# Saliva and tongue coating pH before and after use of mouthwashes and relationship with parameters of halitosis

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## ABSTRACT

Objectives: The aim of this work was to evaluate saliva and tongue coating pH in oral healthy patients with morning bad breath before and after use of different oral mouthrinses. Material and Methods: Saliva and tongue coating pH of 50 patients allocated in 5 groups were measured respectively by a digital pHmeter and color pH indicators, before, immediately after and 30 min after rinsing 5 different mouthrinses: cetilpiridine chloride associated with sodium chloride, triclosan, enzymatic solution, essential oil and distilled water. Results: Only triclosan and essential oil increased salivary pH immediately after rising. The enzymatic solution decreased salivary and tongue coating pH immediately after rinsing. Conclusions: Salivary pH tended to be acidic while tongue coating pH tended to be alkaline, even after rising. Triclosan and essential oil mouthrinses increased salivary pH immediately after rinsing. Enzymatic solution decreased saliva and tongue coating pH immediately after rising.

**Key words:** Halitosis. Saliva. Tongue. Mouthwashes. pH.

## INTRODUCTION

The existence of an association between tongue microorganisms and those present in saliva has been reported[6]. The anaerobic microbiota of the tongue biofilm is one of the main responsible for the release of sulfur-containing compounds (VSC), which are directly involved in the occurrence of halitosis. The origin of halitosis has been localized in the oral cavity in up to 85% of people suffering from this condition. In most cases, it is produced in the mouth by the action of Gram-negative anaerobic bacteria on sulfur-containing proteinaceous substrates in the saliva, such as debris and plaque[10].

The most common source of bad breath in individuals with good oral hygiene and healthy periodontal tissues is from the posterior dorsum of the tongue[10], where the crypts are the favored sites for growth of the anaerobic bacteria responsible for halitosis. Several studies have implicated the dorsum of the tongue as the primary site of microflora putrefaction and the production of VSC[15,17].

Dry mouth is also related with oral malodor[1]. A reduced saliva flow during sleep in healthy patients favors anaerobic bacterial putrefaction, giving rise to so-called "morning breath" or physiological halitosis, a transient condition resulting from decreased salivary flow and decreased activity of tongue and cheek muscles during sleep, which promotes the proliferation of bacteria of the oral cavity that are responsible for the emission of the VSC, which disappears after a meal[10]. Pathologic halitosis is more intense and is not easily reversible[10].

The importance of halitosis has led to the formulation of different commercial products that are claimed to have anti-halitosis effect. The mechanisms of action of these solutions are in general due to their antimicrobial or oxidizing properties or their capacity to inhibit the formation of VSC, even in the presence of oral bacteria.

Antibacterial components such as chlorhexidine, cetylpyridinium chloride, triclosan, essential oils, chlorine dioxide, zinc salts, benzalkonium chloride, hydrogen peroxide and sodium bicarbonate have been used in the treatment of halitosis, either alone or in combination, and either as a single mode of therapy or together with the mechanical treatment of tongue coating.

pH is the major regulating factor in the formation of bad breath[6] and the chemical control of biofilm with antimicrobial solutions may reduce the levels of microorganisms and VSC in patients with halitosis complaint. It is thus important to carry out new studies with these products and their actual influence on salivary and tongue coating pH, as well as investigate the feasibility of the association of these aspects and the development of halitosis. The aim of this work was to evaluate saliva and tongue coating pH in oral healthy patients with physiological halitosis before and after use of different mouthrinses.

## MATERIAL AND METHODS

This study was approved by the Human Research Ethics Committee of Bauru School of Dentistry, University of São Paulo (Process nº 008/2008). Fifty oral and systemic healthy dental students, aged over 18 years, from Bauru Dental School volunteered to participate in this study.

The exclusion criteria were subjects with medical disorders, such as diabetes mellitus, renal disease, gastrointestinal disorders, respiratory diseases[13], evidence of recent bronchitis, sinusitis or tonsillitis[3], pregnant women[3], patients undergoing antibiotic or other antimicrobial therapy, smokers and those who, on pre-study clinical screening, presented a probing depth ≥4 mm, cavitated caries lesion, naso-pharyngeal alterations, mouth breathers and patients with prostheses, orthodontic or dental appliances.

