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ORIGINAL SCIENTIFIC ARTICLE

Are grape juices more erosive than orange juices?

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Abstract

Aims To evaluate the chemical characteristics of grape and orange juices, and their erosive potential in the decrease of microhardness and the loss of enamel structure.

Methods Five grape and orange juices were evaluated for pH, titratable acidity, calcium, phosphate, and fluoride concentration. De-ionised water and Cola soft drink were used as a negative and positive control, respectively. Twelve specimens of bovine enamel were immersed in beverages for 10 min at 37 °C, 3 times/day for 7 days. Erosive potential was quantified using microhardness and loss of enamel structure. Anova One Way, Student's *t* test, Multiple Regression and Spearman Correlation ($p < 0.05$) were used to analyse the results.

Results Powdered grape juice showed the lowest pH (3.18 ± 0.03) and pure grape juice presented the highest titratable acidity (5.48 ± 0.06 mL NaOH/100 mL). Fresh orange juice and soya-based grape juice revealed the lowest calcium (0.77 ± 0.12 mmol/L) and phosphate concentrations (0.35 ± 0.06 mmol/L), respectively. Among juices, powdered orange juice caused the greatest decrease in surface microhardness (SMH) (127.99 ± 40.47 ΔSMH) and grape juice from concentrate caused the greatest loss of enamel structure (13.30 ± 3.56 µm).

Conclusions All of the evaluated juices contributed to dental erosion. Grape juices presented greater erosive potential than orange juices. Pure, powdered and concentrated grape juices showed similar loss of enamel structure to the Cola soft drink. The erosive potential of beverages was statistically correlated to pH, titratable acidity, calcium, phosphate and fluoride concentrations.

Keywords Dental erosion · Chemical properties · Fruit juices

Introduction

Dental erosion is defined as the dissolution of dental tissue by a chemical process without bacteria (Imfeld 1996). Epidemiological studies show a prevalence up to 79% in 5-year-old children (Mantonanaki et al. 2013) and 75% in 12-year-old teenagers (Zhang et al. 2014). High consumption of soft drinks is the most important aetiological factor (Corrêa et al. 2011; Murakami et al. 2011; Chrysanthakopoulos 2012; Kumar et al. 2013), but high consumption of citric fruits (Abu-Ghazaleh et al. 2013; Kumar et al. 2013) and fruit juices (Okunseri et al. 2011; Chrysanthakopoulos 2012; Nayak et al. 2012; Zhang et al. 2014; Kitasako et al. 2015) is also related with dental erosion.

In vitro studies have shown high erosive potential of soft drinks in enamel (Sales-Peres et al. 2007; Borjian et al. 2010), with a loss of microhardness of up to 78% (Sales-Peres et al. 2007). Besides soft drinks, fruit juices also have a high erosive potential. Some studies have shown that these juices can be more erosive than soft drinks because they have a higher titratable acidity (Edwards et al. 1999; Jensdottir et al. 2005) and a higher loss of microhardness in enamel (Lussi et al. 1995). On the other hand, other studies have shown soft drinks with a higher titratable acidity

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(Jensdottir et al. 2006; Ehlen et al. 2008) and a higher erosive potential related to enamel roughness (Fujii et al. 2011). Regarding the erosive potential, some authors have not observed any differences between juices of the same fruit and different forms of preparation, such as from concentrate and powdered (Gonçalves et al. 2012).

Recently the variety of juices has increased greatly. Pure juices, ready to drink, are similar to fresh juices because they are prepared with natural fruit and they do not have preservatives. Soya-based juices have the fruit's pulp and are enriched by soya protein and other vitamins and minerals. Powdered juices are prepared using the dehydrated fruit's pulp and they are fortified with some vitamins and minerals. They are easy to prepare and cheaper than other juices. In addition, the juices from concentrates have lower quantities of fruit pulp, artificial aroma and dye. Of all the juices, only pure juices do not have preservatives. The others have natural and chemical preservatives (as stated on the packaging of juices).

