

RESEARCH ARTICLE



Erosive potential of ice tea beverages and kombuchas

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ABSTRACT

Objectives: Kombuchas and other tea-based beverages are often perceived as healthy products despite the lack of knowledge on their effects on oral health. This *in vitro* study determined the erosive potential of commercial kombuchas, and ice teas compared to cola drinks.

Materials and methods: The pH and fluoride content of 7 kombuchas and 18 tea drinks were measured with ion-selective electrodes. Calcium dissolution from hydroxyapatite grains was quantified by atomic absorption spectroscopy after beverage exposure. The effect of beverages on the enamel surface was visualized by scanning electron microscopy (SEM). Distilled water, and cola drinks were used as negative and positive controls.

Results: The kombuchas exhibited lower pH values (2.82–3.66) than the ice teas (2.94–4.86), but still higher than the cola drinks (2.48–2.54). The fluoride concentration varied between 0.05 and 0.46 ppm and for 7 beverages the concentration was below the detection limit. The calcium release for kombuchas was 198–746 mg/l, for ice teas 16.1–507 mg/l, and for cola drinks 57.7–71.9 mg/l. Twenty-two beverages had a significantly greater calcium release than the cola drinks ($p = .009–.014$). The surface etching of the enamel was seen in the SEM analysis after beverage exposure.

Conclusions: Tea-based beverages have even higher erosive potential than cola drinks. Kombuchas especially, displayed a considerable erosive potential.

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Introduction



Dental erosion is the permanent loss of tooth surface minerals caused by acids or chelating agents such as citric acid. Compared to dental caries, bacteria are not involved in erosive wear [1]. In the oral environment, the amount of erosion and loss of minerals depends on various factors such as the prevalent pH, the concentration of calcium and phosphate, the amount of fluoride, and the duration and frequency of the acid attack as well as the protective influence of saliva. The critical pH is the pH where equilibrium exists between tooth mineral dissolution and precipitation, and for enamel, it is generally accepted to be 5.5 [2,3]. Demineralization in dentin starts at a higher pH and therefore it can dissolve more rapidly than enamel [3].

Dental erosion can stem from internal factors, like reflux disease, cytostatic drug treatment or bulimia [4,5] but commonly the etiology of erosion is explained by dietary habits such as increased intake of fruit juices and soft drinks [5–7]. In Finland the domestic sale of soft drinks has grown 27% from the year 2015 to 2021 [8] and in 2021, the estimated consumption of soft drinks was as high as 54.2 liters/capita [8,9]. However, according to Patenaude et al. [10], the

consumption of sugary drinks like ice tea can be more common than the consumption of traditional refreshments such as juice and lemonade.

Tea, a traditional brewed beverage, is suggested to be a healthy “superfood” since it is claimed to have many beneficial health qualities such as antioxidant, anti-inflammatory [11,12] and even anti-COVID [13] potential. In the oral cavity, tea may protect teeth from erosion and caries enhancing the protective properties of the dental pellicle [14,15] and therefore the supplementation of soft drinks with green tea extract has been suggested to reduce the erosive potential of these drinks [15].

Relatively new products within the beverage industry include kombucha, a fermented tea-based beverage that is claimed to have antimicrobial and antioxidant health benefits. During the fermentation process of kombucha, the pH decreases, and organic acids are produced [16,17]. These acids have the potential to dissolve hydroxyapatite *via* chelating complexes and are therefore assumed to be exceptionally erosive [18]. Nevertheless, the possible threat of kombuchas on oral health is commonly neglected, and they are considered to be healthy drinks.

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The aim of this *in vitro* study was to determine the erosive potential of 25 selected commercial ready-to-drink tea beverages including different variations of kombucha and ice tea. Most of the beverages are commercially available worldwide. The methods included the determination of the fluoride content and pH of the beverages, the calcium dissolution assay from hydroxyapatite grains as well as an examination of the enamel of extracted bovine teeth by scanning electron microscopy (SEM) after exposure to the beverages.

