

## Effects of lactose-containing stevioside sweeteners on dental biofilm acidogenicity

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**Declaration of Interests:** The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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DOI: 10.1590/1807-3107BOR-2014.vol28.0026  
Epub XXX XX, 2014

Submitted: Nov 19, 2013  
Accepted for publication: Mar 04, 2014  
Last revision: June 09, 2014

**Abstract:** The aim of this study was to evaluate the effect of a commercial lactose-containing stevioside sweetener on biofilm acidogenicity in vivo. Nine volunteers refrained from brushing their teeth for 3 days in five phases. On the 4th day of each phase, the pH of the biofilm was measured by the “Strip method”. Interproximal plaque pH was measured before and up to 60 minutes after a 10 mL mouthrinse for 1 minute with the test solutions: I - sweetener with 93% lactose and 7% stevioside; II - sweetener with 6.8% saccharin, 13.6% cyclamate, and 0.82% stevioside; III - 18% sucrose solution (positive control); IV - mineral water (negative control); and V- 93% lactose solution. The results revealed that the most pronounced pH fall was found with sucrose (positive control), followed by the 93% lactose solution, the sweetener with lactose + stevioside, the sweetener with saccharin + cyclamate + stevioside, and finally water (negative control). According to the area under the curve, the two sweeteners containing stevioside were significantly different, and the sweetener with lactose + stevioside was significantly different from water but not from sucrose. The critical pH for dentin demineralization (pH ≤ 6.5) was reached by all volunteers after rinsing with sucrose solution, lactose solution, and the stevioside + lactose sweetener. Analysis of the data suggests that lactose-containing stevioside sweeteners may be cariogenic, especially to dentin.

**Keywords:** Cariogenic Agents; Sweetening Agents; Dental Plaque.

### Introduction

Sucrose is considered the most cariogenic carbohydrate.<sup>1</sup> Searches for alternatives to sucrose have resulted in the development of artificial sweeteners, many of which are considered safe for teeth, such as aspartame, saccharin, cyclamate, xylitol, and manitol.<sup>2,3,4,5</sup> These sweeteners have been used as sugar substitutes for caries-active patients. Non-cariogenic natural products of plant origin have been discovered and accepted for general use to sweeten foods and beverages. Stevioside, a natural sweetener extracted from *Stevia rebaudiana*, offers particular advantages over other non-caloric sucrose substitutes in being heat-stable, resistant to acid hydrolysis, and non-fermentable.<sup>6</sup> Additionally, a caries-preventive action of stevioside extract can be related

to its antimicrobial properties. *In vitro* studies have shown reduced numbers of biofilm-viable cells and polysaccharide formation.<sup>7,8,9,10</sup>

However, commercial products contain several components and formulations that might influence their cariogenicity. These sugars and their derivatives have been added to sweeteners as excipient agents. There is evidence that saccharin, cyclamate, and xylitol are not cariogenic,<sup>11</sup> while lactose shows some cariogenic properties.<sup>12</sup> Commercial products containing lactose plus aspartame show decreased surface microhardness similar to that with lactose solution.<sup>13</sup> A recent *in vitro* study also showed that a commercial aspartame sweetener containing lactose promotes significantly higher biofilm acidogenicity and higher counts of viable *S. mutans* cells than a commercial stevioside sweetener containing lactose.<sup>9</sup> The antimicrobial effect of stevioside could influence the cariogenic properties of commercial sweetening products. There is no *in vivo* study analyzing the cariogenic potential of commercial stevioside products containing lactose. Therefore, the aim of this study was to evaluate the effect of commercial stevioside sweeteners on dental biofilm acidogenicity. Our hypothesis was that commercial sweeteners containing lactose plus stevioside cause less pH drop than with lactose solution.

## Methodology

### Study population

Nine dentists or dental students from the *Universidade Federal do Rio Grande do Sul* (ages 20-31 yrs) participated in the study. Inclusion criteria were that they were in good general and dental health and had not used antibiotics for the 2 months preceding the beginning of the study. The protocol for the current study was approved by the Ethics Committee of the Faculty of Dentistry from the *Universidade Federal do Rio Grande do Sul* (process n° 300/08). Informed and written consent was obtained from all individuals.

