ACTA ODONTOLOGICA SCANDINAVICA
2019, VOL. 77, NO. 5, 340–346
https://doi.org/10.1080/00016357.2019.1570330

ORIGINAL ARTICLE

Erosive potential of soy-based beverages on dental enamel

Elis Janaína Lira Dos Santos[a], Ingrid Andrade Meira[a], Emerson Tavares De Sousa[b], Bennett Tochukwu Amaechi[c], Fábio Correia Sampaio[d] and Andressa Feitosa Bezerra De Oliveira[e]

[a]Department of Prosthodontics and Periodontics, Piracicaba Dental School, University University of Campinas, Piracicaba, SP, Brazil; [b]Department of Pediatric Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil; [c]Department of Comprehensive Dentistry, University of Texas Health Science Center in San Antonio (UTHSCSA), San Antonio, TX, USA; [d]Department of Clinical and Public Health dentistry, Federal University of Paraíba (UFPB), João Pessoa, PB, Brazil; [e]Department of Morphology, Federal University of Paraíba (UFPB), João Pessoa, PB, Brazil

ABSTRACT

Background: This study aimed to investigate the erosive potential of soy-based beverages in comparison to fruit juices of the same flavor.
Methods: Human enamel blocks were randomly divided into 9 groups ($n = 8$), according to the beverage category (soy or non-soy juices). The initial pH, TA and β at the original pH value were measured in triplicate. The composition of calcium, phosphate and total protein was analyzed using the specific colorimetric method. The fluoride analysis was performed using a selective electrode. The degree of saturation (DS) and the critical pH (CpH) of each beverage with respect to hydroxyapatite (HAp) and fluorapatite (FAp) were calculated using the computational software. Enamel samples were immersed into 67.5 mL of each drink for 120 minutes. Enamel surface loss (ESL) and differences in surface roughness ($\Delta Ra_{E\text{-}S}$) were analyzed by a 3D non-contact profilometer.
Results: Non-soy beverages exhibited the lowest pH values (2.93 to 3.40). The highest values of calcium concentration were founded in soy-based formulations. Juices with soy in their composition tend to have high DS when compared with non-soy based beverages ($p = .0571$). Soy beverages produced less ESL than non-soy beverages ($p < .05$). $\Delta Ra_{E\text{-}S}$ was not significantly different between the categories. The ESL and $\Delta Ra_{E\text{-}S}$ were positively correlated with initial pH and buffering capacity in soy-based beverages. On the other hand, in non-soy beverages, the ESL was negatively correlated with the TA to 7.0 and the fluoride composition whereas the $\Delta Ra_{E\text{-}S}$ was negatively correlated with the TA to 5.5.
Conclusions: The erosive potential of soy beverages was lower than non-soy based beverages.

ARTICLE HISTORY
Received 23 May 2018
Revised 28 December 2018
Accepted 2 January 2019

KEYWORDS
Tooth erosion; dental enamel; fruit and vegetable juices; milk substitutes; soy milk

Introduction

Dental erosion is defined as the irreversible dissolution of tooth tissue minerals by acids of non-bacterial origin, which is usually progressive and may compromise both enamel and root surfaces [1]. From a biochemical point of view, this dissolution is explained by frequent episodes of exposure to intrinsic or extrinsic acids, which promotes the exchange of solid components of hydroxyapatite (Ca^{2+}, PO_4^{3-} and OH^- ions). The rate of dissolution as well as the ion activity product of hard dental tissues, depends on many factors as type of substrate (enamel, dentin, cementum), time of exposure (transversal and longitudinal consumption), chemical composition (level of saturation), presence of inhibitors (fluoride calcium, phosphate, magnesium, pyrophosphate, statherin), pH and buffering capacity of the solution, temperature and flow rate (method of consumption) [2,3]. Besides, the chemical dissolution on the tooth surface promotes a softened layer that is more susceptive to the mechanical wear (mainly attrition and abrasion).

The incidence of erosive tooth wear has raised, mainly in children and adolescents [4]. This phenomenon can be explained by significant changes in lifestyle, behavior and habits in relation to the consumption of acidic drinks [5–9]. It is summarized that there are a dose–response relationship between soft drinks, fruit juices, and sports/energy drinks consumptions and dental erosion [4]. In this context, important parameters for the soft drink erosive potential are titratable acidity, pH value and buffering capacity as well as their acidic composition [10–12].

