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Original Article

Buffer Capacity of Saliva as a Function of Time after Consumption of Sugary, Sugar-Free and Probiotic Chewing Gums

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Abstract

Objective: To determine the time required for pH buffering by saliva after use sugary(S), sugar-free (SF) and probiotic (P) chewing gums. **Material and Methods:** Saliva was collected from 12 volunteer dental students at UNESP São José dos Campos / SP, in order to determine salivary flow (SR) rate and initial buffering capacity (BC). Participants presenting $BC \geq 4.0$ were invited to continue the research. Participants chewed different types of gum for 3 consecutive days, and saliva was collected at 0-1min, 1-5min, and 5-10min intervals. The time required to neutralize saliva pH after chewing the different types of gum was analyzed by RM ANOVA and Tukey's test (5%). **Results:** RM ANOVA revealed significant influence on the interaction effect (chewing gum and time) (statistic $F_{df(4,66)} = 4.027$, $p = 0.0055 < 0.05$). According to Tukey's test, differences were observed in the following circumstances: for the 0-1 interval, BC of S differs from SF and P; BC of S differs from SF at 1-5 min and 5-10 min intervals; and, 0-1min interval differs from 1-5 min and 5-10 min intervals for both S and SF. **Conclusion:** Dentistry students showed no increased predisposition to dental caries with a specific type of chewing gum. Although time for pH recovery differed according to gum type, they were all above the critical range for enamel demineralization.

Keywords: Sugar; Saliva; Chewing Gum.

Introduction

Saliva presents a buffering system that neutralizes acids produced by acidogenic microorganisms through the action of inorganic ions (calcium, phosphate, hydroxyl, fluorides). This capacity helps the oral cavity against colonization of potentially infectious microorganisms, and thereby prevents enamel demineralization [1-3]. The buffering capacity of saliva represents one of several factors for dental caries disease. Studies have shown that the use of sugary chewing gum would increase the risk of dental caries, unlike sugar free, natural sugar compounds, xylitol and sorbitol-based gums [4].

Probiotics are defined as live microorganisms administered in adequate quantity. They benefit the health of individuals and they may be, in the near future, a preventive alternative to dental caries when added to chewing gum [5]. Probiotic gums are sugar-free and mostly composed of probiotic bacteria (*Lactobacillus bifidobacterium*). They are indicated to reduce the incidence of dental caries in association with bacteria from saliva, inhibit re-colonization of periodontal pockets by pathogens, reduce bleeding on probing and lead to lower dental plaque index in individuals [6].

Children, youth, adults and seniors make use of chewing gums for pleasure. As a result, salivary flow is increased, which contributes to digestion and to the control of dental caries [7]. Due to the importance of saliva in preventing dental caries, salivary tests (buffering capacity and salivary flow) should be included in routine tests for evaluating patients about the risk of development of the disease [8,9].

The aim of the present study was to determine the influence of probiotic chewing gum on the salivary BC of dentistry students.

Material and Methods

This research included twelve graduate students (aged 23-29 years) in Restorative Dentistry and Oral Biopathology at Unesp in São José dos Campos / SP, after approval by the local Institutional Review Board (CAAE nº14760913.2.0000.0077) and signing the written informed consent form.

Inclusion criteria were: individuals not under any kind of health treatment, individuals not taking any medication (except contraceptives), absence of active dental caries, individuals not under orthodontic treatment and not presenting temporomandibular disorder and/or periodontal disease. Volunteers with low BC were excluded (pH less than 4.0) (detected only after saliva collection).

Subjects were advised to avoid eating any food or drinking 1h before each test session [10]. Chewing gum, cigarette, and coffee were also prohibited during this period. Initially, volunteers rinsed their mouth with deionized water. They chewed latex rubber strip (1 cm long) to generate stimulated saliva. Saliva stimulated at the first minute was discarded, and in the following 5min, stimulated saliva was expectorated into a graduated cylinder [11].

Foam generated by expectoration was precipitated and converted into saliva by using one drop of Simethicone (Laboratório Teuto, Anápolis / GO, Brazil), an anti-gas drug. Salivary flow rate

in mL / min was obtained by the ratio between the total amount expectorated divided by 512. Salivary flow results were classified as normal (greater than 1 mL / min), low (less than 0.7ml / min) and xerostomia (values lower than 0.1 ml / min) [12].

After determining salivary flow, new saliva sample was collected to complete 1 mL in order to determine BC. BC of saliva was evaluated [13] by adding 1 mL of saliva to 3 ml of 0.005 M hydrochloric acid. This solution was manually stirred and allowed to stand in an open container for 10 minutes to eliminate CO₂. The final pH was measured by a previously calibrated portable digital pH meter (HI 8314, Hanna, Mauritius). The results were classified as follows: normal BC (pH between 5.0 and 7.0), borderline BC (pH between 4.0 and 5.0) and low BC (pH less than 4.0).