Before examination, the patients received the following guidelines to improve standardization of data collection: avoid, 24 h before the second consultation, spicy and flavored food, coffee, tea or alcoholic beverage; the night before perform oral hygiene as usual; be fasting for 8 h at the time of consultation; do not perform oral hygiene, use any kind of mint flavoring or rinse solution or drink water before the consultation.

All patients attended two consultations, which were conducted by the same examiner. In the first consultation, prior to the clinical examination, all subjects were asked to fill out a questionnaire[4]. Next, clinical examination was performed, mainly aiming the analysis of oral and systemic health of the patients. In the second consultation, all patients were seen in the morning, at 7 a.m., fasting for at least 8 h and without having performed any oral hygiene procedures on the day of consultation[17], but having performed oral hygiene as usual the previous night at bedtime, with brushing and dental floss.

Each patient underwent three collections of saliva samples: at the beginning of the consultation, immediately after rising a specific solution, and 30 min after rising (phases *before*, *after*, and *30 min,* respectively). Each volunteer was submitted to a type of rinse with a specific oral mouthrinse solution.

The patients were randomly assigned to 5 groups of 10 volunteers each, distributed as follows:

- *SC* Group: rinses with sodium chlorite

**Table 1-** Mean and standard deviation (mean±sd) of saliva pH in the studied groups

| Group | n | Phase | | |
|---|---|---|---|---|
| | | before | after | 30min |
| SC | 10 | 6.63±0.44 | 6.76±0.51 | 6.82±0.43 |
| TS | 10 | 6.58±0.36 | 6.87±0.43 | 6.74±0.26 |
| ES | 10 | 6.80±0.27 | 6.59±0.35 | 6.70±0.37 |
| EO | 10 | 6.62±0.41 | 7.01±0.32 | 6.73±0.31 |
| CTRL | 10 | 6.46±0.48 | 6.70±0.50 | 6.74±0.33 |
| All | 50 | 6.62±0.40 | 6.79±0.44 | 6.75±0.33 |

**Table 2-** Mean and standard deviation (mean±sd) of the tongue coating pH in the studied groups

| Group | n | Phase | | |
|---|---|---|---|---|
| | | before | after | 30min |
| SC | 10 | 7.40±0.52 | 7.10±0.32 | 7.50±0.53 |
| TS | 10 | 7.30±0.48 | 7.50±0.53 | 7.20±0.42 |
| ES | 10 | 7.40±0.52 | 5.90±0.74 | 7.10±0.32 |
| EO | 10 | 7.30±0.48 | 7.40±0.52 | 7.20±0.42 |
| CTRL | 10 | 7.10±0.32 | 7.20±0.42 | 7.40±0.52 |
| All | 50 | 7.30±0.46 | 7.02±0.77 | 7.28±0.45 |

**Table 3-** Multiple comparisons of pH in saliva between groups

| Group | Phase | | |
|---|---|---|---|
| | before | after | 30min |
| 1 | 6.53±0.32$^{a}$ | 6.54±0.33$^{ab}$ | 6.57±0.36$^{a}$ |
| 2 | 6.48±0.23$^{a}$ | 6.71±0.27$^{b}$ | 6.51±0.21$^{a}$ |
| 3 | 6.57±0.28$^{a}$ | 6.33±0.16$^{a}$ | 6.47±0.18$^{a}$ |
| 4 | 6.52±0.19$^{a}$ | 6.78±0.17$^{b}$ | 6.44±0.27$^{a}$ |
| 5 | 6.48±0.25$^{a}$ | 6.52±0.30$^{ab}$ | 6.52±0.21$^{a}$ |

Groups with same letter at each stage have no statistically significant difference between themselves (Tukey's test, p>0.05).

**Table 4-** Multiple comparisons of pH in saliva between phases

| Phase | Group SC | Group TS | Group ES | Group EO | Group CTRL |
|---|---|---|---|---|---|
| Before | 6.53±0.32[a] | 6.48±0.23[a] | 6.57±0.28[b] | 6.52±0.19[a] | 6.48±0.25[a] |
| After | 6.54±0.33[a] | 6.71±0.27[b] | 6.33±0.16[a] | 6.78±0.17[b] | 6.52±0.30[a] |
| 30min | 6.57±0.36[a] | 6.51±0.21[a] | 6.47±0.18[ab] | 6.44±0.27[a] | 6.52±0.21[a] |

Phases with same letter at each stage have no statistically significant difference between themselves (Tukey's test, p>0.05).

