·65	0·74	−7·9	−1·5
Beer, Eichhof	Eichhof/Eichhof Getränke AG	NA	4·9 % alcohol	4·07	18·0	8·1	1·94	9·30	0·06	−6·3	−0·9
Champagner	Freixenet/Grupo Freixenet	NA	12·0 % alcohol	2·99	78·0	35·8	1·90	1·98	0·26	−15·9	−9·0
Red wine, Collivo	Collivo/Italia	NA	13·0 % alcohol	3·43	76·0	54·4	1·25	4·69	0·07	−12·5	−6·4
Red wine, Montagne	Montagne/France	NA	11·7 % alcohol	3·68	63·0	46·5	1·68	2·79	0·11	−10·7	−4·7
White wine	La Côte/France	NA	12·1 % alcohol	3·60	53·0	50·0	1·30	4·42	0·27	−11·3	−4·8
Smirnoff ice vodka	Smirnoff/Diageo plc	Lemon	40·0 % alcohol and lemon juice	3·07	50·0	18·2	0·18	6·54	0·12	−18·8	−12·4
Medications											
Alca-C fizzy tablet	Alca-C/Novartis Consumer Health Schweiz AG	Orange	Acetylsalicylic acid and ascorbic acid	4·20	53·0	45·5	9·03	0·02	0·07	−10·2	−4·8
Alcacyl 500	Alcacyl 500/Novartis Consumer Health Schweiz AG	Sour	Acetylsalicylic acid	6·93	0·5	3·7	1·89	<0·01	0·07	0·4	3·1
Alka-Seltzer fizzy tablet	Alka-Seltzer/Bayer (Schweiz) AG	Sour	Acetylsalicylic acid and citric acid	6·23	14·0	24·9	2·06	0·03	0·08	1·5	5·0
Aspirine-C fizzy tablet	Aspirine-C/Bayer (Schweiz) AG	Sour	Acetylsalicylic acid and vitamin C	5·51	27·5	32·8	2·04	<0·01	0·08	−5·7	−1·5
Berocca fizzy tablet	Berocca/Bayer (Schweiz) AG	Orange	Vitamin C	4·24	59·5	57·2	15·20	0·03	0·12	−8·9	−3·3
Fluimucil 200 fizzy tablet	Zambon	Orange	Tartaric acid, citric acid monohydrate and flavours	4·71	19·5	13·5	1·98	<0·01	0·06	−12·5	−7·6
Neocitran	Neocitran/Novartis Consumer Health Schweiz AG	Sour	Vitamin C	2·85	73·5	27·5	4·63	1·58	0·09	−15·5	−0·9