### Study design

The experiment involved a randomized crossover, double-blind design performed in 5 phases. All participants and the examiner were blinded to the rinses used in each phase. Both crossover and blind-

ing procedures were performed by an operator not involved in the experimental protocol. The rinsing solutions were: (I) sweetener containing 93% lactose, 7% stevioside, and silicon dioxide as an anti-wetting agent (SóStevia, Lowçucar®, Marialva, Brazil), dissolved in deionized distilled water at the ratio of 0.8 g (content of a packet) per 50 mL; (II) sweetener containing 6.8% saccharin, 13.6% cyclamate, and 0.82% stevioside, water, and sodium benzoate preservative (Steva Plus, Lowçucar®, Marialva, Brasil), dissolved in deionized distilled water at the ratio of 6 drops per 50 mL; (III) 18% sucrose (positive control), with 2 teaspoons of sugar dissolved in 50 mL deionized water; (IV) mineral water (negative control); and (V) 93% lactose, which corresponds to 46.5 g of lactose diluted in 50 mL of water. These ratios correspond to a sweetness power of two teaspoons of sucrose used to sweeten a volume of 50 mL (Brazilian small cup of coffee). All participants had refrained from brushing their posterior teeth for 3 days before the experimental rinsing (solutions I to V) but maintained normal oral hygiene for anterior teeth with a conventional dentifrice (> 1000 ppm F). The participants did not receive dietary instructions, since the study followed a crossover design, and all participated in all steps. The participants were instructed not to eat or drink anything at least 1 hour prior to each test session (4<sup>th</sup> day). A 7-day washout period was performed. During the washout periods, the participants brushed their teeth with a conventional dentifrice (> 1000 ppm F). They were not instructed about brushing frequency during the washout period and before the experimental rinsing.

### Calibration

One examiner performed all pH measurements. Intra-examiner calibration of the pH indicator strips was performed with standard solutions. The pH values corresponding to the pH scale of the strips (pH 4.0, 5.0, 6.0, 7.0) were prepared with an ion-specific electrode, Orion 9609 (Orion Research Inc., Beverly, USA), connected to an ion analyzer SA-720 (Procyon Instrumentos Científicos, São Paulo, Brazil). The measurements were performed blinded. The ability of the examiner to detect the pH of the solutions with the use of the indicator strips was measured.

The measurements were performed blinded (pre-coded solutions).

**Test session and plaque pH measurement**

At the 4<sup>th</sup> day, the plaque pH and its response to the respective treatment solutions were determined. The pH measurement was performed with the new so-called “Strip method”<sup>14</sup> based on the usage of pH indicator strips (Merck®, Darmstadt, Germany – pH 4.0-7.0). Briefly, the strips were cut into 4 slices (± 2 mm in width) and inserted into approximal sites during 10 s. The pH value (one decimal) was assessed by comparison of the color of the strip with the color index guide supplied by the manufacturer. The resting pH (0 minute) and the pH curve were measured at 2 approximal sites, between the 2<sup>nd</sup> pre-molar and the 1<sup>st</sup> molar on the right and left sides of the upper jaw.<sup>15</sup> Plaque pH was measured at baseline (0 minute) and at 5, 15, 20, 30, and 60 minutes. Rinsing with solution (10 mL) was performed during the first minute. Relative isolation was performed to avoid saliva bias in the plaque pH.

**Statistical analyses**

Intra-examiner ability to interpret the pH indicator strips was analyzed by the Intra-Class Correlation Coefficient. The mean pH values of the two approximal sites (right and left sides) were calculated.

The mean pH curves, resting pH, minimum pH, and time-point of maximum pH decrease were also calculated. The areas under the curve for pH 6.5 (AUC<sub>6.5</sub>) were determined with the UTHSCA Image Tool computer program (UTHSCSA®, San Antonio, USA). Data from the time-point of maximum pH decrease and AUC<sub>6.5</sub> were analyzed by the Kruskal-Wallis test, followed by the Mann-Whitney test. Data from resting, final, and minimum pH were analyzed by ANOVA. The significance limit was set at 5%. The analyses were performed with the statistical package SPSS 17.0 for Windows (SPSS Inc., Chicago, USA). Power analysis was calculated with the Web site www.openepi.com, and the results of area under the curve at pH 6.5 were compared between the two sweeteners.