On the day after SR and BC determination, sugary (S) Big-Big (Arcor, Brazil), sugar-free (SF) Trident (Adams, Bauru, Brazil) and probiotic (P) Reladent (Verman, Kerava, Finland) (Chart 1) chewing gums were offered to participants on 3 consecutive days (one type of chewing gum per day). Participants were asked to chew for 10 minutes. During this period, saliva was expectorated into 3 different tubes according to periods of collection: 0-1, 1-5, 5-10 min. These assessing periods were determined based on the Stephan's curve [14], in which pH decreases during the first minute, the five minute interval is where pH reaches its maximum drop, and pH is no longer within the critical demineralization zone at the 10-minute period (Figure 1).

Chart 1. Composition of chewing gums used in this study.	
CHEWING GUM	COMPOSITION
Trident	Gum base, sweeteners Natural: Sorbitol and mannitol, humectants: glycerol and Triacetin, Flavors, Emulsifier: Soy Lecithin, Artificial Sweeteners: Aspartame, Acesulfame -K and Sucralose, Artificial dyes: Tartrazine and Blue Bright, phenylalanine
Big-Big	Sugar, glucose syrup, gum base, vegetable oil, stabilizer: soy lecithin, humectant: glycerin, dyes: Red 40 (E129), sunset yellow (E110), and indigotin (E132), flavor
Reladent	Filler (Isomalt), Lactobacillus reuteri Lactobacillus reuteri DSM 17938 and ATCC PTA 5289 (Lactobacillus reuteri Prodentis), hydrogenated palm oil, peppermint flavor, mint flavor, peppermint oil and sweetener (sucralose).

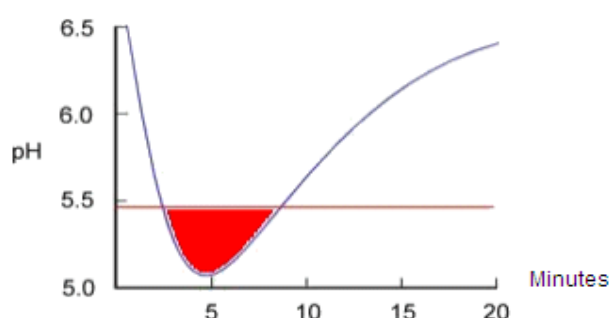


Figure 1. Stephan's curve-analysis of cariogenicity.

Collector tubes were properly identified according to each time for each participant and adapted to pH meter for immediate pH assessment after collection. At the end of 10 minutes, chewing gum was eliminated. Subsequently, analysis was performed to determine the time required

for saliva buffering according to each type of chewing gum. Descriptive statistical analysis consisted of average and standard deviation calculation and inferential analysis consisted of: (1) Repeated Measures two-way analysis of variance (RM ANOVA) (pH and time); and (2) multiple comparison of means, Tukey's test (5 %).

Results

SR values are classified as: normal ($> 1 \text{ mm} / \text{min}$); low ($< 0.7 \text{ mm} / \text{min}$) and xerostomia ($< 0.1 \text{ mm} / \text{min}$) [12]. Among the 12 volunteers, 58 % ($n = 7$) presented normal SR and 33 % ($n = 4$) borderline SR. Only 1 volunteer showed reduced SR.

Buffer capacity is low when $\text{pH} < 4.0$; borderline with pH between 4.0 and 5.0, normal with pH between 5.0 and 7.0 [13]. In relation to BC, all volunteers were classified as normal.

The RM ANOVA'S test (repeated measures ANOVA on both factors) indicated that both factors, time and chewing gum, had $p < 0.0001$ and that the interaction effect (time and chewing gum) was significant (statistic $F_{df(4;66)} = 4.027$; $p\text{-value} = 0.0055 < 0.05$).

The study of the interaction effect (pH x time) of the nine experimental conditions is shown by means of graphics. Significance level of 5 % was adopted (Figure 2).