Materials and methods

Tea beverages and controls

The tea beverages used in the study are listed in Table 1. They were purchased from local supermarkets in Turku, Finland. The studied ready-to-drink ice teas contained, according to the manufacturers' information, black tea, or tea extract in four beverages, green tea or tea extract in six beverages, 'tea extract' without specification in four beverages and roibos tea in one beverage. All the ice teas contained lemon juice or citric acid, except Prykmestar Strawberry Iced Tea which contained malic acid. The other tea drinks contained green tea extract and citric acid (two) or matcha powder and malic acid (one). The tested kombuchas were based on black tea (one), herbal tea (one), green tea (four) or black and green tea (one). Three kombuchas were unseasoned and four were flavored with juice, syrup or aroma. Distilled water, Pepsi and Coca-Cola were used as negative and positive controls, respectively.

pH measurement

Immediately after opening the bottle, the pH of the beverages was measured at room temperature (RT, +20 °C) with a PHM210-pH-combination electrode meter (Meterlab, Germany) connected to an electrode calibrated with a standard solution of pH 4.00 and 7.00 (Reagenan, Toivala, Finland). The pH measurements were conducted two to four times. Because the beverages used in the experiment are often consumed chilled, the pH measurement was also conducted at fridge temperature (FT, 9.5 °C) with selected beverages (7, 14 and 21, Table 1) and the Coca-Cola positive control.

Measurement of fluoride content

The fluoride content was measured with a Thermo Scientific Orion Fluoride Selective Electrode at RT. The samples contained equal volumes of TISAB solution and tea beverage. TISAB solution was prepared by mixing 500 ml deionized water (resistivity 18 MΩ·cm), 57 ml acetic acid and 58 g NaCl (AnalaR NORMAPUR) with a magnetic stirrer. Thereafter, 5 M NaOH was added until the pH of the solution was 5.02 and water was added until the volume was 1000 ml. The pH was measured with an Orion™ Triode™ 3-in-1 pH/ATC Probe connected to a Thermo Scientific Orion Star A111 benchtop pH meter. Calibration was made by using 0.05, 0.1, 5, 50 and

100 ppm fluoride dilutions. The results were calculated using a Nernst equation.

$$E = E^0 + S \times \log(A)$$

E = measured electrode potential; *E*⁰ = the potential of the reference electrode; *S* = slope; *A* = activity level of fluoride.

Measurement of calcium content

The calcium content of the beverages and of the positive controls was determined with an AAnalyst 400 atom absorption spectrophotometer (PerkinElmer, Shelton, CT, USA) with a Win Lab 32 -program. Calibration was made with a CaCO₃ standard (Merck, Darmstadt, Germany).

Measurement of released calcium from hydroxyapatite

Hydroxyapatite grains (Hydroxyapatite DNA Grade Bio-Gel http Gel Bio-Rad Laboratories, Hercules, CA, USA) were used to simulate the enamel. Ten parallel samples of every product were prepared mixing 100 mg hydroxyapatite with 2 ml of the beverage at RT. Five of the parallel samples were mixed in a vertical rotator for one hour and five for 24 h before centrifugation with a Labofuge 4000R (Heraeus Function Line, Osterode, Germany) at 4000 rpm for 10 min. An aliquot of 1 ml was filtered (Jet Biofil Syringe Filter Nylon 0,45 μm sterile) and diluted 1:100 or 1:10 in 0.1% lanthanumoxide solution (Fluka) and centrifuged (2800 rpm, 10 min at RT). The amount of calcium was measured using the AAnalyst 400-atom absorption spectrophotometer as described above. The calcium release was also measured at FT of the samples of selected beverages (7, 10, 14 and 21, Table 1). The protocol was the same as described earlier except for the vertical mixing of the five parallel samples which was executed at FT for one hour. Distilled water was the negative control in all the assays. Coca-Cola was used as a positive control in one-hour RT and FT assays and Pepsi in the one-hour RT assay. The calcium content of the beverages was subtracted from the released value.