**Results**

Intra-examiner ability to reveal the pH of the standard solutions by means of the pH indicator strips was very good (Intra-Class Correlation Coefficient 0.7).

The mean pH curves of the dental biofilm and the AUC<sub>6.5</sub> for the 9 volunteers, obtained with the 5 test solutions, are shown in Figure 1. The most pronounced pH fall was found with sucrose (positive control), followed by the lactose solution, the sweetener with lactose + stevioside, the sweetener with saccharin

**Table 1.** Mean ± SD value of resting pH, final pH, minimum pH, time-point of maximum pH decrease, and areas under the curve for pH 6.5 (AUC 6.5) at the approximal sites in 9 individuals.

	Treatments				
	Lactose + stevioside	Saccharin + cyclamate + stevioside	Sucrose	Water	Lactose
Resting pH	6.91 ± 0.18 <sup>a</sup>	6.85 ± 0.31 <sup>a</sup>	6.90 ± 0.20 <sup>a</sup>	6.98 ± 0.07 <sup>a</sup>	6.61 ± 0.48 <sup>a</sup>
Final pH	7 ± 0 <sup>a</sup>	7 ± 0 <sup>a</sup>	6.89 ± 0.27 <sup>a</sup>	7 ± 0 <sup>a</sup>	6.94 ± 0.17 <sup>a</sup>
Minimum pH	5.77 ± 0.38 <sup>c</sup>	6.31 ± 0.24 <sup>a</sup>	5.24 ± 0.57 <sup>b</sup>	6.58 ± 0.24 <sup>a</sup>	5.71 ± 0.39 <sup>bc</sup>
Time-point of max. pH Decrease					
Mean ± SD	10 ± 6.12 <sup>a</sup>	9.44 ± 6.82 <sup>a</sup>	11.67 ± 5 <sup>a</sup>	6.11 ± 3.33 <sup>a</sup>	7.14 ± 5.67 <sup>a</sup>
Median	5	5	15	5	5
Quartiles (1 <sup>st</sup> - 3 <sup>rd</sup> )	5 - 15	5 - 15	5 - 15	5 - 5	5 - 10
AUC <sub>6.5</sub> Mean	1.0 ± 0.73 <sup>a</sup>	0.16 ± 0.21 <sup>b</sup>	2.03 ± 1.46 <sup>a</sup>	0.03 ± 0.07 <sup>c</sup>	1.44 ± 1.27 <sup>a</sup>
Median	0.88	0.07	2.03	0.0	1.17
Quartiles (1 <sup>st</sup> - 3 <sup>rd</sup> )	0.65 - 1.21	0.01 - 0.31	1.00 - 2.30	0.0 - 0.0	0.47 - 2.40

Values in the same row with the same letter are not significantly different (p > 0.05)

+ cyclamate + stevioside, and, finally, water (negative control). Accordingly, the area under the curve ( $AUC_{6.5}$ ) showed the greatest value with sucrose and the smallest with water ( $p < 0.001$ ;  $AUC_{6.5}$ ) (Table 1). The  $AUC_{6.5}$  values for the two sweeteners containing stevioside were significantly different ( $p = 0.005$ ), and the sweetener with saccharin + cyclamate + stevioside was also significantly different from both lactose ( $p = 0.037$ ) and sucrose ( $p = 0.001$ ). The  $AUC_{6.5}$  for the sweetener with lactose + stevioside was not different from the  $AUC_{6.5}$  of sucrose ( $p = 0.145$ ) and lactose ( $p = 0.626$ ). Water was significantly different from all other groups ( $p < 0.05$ ). The statistical power for comparison of the results of the area under the curve at pH 6.5 between the two sweeteners was 91.27%.

The mean time-point of maximal pH fall varied from 6.11 to 11.67 minutes among the test solutions. For all solutions, pH had returned to the baseline level after 60 minutes (Table 1).

The majority of the volunteers showed a pronounced pH fall ( $pH \leq 5.5$ ) after the sucrose rinse. Two of them presented pH falls below the critical pH for enamel demineralization after rinsing with the stevioside + lactose sweetener, and three showed similar pH falls after rinsing with the lactose sweetener. No drop to  $pH \leq 5.5$  was observed with the stevioside + saccharin + cyclamate sweetener. Regarding pH drop equal to or lower than pH 6, only one participant reached these values after rinsing with saccharin + cyclamate + stevioside, while almost all participants reached these values after rinsing with lactose and lactose + stevioside.

