ORIGINAL ARTICLE

Sour Gummy Candies and their Effect on Salivary pH kinetics

Patrícia Rezende dos Reis Oliveira[1], Flávia Lucisano Botelho Amaral[2], Fabiana Mantovani Gomes França[2], Roberta Tarkany Basting[2], Cecilia Pedroso Turssi[2]

[1]Master's Student, Division of Cariology and Restorative Dentistry, São Leopoldo Mandic Institute and Dental Research Center, Campinas, SP, Brazil.
[2]Assistant Professor, Division of Cariology and Restorative Dentistry, São Leopoldo Mandic Institute and Dental Research Center, Campinas, SP, Brazil.

Author to whom correspondence should be addressed: Cecilia Pedroso Turssi, Instituto e Centro de Pesquisas Odontológicas São Leopoldo Mandic, Rua José Rocha Junqueira, 13, Campinas, SP, Brazil. 13045-755. Phone: +55 19 3211-3774. E-mail: cecilia.turssi@slmandic.edu.br.

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Abstract

Objective: This randomized controlled crossover clinical trial monitored the kinetics of salivary pH over time following the consumption of sour gummy candy. **Material and Methods:** Twenty participants underwent saliva assessment for flow, pH and buffer capacity. Following a two-arm crossover layout, the participants chewed a piece of a sour and a piece of an ordinary (control) gummy candy for 20 seconds. Participants expectorated saliva at 18 time points: immediately after ingesting the candies; then after every 15 second interval, for up to 1 minute; 30 seconds up to 4 minutes; 60 seconds up to 10 minutes; and at 15 minutes. The pH of the collected samples was measured with a pH microelectrode. The data concerning the pH measurements of the whole saliva samples collected over time following chewing of sour and ordinary gummy candies underwent repeated-measures three-way analysis of variance (ANOVA) using a significance level of 5%. **Results:** Repeated-measures three-way analysis of variance demonstrated a significant interaction between the type of candy and time (p<0.001). Tukey's test revealed that with the consumption of sour gummy candy, the salivary pH showed an initial marked exponential drop and remained lower than that observed with the consumption of the ordinary version for up to 120 seconds. **Conclusion:** The consumption of sour gummy candy induces a major, transient fall in salivary pH, which may represent a risk factor for dental erosion.

Keywords: Saliva; Candy; Hydrogen-Ion Concentration; Tooth Wear.

Introduction

Epidemiological studies have indicated that dental erosion is a condition of growing concern that is prevalent in all age groups. Usually researchers have reported that it is present in more than one third of children, adolescents and adults [1-3]. The manifestation of dental erosion is primarily due to non-bacterial acid substances and is regarded as a multifactorial process, triggered by the interplay among chemical, biological and behavioral factors [4]. In fact, the erosive potential of acidic beverages and foodstuffs have been related to their pH, titratable acidity, calcium-chelation ability and adhesiveness [5]. In addition, development and progression of erosive lesions by such extrinsic sources of acid depend on behavioral aspects like the frequency of consumption [5].

To counteract the challenges posed by chemical and behavioral aspects, saliva, as a biological factor, exerts a multitude of important functions against dental erosion [6]. The protective mechanisms played by saliva includes dilution, clearance and neutralization of the erosive agent, formation of the acquired pellicle and mineral deposition on tooth structure [6]. However, the level of protection provided by saliva may not be suffice to prevent the formation and progression of erosive lesions [7].

As part of the initiatives to comprehensively understand the etiology and thereby identify preventative and therapeutic strategies to control dental erosion, an abundance of studies has assessed the erosive potential of acidic beverages. Among such studies are those that focused on monitoring salivary pH after the consumption of acidic drinks, which have demonstrated that saliva pH requires one to fifteen minutes to be recovered [8-10].

In contrast to acidic beverages, there has been scarce research examining the erosive potential of sour foodstuff. If one considers that the greater the adherence of an acidic substance is, the longer the contact time with the tooth surface and the higher the likelihood of erosion [11,12] and that some sour foodstuffs like gummy candies possess high tack to tooth surface, therefore, it seems imperative to obtain a better knowledge about the erosive potential of such confectioneries. This demand gains even more relevance if one considers three further facts: first, in two laboratory investigations sour candies have been shown to be more erosive than acidic drinks, such as orange juice [12,13], whose erosiveness has been extensively proven [14-16]. Second, the findings of an in vitro study have suggested that saliva may offer reduced protection to enamel against the erosive effects of sour candies [17]. Third, sour candies have been significantly associated with dental erosion in children [18] and adolescents [19] in a frequency-dependent manner. In the former quoted survey, children who consumed sour candies more than twice daily, once daily and 2-4 times per week presented, respectively, nearly 24, 18 and 8 times higher risk of having dental erosion compared with those who did not eat sour candies at all.

