

## Salivary flow rate response to stimulation with 2% citric acid in patients with xerostomia

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### Abstract:

**Objective:** Xerostomia is the subjective sensation of dry mouth and it can be an indicator of hyposalivation what would have clinical consequences like increased risk of the development of oral diseases. For this reason, the stimulation of salivary flow with organic acids, such as citric acid, must be considered as a treatment strategy for xerostomic patients with hyposalivation. This study aimed to determine the salivary responses of patients with xerostomia to stimulation on the tongue with 2% citric acid. **Material and Methods:** This study recruited 62 patients with xerostomia. The differences in salivary flow rate (SFR), pH, and buffer capacity values were determined before and after 1, 2, 3, 5, 7, 9, 11, 13, or 15 min of stimulation on the tongue with 2% citric acid. **Results:** Among the recruited patients, 92% were women and 53% had hyposalivation. The average age of the recruited patients was 55 years. The mean basal SFR value was 0.282 ml/min (DS 0.305). SFR ( $p=0.001$ ) increased and pH ( $p=0.000$ ) and buffering capacity ( $p=0.000$ ) decreased at 1 min poststimulation relative their basal values. The values of the three parameters stabilized at 2 min poststimulation and remained constant until the end of the measurement period. **Conclusion:** Citric acid could be used to stimulate salivary flow in xerostomic patients with hyposalivation maintaining salivary pH values and buffering capacity within an acceptable range. However, to be considered a possible therapy for this kind of patients, it is still necessary to perform more studies.

**Keywords:** Xerostomia; Tongue; Citric Acid

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## INTRODUCTION

Saliva is an essential bodily fluid that is involved in oral functions. It facilitates digestion and phonarticulation; lubricates, cleans, and maintains the mucosal integrity of the mouth; and contains bactericidal and antifungal components<sup>1,2</sup>. Salivary secretion maintains the homeostasis of oral tissues, and the decrease and alteration in the qualitative characteristics of salivary secretion are associated with a high risk of developing oral diseases<sup>3</sup>. Xerostomia is the subjective sensation of dry mouth and is not necessarily an indicator of low salivary flow (hyposalivation). It may be induced by changes in salivary pH and buffering capacity<sup>4</sup> and other phenomena, such as aging and drug consumption<sup>5</sup>.

Considering the consequences of xerostomia, especially discomfort<sup>6</sup> and the consequences of hyposalivation like an increased risk of the development of oral diseases, dysphagia, dysphasia, and difficulty wearing dental prosthesis<sup>7</sup>, the stimulation of salivary flow is a fundamental treatment strategy for low salivary flow.

Salivary flow may be stimulated by the local application of natural chemicals and/or pharmaceuticals. Salivary flow stimulation has shown favorable results among patients with xerostomia with and without decreased salivary flow. Nevertheless, the kinetics of stimulated salivary flow has not been documented. Specifically, the amount of saliva produced per minute poststimulation remains unquantified.

The application of citric acid to stimulate salivary secretion<sup>8</sup> has shown good results and could be an alternative therapy for xerostomia<sup>9,10</sup> given its nontoxicity, low cost, accessibility, and potential use without direct medical supervision. However, in addition to the potential increase in salivary flow, the possible variations in salivary pH and buffering capacity induced by citric acid stimulation should be considered given the mineral nature of dental tissues and the acidity of the product.

Studies have indicated that stimulated salivary flow is associated with salivary buffering capacity, which is a protective factor and a facilitator of remineralization<sup>11</sup>. Nevertheless, studies on this association remain scarce. Therefore, this study aims to assess the salivary response of patients with xerostomia after 15 min of stimulation on the tongue with 2% citric acid. Salivary flow, pH, and buffering capacity and their duration were measured.

## MATERIAL AND METHODS

This study is a clinical interventional longitudinal study and did not establish a control group. A convenience sample comprising patients of both sexes and who were diagnosed with xerostomia of any origin on the basis of the Fox questionnaire were recruited through nonprobabilistic sampling from the diagnostic service of the Faculty of Dentistry of the University of Chile. There were considered xerostomic, all patients who answered affirmatively the first question of the questionnaire (do you feel dry mouth?) Or otherwise, affirmatively three of the following questions<sup>12</sup>. Patients with evident lesions of the buccal mucosa and with a history of citric acid hypersensitivity were excluded from the study. This study was approved by the ethics committee of the Faculty of Dentistry of the University of Chile and was performed in accordance with the principles of universal bioethics stated by the Declaration of Helsinki<sup>13</sup>. Each patient signed an informed consent form to participate in the study.