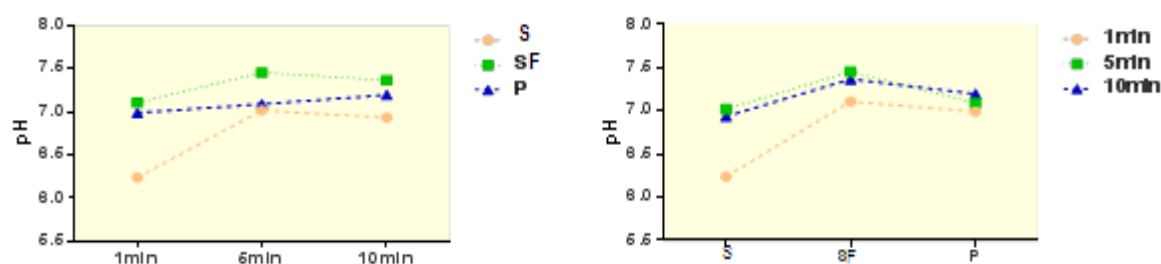


Figure 2. Graph of mean pH values, according to experimental conditions determined by the different types of gum (S = sugary; SF = sugar-free; P = probiotic) and time (0-1 , 1-5 and 5-10 min).

Statistically significant difference was observed between S and SF groups in all three periods evaluated. For P group, there was no statistically significant difference among the three periods evaluated. From the first period, probiotic, gum showed pH value close to 7.0 (neutral) followed by S and SF groups. In the 1-5 minutes interval, there was an increase in pH for S, possibly due to its lower initial pH. In the 5-10 minutes interval, pH remained stable at values close to those found in 1-5min in all groups. The pH has not reached the critical value (< 5.5) in any interval, probably because volunteers have no active caries, they have good brushing and chewed only one gum per day.

According to Tukey's test (5%), statistical differences were verified for the following cases: for the 0-1 minute interval, it was verified that BC for S differs from P and SF; and for the 1-5 and 5-10 min intervals, S differs from SF; 0-1 differs from 1-5 min and 5-10 min for both S and SF groups (Table 1).

Table 1. Mean salivary pH values according to type of chewing gum and time interval.

Chewing Gums	Intervals (min)		
	0-1	1-5	5-10
Sugar	6.24 ^{a A}	7.02 ^{a B}	6.94 ^{a B}
Sugarfree	7.11 ^{b A}	7.45 ^{b B}	7.36 ^{b AB}
Probiotic	6.99 ^{b A}	7.09 ^{ab A}	7.20 ^{ab AB}

*different capital letters mean statistical differences within lines; ** different lowercase letters mean statistical differences within columns.

Discussion

The main benefit of chewing gum for oral health is to stimulate salivary flow through gustatory and mechanical activity while chewing [6,15]. In addition to the increase in salivary flow and consequent increase in the amount of fluoride ion (found in saliva) in the oral cavity, there is interest in knowing whether the presence or absence of substances in the composition of chewing gums would also present anti-cariogenic effect, similarly to fluoride ion [16]. In this study, 58% (n = 7) had normal SR and 33% (n = 4) borderline SR. Only one volunteer showed reduced SR, but the presence of caries or injured oral mucosa has not been verified.

Low chronic salivary flow is one of the strongest salivary risk indicators for developing dental caries [10,17-19]. Oral problems resulting from hypofunction of salivary glands include: changes in oral sensitivity, tasting alteration and dryness of oral mucosa, possibly leading to infection and tooth wear due to abrasion [2,20].

Salivary BC is defined as resistance of saliva to pH changes. It is promoted by carbonate-bicarbonate, phosphate, and proteins systems, the most important buffering agent present in stimulated saliva [21,22]. The bicarbonate system is responsible for approximately 85% of salivary BC in the pH range from 7.2 to 6.8 [20,23]. The concentration of these ions is higher in saliva collected after mechanical stimulation. As salivary flow increases, the concentration of bicarbonate ions also increases, [24,25]. Saliva of individuals with considerable number of carious lesions often presents BC values lower than those who are relatively free of cavities [11,15]. All volunteers had normal BC and no active caries lesions.

In the beginning of the 1940's, it was demonstrated that the increase of caries activity was associated with decrease in in vivo plaque pH after exposure to fermentable carbohydrates. Thus, there is a direct relationship between ingestion of food, pH of the oral cavity and the onset of dental caries [15,26]. The curve (Figure 1) shows that there is a sharp drop in intraoral pH up to values close to 5.0 after food intake, whereas after 20 min, there is gradual pH recovery, reaching values close to those observed at rest. This curve plays an important role as it allows us to extrapolate knowledge for the daily clinical practice. The analysis of pH / time curve shows pH value of 5.5 as a critical value for the onset of dental caries, from early stage on enamel level to a later stage on dentin [27].

A study [28] with children aged 8-10 years living in region with low fluoride concentration in water analyzed pH changes at 5, 10, 20, 30 and 45 min intervals after the use of mouth rinsing

with sucrose solution. Throughout the study, children remained three days brushing teeth only with brush (without toothpaste). Only small numerical differences were observed between intervals, except for 5 min. In the present study, no differences in BC between 5 and 10 min intervals after chewing gum were observed; the difference observed at 1 min corroborates the results of a study [28], in which BC at baseline differed from further intervals of analysis and that prolonged chewing was favorable for salivary pH recovery.