Scanning electron microscopy study

The enamel specimens were prepared from the extracted bovine teeth to demonstrate the erosive changes in the morphology of the enamel structure. The extracted bovine teeth were stored in antibacterial Chloramin T -liquid before the grinding process. The samples were fastened to an acrylic screen and ground using an Exakt grinding system (Kulzer, Kulzer & Co GmbH/Technical Division, Germany) with abrasive paper P2500 (Polishing paper P2500, Exakt, Germany) and polished with Exakt Polishing paper K4000 (Germany) under constant water irrigation. Each specimen was covered with polyvinyl adhesive tape with two standard-size holes made by a rubber dam punch. Beverages 7, 10, 14 and 21 (Table 1) were selected for the scanning electron microscopy examination and distilled water and Coca-Cola were used as controls. A single specimen with

Table 1. The beverages used in the study and their contents.

Controls		
1	Distilled water	
2	Coca-Cola	Carbondioxide, acidity regulator (E338), natural aromas (eg. caffeine)
3	Pepsi	Carbondioxide, acidity regulator (E338), natural aromas (eg. caffeine)
Kombuchas		
4	Puhdistamo Kombucha Cola	Kombucha culture, cola aroma extract, natural lemon aroma, organic green tea
5	Vigo Kombucha Original	Kombucha culture, malic acid, fermented herbal tea extract
6	Smiling Dutchman Kombucha Black Currant	Kombucha culture, blackcurrant juice, green tea, black tea
7	Captain Kombucha Original	Kombucha culture, green tea (31%), tea extract (0,05%)
8	Crazy Croco Organic Kombucha Peach	Kombucha culture, peach syrup, green tea
9	Karma Green Tea Kombucha	Kombucha culture, green tea
10	Karma Kombucha Lemon	Kombucha culture, black tea, lemon juice, lemon extract
Ice teas		
11	San Benedetto Green Iced Tea	Citric acid, green tea extract (0.2%)
12	San Benedetto Lemon Iced Tea	Citric acid, tea leaf extract (0.1%)
13	Arizona Green Tea With Citrus	Citric acid, green tea extract (0.11%)
14	Arizona Iced Tea With Lemon Flavor	Citric acid, black tea extract (0.12%)
15	Marli Ice Tea Elderflower-Blueberry	Citric acid, blueberry juice from concentrate, black tea extract (0.1%)
16	Marli Ice Tea Green Tea and Lime	Citric acid, lime juice from concentrate, green tea extract
17	Paulig Cold Brew Sparkling Rooibos Tea Rhubarb-Lemon	Lemon juice from concentrate, cold-extracted rooibos tea concentrate (3.2%)
18	Paulig Cold Brew Sparkling Green Tea Ginger-Lemon	Lemon juice from concentrate, cold-extracted green tea concentrate (3.5%)
19	Fuze Tea Mango Camomilla	Citric acid, sodium citrate, mango juice from concentrate, green tea extract (0.14%)
20	Fuze Tea Lemon	Citric acid, sodium citrate, lemon juice from concentrate, tea extract (0.12%)
21	Lipton Lemon	Citric acid, sodium citrate, lemon juice from concentrate, black tea extract (0.12%)
22	Lipton Green Ice Tea	Citric acid, sodium citrate, malic acid, green tea extract (0.13%)
23	Prykmestar Strawberry Iced Tea	Strawberry, black tea
24	Rainbow Lemon Iced Tea (sugar free)	Citric acid, sodium citrate, lemon juice from concentrate, tea extract
25	Pirkka Lemon Iced Tea (sugar free)	Citric acid, sodium citrate, lemon juice from concentrate, tea extract
Other		
26	Yoko Matcha Drink	Malic acid, organic matcha tea powder
27	Nocco Caribbean	Citric acid, green tea extract
28	Vitamin Well Prepare	Citric acid, sodium citrate, green tea extract

two exposed area was analyzed for each beverage. The tooth specimens were placed in 20ml of the beverages for 1 h at RT and then washed with distilled water for 15 s. After the exposure, the covering tape was removed. The specimens were placed in deionized water in cold room storage. For the SEM analysis, the specimens were first dried in an exicator for a week and gold coated with a Bal-Tec SCD 050 Sputter Coater and Rowaco Gold Target 54×0.2 mm disc 99.99% Au using 50 mA for 55 s. The surface morphology of the specimens was examined using a scanning electron microscopy JSM-5500 (JEOL, Japan).