In view of the aforementioned factors, it would be valuable to elucidate the underlying physiopathological processes associated with the consumption of sour gummy candies. Toward this aim, this randomized controlled crossover clinical trial was undertaken to monitor the kinetics of salivary pH over time following the intake of a sour gummy candy. The null hypothesis tested was

that intraoral pH would not be lower after consumption of sour gummy candies in comparison to ordinary counterparts.

Material and Methods

Study Outline

This was a randomized controlled crossover clinical trial with both observational and interventional components. The observational side evaluated flow, pH and buffering capacity of unstimulated and stimulated saliva in 20 participants. The interventional side evaluated the salivary pH of the participants after ingestion of sour and ordinary (control) gummy candies over time. All participants, considered as a statistical block, were offered both candies at distinct times, thus characterizing the trial as a two-arm crossover design (Figure 1).
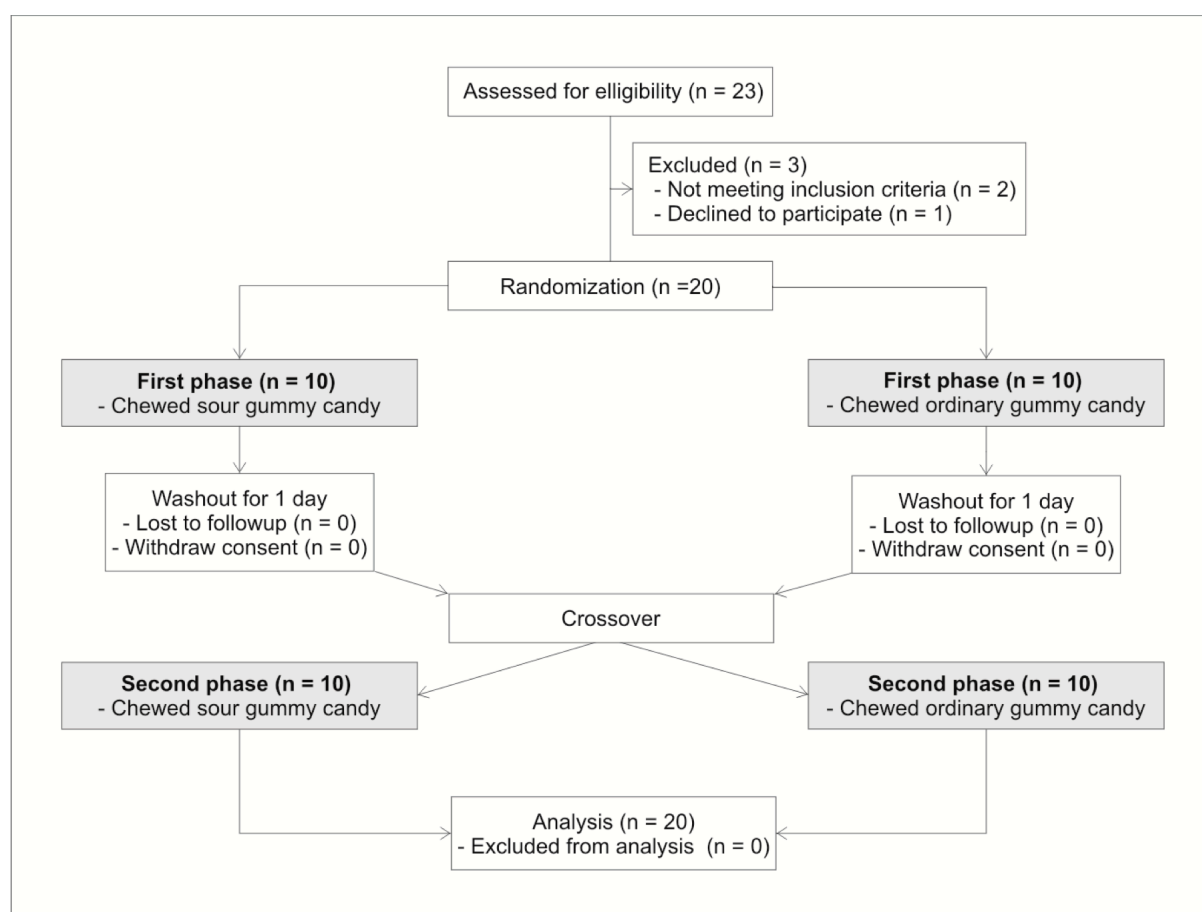

Figure 1. Flow chart of the experiment.