Table 1. *Continued*

Tested agents	Brand name/producer	Flavour	Erosion-related ingredients	pH	mmol OH⁻/l to pH 7·0	β (mmol/l × pH)	[Ca] (mmol/l)	[P$_i$] (mmol/l)	[F] (mg/l)	(pK − pI)$_{HAP}$	(pK − pI)$_{FAP}$
Vitamin C fizzy tablet, Actilife	Actilife/Migros	Orange	Citric acid, vitamin C and flavours	3·86	93·0	58·8	1·90	0·03	0·06	−15·0	−9·4
Vitamin C fizzy tablet, Streuli	Streuli/Streuli Pharma AG	Sour	Vitamin C	3·63	85·0	42·6	1·78	2·01	0·06	−11·4	−5·6
Siccoral	Siccoral/Drossa Pharma GmbH	NA	NA	5·41	2·5	2·0	0·15	0·12	0·03	−7·4	−3·6
Yogurt											
Kiwi Tropicana	Hirz/Nestlé Switzerland	Kiwi	Kiwi and exotic fruits	3·99	124·5	111·1	45·83	33·82	0·04	0	5·3
Nature	n/a /Migros	Milky	NA	3·91	120·0	95·5	43·33	34·34	0·04	−0·6	4·7
Slimline	n/a /Migros	Milky	NA	4·03	133·5	100·0	56·33	38·74	0·03	0·8	5·9
Forest berries	n/a /Migros	Berries	Forest berries	3·77	159·0	200·0	45·50	36·81	0·05	−1·4	4·1
Mineral water											
Henniez	Henniez/Nestlé Waters	NA	NA	7·68	N.A	2·0	2·48	<0·01	0·10	2·4	4·5
Henniez sparkling	Henniez/Nestlé Waters	NA	NA	6·13	4·0	4·2	2·40	<0·01	0·09	−6·2	−2·6
Valser	Valser/Valser Mineralquellen AG	NA	NA	5·63	12·5	10·9	9·93	<0·01	0·60	−2·8	2·1
Valser Viva Lemon	Valser/Valser Mineralquellen AG	Lemon and herbs	Lemon and herbs	3·31	40·0	21·1	9·75	0·08	0·63	−14·7	−7·6
Tea											
Rose hip	n/a /Migros	Rose hip	Rose hip	3·15	19·5	19·7	2·65	0·42	0·05	−16·0	−10·0
Pepper mint	n/a /Migors	Pepper mint	Pepper mint	7·51	N.A	2·9	1·93	0·35	0·05	11·8	13·8
Black tea	n/a /Coop	Black tea	Black tea	6·59	1·5	2·6	1·10	0·27	1·63	5·6	10·0
Wild berries	Lipton/Unilever	Berries	Hibiscus bloom, apple, strawberry, currant and blackberry	6·78	1·0	2·6	1·10	0·24	0·78	6·6	10·5
Coffee											
Espresso	Nestlé /Nestlé Switzerland	Coffee	NA	5·82	3·0	2·2	0·69	0·63	0·07	0·6	4·5
Salad dressing											
Thomy French Classic	Thomy/Nestlé Switzerland	Creamy and sour	Vinegar and lemon juice	4·04	141·0	111·1	20·50	0·46	0·11	−6·1	−0·5
Thomy French Light	Thomy/Nestlé Switzerland	Creamy and sour	Vinegar	3·85	145·0	100·0	40·00	1·14	0·11	−5·3	0·5

pK − pI, degree of saturation; HAP, hydroxyapatite; FAP, fluorapatite; NA, not available.

* Titratable acidity, mmol OH⁻/l to pH 7·0; β, buffering capacity at the pH value; pK − pI with respect to HAP and pK − pI with respect to FAP.

procedures, the resin disks were further polished with a 1 μm diamond abrasive for 1 min (LaboPol-6, DP-Mol Polishing, DP-Stick HQ; Struers, Copenhagen, Denmark), which assured the removal of the possible remnants from storage.

Before the erosive challenge, enamel specimens were immersed in 20 ml of freshly collected human saliva for 3 h to form a salivary pellicle. The saliva, stimulated by paraffin wax (Fluka; Sigma-Aldrich Chemie GmbH, Munich, Germany), was collected in an ice-cooled tube from a single healthy donor at least 1 h after any intake of drink or food[20,21]. She gave informed consent, and saliva collection was performed in accordance with the protocol approved by the University of Bern (Bern, Switzerland). After being carefully rinsed with tap water for 50 s, with deionised water for 10 s and then dried for 5 s with oil-free air, the SH baseline of the samples was measured. Afterwards, the resin disks with five enamel specimens each were individually placed in 60 ml (or g) of the appropriate solution under constant agitation (95 rpm) at 30°C (shaking bath Salvis; Renggli AG, Rotkreuz, Switzerland). After immersion for 2 and 4 min, the resin disks were taken out of the solution, and the SH measurement was performed once again.