Statistical analysis

Spearman's correlation coefficients were calculated to evaluate the associations of pH and calcium release in one hour as well as the fluoride concentration and calcium release in one hour. The pH values were compared between the beverage groups (kombuchas, ice teas and cola drinks) with a Mann-Whitney *U*-test.

The difference in calcium release between 1 h and 24 h was analyzed with a Mann-Whitney *U*-test. The comparison of calcium release in tea beverages to the negative control and positive controls was analyzed with a Mann-Whitney *U*-test. The calcium release in one hour at RT was compared to the 1 h release at FT with a Mann-Whitney *U*-test.

The normal distribution of the variables was evaluated visually together with Kolmogorov-Smirnov test. *p*-values <.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 27.0.1.

Results

pH

As a group, kombuchas had significantly lower pH than ice teas ($p=.008$). At RT, the mean pH values of the kombuchas varied between 2.82 and 3.66 and the ice teas between 2.94 and 4.86. Distilled water had a pH of 5.71 and the pH of cola drinks used as positive controls were 2.54 (Coca-Cola) and 2.48 (Pepsi). At FT, the pH of Coca-Cola was 2.63, for the distilled water 5.78 and for three beverages (7, 14 and 21) it was 3.05–3.39 (Table 2).

Fluoride and calcium content

Fluoride concentration was measured from beverages 4–6 and 11–28. In 7 beverages the fluoride content was below the detection limit (0.05 ppm). The highest fluoride concentration for the kombucha group was 0.08 ppm and for the tea drinks 0.46 ppm. The calcium content of the kombuchas varied between 6.74 and 38.2 mg/l and for the tea drinks 1.76 and 40.4 mg/l (Table 2).

Calcium release

The calcium release from the hydroxyapatite grains after one hour and 24 h exposure to the beverages is shown in Table 3. The mean calcium release at RT of ice teas and of kombuchas varied between 16.1 and 917 mg/l at one hour and between 20.8 and 912 mg/l at 24 h. The mean calcium release

Table 2. The mean pH, fluoride, and calcium concentration of each beverage. The measurements were done from one (fluoride and calcium) or two to four (pH) beverage containers at room temperature (RT, +20°C). The mean pH of selected beverages at fridge temperature (FT, 9.5°C).

Beverage	pH at RT	pH at FT	Fluoride concentration ppm	Calcium concentration mg/l
1 Distilled water	5.71	5.78	NM*	NM
2 Coca-Cola	2.54	2.63	NM	13.6
3 Pepsi	2.48	NM	NM	2.63
4 Puhdistamo Kombucha Cola	3.28	NM	0.03	6.74
5 Vigo Original Kombucha	3.17	NM	BD**	11.5
6 Smiling Dutchman Black Currant Kombucha	3.16	NM	0.08	18.9
7 Captain Kombucha Original	3.16	3.67	NM	7.45
8 Crazy Croco Organic Kombucha Peach	3.06	NM	NM	30.4
9 Karma Green Tea Kombucha	3.02	NM	NM	38.2
10 Karma Lemon Kombucha	3.00	NM	NM	36.3
11 San Benetto Green Iced Tea	3.28	NM	BD	40.4
12 San Benetto Lemon Iced Tea	3.27	NM	BD	37.4
13 Arizona Green Tea Citrus	3.43	NM	0.14	7.25
14 Arizona Iced Tea With Lemon Flavor	2.94	3.05	0.07	4.59
15 Marli Ice Tea Elderflower-Blueberry	2.95	NM	0.05	15.1
16 Marli Ice Tea Green Tea and Lime	3.28	NM	0.05	17.0
17 Paulig Cold Brew Sparkling Rooibos Tea Rhubarb-Lemon	3.34	NM	BD	23.9
18 Paulig Cold Brew Sparkling Green Tea Ginger-Lemon	3.47	NM	0.17	19.2
19 Fuze Tea Mango Camomilla	3.67	NM	0.32	19.2
20 Fuze Tea Lemon	3.68	NM	0.46	20.5
21 Lipton Lemon	3.57	3.39	BD	2.10
22 Lipton Green Ice Tea	3.55	NM	0.18	1.76
23 Prykmestar Strawberry Iced Tea	4.86	NM	0.34	8.01
24 Rainbow Lemon Iced Tea (sugar free)	3.39	NM	0.18	26.9
25 Pirkka Lemon Iced Tea (sugar free)	3.38	NM	0.22	28.2
26 Yoko Matcha Drink	3.58	NM	0.11	35.3
27 Nocco Caribbean	3.32	NM	BD	28.0
28 Vitamin Well Prepare	3.48	NM	BD	7.92

*NM: not measured.