In the same study with children living in areas with low concentration of fluoridated water [28], those presenting previous tooth demineralization received the same brushing protocol and sucrose solution for 1 min. The plaque pH recovery time was 10 min and pH remained close to 6.5 up to 45 min. In this study, in which volunteers had no dental caries and brushed regularly with toothbrush and toothpaste before the survey, after chewing gum for 10 min, pH returned to normal after 5 min and remained close to 7.0 at 10min for S. The pH recovery time for biofilm [28] was 10 min, while the salivary pH recovery in the present study was at 5 min.

People with high dental caries rates would possibly present slower saliva buffering with any of the three types of chewing gums used in the present study, with longer recovery time (favoring demineralization) in relation to people with low dental caries rate.

Natural sweeteners such as xylitol, sorbitol and mannitol, derived from plants, fruits and vegetables, have sugary flavor and should be used by diabetics, as they are not dependent of insulin to metabolize. They inhibit the growth of *Streptococcus mutans* and thereby reduce the prevalence of carious lesions. Xylitol-based gums²⁹ have demonstrated better results when compared to sorbitol-based gums. The mechanism of action of xylitol is understood as they enter the bacterial cell via fototransferase fructose system. Xylitol-5-P, a metabolite from xylitol, seems to be associated to inhibition of bacterial metabolism, once they establish an energy expenditure cycle and inhibit glucose recognition and its mechanism. Bacteria need to spend a large amount of energy to absorb xylitol, and yet they fail to metabolize them. Therefore, the use of chewing gum with natural sweeteners would be indicated as a tool for decreasing the risk of dental caries. If the chewing gum habit persists, it is interesting to replace sucrose-based chewing gum to xylitol or sorbitol-based chewing gum because they prevent the formation of plaque and the degradation of polysaccharides present in plaque [31].

In the present study, S had the lowest salivary pH in the first minute. This result was expected due to the presence of fermentable carbohydrates, which are substrate for bacteria that cause dental caries. On the other hand, the behavior of P was similar to SF, demonstrating its possible non-cariogenic or even anticariogenic characteristics of microorganisms present in their composition and also the salivary pH of individuals close to 7.0 already in the first minute of assessment.

For SF, although presenting basic pH at 1min interval, pH increase was detected after 5 min. This was probably due to natural sugars and their ability to inhibit acidity generated by the

metabolism of *Streptococcus mutans*. It is believed that, in people with high risk of dental caries, the ability of inhibiting the metabolism of cariogenic bacteria would be even more important.

Probiotics have inhibitory action on the pathogenic bacterial environment, through the competition of binding sites of host tissues by bacteria and competition for nutrients, once probiotics are able to use common nutrients of pathogens (carbohydrates, for example), leading to decrease of acid production [5,32,33]. In vitro experiments have shown that *Lactobacillus acidophilus* is the most suitable species for developing a new chewing gum. As a result of applied technology, probiotic is able to survive processing conditions, to remain alive in the gum (without refrigeration), to stand the greatest possible storage period, to meet certain requirements of individual perception (taste, texture, color and odor) and finally to be released in the mouth, producing compounds that fight *Streptococcus mutans*, one of the main pathogens of dental caries [5,32]. An interesting point of probiotic gum is its local action, which means that it does not have a systemic action, which necessarily involves effect from probiotic microorganism adhesion at intestines [34].

In this study, salivary pH remained constant at all intervals for P group. Thus, it is believed that this type of gum would be ideal especially for individuals with high levels of dental caries because it could reduce and even prevent caries activity. Salivary pH for S was lower than for SF and P in the first minute. This was expected due to the high sucrose contents released in the first minutes of chewing. S presented similar salivary pH to P and lower than SF in both 5 and 10 min. It is believed that there is a possible residual effect of sugar-free gums, a fact that would be interesting if incorporated into probiotic gums.

A careful selection of probiotic agents to be used is required, including type, time of use, mode of administration, and individual's age and health status. It is a challenge to create effective probiotics regarding retention time, exposure for effective colonization of target site, and thus ensuring effectiveness of its mechanism of action [35,36]. Despite the limitations of the present study, namely the restricted age range of volunteers (23-28 years) and lack of active and inactive dental caries, the results of this study indicated that an extended chewing time was favorable for salivary pH recovery after use of the three different types of chewing gum tested.

Conclusion

For young dentistry students, the time for saliva buffering in the 3 groups was approximately 1 min and therefore, there is no higher risk of dental caries according to groups. Probiotic therapy using gum, now widely used, had no cariogenic potential in the study population.

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