

Titratable acidity of beverages influences salivary pH recovery

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Abstract: A low pH and a high titratable acidity of juices and cola-based beverages are relevant factors that contribute to dental erosion, but the relative importance of these properties to maintain salivary pH at demineralizing levels for long periods of time after drinking is unknown. In this crossover study conducted *in vivo*, orange juice, a cola-based soft drink, and a 10% sucrose solution (negative control) were tested. These drinks differ in terms of their pH (3.5 ± 0.04 , 2.5 ± 0.05 , and 5.9 ± 0.1 , respectively) and titratable acidity (3.17 ± 0.06 , 0.57 ± 0.04 and < 0.005 mmols OH⁻ to reach pH 5.5, respectively). Eight volunteers with a normal salivary flow rate and buffering capacity kept 15 mL of each beverage in their mouth for 10 s, expectorated it, and their saliva was collected after 15, 30, 45, 60, 90, and 120 s. The salivary pH, determined using a mini pH electrode, returned to the baseline value at 30 s after expectoration of the cola-based soft drink, but only at 90 s after expectoration of the orange juice. The salivary pH increased to greater than 5.5 at 15 s after expectoration of the cola drink and at 30 s after expectoration of the orange juice. These findings suggest that the titratable acidity of a beverage influences salivary pH values after drinking acidic beverages more than the beverage pH.

Keywords: Carbonated Beverages; Citric Acid; Tooth Erosion; Buffers; Saliva.

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Introduction

Dental erosion often is associated with frequent consumption of acidic beverages,^{1,2,3} of which orange juice and cola-based drinks are reported to be among the most consumed by young adults.⁴ Although both types of beverages are acidic, their pH values and their capacity to maintain a low pH, *i.e.*, the titratable acidity, are different. Cola-based soft drinks contain phosphoric acid as the main buffer, presenting a low initial pH (approximately 2.5), but a low titratable acidity in this pH range. In contrast, commercial orange juice presents a higher pH (approximately 3.5-4.0) but a high titratable acidity at this pH due to the presence of citric acid, which is naturally found in oranges.

Although it is accepted that both the pH and the titratable acidity of acidic beverages influence their erosive potential,^{3,5,6,7,8} most of the available studies have tested both effects only *in vitro*. Such experimental designs have limitations as surrogates of *in vivo* tests of erosion because in the mouth, salivary flow and buffering capacity influence the clearance rate

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of acidic beverages,⁹ which could reduce the impact of these exogenous acids.^{10,11} However, no previous study has compared the salivary pH after ingestion of acidic beverages with distinct pH values and titratable acidities.

Therefore, the aim of this study was to assess, *in vivo*, the time needed for the salivary pH to return to baseline after exposure to orange juice, which has a higher titratable acidity, compared to a cola-based soft drink, which has a lower pH.

Methodology

Experimental design

This crossover study (Figure 1) was conducted *in vivo* in three experimental phases to test the salivary pH after oral exposure to the following test solutions: 10% sucrose solution (control group, similar to the sucrose concentration found in beverages, to account for chemical stimulation of salivary flow), a cola-based soft drink (Coca Cola®, São Paulo, Brazil), and orange juice (Minute Maid®, São Paulo, Brazil). The eight participating volunteers signed a term of consent under a protocol approved by the Research and Ethics Committee of Piracicaba Dental School (#096/2008). The study was not blinded because the test solutions were identifiable through color and taste

by the volunteers, and the saliva samples with remnants of the solutions could be identified by the operator. A one-week interval was maintained between each phase. All experiments were carried out in the afternoon at the same time to avoid salivary flow variability.

Determination of pH and titratable acidity of the tested beverages

The pH of each beverage (50 mL) was determined by using a glass pH electrode (Orion 8102, Waltham, USA) coupled to a potentiometer (Procyon SA-720, Olímpia, Brazil), previously calibrated with pH 4.0 and 7.0 buffers. The titratable acidity was then measured by adding 1.0-mL aliquots of 0.1 M NaOH to each beverage until the pH reached 7.0. The amount of base (mmol) required to reach pH 5.5 and pH 6.5 in 1 L of the tested solution was calculated (Table 1). Due to the absence of weak acids in the control group, only one aliquot (50 µL) of the base was added.