Statistics

Wilcoxon's signed rank tests were calculated in an attempt to compare the SH values before and after immersion. The relationship between the changes in SH (ΔSH) within the first 2 min ($\Delta SH_{2-0} = SH_{2\,min} - SH_{baseline}$) and the second 2 min ($\Delta SH_{4-2} = SH_{4\,min} - SH_{2\,min}$) immersion (dependent variables) and pH, buffering capacity, and Ca, P_i and F concentrations (independent variables) was investigated using multiple linear regression (backward selection) analyses. Only variables independent from each other were included. The $pK - pI$ and the titratable acidity were not eligible for inclusion. To assess the bivariate associations between different chemical properties and ΔSH after 2 or 4 min exposure, Spearman's correlation coefficients were used. The statistical calculations were performed using SAS Enterprise Guide 4.1 software. The significance level was set at 0·01 in Wilcoxon's signed rank tests and at 0·05 in multiple linear regression and Spearman's correlation analyses.

Results

Changes in the surface hardness of enamel

In Table 2, Wilcoxon's signed rank tests revealed a significant reduction ($P < 0.01$) of ΔSH_{2-0} for soft drinks, sports drinks, the energy drink (Red Bull), juices (except for carrot juice), fruits and salad dressings. Except for Isostar (sports drink) and Thomy French Classic salad dressing, these substances presented a trend towards further decrease in ΔSH_{4-2}. On the contrary, no statistically significant change was found for coffee, most mineral waters, teas and yogurts in both ΔSH_{2-0} and ΔSH_{4-2}. Exceptions were rose hip tea, forest berries yogurt and Valser Viva Lemon mineral water that had a similar erosive effect as soft drinks. A complicated erosive pattern was observed in the medication and alcoholic drink groups. For

example, Alca-C, Alcacyl 500 and Berocca fizzy tablets did not induce a significant decrease in SH, while the reduction was observable within the first 2 min for Aspirine-C fizzy tablet and within the second 2 min for Siccoral, Alka-Seltzer and Fluimucil 200 fizzy tablets. In the alcoholic drink group, by the end of the experiment, Cynar, Carlsberg beer and Montagne red wine did not produce any significant changes in SH of enamel specimens, whereas Eichhof beer demonstrated erosive potential within the second 2 min. It is worth noting that as no adjustment for multiple testing was done, the present results can only be taken into exploratory consideration.

Influence of different chemical properties on changes in surface hardness

Table 1 also gives an overview of the chemical properties of all tested agents.

Coffee, teas (except for rose hip tea), mineral waters (except for Valser Viva Lemon mineral water) and some medications (Alcacyl 500, Alka-Seltzer and Aspirine-C fizzy tablets) had the highest pH values, above 5·5. The lowest pH values, varying between 2·4 and 3·3, were mostly found in the soft drinks and the energy drink (Red Bull).

The larger titratable acidity was found for fruits, salad dressings, yogurts as well as for grapefruit and orange juices (>100 mmol/l). The buffering capacity ranged from 2·0 to 200 mmol/l × pH. The highest values were observed for yogurts, fruits (except for orange) and salad dressings (>95 mmol/l × pH), the lowest values for Siccoral, Henniez mineral water, coffee and tea (except for rose hip tea) (<3 mmol/l × pH).

Yogurts contained the highest concentrations of Ca (>43 mmol/l) and P_i (>33 mmol/l). Black tea contained the highest concentration of F (1·63 mg/l), whereas F concentration in other agents normally varied between 0 and 1 mg/l.

Many of the test agents under study were undersaturated with respect to both HAP and FAP. Exceptionally, Henniez mineral water, Alcacyl 500 and Alka-Seltzer fizzy tablets, Kiwi and Slimline yogurts, coffee and teas (except for rose hip tea) were supersaturated with respect to both minerals. Valser mineral water, natural and forest berries yogurts, Thomy French Light salad dressing were undersaturated with respect to HAP but supersaturated with respect to FAP.

Table 3 shows the chemical properties with a significant impact on ΔSH after a 2 and 4 min immersion in the multiple linear regression analysis. In this analysis, 52 % of the variation of ΔSH after 2 min immersion and 61 % of the variation after 4 min immersion could be explained by pH, buffering capacity, Ca and F concentrations ($P < 0.05$).