The degree of undersaturation of acidic-beverages (lack of calcium, phosphate or both) determines the magnitude of the dental hard tissues dissolution, and probably can be used to explain why some products have more erosive potential. Thus, the addition of calcium, fluoride and phosphate has been shown to reduce the erosive potential of acidic drinks [3,8,13]. Moreover, the addiction of some proteins, as soy, on the juice composition may alter the chemical behavior of these juices and promote changes in their erosive potential [13]. This trend was explored by Brito et al. [13] who analysed quanti- and qualitatively the demineralizing effect of industrialized fruit juices plus soybean extract.

CONTACT Andressa Feitosa Bezerra De Oliveira andressafeitosa@msn.com Federal University of Paraíba (UFPB), Av. Cabo Branco, 1850/201 Cabo Branco, João Pessoa, PB 58045-010, Brazil

Supplemental data for this article can be accessed here.

Nevertheless, as soymilk, presents calcium and protein in its composition [13], we hypothesized that possibly this type of formulation may cause less erosive tooth wear. Given this background, we were not able to find any study that properly investigated the erosive potential of commercially available beverages of similar type, with and without soybean extract.

Soy products have attracted interest because of the health benefits attributed to the consumption of this legume. Hence fruit juice with added soymilk has become increasingly popular, basically for being tasteful and relatively cheap [14]. Therefore, the aim of this study was to investigate the erosive potential of soy-based beverages in comparison to fruit juices of the same flavor.

Materials and methods

Ethical considerations

Before the study was initiated, ethical approval was obtained by the Ethics Committee in Research of Federal University of Paraiba (CAAE: 17581413.4.0000.5188). The goals and the study entire procedure were explained as well as the possible use of their teeth. In case of agreement, the volunteers signed an informed consent. The experiment was conducted in accordance with the Declaration of Helsinki and following the guidelines of Good Clinical Practice.

Experimental design

This is an *in vitro* study performed on human enamel specimens. The study was conducted in two phases:

- Phase 1 (Beverages selection and Characterization of the beverages). In this phase, the beverages were selected and analyzed regarding pH, titratable acidity (TA), buffering capacity (â) as well as calcium, phosphate and fluoride composition.
- Phase 2 (Erosive challenge and determination of the erosive potential of the beverages). Human enamel blocks were randomly assigned into 9 experimental groups ($n = 8$): (1) Soy grape juice, (2) Soy orange juice, (3) Soy peach juice (4) Soy strawberry juice, (5) Grape juice, (6) Orange Juice, (7) Peach juice, (8) Strawberry juice and (9) Coca-Cola soft drink (positive control). The response variables were the enamel surface loss (ESL) and surface roughness (SR), both analyzed by an optical profilometry.

Beverages selection

Nine commercially popular bottled drinks were selected and purchased for analysis (Box 1). They were separated into two categories, with or without soy additives. The Coca-Cola® soft drink was used as a positive control for erosion production. All beverages were stored according to the manufacturer's instructions.

Box 1. Compositions of the experimental beverages as listed on their respective packaging.

Beverages	Ingredients	Brand name/ producer
Soy Grape juice	Water, soy extract, liquid invert sugar, grape juice concentrate, sugar, maltodextrin, vitamins (C, B3, B6, B2, folic acid and B12), mineral (iron and zinc), pectin, guar gum, citric acid, carmine, malic acid and sucralose.	Addes®/ Unilever©
Soy Orange juice	Water, soy extract, liquid invert sugar, orange juice concentrate, sugar, maltodextrin, vitamins (C, B3, B6, B2, folic acid and B12), mineral (iron and zinc), pectin, guar gum, citric acid, annatto and turmeric dye and sucralose.	Addes®/ Unilever©
Soy Peach juice	Water, soy extract, liquid invert sugar, maltodextrin, sugar, peach juice concentrate, vitamins (C, B3, B6, B2, B12 and folic acid), zinc mineral, pectin, guar gum, citric acid, malic acid, carmine and annatto dye and sucralose.	Addes®/ Unilever©
Soy Strawberry juice	Water, soy extract, liquid invert sugar, maltodextrin, sugar, strawberry juice concentrate, vitamins (C, B3, B6, B2, B12 and folic acid), minerals (zinc and iron), pectin, guar gum, citric acid, carmine and annatto dye and sucralose.	Addes®/ Unilever©
Grape juice	Water, sugar, grape, apple, carrot and blueberry juice, vitamins (C, E, B3, A, D, B6 and B12), grape synthetic aroma, citric acid and guar gum.	Del Valle Kapo®/ The Coca-Cola Company ©
Orange juice	Water, orange juice concentrate, sugar, natural flavor, citric acid and antioxidant ascorbic acid.	Valle®/The Coca-Cola Company©
Peach juice	Water, peach juice concentrate, sugar, citric acid, beta-carotene, peach flavoring, xanthan gum and vitamin C.	Dafruta®/Ebba©
Strawberry juice	Water, strawberry juice concentrate, sugar, citric acid, beta-carotene, strawberry flavoring, xanthan gum and vitamin C.	Dafruta®/Ebba©
Coca-Cola (soft drink)	Carbonated water, sugar, kola nut extract, caffeine, caramel coloring IV, acidifying phosphoric acid and natural aroma.	Coca-Cola®/The Coca-Cola Company©