## Discussion

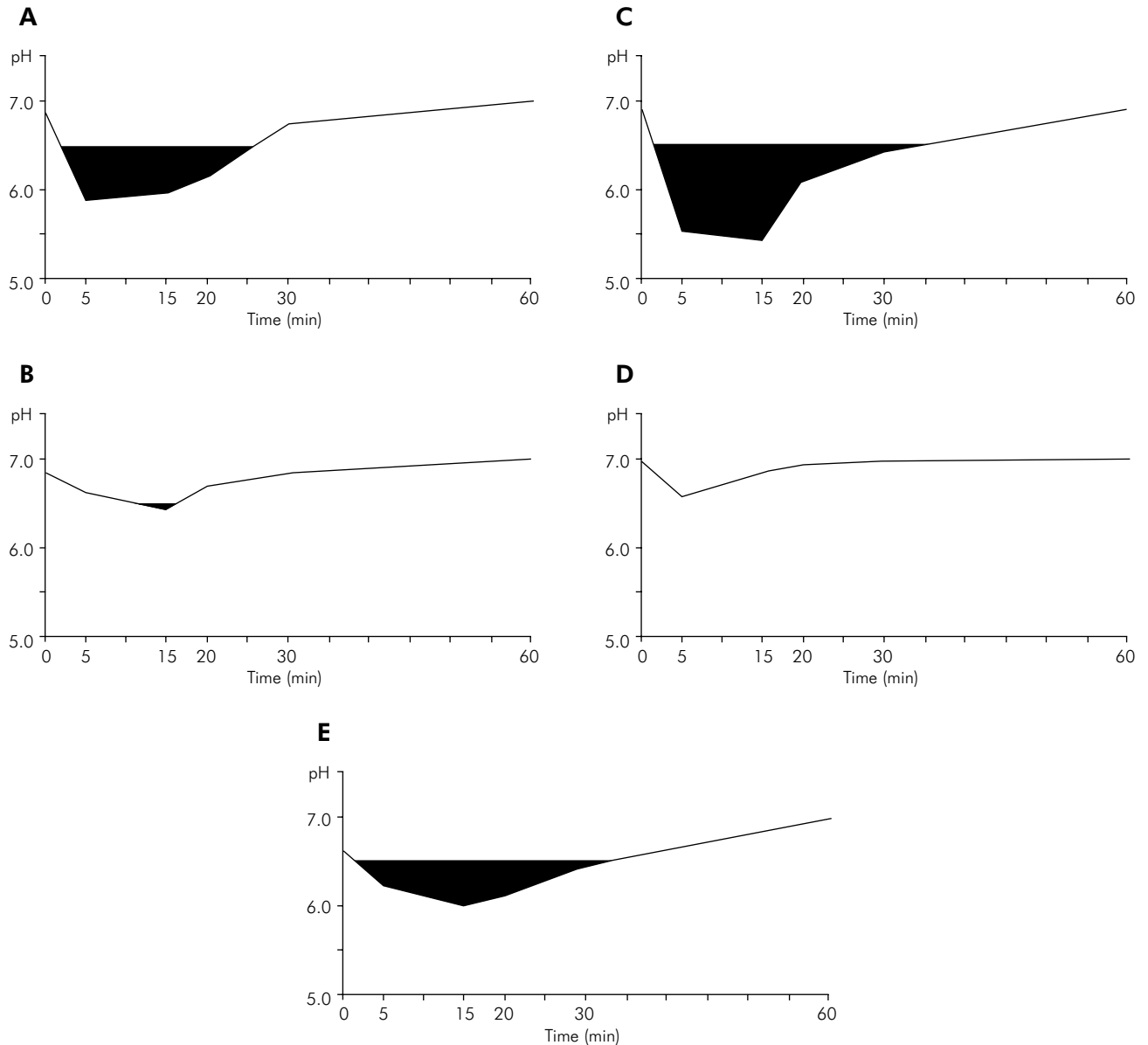
In the present study, it was found that commercial stevioside sweeteners containing lactose as the excipient agent showed an  $AUC_{6.5}$  greater than that of those containing non-fermentable sweeteners such as saccharin and cyclamate. The mean  $AUC_{6.5}$  value from the stevioside sweeteners containing lactose was similar to that reached after participants rinsed with 18% sucrose and 93% lactose solutions. These findings reject our hypothesis, showing that the commercial sweetener containing lactose plus stevioside does not cause less pH drop than the lactose solution.