There were high bivariate correlations between ΔSH and the pH, the $(pK - pI)_{HAP}$ and $(pK - pI)_{FAP}$ (Table 4). However, the concentrations of Ca, P_i and F, the titratable acidity and the buffering capacity showed small bivariate correlations with ΔSH.

Discussion

In agreement with previous studies[4,5,22,23], the present study indicated that soft drinks, energy drinks (Red Bull), sports

Table 2. Original surface hardness (SH$_{baseline}$) of specimens, and the changes within the first 2 min ($\Delta SH_{2-0} = SH_{2\,min} -$ SH$_{baseline}$) and the second 2 min ($\Delta SH_{4-2} = SH_{4\,min} - SH_{2\,min}$) incubation in different dietary agents and medications

(Mean values with their standard errors)

	SH$_{baseline}$		ΔSH_{2-0}			ΔSH_{4-2}		
	Mean	SEM	Mean	SEM	*P*	Mean	SEM	*P*
Soft drinks								
Carpe Diem Kombucha fresh	526·8	6·5	− 190·2	8·0	0·002*	− 143·5	10·4	0·002†
Coca-Cola	513·4	7·7	− 157·4	21·1	0·002*	− 153·3	15·9	0·002†
Coca-Cola light	600·3	16·1	− 276·7	17·0	0·002*	− 147·0	13·8	0·002†
Fanta regular orange	513·3	10·0	− 244·8	12·4	0·002*	− 136·9	7·3	0·002†
Ice tea classic	517·0	13·9	− 84·3	12·6	0·002*	− 99·3	14·9	0·002†
Ice tea lemon	511·7	6·9	− 86·1	8·0	0·002*	− 106·8	8·7	0·002†
Ice tea peach	541·0	14·8	− 82·4	14·5	0·002*	− 158·7	11·8	0·002†
Pepsi Cola	563·3	16·9	− 190·8	12·8	0·002*	− 106·8	10·7	0·002†
Pepsi Cola light	512·7	7·7	− 180·4	8·0	0·002*	− 110·0	9·2	0·002†
Rivella blue	530·2	16·4	− 253·8	17·8	0·002*	− 138·9	5·6	0·002†
Rivella green	505·6	18·3	− 144·9	19·4	0·002*	− 142·7	22·6	0·002†
Rivella red	532·0	16·4	− 211·1	10·8	0·002*	− 136·0	10·9	0·002†
Sinalco	514·3	4·8	− 166·5	7·8	0·002*	− 120·5	9·8	0·002†
Sprite	513·2	12·9	− 192·9	19·6	0·002*	− 119·5	11·9	0·002†
Energy drink								
Red Bull	534·9	18·7	− 88·7	22·5	0·009*	− 135·7	10·2	0·002†
Sports drinks								
Gatorade	513·2	7·3	− 124·7	9·3	0·002*	− 107·5	13·6	0·002†
Isostar	539·9	11·1	− 35·4	5·5	0·002*	− 0·2	7·6	1·000
Powerade	510·0	6·3	− 62·7	6·0	0·002*	− 96·3	11·9	0·002†
Juice								
Apple juice	560·9	26·5	− 145·4	13·6	0·004*	− 145·8	16·1	0·002†
Carrot juice	531·9	12·5	− 13·4	5·5	0·037	− 10·5	3·4	0·020
Grapefruit juice	491·0	8·6	− 152·8	7·6	0·002*	128·9	9·7	0·002†