There are some important characteristics that define the erosive potential of foods and beverages: type of acid (West et al. 2000; Hannig et al. 2005), pH, titratable acidity, calcium, phosphate and fluoride concentrations (Lussi et al. 1993, 2012; Jensdottir et al. 2005). Hannig et al. (2005) verified that different types of acid interfere differently in the loss of minerals from dental tissues. The lower the pH, the higher the titratable acidity and the higher the erosive potential related to loss of microhardness in enamel (Lussi et al. 1993). The higher the calcium, phosphate and fluoride concentration, the lower the erosive potential related to loss of enamel weight, loss of calcium ions (Jensdottir et al. 2005), and loss of microhardness in enamel (Lussi et al. 2012).

Due to the variety in the forms of preparation of juices available in the market, it is important to know the characteristics that can contribute to their erosive potential and their erosive behaviour in dental tissues. The aims of this study were to evaluate pH, titratable acidity, calcium, phosphate and fluoride concentrations of grape and orange fruit juices, compare these juices to themselves and with a cola soft drink, and also to evaluate the decrease in microhardness and loss of enamel tissue.

Materials and methods

In the present study, five different forms of preparation of grape and orange juices that contained tartaric acid and citric acid, respectively, were analysed. These 10 juices were compared with Coca-Cola® (positive control) and deionised water (negative control) (Table 1). This study was in two parts: chemical and erosive analysis potential of beverages in bovine enamel.

Beverage preparation

The pure juices, soya-based juices and juices from concentrates were ready for consumption. The fresh juices were prepared with fresh fruits. To prepare the fresh orange juice only the fruit pulp was used. To prepare the fresh grape juice, 200 mL of filtered water was added to 100 g of fruit pulp. Powdered juices were dissolved in filtered water per package instructions. Both, fresh juices and powdered juices were prepared immediately before the experiment.

Beverage chemical analysis

The pH and titratable acidity (quantity of bases added to neutralise the pH) were measured using a digital pH meter (pHS-3B, pHTek, Curitiba, PR, Brazil) in a solution with 10 mL of beverage and 90 mL of Milli-Q ultrapure water. To measure the titratable acidity aliquots of 200 µL of NaOH 0.1 M were added to the beverages in containers with magnetic stirring at room temperature until a pH of 7.0 was reached.

All beverages were analysed spectrophotometrically, to determine calcium and phosphate concentrations, in an auto-analyser (Cobas Mira Plus, Roche Diagnostics, Indianapolis, IN, USA) at 660 and 340 nm wavelengths, respectively. Fluoride concentration was measured using an ion-specific electrode (ExStik II, Extech Instruments, Nashua, NH, USA). To determine fluoride concentration, a total ionic strength adjusting buffer (TISAB) tablet was added. The calcium and phosphate concentrations were expressed in mmol/L and fluoride concentration in mg/L (ppm).

To ensure reliability to this study, all of the chemical analyses were performed for six different samples of each beverage. Instead of taking one sample of the same beverage preparation, which is the most usual methodology found in the literature, an unusual approach was used. The fresh juices were prepared using fresh fruits for each of the six separate samples of grape and orange juice, as explained above. There were five different packages of industrialised beverages that were used at the time of experiment.

Specimen preparation

Bovine incisors (124) were selected which were free of cracks when viewed stereomicroscopically at 25× magnification (Medilux, MDL-DS4-BI, Biosystems, Curitiba, PR, Brazil). After disinfection for 48 h in a 0.1% thymol solution, the crowns were separated from the roots and cut into 4 × 4 mm blocks (Isomet 1000, Buehler, Illinois, USA). The blocks were set in polyester resin (central fibreglass, Florianópolis, SC, Brazil) and the surfaces were

Table 1 The selected beverages, commercial brand, form of presentation and ingredients as listed on the beverage packaging