pH, titratable acidity (TA) and buffering capacity (β) of the beverages

Immediately after opening each beverage, pH and titratable acidity were measured in triplicate, at room temperature. The pH was measured with a previously calibrated pH meter (pH 20, Hanna Instruments, Woonsocket, RI) placed in a beaker and stirred using a non-heating magnetic stirrer until a stable reading was observed.

The *TA* was determined as the volume of a standard 1 M NaOH solution required to increase the pH of 50 mL of each beverage to 5.5 and 7.0. This was added in 0.2 ml increments while stirring with a non-heating magnetic stirrer, until a stable pH reading was obtained. The values were then converted to mmol of OH^- per liter to the pH of the drinks. The buffering capacity (β) was calculated according to Lussi et al. [15], using the following equation: $\beta = \Delta C/\Delta pH$, where ΔC is the total amount of base used to raise the initial pH to 7.0 and ΔpH is the change in the pH of the solution.

Assessment of calcium, phosphate and fluoride composition

Calcium concentration was analyzed by the direct colorimetric method. In this technique, calcium (Ca^{2+}) was quantified by a mass spectrophotometer on an automatic microplate reader with calcium sensitive reagent (Arsenazo III). The reader was pre-calibrated with a standard solution (Calcium carbonate: 100 µg/ml) and the absorbance of λ 650 nm [16].

The phosphate ($PO_3{}^{-4}$) was determined by colorimetry, which has the principle that the phosphorus of the mineral phosphates is converted to phosphomolybdate. The phosphomolybdate was reduced by the alpha-aminonaphthol sulfonic acid to produce a product colored with blue. The color intensity was measured in a Beckman DU – 70® at 660 nm by a mass spectrophotometer, previously calibrated with standards of 3–24 µg phosphor/mL [17].

Fluoride concentration was measured by microdiffusion method [18]. A fluoride-ion-specific electrode (BN Model 9409, Orion, Cambridge, MA) and a potentiometer (Model 720 Orion, Cambridge, MA) were used for fluoride analysis, and each sample was analyzed in triplicates. The limit of readings repeatability was 95%. Calibration curves were performed using fluoride standards of 0.02–2.56 µg of F/mL under the same conditions as samples. Standard samples were prepared by serial dilution of a stock solution containing 0.1 MF (Orion 940906). The millivolt readings were converted to fluoride ion concentration using a standard correlation curve ($r^2 > .99$) based on linear equation ($y = ax + b$).

Values of calcium, phosphate and fluoride concentrations were expressed in µg/mL.

Assessment of degree of saturation and critical pH of juices

The degree of saturation (DS) of the juices with respect to hydroxyapatite (HAp) and fluorapatite (FAp) was calculated from solubility product of HAP and FAP, the pH and the concentrations of Ca, Pi and F. The Critical pH (CpH) of juices was assessed through the simulation of the DS with respect to HAP and FAP at several pH values. Thus, the pH value at which the solution is exactly saturated with respect to HAP and FAP (DS = 1), is considered as the CpH of the solution. These calculations were performed using the computer program IONPRODUCT developed by Shellis [19]. This program considers that the solubility product for HAP and FAP is $10^{-58.5}$ and $10^{-59.6}$, respectively.

Assessment of protein concentration

The protein concentration was determined by the Bradford colorimetric method [20], using Bradford's reagent (Sigma Aldrich, Jurubatuba-SP, Brazil). The bovine serum albumin (Sigma Aldrich, Jurubatuba-SP, Brazil) was used as the standard at concentrations of 1–8 µg/µL. Briefly, samples (10 µL) were added to a 96-well plate containing 200 µL Bradford reagent. This content was be incubated at 22 °C for 5 minutes and then analyzed by an Elisa plate reader (EPOCH, Biotek, Winooski, VT) at 595 nm of absorbance.