Orange juice, Del Monte	500·6	6·1	− 35·2	5·4	0·002*	− 72·2	8·6	0·002†
Orange juice, Hohes C	590·8	11·8	− 59·8	8·7	0·002*	− 100·3	15·0	0·002†
Fruit								
Apricot	519·1	5·9	− 120·3	9·2	0·002*	− 103·6	7·5	0·002†
Kiwi	499·8	6·2	− 116·8	9·2	0·002*	− 110·3	6·4	0·002†
Orange	561·2	13·1	− 97·4	7·2	0·002*	− 101·9	14·6	0·002†
Alcoholic drinks								
Bacardi Breezer orange	572·1	17·0	− 224·9	13·3	0·002*	− 130·0	8·5	0·002†
Cynar	519·1	8·4	+ 1·2	5·4	0·695	+ 9·1	7·8	0·275
Beer, Carlsberg	511·1	10·4	− 1·6	5·5	1·000	− 5·0	3·5	0·124
Beer, Eichhof	520·6	10·1	+ 0·5	8·0	0·846	− 13·8	3·6	0·006†
Champagner, Freixenet	531·3	8·0	− 126·9	7·3	0·002*	− 98·8	10·0	0·002†
Red wine, Collivo	543·4	22·3	− 31·1	5·6	0·002*	− 33·4	5·6	0·002†
Red wine, Montagne	556·2	12·5	− 20·5	7·4	0·027	− 35·9	11·4	0·557
White wine, La Côte	505·0	8·1	− 24·9	3·9	0·002*	− 38·1	5·5	0·002†
Ice vodka, Smirnoff	565·3	11·1	− 173·9	9·0	0·002*	− 132·4	10·1	0·002†
Medications								
Alca-C fizzy tablet	533·6	11·3	− 13·2	5·0	0·037	− 7·5	6·1	0·160
Alcacyl 500	527·8	11·8	− 2·3	5·5	0·492	+ 2·7	3·7	0·557
Alka-Seltzer fizzy tablet	512·3	7·7	− 3·9	4·8	0·492	− 15·0	4·2	0·009†
Aspirine-C fizzy tablet	534·6	7·1	− 17·4	4·7	0·006*	− 8·4	3·8	0·049
Berocca fizzy tablet	511·4	5·3	− 1·7	7·0	0·846	+ 1·2	4·4	0·846
Fluimucil 200 fizzy tablet	530·8	9·5	− 9·4	9·7	0·322	− 19·1	5·4	0·009†
Neocitran	541·5	13·3	− 249·5	10·9	0·002*	− 119·8	5·0	0·002†
Vitamin C fizzy tablet, Actilife	509·5	8·3	− 88·2	7·1	0·002*	− 98·6	4·9	0·002†
Vitamin C fizzy tablet, Streuli	549·4	19·5	− 139·1	11·3	0·002*	− 147·7	8·8	0·002†
Siccoral	525·7	16·6	− 7·4	6·0	0·322	− 21·0	6·1	0·002†
Yogurt								
Kiwi Tropicana	548·3	8·7	+ 7·3	10·3	0·770	+ 18·1	16·1	0·492
Nature	524·3	4·3	+ 2·8	3·6	0·375	− 8·5	4·3	0·160
Slimline	573·7	18·5	− 3·3	10·2	0·105	− 3·0	7·1	0·275
Forest berries	525·0	8·7	− 6·3	2·7	0·049	− 5·6	1·8	0·009†
Mineral water								
Henniez	543·0	11·9	+ 3·8	10·6	1·000	− 9·5	5·1	0·131
Henniez sparkling	501·3	7·4	− 1·0	3·2	0·760	+ 0·4	3·1	0·846
Valser	491·7	5·0	− 1·5	2·3	0·625	− 4·3	3·1	0·275
Valser Viva Lemon	506·3	11·6	− 81·0	10·9	0·002*	− 89·7	6·7	0·002†
Tea								
Rose hip	545·6	16·2	− 181·1	19·6	0·006*	− 117·8	5·9	0·006†
Pepper mint	519·5	6·7	+ 0·8	5·2	0·922	+ 7·2	5·0	0·155