Participants and Ethical Aspects

Twenty participants (5 men and 15 women) ranging from 18 to 32 years of age volunteered for this study after it was approved by the local Institutional Review Board (#2012/0324). Informed consent was obtained from all participants prior to their entry into this study. Volunteers were eligible if they exhibited good oral hygiene condition, no tooth wear lesions, no caries activity, no periodontal disease or reflux disease and showed mean stimulated saliva flow rate ≥ 0.7 mL/min.

Ineligible were subjects wearing removable orthodontic appliances and those taking medicines, as were those with systemic diseases. Smokers and patients suffering from alcoholism were not recruited. Women who were pregnant or breastfeeding were not included as well.

Flow Rate, pH and Buffering Capacity of Saliva

Two days prior to saliva collection, participants were required to use only the toothpaste (Colgate Maximum Anti-caries Protection, 1450 ppm of fluoride as MFP, Colgate-Palmolive Industrial Ltda., São Bernardo do Campo, SP, Brazil) and soft-bristled toothbrush (Oral B Indicator Plus, Procter & Gamble, São Paulo, SP, Brazil) provided, while refraining from using any other oral product.

Saliva was collected between 8:30 and 11:30 a.m. All volunteers abstained from eating, drinking and oral hygiene for 2 hours prior to collection. Once seated upright in a chair, the volunteers relaxed for 5 min and were instructed to make as few movements as possible, including swallowing, during the collection. All volunteers contributed one sample of unstimulated and stimulated whole saliva. Before the collection, disposable cups were weighed electronically (ASF11, Marte Ltda., São Paulo, SP, Brazil).

For unstimulated saliva, volunteers were instructed to seat with their heads slightly down and drain their saliva into one of the pre-weighed disposable cups as passively as possible. After 5 min of collection, the disposable cup was reweighed. The flow rate was calculated in g/min, which is equivalent to mL/min. After collection, unstimulated saliva was also evaluated for pH, measured using a 3-mm diameter calomel microelectrode (EW-55500-45, Accumet Cole-Parmer, Chicago, IL, USA) connected to a digital pH meter (W3B, Bel Engineering, Piracicaba, SP, Brazil).

To estimate the saliva buffering capacity, 3 mL of a 5 mM HCl solution was added to 1 mL of whole saliva. The sample was vortexed and allowed to stand for 10 min to eliminate CO_2. The pH of the saliva/HCl mixture was measured using the aforementioned calomel microelectrode.

After collecting the unstimulated saliva, volunteers were instructed to chew for 30 seconds on a 5 x 5 cm piece of paraffin. The saliva produced during this time was then swallowed before the collection was started. During the next 5 min, in which the chewing was resumed, saliva was spat out at short intervals into the pre-weighed disposable cups. Samples of stimulated saliva were also measured for flow rate, pH and buffering capacity.

Candy Consumption and Monitoring of Salivary pH

The sour candy used in this study had fumaric acid to provide sourness (pH 2.09). The composition of the ordinary candy (pH 3.07) was identical except that it did not contain fumaric acid. In the first arm, according to a computer-generated randomization list, half of the volunteers (n = 10) was randomly allocated to consume a piece of sour gummy candy, while the remainders (n = 10) consume a piece of the ordinary version. Immediately after consuming the designated candies, the participant spat their saliva into a funnel placed over a conical tube with a screw-on lid. This sample

was considered baseline (t0). Further aliquots of saliva were then collected every 15 seconds until one minute (t15, t30, t45, t60), every 30 seconds for the next 4 minutes (t90, t120, t150, t180, t210, t240), every 60 seconds up to 10 minutes (t300, t360, t420, t480, t540, t600), and finally after 15 minutes had lapsed (t900), therefore totaling 18 samples. The pH of each aliquot was measured using a calomel microelectrode (EW-55500-45, Accumet), attached to a digital pH meter (Model W3B, Bel Engineering). This protocol has been employed elsewhere 12. After a 2-day washout period, participants were crossed over to the second arm of the study to ingest the alternate gummy candy. Monitoring of salivary pH followed exactly the same procedures as those specified in the arm 1.