Determination of salivary flow and buffer capacity of the volunteers

On three different days prior to the *in vivo* test, chewing gum-stimulated saliva was collected for 5 min from the volunteers. During the first 30 s of

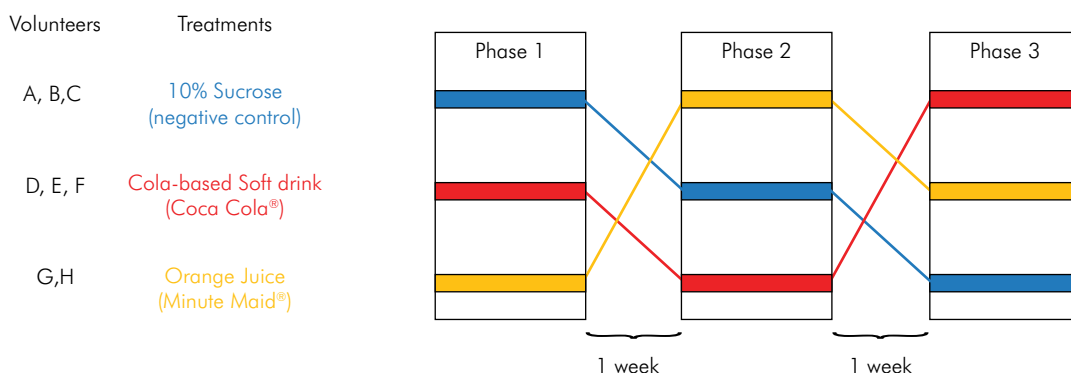


Figure 1. Schematic representation of the experimental design.

Table 1. Titratable acidity of the tested beverages (average ± standard deviation)

Beverage	pH	mmol of OH ⁻ to reach pH 5.5	mmol of OH ⁻ to reach pH 6.5
10% Sucrose (n = 5)	5.9 ± 0.1	< 0.005	< 0.005
Cola-based soft drink (n = 5)	2.5 ± 0.05	0.57 ± 0.04	1.36 ± 0.23
Orange juice (n = 3)	3.5 ± 0.04	3.17 ± 0.06	4.33 ± 0.12

chewing, the saliva was swallowed. During the next 5 min, the saliva was collected in previously weighed plastic cups. To obtain the salivary flow (mL/min), the saliva volume was determined by weighing the cups again (assuming a density of 1 g/mL).

To assess buffer capacity,¹² 0.5 mL of stimulated saliva was mixed with 1.5 mL of 5 mM HCl in a plastic tube, and the mixture was left to rest for 5 min to release CO₂. The final pH of this mixture was determined by using a pH meter as described above. The results of the three flow rate and buffer capacity tests for each volunteer were averaged.

In vivo test

Unstimulated saliva was collected from all volunteers to determine the baseline pH, before beverage consumption. The volunteers placed 15 mL of one of the beverages in their mouth; and after 10 s, the mixture of saliva and beverage was expectorated at once in plastic cups for pH determination. Saliva samples were subsequently collected at 15, 30, 45, 60, 90, and 120 s after beverage expectoration by asking the volunteers to spit the residual amount of saliva in their mouth into microcentrifuge tubes at the determined times. Between each collection, the volunteers were allowed to swallow. At each timepoint, the amount of saliva collected was small; therefore, a mini pH electrode (Cole-Parmer Accumet, model 5500-45, Vernon Hills, USA) was used to determine the pH of the samples. This electrode allows the measurement of samples with volumes as low as 0.1 mL. To avoid the effect of CO₂ loss on the sample pH, collection tubes were kept closed and the pH measurements were performed shortly after collection.

Statistical analysis

Data were statistically analyzed using analysis of variance, considering volunteers as statistical blocks. A paired t-test was used to check for differences between the baseline saliva pH and the saliva pH at each collection timepoint. The SAS system (SAS Institute Inc., version 9.2, Cary, USA) was used at a significance level of 5%.

Results

All volunteers presented a normal stimulated saliva flow rate (2.11 ± 0.56 mL/min) and 'saliva buffer capacity' (pH 5.88 ± 0.81). For the tested beverages, the initial pH and titratable acidities are shown in Table 1. Only 5 μ mol of base increased the pH of the negative control group to values greater than 7. Conversely, the titration curves of the orange juice and cola-based drink (Table 1 and Figure 2) illustrate that orange juice, although it has a higher initial pH, required a larger amount of base to reach pH 7 than the cola-based soft drink, which had a lower initial pH.

In this *in vivo* study, the initial baseline salivary pH (6.96 ± 2.09) of the volunteers was similar in all test groups (Figure 3). The pH values of the expectorated beverages (time 0 in Figure 3) demonstrate that, during the 10 s in the mouth, the beverage pH did not change noticeably, except for the negative control, whose pH increased from 5.9 (Table 1) to 6.7 ± 0.2 (Figure 3). At this timepoint, the pH values of the samples from various groups differed significantly from each other ($p < 0.05$).