Table 2. *Continued*

	SH$_{baseline}$		ΔSH$_{2-0}$			ΔSH$_{4-2}$		
	Mean	SEM	Mean	SEM	*P*	Mean	SEM	*P*
Black tea	507·4	8·5	− 1·2	5·7	1·000	+2·4	2·8	0·625
Wild berries	603·7	9·5	+2·1	7·4	0·846	+5·3	5·3	0·322
Coffee								
Espresso	516·5	7·1	+3·7	5·0	0·492	+0·5	5·8	0·846
Salad dressing								
Thomy French Classic	548·6	9·5	− 21·2	5·8	0·002*	−4·0	6·7	0·492
Thomy French Light	509·1	14·1	− 32·6	4·0	0·002*	− 61·4	8·5	0·002†

* Mean values were significantly different in SH within the first 2 min of erosive challenge.
† Mean values were significantly different in SH within the second 2 min of erosive challenge.

drinks, juices, fruits, and some medications and alcoholic drinks caused statistically significant decrease in SH of enamel samples. Yogurts, teas, mineral waters and coffee, except for those that were flavoured with acidic additives, did not have a detrimental effect on enamel SH.

The results highlight the role of acidic additives in increasing erosive capacity of potentially erosive agents. The fruit-based or other acidic flavourings added to 'plain' or 'flat' drinks and foods, which are intended to stimulate taste, contribute to lower acidity and, consequently, induce erosion. Yogurt is a good example for demonstrating the effect of acidic additives. Natural yogurt caused no erosion in spite of its low pH value (3·91). This can be attributed to its high (pK − pI)$_{HAP}$ resulting from high concentrations of Ca and P$_i$. The addition of berries (forest berries yogurt) caused a clinically not relevant reduction in SH within the second 2 min exposure. Even though this brand had higher Ca and P$_i$ concentrations than natural yogurt, its pH of 3·77 was too low for it to be supersaturated with respect to HAP. These findings are in accordance with other studies[24,25]. Similarly, compared with plain mineral water or tea, flavoured products, such as Valser Viva Lemon mineral water and rose hip tea, had much lower pH and negative (pK − pI)$_{HAP}$, and hence caused a statistically significant reduction in SH. Moreover, it has been suggested that fruit-based acids might enhance the buffering capacity[12], which perhaps explains the higher buffering capacity and titratable acidity for the flavoured liquids (Table 1). Therefore, the above-mentioned flavoured products, from the chemical composition point of view, should be classified as soft drinks. Their erosive potential would be expected to be much closer to erosive drinks than to plain products[6,26].

The pK − pI with respect to tooth mineral, determined by the pH value and the concentrations of Ca, P$_i$ and F in a solution, is the driving force for mineral dissolution. When (pK − pI)$_{HAP}$ < 0, the solution is undersaturated with respect to HAP, which chemically and structurally resembles natural tooth[27]. In acidic media, the value of the ion activity product for HAP was a good predictor of enamel lesion[28,29]. Therefore, this solution may induce demineralisation of the enamel. When (pK − pI)$_{HAP}$ > 0, the solution is supersaturated, so favours remineralisation[30]. Previous studies have observed that (pK − pI)$_{HAP}$ plays an important role in tooth dissolution. A small change in (pK − pI)$_{HAP}$ might result in a

marked difference in the dissolution rate of enamel[13,31,32]. As (pK − pI)$_{HAP}$ is dependent on pH and Ca and P$_i$ concentrations, it was not included in the multiple regression analysis. However, there was a negative and strong bivariate correlation between both (pK − pI)$_{HAP}$ and (pK − pI)$_{FAP}$ and ΔSH after both 2 and 4 min. In general, bivariate analyses may be misleading because possible interactions between variables are neglected. Interestingly, the concentrations of Ca, P$_i$ and F alone had a weak correlation with ΔSH[0], whereas the pK − pI defined by the combination of these variables (and the pH) showed a strong correlation.