**BD: below detection. Detection limit 0.05 ppm.

in 1 h of the kombucha group (beverages 4–10) was 457 mg/l and of the ice tea group (beverages 11–25) 211 mg/l.

All tested beverages had a significantly greater release of calcium than distilled water which was used as a negative control ($p = .009-.014$). Twenty-two of the 25 beverages had a significantly greater release of calcium than the cola drinks ($p = .009-.014$). Beverages 13 ($p = .009$) and 23 ($p = .009$) had a significantly lower release of calcium than cola drinks. Sample 16 did not differ statistically from the cola drinks.

Calcium release of distilled water and 21 beverages (4–6 and 11–28) was compared between 1 h and 24 h (Table 3). Ten of the 21 beverages (4, 6, 13, 17, 18, 19 and 23–26) had a significantly greater calcium release in 24 h than in one hour ($p = .009-.047$). Calcium release did not differ at different timepoints for the remaining 11 beverages and negative control.

Calcium release at FT in one hour was measured for four of the beverages (7, 10, 14 and 21) and compared to calcium release at RT (Table 3). Two had a greater calcium release in FT than RT while for two others the calcium release did not differ between the tested temperatures.

In the analysis of all 25 beverages, there was a significant negative correlation between pH and calcium release in one hour at RT ($r_s = -0.406$, $p = .032$). The beverages with a lower pH had a greater release of calcium. The correlation between fluoride concentration and calcium release in 1 h was not statistically significant ($r_s = -0.202$, $p = .379$).

SEM images

The SEM images with a magnification of $\times 2000$ demonstrated the enamel surface etching after exposure to the selected drinks. In the hydroxyapatite assay, all the selected beverages released a significant amount of calcium from the grains. Irrespective of the studied beverage (excluding distilled water), the erosive impact can be seen in the SEM images. Figure 1(B,F) show the typical honeycomb pattern of the eroded enamel tissue. Figure 1(C,D) show exposed enamel prism heads and a minor demineralization of the interprismatic space. Minor demineralization is seen in Figure 1(E).

Discussion

The food and drink industry undergoes constant changes to keep pace with evolving consumer demands and new trends. Particularly healthy, natural – or naturally perceived products are currently on the rise. For example, tea is widely professed to be good for health, thus ready-to-drink ice teas might tempt even the health-conscious consumer despite the high amounts of carbohydrates and citric acid. Furthermore, the global markets have discovered a relatively new tea-based beverage, kombucha, the consumption of which has grown exponentially within the last few years across the globe [19]. Although kombuchas have gained a reputation as health-promoting beverages [16,20], very little information was to be found in the literature on the question of their

effect on oral health. In recent research in the field of restorative materials, some evidence was found of the eroding effect of kombucha, however, caution was suggested due to the small sample size and the rarity of related studies [21]. Therefore, to our knowledge, this is the first study to assess the erosive potential of kombuchas.

Our results indicate that both the studied kombuchas and ice teas have strong erosive potential. The studied kombuchas showed a remarkably high release of calcium even when compared to the cola drinks which are extremely erosive [22]. The results also demonstrated that all the examined beverages, had a pH under the critical pH 5.5. The kombuchas had a greater release of calcium and significantly lower pH (2.82–3.66) than the evaluated ice teas.

The high erosive potential of kombuchas may be explained by the acid composition. Organic acids, which are the main acids of kombuchas [16,17], are shown to be more erosive *in vitro* than the phosphoric acid [23] of the cola drinks (Table 1) and the citric acid [23,24] of the ice teas

(Table 1). Consequently, the analysis of titratable acidity together with buffering properties would provide additional information about the chemical behavior of these drinks and might further explain noted differences in the erosive potentials. However, it must be emphasized that kombucha is a living product, hence, the final composition of kombucha varies and every manufacturing lot can differ [16,17,25].