The pH values of the samples returned to baseline (paired t-test, $p > 0.05$) at 30 s after expectoration of the cola-based soft drink and at 90 s after expectoration of the orange juice. The salivary pH increased to greater than 5.5 at 15 s after expectoration of the cola drink and at 30 s after expectoration of the orange juice.

Discussion

Although both the pH and titratable acidity of acidic beverages may affect their erosive potential,^{5,6,7,8} the importance of these properties on the *in vivo* salivary pH after beverage consumption is not clear. In the present study, the titratable acidity of commercial beverages was shown to affect the salivary pH for a longer time than their initial pH value. Thus, despite the lower pH of the cola-based soft drink compared to that of the orange juice, the lower titratable acidity of the former resulted in a faster neutralization of the pH due to salivary clearance and buffering. However, during intraoral exposure (while the beverage was in the mouth), the volume of residual saliva present and/or secreted was too small to induce a significant change in the beverage pH. Therefore,

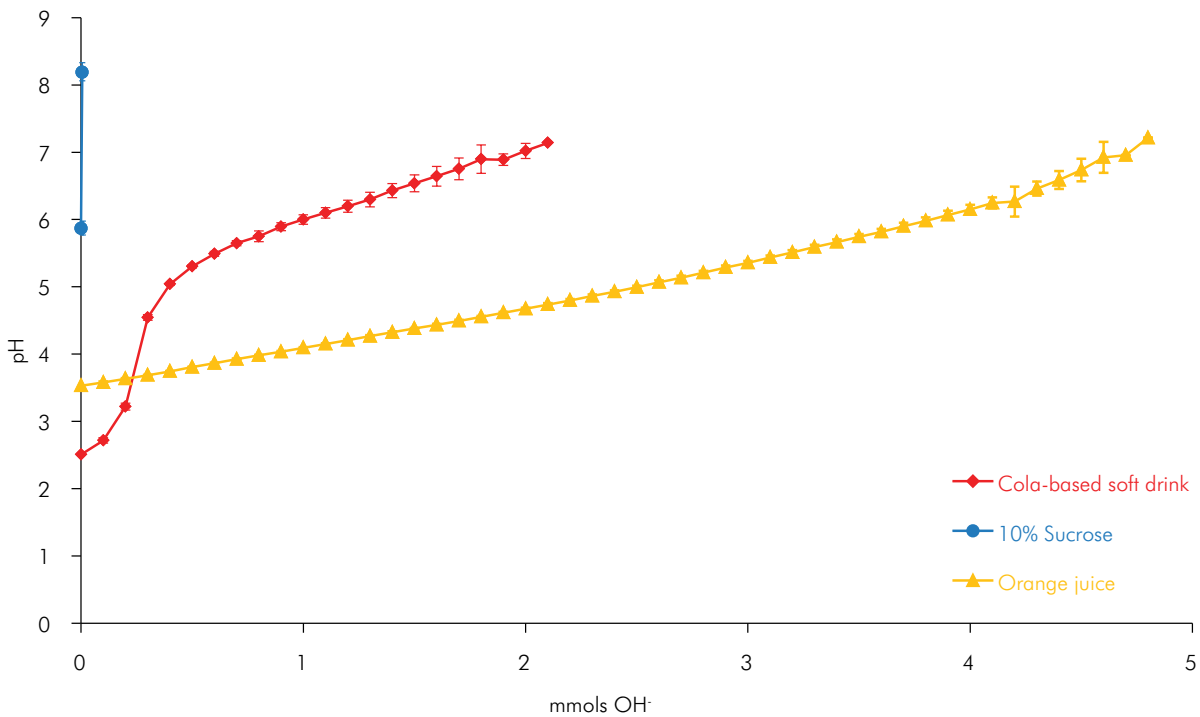


Figure 2. pH changing according to amount of mmols of NaOH added to 50 ml of beverage (mean, n = 5).