**Measurement of salivary flow rate.** A trained dental surgeon examined the patients' mouths to exclude the presence of mucosal lesions. Salivary flow rate (SFR) was determined between 8 and 11 A.M. The individual remained seated in the relaxed (coachman) position with eyes open and head slightly bent forward in accordance with the protocol described by Navazesh et al.<sup>14</sup>.

SFR was measured through the unstimulated total saliva test<sup>15</sup>. The patient was asked to rinse their mouth with distilled water and refrain from consuming food for 1 h prior to the measurement of SFR. The patient was asked to deposit the saliva they produced within 5 min in a previously weighed and labeled sterile vessel (Falcon tube with a 50 ml volume).

The samples were stored at 4°C and transported maximum 15 minutes after the collection to the Laboratory to determine salivary buffering capacity, pH and salivary flow rate. Hyposalivation was defined like an Abnormally low unstimulated salivary flow rate with values equal to or less than 0.1 ml/min<sup>16</sup>. Patients who failed to produce sufficient amounts of saliva were considered as asialics. After the determination of resting SFR, the effect of 15 min of stimulation on the tongue with 2% citric acid was determined. The patient remained in the previously described body position. Saliva produced within 1 min was collected in a preweighed receptacle. Then, 2% citric acid solution was applied with cotton bud in the anterior third of the dorsum of the

tongue. Subsequently, saliva produced at 1, 2, 3, 5, 7, 9, 11, 13, and 15 min after stimulation were consecutively collected and stored in different containers.

Measurement of salivary pH. The pH of the samples was measured before and after citric acid stimulation by using a digital pH-meter previously calibrated with pH=4, pH=7 and pH=10 (Model PL-600 EZDO-OMEGA that complies with ISO-9001), which automatically provided the pH value in digital form with 2 decimals. All measurements were taken by the same operator and with the same methodology, as follows: a) calibration of the pH meter, b) immersion of the electrode in the saliva collection tube, c) reading of the pH value of the sample 5 s after stabilization, d) washing of the electrode with distilled water, and e) preservation of the electrode in buffer solution.

Measurement of salivary buffering capacity. The Ericsson method was used to determine the buffering capacity of saliva at rest and before and after citric acid stimulation<sup>16</sup>. Saliva samples collected at each time point were pooled in an Eppendorf tube and centrifuged at 2,000 rpm in a microcentrifuge. Then, 1 ml aliquots of the supernatants were mixed with 3 ml of 0.005 M HCl to prevent foaming. Next, the samples were mixed through 20 min of shaking<sup>17</sup>. pH was determined with a digital pH meter in the same manner as the above procedure. The buffering capacity of all saliva samples with pH values greater than 5.5, between 5.5 and 4.5, and less than 4.5 was classified as high, medium, or low, respectively.

### Statistical analysis

Paired *t*-test and Mann-Whitney test were performed to analyze differences between salivary pH and buffering capacity and basal and stimulated SFR. To correlate SFR with buffer capacity of saliva, Pearson correlation test was used. Data were analyzed using STATA 11 software. Statistically significant differences were accepted with an alpha error equal to or less than 5% and a 95% confidence interval.

## RESULTS

Out of the 62 evaluated individuals, 57 (92%) were women and five (8%) were men. The minimum age was 18 years and the maximum was 80, with an average of  $55 \pm 13.3$  years. Thirty-three individuals (53%) had hyposalivation, and four of the individuals did not manage to collect saliva. The resting SFR of the subjects ranged from 0 ml/min to 1.478 ml/min with

a mean of 0.282 ml/min (DS 0.305). 50 subjects were consuming at least one xerostomizing drug, such as antidepressants, diuretics, antihypertensives, anxiolytics, or anticonvulsants. Specifically, 16 of them were taking antidepressants and anxiolytic drugs.

Table 1 shows the basal mean values of unstimulated SFR (0.331 ml/min., DS 0.339), pH (7.41, DS 0.56), and buffering capacity (4.30, DS 0.90). The table provides the values of these parameters after stimulation with citric acid. Values were obtained over the duration of 15 min at 1 min intervals. The three parameters obtained at 1 min poststimulation were significantly different from those obtained prior to stimulation (SFR=0.655 ml/min, DS 0.541; pH=7.08, DS 0.76; and buffering capacity=4.03, DS 0.81).