According to our results, all the studied beverages exhibited lower pH values than the distilled water. Albeit the pH of the distilled water was slightly acidic, it was still above the critical level for enamel which enables its use as a negative control. The beverages with a lower pH had a greater release of calcium. Consequently, the only studied ice tea which contained pure tea instead of tea extract (sample 23) had the highest pH (4.86) and it released noticeably less calcium than the other experimented beverages. Nevertheless, almost all the ice teas included in the study showed increased calcium release potential compared to the cola drinks. Although the specific content of the ready-to-drink ice teas

Table 3. The calcium release (mg/l) of the beverages at room temperature (RT, +20 °C) and for selected beverages at fridge temperature (FT, 9.5 °C).

Beverages	RT			FT		RT	
	One hour (mg/l)	One hour compared to controls		One hour (mg/l)	RT compared to FT	24 h (mg/l)	One hour compared to 24 h
	Mean (SD)	Water <i>p</i> -value	Cola drinks <i>p</i> -value	Mean (SD)	<i>p</i> -value	Mean (SD)	<i>p</i> -value
1 Distilled water	10.1 (0.68)			6.47 (0.69)	.006	9.68 (0.84)	.347
2 Coca-Cola	57.5 (1.58)			71.9 (4.69)	.009	NM*	
3 Pepsi	61.3 (2.05)			NM		NM	
4 Puhdistamo Kombucha Cola	199 (14.3)	.009	.009	NM		217 (11.8)	.028
5 Vigo Original Kombucha	344 (3.83)	.014	.014	NM		356 (7.87)	.086
6 Smiling Dutchman Black Currant Kombucha	702 (30.9)	.009	.009	NM		746 (34.4)	.047
7 Captain Kombucha Original	332 (23.8)	.009	.009	310 (3.70)	.142	NM	
8 Crazy Croco Organic Kombucha Peach	365 (20.4)	.009	.009	NM		NM	
9 Karma Green Tea Kombucha	686 (35.0)	.009	.009	NM		NM	
10 Karma Lemon Kombucha	575 (34.9)	.009	.009	815 (14.7)	.009	NM	
11 San Benetto Green Iced Tea	172 (3.32)	.009	.009	NM		166 (11.0)	.465
12 San Benetto Lemon Iced Tea	168 (13.2)	.009	.009	NM		175 (29.4)	.917
13 Arizona Green Tea Citrus	48.9 (3.23)	.009	.009	NM		56.2 (2.18)	.016
14 Arizona Iced Tea With Lemon Flavor	480 (90.0)	.009	.009	428 (26.7)	.465	507 (34.8)	.347
15 Marli Ice Tea Elderflower-Blueberry	295 (8.11)	.009	.009	NM		300 (12.5)	.465
16 Marli Ice Tea Green Tea and Lime	58.9 (1.40)	.009	.175/.117**	NM		60.2 (1.87)	.251
17 Paulig Cold Brew Sparkling Rooibos Tea Rhubarb-Lemon	148 (4.03)	.009	.009	NM		161 (11.3)	.028
18 Paulig Cold Brew Sparkling Green Tea Ginger-Lemon	106 (15.5)	.009	.009	NM		125 (8.03)	.047
19 Fuze Tea Mango Camomilla	154 (4.90)	.009	.009	NM		164 (6.84)	.047
20 Fuze Tea Lemon	278 (13.0)	.009	.009	NM		293 (5.02)	.076
21 Lipton Lemon	236 (9.37)	.009	.009	313 (20.4)	.009	245 (11.6)	.347
22 Lipton Green Ice Tea	165 (14.6)	.009	.009	NM		173 (4.75)	.175
23 Prykmestar Strawberry Iced Tea	16.1 (0.37)	.009	.009	NM		20.8 (1.43)	.009
24 Rainbow Lemon Iced Tea (sugar free)	411 (4.09)	.009	.009	NM		438 (6.52)	.009
25 Pirkka Lemon Iced Tea (sugar free)	431 (9.45)	.009	.009	NM		450 (8.35)	.028
26 Yoko Matcha Drink	105 (2.21)	.009	.009	NM		112 (3.89)	.016
27 Nocco Caribbean	917 (30.4)	.009	.009	NM		912 (33.7)	.754
28 Vitamin Well Prepare	196 (4.27)	.009	.009	NM		193 (9.41)	.465