Form of preparation	Fruit	Commercial brand	Form of presentation	Ingredients
Fresh	Grape	N/A	Fresh juice	Grape pulp and water
Fresh	Orange	N/A	Fresh juice	Orange pulp
Pure	Grape	Aurora® (Bento Gonçalves, RS, Brazil)	Glass bottle	Grape pulp, potassium sorbate, sulphur dioxide
Pure	Orange	Macrovita® (Braço do Norte, SC, Brazil)	Plastic bottle	Orange pulp
Soy	Grape	Ades® (Unilever, São Paulo, SP, Brazil)	Box	Grape pulp, citric acid, malic acid, soya, sugar, water
Soya	Orange	Ades® (Unilever, São Paulo, SP, Brazil)	Box	Orange pulp, citric acid, malic acid, soya, sugar, water
Powdered	Grape	Tang® (Mondelez, Curitiba, PR, Brazil)	Package	Grape pulp dehydrated, citric acid, fumaric acid, ascorbic acid, tricalcium phosphate, sugar
Powdered	Orange	Tang® (Mondelez, Curitiba, PR, Brazil)	Package	Orange pulp dehydrated, citric acid, potassium citrate, ascorbic acid, tricalcium phosphate, sugar
From concentrate	Grape	Sufresh® (Wow Nutrition, Caçapava, SP, Brazil)	Box	Grape pulp, citric acid, ascorbic acid, tartaric acid, sugar, water
From concentrate	Orange	Sufresh® (Wow Nutrition, Caçapava, SP, Brazil)	Box	Orange pulp, citric acid, ascorbic acid, sugar, water
Cola soft drink	N/A	Coca-Cola® (Antônio Carlos, SC, Brazil)	Plastic bottle	Cola nut extract, phosphoric acid, sugar, sparkling water
Water	N/A	N/A	N/A	De-ionised water

NA not applicable

flattened and polished using 600, 800 and 1200 grit silicon carbide paper (Wet and Dry, 3M, São Paulo, SP, Brazil) and 1, 0.3 and 0.05 μm diamond suspension (Fortel Ltda, São Paulo, SP, Brazil) in a polishing machine (DPU 10, Struers, Denmark) with water for 1 min each. Debris was removed between each segment with ultrasonic cleaning for 5 min. The specimens were randomly divided into 12 groups ($n = 12$) and one half of each specimen (2×2 mm) was covered with nail varnish.

Study design

Before the erosive challenge, the specimens were immersed for 24 h in artificial saliva with the following formulation (Söderholm et al. 1996): 0.1 L each of 25 mM K_2HPO_4, 24 mM Na_2HPO4, 150 mM $KHCO_3$, 100 mM NaCl, and 1.5 mM $MgCl_2$. To this were added 0.006 L of 25 mM citric acid and 0.1 L of 15 mM $CaCl_2$. The pH was then adjusted to 6.7 with NaOH and the volume made up to 1 L. To avoid bacterial growth 0.05% by weight thymol was added to the artificial saliva.

After, the specimens were washed for 30 s in de-ionised water and, each group, of 12 specimens was immersed in 250 mL of new beverage for 10 min at 37 °C. These steps were repeated 3 times a day (at 9 am, 12 and 3 pm) for 7 days. The specimens were immersed in artificial saliva between beverage exposures and overnight at room temperature.

Profilometer (μm) and surface microhardness (SMH) analysis

After the nail varnish was removed with acetone, the loss of enamel structure was observed with a digital profilometer (DektakXT Entry System, Bruker, Mass, US). The needle went across the specimen at three different sites (upper, medium, lower) for 20 s, at 520 μm and maximum height. The step between the control side and the test side was measured using a software package (Vision64, Bruker, Mass, US), and the mean from the two sites was calculated. The loss of enamel structure was expressed in μm.

The Vickers microhardness was determined using an automated microhardness indenter (LM100AT, Leco®-CORP, St. Joseph, MI, USA), taking 3 indentations on the control side and 3 indentations on the test side, with 100 µm distance between each indentation using a 50 g load for 10 s. The Vickers SMH values were determined using Amh43 software (Leco®CORP, St. Joseph, MI, USA), the mean was taken between the control and the test side, and then the decrease in microhardness was calculated ($\Delta SMH = SMH_c - SMH_t$).

Statistical analysis

The data were analysed using SPSS 21 (IBM, NY, USA). Data homogeneity and normality tests were applied. ANOVA one way and Tukey were used to compare the different forms of preparation of juices with the same fruit for each one of the variables (pH, titratable acidity, calcium, phosphate and fluoride concentration, loss of enamel structure and microhardness). Student's *t* test and Mann–Whitney were applied to compare the different fruits with the same form of preparation for each of the variables cited above. Multiple regression (backward selection) was used to analyse the relationship between loss of enamel structure (dependent variable) with pH, titratable acidity, calcium, phosphate and fluoride concentrations (independent variables). Spearman correlation was used to evaluate the correlation between loss of enamel structure and microhardness. The significance level was 5%.