**Table 1.** Average SFR, pH, and buffering capacity per minute of samples

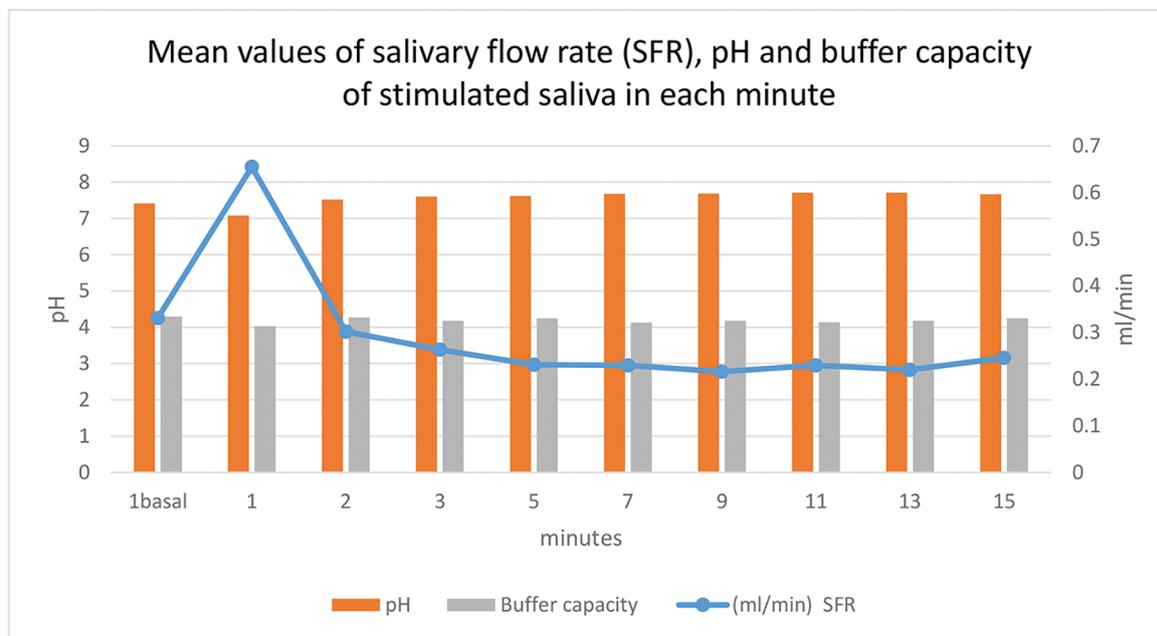
Minute	SFR (ml/min) (x ± SD)	pH (x ± SD)	Buffer capacity (x ± SD)
1 Basal	0.331±0.339	7.41±0.56	4.30±0.90
1	0.655±0.541 <sup>A</sup>	7.08±0.72 <sup>B</sup>	4.03±0.81 <sup>B</sup>
2	0.302±0.291	7.52±0.54	4.27±0.81
3	0.263±0.266	7.60±0.51 <sup>B</sup>	4.18±0.82
5	0.231±0.223	7.62±0.52 <sup>B</sup>	4.25±0.86
7	0.230±0.231	7.68±0.52 <sup>B</sup>	4.13±0.91 <sup>B</sup>
9	0.216±0.206	7.69±0.51 <sup>B</sup>	4.18±0.85
11	0.230±0.267	7.71±0.50 <sup>B</sup>	4.14±0.80 <sup>B</sup>
13	0.220±0.252	7.71±0.48 <sup>B</sup>	4.18±0.79
15	0.246±0.304	7.67±0.50 <sup>B</sup>	4.25±0.80

<sup>A</sup> Mann-Whitney test  $p < 0.05$

<sup>B</sup> Teste-t Paired  $p < 0.05$

However, the values of the three parameters immediately stabilized at 2 min poststimulation (SFR=0.302 ml/min, DS 0.29; pH=7.52, SD 0.54; and buffering capacity=4.27, DS 0.81) and were maintained until the end of the measurement period without presenting any significant differences from the baseline values. Salivary flow dynamics, pH, and buffering capacity during 15 min after stimulation with 2% citric acid 15 minutes are summarized in Figure 1. The parameters of xerostomic patients with and without hyposalivation were not significantly different.

SFR and buffering capacity values at 1 min poststimulation were weakly and negatively correlated with SFR and buffer capacity at 15 min poststimulation. Specifically, at 1 min poststimulation, SFR decreased and buffer capacity was maintained ( $r=0.35$ ;  $p < 0.05$ ).



**Figure 1.** Mean values of salivary flow rate (SFR), pH, and buffering capacity at each time point after stimulation on the tongue with 2% citric acid. SD (standard deviation).