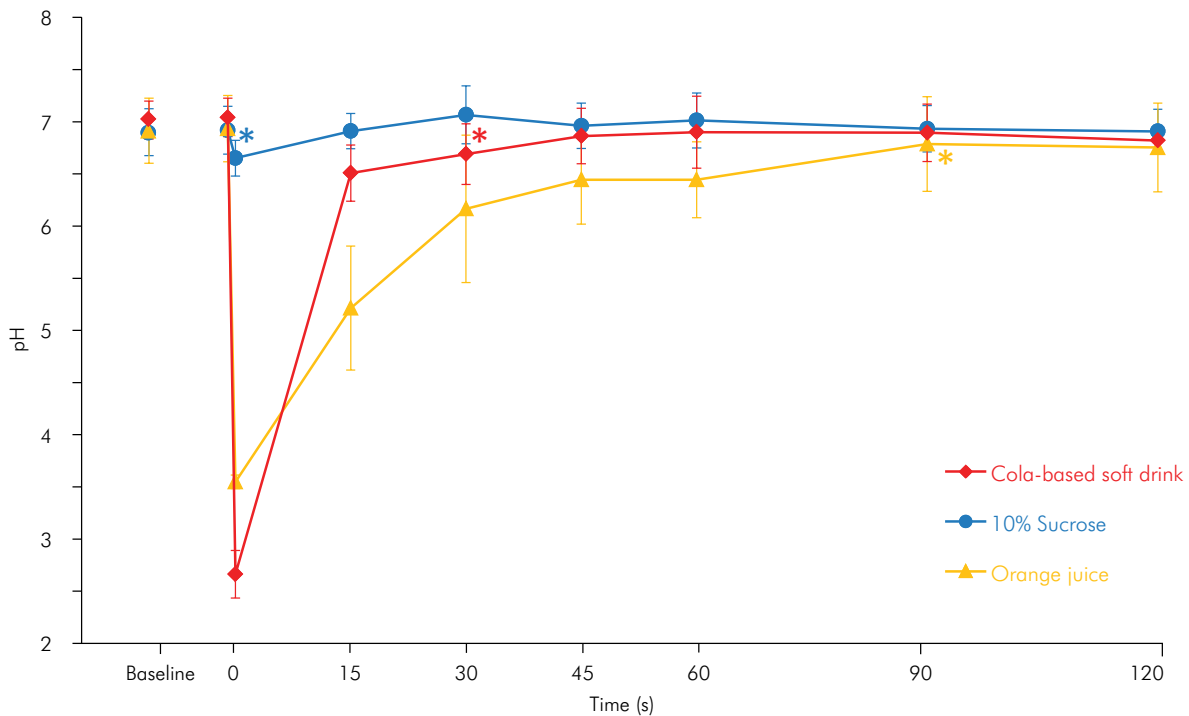


Figure 3. Saliva pH at baseline, mixed with beverage (time zero), and after expectoration of the beverage (10 to 120 s) (average \pm standard deviation; n = 8). Note, for the orange juice sample at 15 s, one outlier was removed; pH = 6.6. The asterisk represents the timepoint when the pH returned to baselines values (paired t-test, $p < 0.05$).

the beverage pH influences the erosive potential of the beverage while it is being consumed;⁵ whereas after ingestion, the titratable acidity is responsible for the time that the salivary pH is maintained at a low level in the mouth. However, in the present study, only one short exposure to the acidic beverages was simulated. Under clinical conditions in which a beverage is continuously sipped for minutes, the effects of pH and titratable acidity on the maintenance of a low salivary pH may be different, which is currently under study.

Comparisons of the erosive potential of cola-based soft drinks and citric juices (or their acid contents) are available in the literature, suggesting that both pH and titratable acidity should be considered when assessing their erosive potential.^{13,14,15} Most studies have been performed *in vitro*; therefore, the results observed may be only due to the effects of pH and titratable acidity of the drink itself, without considering salivary clearance. Moreover, in many studies assessing the erosive potential of acidic beverages^{13,16,17,18,19} or other dietary substances and medications,³ the counter effects of salivary clearance and buffering have not been considered. Our results showed that the salivary pH recovery is affected by the titratable acidity of the beverages, which should be considered in the models that study erosion.

Although this study provides useful information on the buffering effect of saliva when distinct types of commercial acidic beverages (*i.e.*, a fruit juice and a soft drink) are consumed, it was not able to

predict their erosive effects. The time needed for the pH to increase above demineralizing values (> 5.5) was short; therefore, the protective effect of the salivary pellicle may overcome the difference between beverages.²⁰ Also, only whole saliva pH was measured, without considering specific sites of dentition where different pH recovery periods might be observed.²¹ In future research, the pH of the tooth surface should be measured²² in a wide range of volunteers with different salivary flow rates and buffering capacities, and sequential sipping of acidic beverages should be evaluated to simulate clinical conditions. Moreover, clinical studies evaluating dental erosion are necessary to show the impact of titratable acidity on this process.

Conclusion

The results of the present study suggest that, after drinking one sip, the high titratable acidity of orange juice plays a more important role than the low pH of a cola-based soft drink in prolonging the effects of acid in the mouth.

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