The pH measurements were performed by the newly introduced “strip method” for plaque-pH examinations. This method has been shown to give approximate plaque-pH values equal to those obtained with the microtouch method in individuals after rinsing with 10% sucrose.<sup>14</sup> The fact that both the strip method and the microtouch method disrupt the biofilm and may remove some biofilm from the interproximal site could be a limitation. However, Lingström *et al.*<sup>15</sup> evaluated plaque pH measurements by the telemetric method, which uses a pH electrode under an undisturbed biofilm, and by the microtouch method and found a similar trend in the pH curves between the two methods. To minimize possible biofilm disruption, pH measurements were limited to 6 time-points. The pH strip used in this study (pH 4.0 to 7.0) has a smaller color shift for pH intervals  $> 6$  than in the pH 4 to 6 intervals.<sup>14</sup> This could result in a less precise assessment for higher pH values. However, in this study, the cutoff point for AUC analysis was 6.5 (Figure 1).

Stevioside is heat-stable, resistant to acid hydrolysis, and non-fermentable for oral bacteria.<sup>16</sup> Therefore, it is considered to be a safe product for teeth.<sup>6</sup> However, due to the fact that this intense sweetener is  $\geq 300$  times sweeter than sucrose, it has been used in very small quantities and mixed with other sweeteners or their derivatives in foods.<sup>11</sup> In the present study, the commercial product containing stevioside mixed with the artificial substitutes saccharin and cyclamate showed pH falls almost similar to those with the negative control (water). The small but significant difference observed in the AUC between these two solutions could be due to other components present in the commercial formulation, such as the preservative sodium benzoate, which has bacteriostatic and fungistatic properties under acidic conditions.<sup>11,17</sup>

Lactose is a fermentable carbohydrate, although it is less cariogenic than sucrose.<sup>2,13,18</sup> Our findings confirmed that sucrose can cause pronounced pH drops in the dental plaque,<sup>19</sup> reaching the critical pH for both dentin (6.5) and enamel (5.5). Similar results have been observed in a study with a lactose-containing nitroglycerin tablet that also produced a marked pH drop in dental plaque.<sup>18</sup>

The sweetener with lactose seems to be safe for enamel but could cause pH drops in dental plaque<sup>18</sup>



**Figure 1.** Mean pH drop of the following solutions: (A) sweetener with 93% lactose and 7% stevioside; (B) sweetener with 6.8% saccharin, 13.6% cyclamate, and 0.82% stevioside; (C) 18% sucrose solution; (D) water; and (E) 93% lactose solution. Dark areas represent  $AUC_{6.5}$ .

and demineralize dentin *in situ*.<sup>13</sup> This is consistent with our findings of a more pronounced pH fall ( $AUC_{6.5}$ ) for the lactose-containing sweetener compared with that for the stevioside sweetener containing saccharin and cyclamate. The stevioside extract present in the commercial stevioside sweetener containing lactose was unable to promote reduced biofilm acidogenicity after a 1-minute rise *in vivo*, since no difference in pH drop was found between this commercial sweetener and the

93% lactose solution. A previous *in vitro* study has also shown that a commercial stevioside sweetener containing lactose promoted significantly less biofilm acidogenicity and fewer viable cells than a 10% sucrose solution.<sup>9</sup> However, the present *in vivo* study found that the commercial stevioside sweetener containing lactose promoted dental biofilm acidogenicity ( $AUC_{6.5}$ ) similar to that of the 18% sucrose solution, despite differences in the minimum pH values.

## Conclusion

Analysis of the data showed that the cariogenic potential of different commercial stevioside sweeteners differs. The commercial stevioside sweetener containing 93% lactose may be cariogenic, especially to dentin.

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## Acknowledgements

The authors are not professionally affiliated with any corporation. We thank Lowçucar®, the sweetener company, for product donations, the *Universidade Federal do Rio Grande do Sul* (UFRGS), and all volunteers for their dedication.