Sample size, tooth selection and sample preparation

The sample size calculation was performed to compare the least difference in enamel mineral loss between soy-based beverages of different flavor, based on the research of Beltrame et al. [11]. The number of enamel samples required in each group is given by the following formula [21]:

$$n = f(\alpha, \beta) x \frac{2\theta^2}{(\mu 1 - \mu 2)^2}.$$

where, $f(\alpha, \beta)$ is a function of α (0.05) and β (0.05), $\mu 1 - \mu 2$ is the smallest difference in means between Grape (0.5) and Orange (0.7) group, and θ is the standard deviation of the Grape Juice group (0.1). The final sample size was 6.5 enamel blocks in each group. This number was raised by 20% for compensate possible losses or failures during the experiment. Thus, the required enamel blocks that should be used to access enamel mineral loss was 8 in each experimental group.

Thirty-six human third molars were collected. Following tooth extraction, soft connective tissues adhering to the tooth surfaces were carefully scraped off using a sterile curette and stored in 10% (v/v) of formalin (pH 7.0) solution prior to use at room temperature. Using a 5× magnification prism loupe, teeth without cracks, caries and any enamel malformation were selected for the study.

A total of 72 enamel blocks (3 × 3 × 2 mm) were obtained, from the buccal and lingual surfaces of the selected teeth, using a double-sided diamond disk in a precision cutting machine (Labcut 1010, Extec, Enfield, CT) under slow-speed and constant irrigation. The specimens were embedded in self-cured acrylic resin circular using molds of 16 mm diameter and 3 mm deep. The outer enamel surface was ground flat with grit papers (600–1500 grades) under water refrigeration and polished with 1 µm diamond paste (Extec Corporation, Enfield, CT) in a rotating polishing Machine PSK-2V (Skill-tec Comércio e Manutenção Ltda, São Paulo, SP, *Brazil*). Following 5 min sonication in water using an ultrasonic device, two halves edges of the exposed enamel surface were covered with two layers of nail varnish (Risque, Niasi, Taboão da Serra, São Paulo, *Brazil*) as a reference area for profilometry analysis. The central portion (1 mm) remained uncovered for erosion assay. Each tooth represents an independent experimental unit.

Erosive challenge

For erosion production, the samples in each group were immersed into 67.5 ml (7.5 ml of the beverage/mm² exposed enamel specimen), at room temperature (22–25 °C), for 120 min [9], under gentle agitation. At the end of the exposure time, the specimens were rinsed with deionized water and stored in a humidity-controlled environment to prevent drying until further analysis.

Table 1. Initial pH, titratable acidity (mmol/l NaOH to reach pH 5.5 and 7.0), buffering capacity (β), inorganic composition (μg/mL), DS and CpH with respect to HAp and FAp, and protein concentration (μg/μL) of each tested beverage.

		TA										
	pH	→ pH 5.5	→ pH 7.0	β	Pi	Ca^{2+}	F^-	DS HAp	DS FAp	CpH HAp	CpH FAp	Protein concentration
Soy juices												
Grape	3.88	0.92	1.30	8.11	63.30	100	0.08	0.031	0.133	5.84	5.18	0.012
Orange	3.88	1.12	1.67	10.3	67.50	10.61	0.10	0.009	0.040	6.59	5.99	0.014
Peach	4.23	0.88	1.47	10.2	79.80	110.44	0.02	0.065	0.224	5.77	5.20	0.016
Strawberry	3.99	0.88	1.43	9.38	53.10	107.41	0.09	0.037	0.159	5.84	5.18	0.013
Non-soy juices												
Grape	2.93	0.88	1.15	5.53	22.20	57.79	0.12	0.003	0.015	6.18	5.55	0.001
Orange	3.40	3.40	4.59	24.34	126.0	72.92	0.44	0.013	0.075	5.84	5.06	0.008
Peach	3.10	1.83	2.43	12.3	56.40	55.98	0.03	0.005	0.023	6.04	5.48	0.001
Strawberry	3.26	1.38	1.80	9.28	34.80	51.44	0.04	0.006	0.026	6.15	5.58	0.003
Control												
Coca-Cola®	2.47	0.63	1.97	8.36	73.80	64.90	0.06	0.002	0.009	5.96	5.35	0.005