Results

The chemical characteristics, the decrease in microhardness, and the loss of enamel structure produced by the grape and orange fruit juices are shown in Tables 2 and 3, respectively. The chemical characteristics, the decrease in microhardness, and the loss of enamel structure for the same form of preparation and different fruits are shown in Fig. 1.

Among the fresh juices, the grape flavour displayed lower pH and lower phosphate concentrations than orange flavour. The pure grape juice also displayed lower pH and higher titratable acidity than the orange juice. The soya-based orange juice resulted in a higher titratable acidity than the grape juice. Between the powdered juices, the grape juice had lower calcium and phosphate concentrations than the orange juice. The grape juice from concentrate had a lower pH than the orange juice from concentrate. The grape juices had higher erosive potentials in relation to the loss of enamel structure than the orange juices, with the exception of the soya-based juices.

The multiple regression analysis showed that 86% of the change in loss of enamel structure was related to pH, titratable acidity, calcium, phosphate and fluoride concentration variations. Spearman correlation showed a decrease in microhardness and the loss of enamel structure ($p < 0.01$).

Discussion

The loss of tooth structure is not only strongly related to the soft drink consumption (Sales-Peres et al. 2007; Borjian et al. 2010; Murakami et al. 2011) but also to natural and artificial juice consumption (Edwards et al. 1999; Jensdottir et al. 2006). Many characteristics can increase or decrease the erosive potential of these beverages (Lussi et al. 2012; Carvalho et al. 2017). Most studies did not compare the erosive potential of different flavours and forms of preparation of juices. In the present study pH, titratable acidity, calcium, phosphate and fluoride concentrations were determined for different forms of preparation of grape and orange juices. These chemical characteristics of the juices

Table 2 The mean, standard deviation and statistical analysis of the characteristics and erosive potential of grape juices

Form of preparation	pH	Acidity mL of NaOH/100 mL	Ca mmol/L	P mmol/L	F ppm	ΔSMH	Mineral loss µm
Fresh	3.4 ± 0.0^{b}	2.2 ± 0.3^{b}	1.8 ± 0.7^{b}	1.5 ± 0.2^{bc}	0.5 ± 0.0^{b}	100.7 ± 43.2^{c}	8.6 ± 0.8^{b}
Pure	3.5 ± 0.0^{c}	5.5 ± 0.1^{e}	3.3 ± 0.3^{c}	5.3 ± 0.3^{d}	0.1 ± 0.1^{a}	70.9 ± 27.7^{b}	12.0 ± 2.0^{c}
Soya	4.3 ± 0.0^{d}	1.5 ± 0.1^{a}	3.2 ± 0.1^{c}	0.3 ± 0.1^{a}	0.1 ± 0.0^{a}	6.4 ± 10.4^{a}	0.5 ± 0.1^{a}
Powdered	3.2 ± 0.0^{a}	3.0 ± 0.1^{c}	3.4 ± 0.4^{c}	1.8 ± 0.2^{c}	0.5 ± 0.1^{b}	112.4 ± 21.5^{c}	12.1 ± 2.9^{c}
From concentrate	3.2 ± 0.0^{a}	3.4 ± 0.1^{d}	1.3 ± 0.2^{b}	1.1 ± 0.1^{b}	0.2 ± 0.0^{a}	81.0 ± 31.9^{bc}	13.3 ± 3.6^{c}
Cola soft drink	3.4 ± 0.0^{b}	1.3 ± 0.2^{a}	0.3 ± 0.3^{a}	5.8 ± 0.3^{e}	0.1 ± 0.1^{a}	100.6 ± 22.4^{c}	12.8 ± 1.9^{c}
De-ionised water	N/A	N/A	N/A	N/A	N/A	11.2 ± 25.4^{a}	0.6 ± 0.2^{a}

Ca calcium concentration, *P* phosphate concentration, *F* fluoride concentration, Δ*SMH* decrease of surface microhardness, *N/A* not applicable

Different letters in the columns indicate statistically significant differences ($p \leq 0.05$)

Table 3 The mean, standard deviation and statistical analysis of the characteristics and erosive potential of orange juices