The comparisons of calcium release at RT in one hour to distilled water, Coca-Cola, and Pepsi, at RT to FT, and at RT in one hour to 24h. Results are expressed as means (SD), *n*=4–6.

*NM: not measured.

***p* = .175 Coca-Cola, *p* = .117 Pepsi.

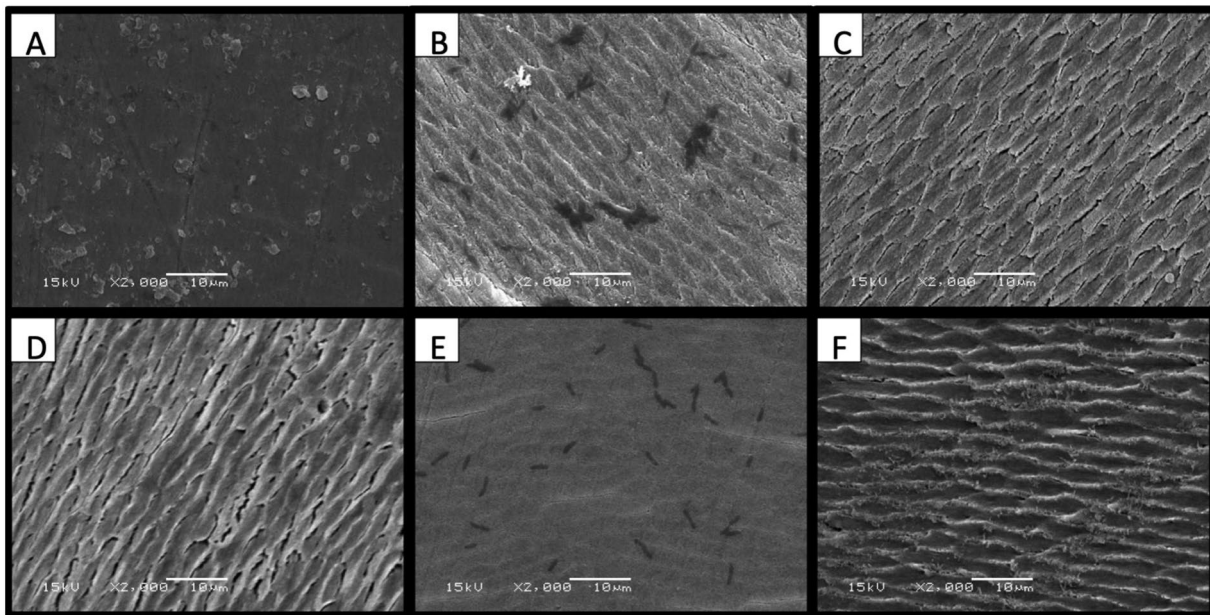


Figure 1. SEM images of enamel specimens with a magnification of $\times 2000$. (A) Distilled water. No erosion is seen compared to other studied beverages B-F; (B) Coca-Cola. The image shows demineralized enamel with a honeycomb pattern; (C) Arizona Lemon. The image shows demineralized enamel with a sharp prismatic pattern and slightly eroded interprismatic spaces; (D) Lipton Lemon. The image shows demineralized enamel with a prismatic pattern and a fused appearance; (E) Captain Kombucha Original. The image shows flattened, slightly demineralized enamel; (F) Karma Kombucha Lemon. The image shows the honeycomb appearance of the enamel surface with a selective demineralization.

is not declared on any of the manufacturer's information, the amount of tea or tea exact seemed to be only minute and the main ingredient of the beverages seems to be fruit juice (Table 1). These juices can resist pH changes and thereby induce a prolonged erosive period in the oral cavity [26]. Furthermore, *in vitro* studies have indicated that the erosive potential of fruit-flavored drinks was not effectively reduced by adding fluoride [27] or diluting the drink with water [28]. Thus, ice teas are expected to also have a strong erosive potential *in vivo*.