Analysis of enamel surface loss and roughness

A 3D non-contact profilometry (Proscan 2000, Scantron, Taunton, *England*) was used to measure the enamel surface loss and surface roughness. The nail varnish was carefully removed using acetone solution (1:1 water). An area of 2 mm long (X) × 1 mm wide (Y) was scanned, covering treated and reference surfaces. The step size was set 0.01 mm (X axle) and 0.1 mm (Y axle) and the number of steps 200 in the (X) axle and 10 in the (Y) axle. The unprocessed data file for each specimen was saved. With the use of software, the ESL (μm) in the treated area was calculated based on the reference surfaces. A 3-point height tool was applied. The enamel surface roughness (Ra, μm) was measured using the same software. Three measurements in each of the treated (Ra_E) and reference (Ra_S) areas were collected from the unprocessed saved data file. A surface roughness difference was calculated ($\Delta Ra_{E\text{-}S}$) based on sound (Ra_S) and eroded (Ra_E) surface roughness values (Supplementary material). A mean of Ra_S, Ra_E and $\Delta Ra_{E\text{-}S}$ was used for each sample.

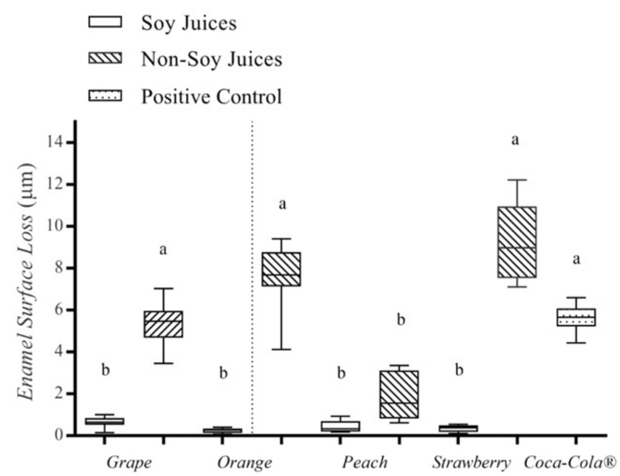

Figure 1. Enamel mineral loss (μm) according to each experimental beverage (n = 8). Distinct letters are statistically different by Kruskal-Wallis followed by Student-Newman-Keuls test (p<0.05).

Statistical analysis

Statistical procedures were performed with the SPSS statistical software (SPSS, Inc., Chicago, IL) and the GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA). The assumptions of normal distributions were checked for all the variables (Shapiro–Wilk test) and data did not have a Gaussian distribution. The median and interquartile range (IQR) for enamel surface loss and enamel surface roughness were calculated. Multiple comparisons were performed using Kruskal–Wallis, followed by Student–Newman–Keuls *post-hoc* for intergroup comparisons. The difference between soy based and non-soy based beverages was assessed using the Mann–Whitney test. Spearman's rank correlation was used to correlate the ESL and the $\Delta Ra_{E\text{-}S}$ with initial pH, TA for pH 5.5 and 7, β, inorganic composition (Pi, Ca^{2+} and F^-), DS with respect to FAp and Hap, and the protein concentration of soy and non-soy beverages. The level of significance established was 5%.

Results

Table 1 summarized that the pH of the beverages ranged from 2.93 (grape juice) to 4.23 (Soy peach juice) with the non-soy juices having the lowest pH values (2.93–3.40; $p = .035$). All tested beverages had initial pH values below 5. The TA to 5.5 and to 7.0, as well as the buffering capacity, did not demonstrate difference between soy-based and non-soy juices ($p > .05$). The concentration of inorganic phosphate, calcium and fluoride ions vary between the soy-based and the non-soy based juices; however, the statistical analysis did not show any significant difference. Although the juices with soy in their composition tend to have high DS when compared with non-soy based beverages ($p = .0571$), all beverages under study were undersaturated with respect to both HAP and FAP (DS < 1). The average of protein concentration was respectively 0.015 and 0.003 for soy-based and non-soy based juices ($p = .000$).

Figure 1 demonstrates that soy beverages produced less ESL than non-soy beverages ($p < .05$). Besides, the ESL among non-soy based beverages was not different from the positive control (Coca-Cola®).

Figure 2 shows that the $\Delta Ra_{E\text{-}S}$ was not significantly different between soy and non-soy beverages, except non-soy strawberry juice, which exhibited greater $\Delta Ra_{E\text{-}S}$ value than the control ($p < .001$).