Form of preparation	pH	Acidity mL of NaOH/100 mL	Ca mmol/L	P mmol/L	F ppm	ΔSMH	Mineral loss µm
Fresh	4.0 ± 0.2^{d}	4.2 ± 0.7^{c}	0.8 ± 0.1^{b}	3.2 ± 0.5^{d}	0.1 ± 0.0^{a}	63.3 ± 20.3^{b}	2.4 ± 0.5^{b}
Pure	3.9 ± 0.0^{c}	4.9 ± 0.1^{d}	0.9 ± 0.0^{b}	2.7 ± 0.1^{c}	0.2 ± 0.1^{a}	84.0 ± 31.0^{bc}	3.2 ± 0.7^{b}
Soya	4.2 ± 0.0^{e}	1.7 ± 0.1^{a}	3.3 ± 0.3^{c}	0.4 ± 0.0^{a}	0.1 ± 0.1^{a}	-1.5 ± 24.7^{a}	0.7 ± 0.2^{a}
Powdered	3.4 ± 0.1^{a}	3.6 ± 0.1^{b}	6.4 ± 0.4^{d}	3.4 ± 0.2^{d}	0.6 ± 0.0^{b}	128.0 ± 40.5^{d}	6.1 ± 1.2^{d}
From concentrate	3.7 ± 0.0^{b}	3.3 ± 0.1^{b}	1.0 ± 0.3^{b}	1.2 ± 0.1^{b}	0.2 ± 0.1^{a}	75.4 ± 32.2^{bc}	4.5 ± 0.7^{c}
Cola soft drink	3.4 ± 0.0^{a}	1.3 ± 0.2^{a}	0.3 ± 0.3^{a}	5.8 ± 0.3^{a}	0.1 ± 0.1^{a}	100.6 ± 22.4^{cd}	12.8 ± 1.9^{e}
De-ionised water	N/A	N/A	N/A	N/A	N/A	11.2 ± 25.4^{a}	0.6 ± 0.2^{a}

Ca calcium concentration, *P* phosphate concentration, *F* fluoride concentration, *ΔSMH* decrease of surface microhardness, *N/A* not applicable

Different letters in the columns indicate statistically significant differences ($p \leq 0.05$)

were compared to each other and to to Coca-Cola. Also, the erosive potential of these beverages, represented by the decrease in SMH and the loss of enamel structure in bovine enamel were determined.

Among the evaluated characteristics, pH seemed to be the most important characteristic related to the decrease in enamel SMH (Sales-Peres et al. 2007) and to the tooth loss of calcium (Jensdottir et al. 2006). In the present study, the lowest pH value was observed for the powdered grape juice which had the greatest decrease in SMH and loss of enamel structure. In addition, the soya-based juices and the fresh orange juice showed the highest pH and the lowest erosive potential. Sales-Peres et al. (2007) observed that the pH might have influenced the decreasing microhardness after evaluating the chemical characteristics and erosive potential of five types of soft drinks. The pH was also strongly related with tooth loss of calcium, especially in the first 30 min after acidic beverage exposure (Jensdottir et al. 2006).

With regard to titratable acidity, which is related to apatite dissolution (Larsen and Nyvad 1999) pure grape juice showed the greatest measurement and also contributed to a substantial loss of enamel structure, similar to the powdered grape juice, despite its high calcium and phosphate concentration. However these characteristics could have partially protected its erosive potential as the pure grape juice resulted in a lower decrease of microhardness than for the other drinks, except for the soya-based juices. Lussi et al. (1993) observed a noticeable decrease in enamel microhardness with grape juice, which also resulted in a higher titratable acidity.

The soya-based juice presented the lowest loss of enamel structure and the lowest decrease in microhardness. This result differs from Brito et al. (2016), where a high erosive potential was observed in a soya-based juice. However, in their study, the specimens were submitted to 15 days of erosive challenge and did not undergo remineralisation in saliva between cycles. In this study, the soya-based juices showed a high calcium concentration, the highest pH value and the lowest titratable acidity. Some authors have shown that the addition of calcium to an acidic beverage could reduce the erosive potential of the beverage (Hughes et al. 2000; Jensdottir et al. 2005). Further, the high calcium and phosphate concentration of natural yogurt could explain it's low erosive potential even with a very low pH (3.91) (Lussi et al. 2012). Although Larsen and Nyvad (1999) did not observe any difference in the titratable acidity between orange juices with or without calcium and phosphate supplement (43 and 31 mmol/L, respectively), they observed that the orange juice with supplement did not promote erosion while the orange juice without supplement promoted erosive wear similar to the soft drinks (Larsen and Nyvad 1999). In the present study, the powdered orange juice had the highest calcium concentration, but, probably, this was not enough to counter its low pH and high titratable acidity, which contributed to the greater decrease in microhardness and loss of enamel structure compared to the other orange juices.