Many studies have demonstrated that pH is a good predictor of dental erosion: as the pH of the investigated product decreases, there is an increased amount of erosion, independent of the way in which erosion is measured[22,33]. The buffer properties (buffering capacity or titratable acidity) have also been considered to be important[5], even more than pH[34,35], in predicting the erosive potential because it maintains the H$^+$ concentration available for the interaction with the tooth surface[1]. The effect of buffering might, however, vary with pH. Because erosive demineralisation takes place at least partly beneath the enamel surface, buffering capacity may become increasingly important as pH falls, since this is accompanied by an increase in dissolution rate. Consequently, while diffusion may be capable of supplying sufficient H$^+$ ions at higher pH (slow dissolution), increased buffering will be required at lower pH in order to maintain the supply of H$^+$ ions[36]. However, the relative importance of pH and buffering properties could depend on factors such as exposure time and the ratio of the volume of solution to the area of exposed tooth surface. In an *in vitro* study using a low ratio of solution to specimen area, Jensdottir *et al.*[23] reported a significant correlation between buffer properties, titratable acidity, buffering capacity and tooth tissue dissolution after exposure to selected soft drinks for a long time (24 h), while after a short-term exposure (3 min), erosion was associated with pH but not with titratable acidity[37]. They speculated, therefore, that titratable acidity was the better predictor of erosive potential during longer erosive challenges and pH was better for short challenges. However, Hara & Zero[7] observed that after 2 h exposure, titratable acidity showed a low-to-moderate correlation with enamel demineralisation, while pH value was the best predictor for erosion. They ascribed this result to the relatively high

Table 3. Multiple linear regression analysis of the changes in surface hardness (ΔSH) of all specimens after immersion in all agents for 2 and 4 min*
(β Coefficients)

ΔSH	pH		Buffering capacity		Ca concentration		F concentration		Intercept	
	P	β	*P*	β	*P*	β	*P*	β	*P*	β
ΔSH_{2-0} (R^2 0·52)†	<0·0001	−46·5	<0·0001	−0·5	<0·0001	−1·2	0·0006	−34·2	<0·0001	300·3
ΔSH_{4-0} (R^2 0·61)‡	<0·0001	−81·0	<0·0001	−0·6	<0·0001	−2·6	0·0055	−39·9	<0·0001	521·1

* *P* values (β: estimate) are listed for those variables with a significant impact on ΔSH.
† $\Delta SH_{2-0} = SH_{2\,min} - SH_{baseline}$.
‡ $\Delta SH_{4-0} = SH_{4\,min} - SH_{baseline}$.

volume (30 ml) used in their study. Buffering properties are likely to be relatively more important when a low volume of solution is used, as the pH would be raised more easily by mineral dissolution[7]. The dependence of tooth erosion on both pH value and buffering capacity observed in the present study, and the lack of a significant effect of titratable acidity could thus be due to our use of short erosive challenges and an adequate, well-stirred volume of the test product.

The literature is contradictory with regard to the erosive potential of acidic drinks and foods containing F[4,5,22]. Previous studies have shown that the erosive capacity of different drinks was significantly and negatively associated with their original F concentration[4,5]. This observation was confirmed in the present study. In contrast, Larsen & Nyvad[22] reported that F concentration in eighteen soft drinks had no effect on the depth of tooth erosion. Furthermore, a study by Larsen & Richards[38] showed that in drinks with pH above 3, F concentrations reduced the *in vitro* development of erosion by 28%; in drinks with pH below 3, erosion was not affected, despite total F concentrations of 20 parts per million and saturation with calcium fluoride. It is worth noting that in those studies, severe acid attacks with surface loss was chosen, while in the present study initial erosion (softening) caused by various agents was assessed.