Statistical Analysis

The data concerning the pH measurements of the whole saliva samples collected over time following chewing of sour and ordinary gummy candies underwent repeated-measures three-way analysis of variance (ANOVA) using a significance level of 5%. Statistical calculations were performed on SPSS 20 (SPSS Inc., Chicago, IL, USA).

Results

Repeated-measures three-way ANOVA demonstrated a significant interaction between the type of gummy candy consumed and time ($p < 0.001$). Figure 2 illustrates that after the consumption of sour gummy candy the salivary pH showed an initial marked exponential drop, which was succeeded by a pH rise. The pH was recovery to a value above the assumed critical pH for enamel (5.5) and dentin (6.2-6.5) after 30 and 60 seconds, respectively. The pH rose to a steady state plateau, where saliva was recovery to basal pH values. Table 1 shows that with the ingestion of the sour gummy candy, the salivary pH was significantly lower than in the control group (ordinary gummy candy) for up to 120 seconds. Fifteen seconds after the ingestion the ordinary gummy candy, the salivary pH was already above the assumed critical pH for dentin, showing a rapid return to its basal value (Figure 2).
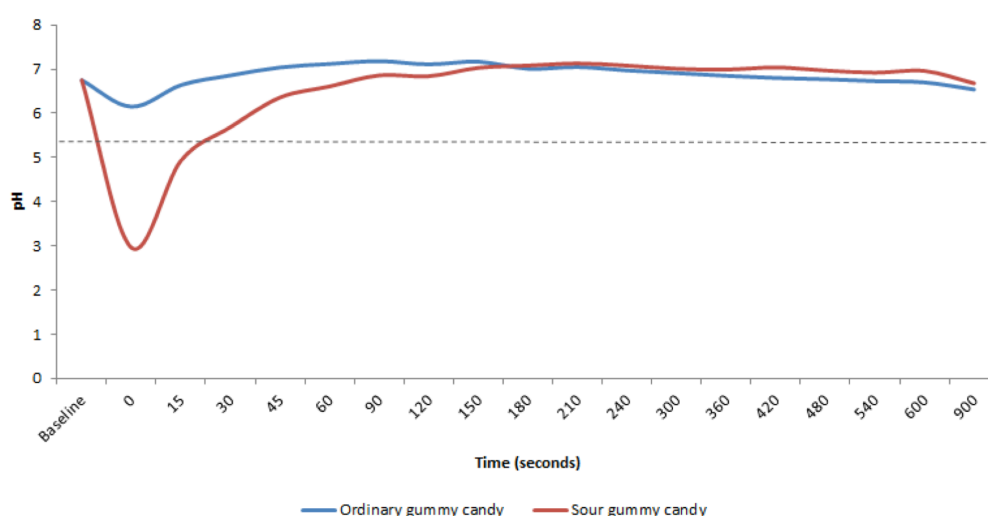

Figure 2. Line chart of the salivary pH kinetics during the consumption of regular and sour gummy candies.

Table 1. Salivary pH kinetics during the consumption of regular and sour gummy candies.

Time (Seconds)	Sour candy	Ordinary candy
0	2.96 (0.22) [b]	6.15 (0.51) [a]
15	4.93 (1.36) [b]	6.64 (0.64) [a]
30	5.69 (1.07) [b]	6.86 (0.45) [a]
45	6.36 (0.87) [b]	7.04 (0.47) [a]
60	6.61 (0.89) [b]	7.12 (0.42) [a]
90	6.86 (0.57) [b]	7.18 (0.35) [a]
120	6.84 (0.54) [b]	7.11 (0.33) [a]
150	7.03 (0.53) [a]	7.17 (0.24) [a]
180	7.08 (0.39) [a]	7.01 (0.25) [a]
210	7.13 (0.36) [a]	7.05 (0.28) [a]
240	7.08 (0.43) [a]	6.97 (0.33) [a]
300	7.01 (0.36) [a]	6.91 (0.29) [a]
360	6.99 (0.35) [a]	6.85 (0.31) [a]
420	7.04 (0.35) [a]	6.80 (0.27) [a]
480	6.97 (0.38) [a]	6.77 (0.26) [a]
540	6.92 (0.44) [a]	6.73 (0.28) [a]
600	6.96 (0.43) [a]	6.70 (0.28) [a]
900	6.68 (0.38) [a]	6.54 (0.34) [a]

Means followed by different superscript letters indicate a significant difference within the same row.