All of the tested juices had low fluoride concentrations, although the fresh grape juice and the powdered juices showed a higher fluoride concentration because they were prepared with filtered water, which contained fluoride. This could have been the reason why fresh grape juice protected the enamel blocks even though the pH, calcium and phosphate concentrations were low, it caused lower loss of enamel structure than any of the grape juices, except for the soya-based juice. Even with low concentration, the beverages with the highest fluoride concentration (0.18–0.24 ppm) caused the lowest reduction in SMH (Lussi et al. 1995). Larsen and Nyvad (1999) did not observe any erosive wear when they evaluated a beverage with 1.8 ppm of fluoride, compared to two other beverages with similar pH and lower fluoride concentrations (0.2–0.3 ppm).

Coca-Cola's erosive potential related to the loss of enamel structure was greater than all the other orange

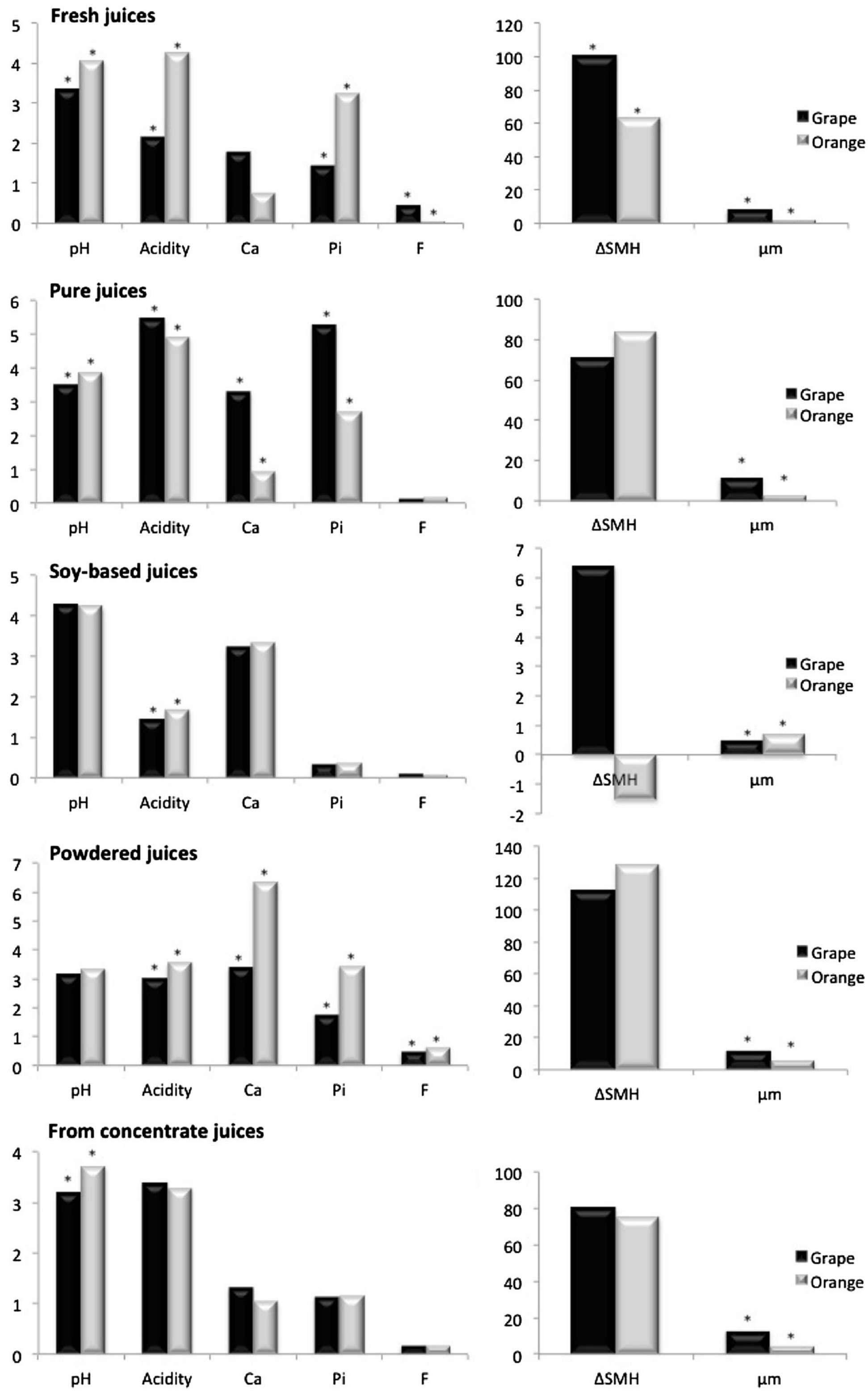

Fig. 1 Graphs of the chemical characteristics and erosive potential for different preparations of grape and orange juices. *Ca* calcium concentration, *P* phosphate concentration, *F* fluoride concentration, Δ*SMH* decrease of surface microhardness, *µm* mineral loss;*Statistically significant ($p \leq 0.05$)

juices. Grape juice (pure, powdered and from concentrate) promoted loss of enamel structure similar to Coca-Cola. Pure grape, fresh orange and soya-based juices promoted lower decrease in SMH than Coca-Cola, and all of the other juices promoted a similar decrease. In this study, Coca-Cola's erosive potential was related to its low pH and its low calcium and fluoride concentrations. This result was in agreement with Jensdottir et al. (2005), in which Coca-

Cola promoted a tenfold greater loss of calcium than the orange juices, a result attributed to the low pH value. Lussi et al. (2012) showed that even though the grape and orange juices had higher titratable acidity than Coca-Cola a greater decrease in SMH was observed.

Tartaric and citric acid are present in grape and orange fruits. Hannig et al. (2005) showed that these acids had the highest titratable acidity with pH 3.0: 5.3 mmol NaOH/L for citric acid and 2.8 mmol NaOH/L for tartaric acid except for acetic acid (36 mmol NaOH/L). In the present study, it was observed that some types of orange juices, which contain citric acid, resulted in higher