The ESL and $\Delta Ra_{E\text{-}S}$ were positively correlated with initial pH and buffering capacity in soy-based beverages. On the

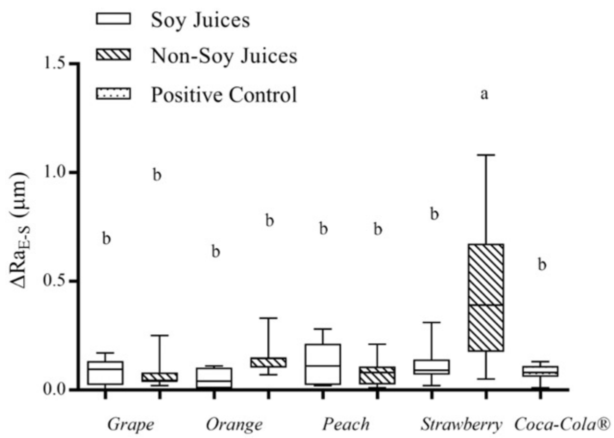

Figure 2. Difference between surface roughness (△RaE-S) of sounded (RaS) and eroded (RaE) enamel according to each experimental beverage (n=8). Distinct letters are statistically different by Kruskal-Wallis followed by Student-Newman-Keuls test ($p<0.05$).

Table 2. Enamel mineral loss and $\Delta Ra_{E\text{-}S}$ correlations according initial pH, TA for pH 5.5 and, BC and inorganic composition of soy and non-soy beverages.

	Soy beverages				Non-soy beverages			
	Mineral loss		$\Delta Ra_{E\text{-}S}$		Mineral loss		$\Delta Ra_{E\text{-}S}$	
	ρ	p	ρ	p	ρ	p	ρ	p
pH	.54	.00*	.42	.02*	−.04	.82	.34	.06
TA 5.5	−.05	.79	.05	.78	−.12	.53	−.37	.04*
TA 7.0	−.05	.79	.05	.78	−.48	.00*	−.12	.53
β	.81	.00*	.44	.02*	.10	.60	.00	.99
Pi	−.48	.00*	−.08	.66	−.10	.60	.00	.99
Ca^{2+}	−.11	.55	−.28	.13	−.22	.24	.28	.13
F^{-}	.48	.00*	.08	.66	−.37	.04*	−.28	.13
DS HAp	.13	.46	.37	.03*	.54	.00*	.42	.02*
DS FAp	.13	.46	.37	.03*	.54	.00*	.42	.02*
Protein concentration	.41	.02*	−.02	.89	.80	.00*	.43	.01*

other hand, in non-soy beverages, the ESL was negatively correlated with the titratable acidity to 7.0 and the fluoride composition whereas the $\Delta Ra_{E\text{-}S}$ was negatively correlated with the titratable acidity to 5.5 – Table 2.

Discussion

The present *in vitro* study investigated the influence of soy addition into commercial acidic beverages on the dental erosion using human tooth enamel as the substrate. Here, it was evaluated the erosive effect of soy and non-soy juices on the enamel surface, considering physicochemical factors related to the beverages such as pH, TA, buffering capacity, and composition of calcium, phosphate, and fluoride. Given this context, the interesting finding of our study is that although all the tested beverages present erosive potential, fruit juices with soy extract in their composition promoted less dental erosion when compared to the beverages without soy. Besides, it was demonstrated that some chemical aspects of soy and non-soy beverages can act modulating the enamel erosion.

The effect of acidic beverages on the enamel erosion was quantified thought non-contact profilometry considering the ESL and the $\Delta Ra_{E\text{-}S}$. These analyses provide a highly sensitive and accurate quantification of the reduction of mineral content plus the changing on surface texture after the erosive challenger [22]. In this study, it was evidenced that soy beverages produced less ESL than non-soy beverages ($p < .05$), and that the ESL of non-soy beverages did not differ of the positive control (Coca-Cola®). On the other hand, despite the absence of significant structural difference between soy and non-soy based beverages, the 3D surface roughness measurements give an indication of more pronounced erosive wear in specimens submitted to non-soy based erosive challenge. These results can be explained by the variable chemical composition of commercial beverages can provide a susceptible environment to the erosion process when in contact with the enamel surface.

All analyzed beverages had an initial pH below the critical level (5.5) for HAp demineralization. Thus it was not surprising that both the soy and non-soy added drinks investigated in the present study caused enamel surface loss, and this can be attributed to the acidic compounds such as citric, malic and phosphoric acid found naturally or added to these drinks [12,13,22]. Besides, our experiment demonstrated that initial pH and buffering capacity was correlated with ESL and $\Delta Ra_{E\text{-}S}$ in soy beverages whereas this tendency cannot be observed in non-soy beverages. Thus, this phenomenon demonstrates that the enamel dissolution rate surrounding the enamel is complex and signal that the erosive potential of these commercial products can vary over a wide range according to the predominant composition.