West et al. [29] found that the erosion rate increases with a rise in temperature. Hence, it might be assumed that if acidic soft drinks are consumed at low temperatures, less erosion occurs. The study from Eisenburger and Addy [30] sustains the allegation. In our *in vitro* experiment, however, the calcium release was significantly higher at the chilled temperature for Karma Kombucha Lemon, Lipton Lemon, and the positive control Coca-Cola. The measured pH-values of the chilled beverages were very similar to the ones at RT, and therefore do not explain the increased calcium release. The discrepancy between our study and previous results could be attributed to the differences in the study protocols. The results of the aforementioned studies [29,30] were accumulated using pure citric acid instead of commercial products, the temperature changes transpired within a wide scale and the rate of erosion was evaluated with profilometric measurements.

The fluoride level of a tea beverage depends on the concentration of fluoride in the tea leaves and the amount of tea extract as well as the other supplements utilized in the manufacturing process. The fluoride concentration in black tea is higher compared to green tea, whereas herbal and

rooibos teas contain no tea leaves at all and hence, are fluoride-free. [25,31,32]. Kombuchas are suggested to have high fluoride contents, up to 0.93 mg/ml [25], but the kombuchas in the present study contained a fluoride maximum of 0.08 ppm. However, the high fluoride concentrations in the previous study [25] were found in kombuchas fermented from tea leaves using a kombucha consortium whereas the kombuchas in our study were purchased as processed ready-to-drink beverages. The ascertained fluoride concentrations of the ice teas (below detection limit 0.05–0.46 ppm) were approximately the same level (0.03–1.79 ppm) as reported earlier [32,33]. However, there was no significant correlation between fluoride concentration and calcium release, hence fluoride seems unlikely to have any effect on the erosive potential of the analyzed products.

Using SEM we illustrated that the erosive damage also occurs on the enamel surface, as presented earlier by other acidic beverages [22,34,35]. The erosion patterns of the used ice teas were similar to each other but slightly variable in the kombuchas. The differences between the appearance of these patterns can be partly explained by grinding factors and variances in scanning directions. The flattening and polishing of the enamel specimens can sensitize the enamel surface to acid dissolution due to the fact that the hardness of the enamel decreases when the distance from the surface increases [3]. However, although the quantity of samples was low and there is a lack of parallel controls, the difference in the enamel structure is clear between the studied beverages and the negative and positive controls.

Because dental erosion is caused by many modifying factors, an inference from *in vitro* to *in vivo* is only approximate. The main drawback of laboratory-based experiments is that

such studies lack critical oral cavity conditions, such as the influence of the salivary flow and especially the acquired salivary pellicle. Furthermore, the 60 min used for the erosive challenge can be considered unreasonably long. Nonetheless, cooled refreshments, such as kombucha and ice tea, are often consumed over a prolonged period of time, and continuous sipping extends the duration of the erosive interlude in the mouth [3]. However, the main aim of the current study was to explore the erosive potential that kombuchas and other widely used tea-based drinks may have. This aim was conducted by evaluating the calcium dissolution from the hydroxyapatite and pH measurements. The techniques such as profilometry and surface microhardness of the bovine tooth samples could further help to quantify the rate of surface erosion. In addition, the pH value only displays the hydrogen ion concentration, therefore it does not indicate the overall acidic content. However, a low pH and an ability to dissolve calcium from hydroxyapatite are qualities known to expose to erosion and fluoride an agent against it [36]. Thus, in spite of its limitations, the present results add to our understanding of the erosive nature of tea beverages and kombuchas.

Conclusion

The present study indicates an erosive potential for all of the tested ready-to-drink kombuchas and ice teas. The analyzed kombucha beverages had a remarkably low pH and released high amounts of calcium even when compared to cola drinks. As the selection of commercial healthy-promoted beverages grows, it is particularly relevant to increase awareness of their detrimental erosiveness because the prevention of erosion is effective and always the best treatment.

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Disclosure statement

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