## DISCUSSION

We aimed to determine the salivary responses of patients with xerostomia after stimulation on the tongue with 2% citric acid. We used SFR and salivary pH and buffering capacity as indices of salivary responses and measured the duration of responses. Normal resting SFR values range from 0.29 ml/min to 0.41 ml/min<sup>18,19</sup>. In our study, SFR values were lower than those described for the general population because 53% of the patients had hiposaliva<sup>20,21</sup> and confirmed previous reports stating that xerostomia is not necessarily an indicator of decreased salivary flow<sup>1,22,23</sup>.

Xerostomia is associated with anxiety and depression, which alter the systemic state of patients and require the use of medications<sup>24</sup>. Although drug use is the most important factor in the occurrence of hyposalivation, psychological factors may also be relevant for xerostomia<sup>25</sup>. In our study, the vast majority of subjects consumed at least one xerostomizing drug, such as antidepressants, diuretics, antihypertensives, anxiolytics, or anticonvulsants. Specifically, 16 of the patients were taking antidepressants and anxiolytic drugs, which could have affected our results.

SFR value immediately peaked at 1 min after the topical application of citric acid, decreased to below nonstimulated basal levels, and linearly stabilized at values close to 0.25 ml/min throughout the 15 min measurement period. A previous study on subjects with

xerostomia<sup>10</sup> indicated that stimulation with 3% citric acid produces immediate effects (15 min) that persist for 1 hour. However, we only observed an increase in salivary flow at 1 min poststimulation during our study.

Variations in salivary pH should be considered when stimulating salivary flow with organic acids, such as citric acid, in the treatment of xerostomic patients with hyposalivation given the mineral nature of dental enamel. We observed that salivary pH first decreased at 1 min after stimulation, increased to values even higher than the basal value, and later stabilized at values close to 7.6. This value would not present a therapeutic problem. Salivary pH values ranging from 4.5<sup>26</sup> and 5.5<sup>27</sup> are required to induce the demineralization of hydroxyapatite. Therefore, although the application of citric acid decreased salivary pH, this effect would not adversely affect dental enamel and lasted only for 1 min.

SFR and buffering capacity immediately increased at 1 min poststimulation and were negatively associated over the 15 min measurement period. Specifically, as SFR decreased, the buffering capacity increased. This phenomenon could account for the protective behavior of saliva against the acidic stimulus applied to increase SFR and could be related to the protective activity of the parotid gland<sup>28</sup>. Similar results have been described in healthy patients<sup>27</sup>.

The buffering capacity of saliva tended to decrease and remained low under stimulation with citric acid. The

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values of salivary buffering capacity presented a sinuous curve during the first minutes after stimulation until stabilizing during the final minutes of the measurement period. The salivary buffering capacity remained low before and after stimulation.

Buffering capacity is a parameter that tends to remain stable despite exhibiting some fluctuations. Buffering capacity values of less than 5.5 have considerable clinical importance and are associated with the high risk of developing caries. Such values, however, were not observed in our study. This result could be associated with the nature of the stimulus (2% citric acid) and the protective response of saliva to neutralize the stimulus to normal salivary pH values. These results indicated that the protective function of saliva is conserved in response to stimulation with citric acid.

Our results indicated that 2% citric acid could be eventually used to stimulate SFR in xerostomic patients with hyposalivation. The application of 2% citric acid maintains salivary pH and buffering capacity values within a normal range. Nevertheless, given the immediate but temporary effect of citric acid application, the frequency of its use should be evaluated and monitored in accordance with the needs of each patient.

Although the results of this study may be encouraging in the search for a therapy to treat hyposalivation, we must consider that the increase in SFR only occurred in the first minute after stimulation and citric acid application was performed only in a moment. It would be interesting in future studies to consider other times of application to consider the potential of this product as a permanent therapy for hyposalivation in patients with xerostomia. In addition, it is necessary to compare the behavior of 2% citric acid with pilocarpine, that is the golden pattern of stimulated saliva.

The long-term response for pH and buffer capacity to prolonged citric acid stimulation needs to be evaluated in future studies too. Anyway, given the present results, the application of citric acid in other forms, such as sprays, is an interesting research topic. Future studies should analyze the etiology of xerostomia in each patient to aid the development of individualized treatment.

The presence of some systemic diseases, such as Sjögren's syndrome; the use of drugs; and the presence of some psychological factors, especially chronic anxiety, excessive stress, and depression, should be considered too in the individualized treatment of hyposalivation in xerostomic patients.

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