It is important to notice that the erosive potential of acidic solutions can be influenced by either titratable acidity or acid type (citric acid – tribasic, malic acid – dibasic, lactic acid – monobasic) [12]. In a previous study, a significant correlation was observed between the titratable acidity, buffering capacity and enamel erosion after exposure to a soft drink [23]. Regarding the TA to 5.5 or 7.0, our experiment finds correlations between TA 7.0 and ESL, and between TA to 5.5 and $\Delta Ra_{E\text{-}S}$ in non-soy beverages, which represent the experimental groups with greater erosion. In light of this, it should be pointed out that the titratable acidity is a better indicator of erosive potential at longer challenges, while the pH is a more relevant parameter in shorter challenges [23,24].

Considering the inorganic concentration of calcium, phosphate and fluoride, all beverages that were undersaturated with respect to HAp and FAp can produce erosion (DS $<$ 1). In other words, the ionic product activity of the tested beverages was lower than the product of solubility of HAP and FAP. In this perspective, our research demonstrated that although the composition of calcium, phosphate and fluoride vary among the tested beverages, the majority of the soy-based juices had high levels of calcium and phosphate. Thus, the ionic strength of calcium and phosphate reduces the CpH for enamel demineralization. It has been shown that calcium addition to acidic beverages can reduce their erosive potential [3,8,25,26]. Soluble calcium salts and calcium phosphates should have an anti-erosive effect by increasing the calcium concentration gradient within the immediate environment of the tooth, thereby slowing down the dissolution of the HAp of the tooth tissue [3].

The present study further confirmed the evidence of the high inorganic composition reduces the ability of the solutions to demineralize enamel, preventing enamel erosion [3]. Thus, the high concentration of electrolytes (calcium, phosphate and fluoride) raise the DS of HAp, and consequently, providing a reduction of the critical pH. However, the comparison between the orange juice with and without soy counterpose this statement, mainly because their non-soy based version had high levels of fluoride, calcium and phosphate, low CpH and more pronounced enamel mineral loss. In this context, it should be pointed out that in the oral environment, the interactions between some electrolytes and proteins are critical to the ability of formation of a protective pellicle against erosive challenge [26]. Besides, it was demonstrated experimentally that the protein concentration modifies the kinetics of HAp demineralization, changing the chemical properties of solutions or affecting the surface energy of HAp [27,28]. In our investigation, the protein concentration is higher in the group of soy-based juices ($p = .000$). Therefore, it is hypothesized that the protein concentration in each beverage is an important factor to prevent dental erosion. In this regard, is it possible that the soy extract targeted the tooth surface and exert some protective effect? Future studies should be conducted to test this theory.

Given the laboratory nature of the present study, the influence of biological factors (saliva characteristics, acquired pellicle and surrounding soft tissues) and behavioral (frequency of exposure, dietary lifestyle and oral hygiene practice) related to dental erosion was not evaluated. Additionally, the erosive challenge did not consider the damage caused by the combination of acid challenge and abrasion, also did not consider the repair processes *in vivo*. Despite the limitations of this *in vitro* study, the results suggested that the erosive potential of processed juices, which are often consumed by children and teenagers, should be brought to the knowledge of the public. This research further supports the fact that the increasing prevalence of erosion is associated with the high consumption of these drinks [29–31].

Conclusions

The erosive potential of commercially soy beverages was lower than non-soy based beverages, which is probably explained by the innate composition and the chemical behavior of these acidic beverages.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Brazilian National Council for Scientific and Technological Development (CNPq) [139146/2013-0].

ORCID

Elis Janaína Lira Dos Santos http://orcid.org/0000-0002-0324-3863
Ingrid Andrade Meira https://orcid.org/0000-0002-3631-0030
Emerson Tavares De Sousa http://orcid.org/0000-0002-4009-8597
Bennett Tochukwu Amaechi https://orcid.org/0000-0002-5097-4466