Discussion

The comprehension of the erosive potential of beverages and foodstuffs under conditions that allow saliva to exert its protective roles represents an important step in advancing research into the physiopathological process involved in dental erosion. This randomized controlled crossover clinical trial was designed to move a step forward towards the understanding of the salivary pH kinetics after the consumption of sour candies, whose erosive potential have been demonstrated in an observational survey [18,19] and in laboratory experimental investigations [17,20].

Although sour and ordinary candies can provide mechanical and gustatory stimuli for saliva [21], which remarkably increases its buffer capacity, the hypothesis tested in this study was rejected. Our findings showed that during the consumption of the sour candy there was a sharp exponential drop in salivary pH. In fact, salivary pH reached values as low as 2.96 immediately after the consumption of the sour candy, while with the ordinary version pH was 6.15. Therefore, despite the slight difference in the pH values found for the sour and ordinary candies (2.09 and 3.07, respectively), a substantial difference in the kinetics of the salivary pH was noticed as a result of the consumption of such candies. This finding may be essentially attributed to the presence of fumaric acid in the sour gummy candy, which may have buffer capacity higher than citric acid. In fact, a concern related to fumaric acid is the fact that its erosive potential has been proven to be higher than that of citric acid [22].

In contrast to the salivary pH observed during the consumption of the sour candy, with the consumption of the ordinary version of the gummy candy there was only a slight drop in salivary pH, which was likely caused by the presence of citric acid used as a preservative in the ordinary version. Shortly after the ingestion of the ordinary candy, salivary pH got back to the basal values (Figure 2).

After the ingestion of the sour candy, it was seem a gradual increase in salivary pH but in comparison to the values observed after the consumption of the ordinary candy, the sour version provided a significant decrease in salivary pH over 120 seconds (Table 1). This time was twice that needed for saliva to clear, dilute and neutralize orange juice in a previous study using the same methodology [11]. In the current study, 30 seconds after consuming the sour gummy candy, the salivary pH was above the assumed critical pH for enamel (5.5). On the other hand, it took 15 seconds for saliva to reach a pH higher than 5.5 when orange juice was consumed. Apart from the different type of the acid contained in the sour candy and in the orange juice, this finding may be ascribed to the fact that the sour candies remain for a more prolonged time on tooth surface than the orange juice.

It was not until 60 seconds had lapsed after ingesting the sour gummy candy that the salivary pH returned above the critical pH for dentin dissolution. Therefore, it should be considered that in situations of dentin exposure in the oral cavity, damage would be more pronounced.

Owing to the fact that there is solid evidence that orange juice causes erosion, if one considers that saliva demands twice the time to be recovery after the consumption of sour candy, it seems reasonable to point out that the current findings confirmed previous in vitro investigations indicating that sour candies are more erosive than orange juice [12,13].

It is appropriate to highlight that in this study, only one sour gummy candy was consumed on only one occasion, whereas normally several candies would be ingested. This would cause frequent prolonged drops in intraoral salivary pH, leading to a more erosive scenario. Therefore, further studies are required that focus specifically on confirming the effect of quantity and frequency of sour gummy candy consumption on intraoral pH and tooth surface loss.

This in vivo study was carried out using young adult volunteers but children and adolescents are the mainly consumers of sour candies. As in children the volume of saliva [23,24], salivary calcium content [25] and salivary pellicle thickness [26] are reduced in comparison to adults, one may speculate that the consumption of sour candies will result in an more erosive scenario as it would be more challenging for saliva to exert its protective roles. Another issue that warrants investigation is the impact of the consumption of sour gummy candies on tooth surface rather than on whole saliva. That is because residues of sour gummy candies are expected to remain for longer periods on tooth surface than in whole saliva.

Overall, understanding the pH kinetics of saliva following the intake of sour gummy candies may offer perspectives not only for elucidating the physiopathological processes associated with the consumption of such confections, but also for developing preventative measures in the clinical setting.

Conclusion

The substantial, transient drops in intraoral pH after consumption of sour gummy candies may represent a risk factor for dental erosion and may suggest the need to establish health education and preventative strategies against the risk of wearing teeth away.

Acknowledgments

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