titratable acidities than for the grape juices, which contain tartaric acid. However, the grape juices had greater erosive potentials for loss of enamel structure than the orange juices, probably due to their lower pH, with the exception of the soya-based juices. Hannig et al. (2005) showed that phosphate loss for both citric and tartaric acids resulted in similar results between these acids and tartaric acid was also similar to acetic acid. It is important to note that the juices analysed in this study, with the exception of the fresh and the pure juices, had other acid ingredients in addition to the fruit's natural acids (Table 1). Those additional acids contributed to the decreased pH and increased titratable acidity. As a result, the juices with additional acids (e.g. citric, tartaric, malic, fumaric, ascorbic) had better erosive potentials, with the exception of the soya-based juices.

The present study results support other studies that showed that the erosive potential of the beverages was related to a set of characteristics and not only to one single characteristic. Nevertheless, it is important to emphasise that even though the fresh and pure juices showed some erosive potential, the nutritional aspect was significant, especially when recommended for children.

Conclusions

All the evaluated juices had characteristics that contributed to dental erosion and induced a decrease in SMH and loss of enamel structure. Grape juices had greater erosive potentials than orange juices. The soya-based juices produced less loss of enamel structure. Pure, powdered and concentrate grape juices showed similar loss of enamel structure to Coca-Cola soft drink. The erosive potential of beverages had a clear relationship with the pH, titratable acidity, calcium, phosphate and fluoride concentrations. Dentists should be well informed about the erosive potential of beverages which are consumed by their child patients in order to guide them in the prevention and/or treatment of dental erosion.

Compliance with ethical standards

Conflict of interest Beltrame APCA declares that she has no conflict of interest. Noschang RAT declares that he has no conflict of interest. Lacerda DP declares that she has no conflict of interest. Souza LC declares that she has no conflict of interest. Almeida ICS declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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