References

[1] Larsen MJ. Chemical events during tooth dissolution. J Dent Res. 1990;69:575–580; discussion 634–576.
[2] Lussi A, Schlueter N, Rakhmatullina E, et al. Dental erosion – an overview with emphasis on chemical and histopathological aspects. Caries Res. 2011;45:2–12.
[3] Shellis RP, Featherstone JD, Lussi A. Understanding the chemistry of dental erosion. Monogr Oral Sci. 2014;25:163–179.
[4] Lussi A, Ganss C, editors. Erosive tooth wear. Vol. 25, Monogr Oral Sci. Basel: Karger; 2014, p. 74–98.
[5] Schlueter N, Luka B. Erosive tooth wear – a review on global prevalence and on its prevalence in risk groups. Br Dent J. 2018; 224:364–370.
[6] Jager DHJ, Vieira AM, Ligtenberg AJM, et al. Effect of salivary factors on the susceptibility of hydroxyapatite to early erosion. Caries Res. 2011;45:532–537.
[7] Salas MM, Nascimento GG, Huysmans MC, et al. Estimated prevalence of erosive tooth wear in permanent teeth of children and adolescents: an epidemiological systematic review and meta-regression analysis. J Dent. 2015;43:42–50.
[8] Hara AT, Zero DT. Analysis of the erosive potential of calcium-containing acidic beverages. Eur J Oral Sci. 2008;116:60–65.
[9] Cochrane NJ, Cai F, Yuan Y, et al. Erosive potential of beverages sold in Australian schools. Aust Dent J. 2009;54:238–244.
[10] Lussi A, Megert B, Peter Shellis R, et al. Analysis of the erosive effect of different dietary substances and medications. Br J Nutr. 2012;107:252–262.
[11] Beltrame APCA, Noschang RAT, Lacerda DP, et al. Are grape juices more erosive than orange juices? Eur Arch Paediatr Dent. 2017;18:263–270.
[12] Shellis RP, Barbour ME, Jesani A, et al. Effects of buffering properties and undissociated acid concentration on dissolution of dental enamel in relation to pH and acid type. Caries Res. 2013;47:601–611.
[13] Brito JS, Santos Neto A, Silva L, et al. Analysis of dental enamel surface submitted to fruit juice plus soymilk by micro x-ray fluorescence: in vitro study. Sci World J. 2016;2016:8123769.
[14] Badger TM, Ronis MJ, Hakkak R, et al. The health consequences of early soy consumption. J Nutr. 2002;132:559S–565S.
[15] Lussi A, Jaeggi T. Chemical factors. Monogr Oral Sci. 2006;20:77–87.
[16] Brown HM, Rydqvist B. Effect of pH, ionic strength, and Arsenazo III concentration on equilibrium binding evaluated with Ca^{2+} ion-sensitive electrodes and absorbance measurements. Biophys J. 1981;36:117–137.
[17] Chen PS, Toribara TY, Warner H. Microdetermination of phosphorus. Anal Chem. 1956;28:1756–1758.
[18] Martínez-Mier EA, Cury JA, Heilman JR, et al. Development of gold standard ion-selective electrode-based methods for fluoride analysis. Caries Res. 2011;45:3–12.
[19] Shellis RP. A microcomputer program to evaluate the saturation of complex solutions with respect to biominerals. Comput Appl Biosci. 1988;4:373–379.
[20] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–254.
[21] Jain S, Gupta A, Jain D. Estimation of sample size in dental research. Int Dent Med J Adv Res. 2015;1:1–6.
[22] Field J, Waterhouse P, German M. Quantifying and qualifying surface changes on dental hard tissues in vitro. J Dent. 2010;38:182–190.
[23] Zero DT, Lussi A. Erosion-chemical and biological factors of importance to the dental practitioner. Int Dent J. 2005;55: 285–290.

[24] Jensdottir T, Bardow A, Holbrook P. Properties and modification of soft drinks in relation to their erosive potential in vitro. J Dent. 2005;33:569–575.
[25] Mita H, Kitasako Y, Takagaki T, et al. Development and evaluation of a low-erosive apple juice drink with phosphoryl-oligosaccharides of calcium. Dent Mater J. 2013;32:212–218.
[26] Stefański T, Postek, Stefańska L. Possible ways of reducing dental erosive potential of acidic beverages. Aust Dent J. 2014; 59: 280–288.
[27] Jensdottir T, Holbrook P, Nauntofte B, et al. Immediate erosive potential of cola drinks and orange juices. J Dent Res. 2006;85: 226–230.
[28] Baumann T, Kozik J, Lussi A, et al. Erosion protection conferred by whole human saliva, dialysed saliva, and artificial saliva. Sci Rep. 2016;6:34760.
[29] Kosoric J, Hector MP, Anderson P. The influence of proteins on demineralization kinetics of hydroxyapatite aggregates. J Biomed Mater Res A. 2010;94:972–977.
[30] Gurgel CV, Rios D, de Oliveira TM, et al. Risk factors for dental erosion in a group of 12- and 16-year-old Brazilian schoolchildren. Int J Paediatr Dent. 2011;21:50–57.
[31] Okunseri C, Okunseri E, Gonzalez C, et al. Erosive tooth wear and consumption of beverages among children in the United States. Caries Res. 2011;45:130–135.