A higher concentration of the Ca and/or P_i in a solution will increase the $pK - pI$ with respect to dental mineral, so that the presence of suitable concentrations of Ca and P_i may counteract tooth erosion caused by acidic drinks and foods. Some studies have proved that lower levels of enamel demineralisation were found in Ca-containing drinks than in those without Ca[23,39–41]. The relatively higher concentrations of Ca and P_i are most probably responsible for the less erosive effect of Isostar compared with other sports drinks. Isostar does not contain other protective ingredients, such as casein. The results of multiple linear regression analyses indicated a significant relationship between Ca concentration and erosion. However, there was no evidence of a relationship between P_i and tooth erosion. There are four species of inorganic P_i, namely H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}, in a given solution and their proportions depend on the pH[42]. At the pH of erosive drinks (approximately 2–4), only a minute fraction of the total P_i (of the order of 10^{-13}) is in the form of PO_4^{3-} ions[42], which are the only important P_i species in the ion activity product of HAP and FAP. Therefore, enormous quantities of P_i are required to raise the degree of saturation of the solution. This may be the reason why P_i is ineffective in the present study.

The formation of a pellicle with human saliva as well as the exposure time scale of a few minutes used in the present study is of particular physiological relevance and clinical interest. First, this exposure time is comparable with clearance time of acids in the mouth[23]. Second, in the early stage, acids diffuse into the tooth and remove Ca and P_i from the outer few micrometres of hard tissues, forming a demineralised, weakened layer. Remineralisation is possible in this stage, since the remaining enamel can serve as framework in which minerals can be deposited again[43].

The present *in vitro* study, however, cannot totally reproduce the clinical conditions, and should only be interpreted as a prediction of the relative erosive potential of a dietary substance or a medication. Erosion is a multifactorial condition, and its occurrence and development depend on many risk and protective factors as well as on their interplay[44]. In addition to the erosive potential of dietary substances and medications, a variety of factors, for example frequency of acid intake, individual dietary habits (sipping, gulping, frothing or use of a straw)[45], the physical properties (the adhesiveness and displacement) of these agents[46], the flow rate, composition and clearing capability of the saliva, may influence the progress of tooth erosion[47]. However, an investigation of the parameters associated with the erosive potential of dietary substances and medications could act as a significant screening test through which dentists can provide instructional recommendations for patients at high risk of dental erosion. In addition, the present study covered a wide range of tested agents with various chemical and physical properties. Some components in these agents may have an

Table 4. Spearman's correlation coefficients: all chemical properties *v.* the changes in surface hardness (ΔSH) and the respective *P* values

Chemical property	ΔSH_{2-0}*		ΔSH_{4-0}†	
	R^2	*P*	R^2	*P*
pH	−0·83	<0·0001	−0·86	<0·0001
Titratable acidity	0·14	0·0006	0·16	0·0001
Buffering capacity	0·04	0·321	0·06	0·154
Ca concentration	−0·27	<0·0001	−0·28	<0·0001
P_i concentration	−0·13	0·0009	−0·14	0·0005
F concentration	−0·11	0·006	−0·11	0·005
$(pK - pI)_{HAP}$‡	−0·75	<0·0001	−0·78	<0·0001
$(pK - pI)_{FAP}$§	−0·70	<0·0001	−0·73	<0·0001

$pK - pI$, degree of saturation; HAP, hydroxyapatite; FAP, fluorapatite.
* $\Delta SH_{2-0} = SH_{2\,min} - SH_{baseline}$.
† $\Delta SH_{4-0} = SH_{4\,min} - SH_{baseline}$.
‡ $pK - pI$ with respect to HAP.
§ $pK - pI$ with respect to FAP.

influence on salivary pellicle and thus interfere in the correct assessment of tooth erosion. For example, black tea and red wine have been shown to have a profound effect on *in vitro* pellicle maturation, causing thickened layers of stained material to build up, which were not readily removed. The mechanism behind this effect was ascribed to the polyphenols contained[48]. Salivary proline-rich proteins, particularly basic proline-rich proteins, via the proline rings[49], have a particularly high affinity for dietary polyphenols[50,51], as do histatins[52,53].

In conclusion, the present study confirmed the erosive potential of a wide range of dietary substances and medications. Tooth erosion had a significant relationship with pH, with buffering capacity, F and Ca concentrations. The degree of saturation with respect to HAP and FAP, illustrating the combined effect of these parameters, showed a high bivariate correlation with tooth erosion.

Acknowledgements

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