**Table 5-** Multiple comparisons of pH in tongue coating between groups

| Group | Phase | | |
|---|---|---|---|
| | before | after | 30min |
| SC | 7.40±0.52[a] | 7.10±0.32[b] | 7.50±0.53[a] |
| TS | 7.30±0.48[a] | 7.50±0.53[b] | 7.20±0.42[a] |
| ES | 7.40±0.52[a] | 5.90±0.74[a] | 7.10±0.32[a] |
| EO | 7.30±0.48[a] | 7.40±0.52[b] | 7.20±0.42[a] |
| CTRL | 7.10±0.32[a] | 7.20±0.42[b] | 7.40±0.52[a] |

Groups with same letter at each stage have no statistically significant difference between themselves (Tukey's test, p>0.05).

combined with cetylpyridinium chloride - chlorine dioxide (Saúde Bucal®; Embatek Technology in Cosmetics Ltd., São Paulo, SP, Brazil);

-*TS* Group: rinses with combined solution of triclosan (0.03%), sodium fluoride (225 ppm of fluorine) and copolymer PVM/MA (0.20%) *Gantrez* (Colgate Total Plax Classic® 250 mL; Colgate-Palmolive and Industry Trade Ltd., São Bernardo do Campo, SP, Brazil);

-*ES* Group: rinses with enzyme solution – Lysozyme, Lactoferrin, Glucose Oxidase and Lactoperoxidase (Biotène Mouthwash® 240 mL; Laclede, East University Drive, Rancho Dominguez, CA, USA);

-*EO* Group: rinses with essential oil (Listerine Cool Mint® Oral antiseptic 1.5 L; Warner-Lambert Co., Morris Plains, NJ, USA);

-*CTRL* Group (control): rinses with distilled water (placebo).

Each volunteer rinsed with 20 mL of the solution for 30 s followed by gargling for 10 s.

Before saliva collection, patients were kept seated for 5 min, relaxed and without talking[12]. Unstimulated saliva was collected over a period of 5 min. Before collection, the mouth was emptied by an initial swallow[9]. The examinator asked the subjects to spit out the produced saliva each 30 s in a plastic container (J-10; Injeplast®, São Paulo, SP, Brazil)[14]. This procedure was performed before and after rising, and after 30 min.

Salivary pH was measured by a digital pH meter (Sentron Model 1001 pH System; Sentron Incorporated, 33320, USA), calibrated with standard solutions of pH 4.0 and 7.0. The electrode was washed with distilled water and dried with absorbent paper after each analysis[8].

In the same consultation, tongue coating pH was measured using pH indicator strips (pH 0-14; Merck, Darmstadt, Germany). One strip was placed on posterior tongue region, with the patient with the mouth opened, for 1 min. The color change in the strip indicated tongue coating pH. The measurements were performed before, immediately and 30 min after rising.

Data were analyzed statistically by two-way ANOVA and Tukey's test. A significance level of 5% was set for all analyses.

## RESULTS

The measurements of salivary and tongue coating pH in the 5 groups are presented in Tables 1 and 2. There was no statistically significant difference (p>0.05) in the salivary pH between the groups in the *before* and *30 min* phases. In the *after* phase, the *ES* group presented mean values smaller than the *EO* and *TS* groups (Table 3). In the groups *CTRL* and *SC* there was no statistically significant difference (p>0.05) in salivary pH between the 3 phases. In the *TS* and *EO* groups, the mean pH values in the *after* phase were higher than the mean values obtained in the *before* and *30 min* phases. In the *ES* group, the mean pH values in the *before* phase were higher than in the *after* phase (Table 4).

In the tongue coating pH analysis, there was no statistically significant difference (p>0.05) between groups in the *before* and *30 min* phases. In the *after* phase, the *ES* group presented smaller mean values than all other groups (Table 5). In the *SC, TS, EO* and *CTRL* groups, there were no statistically significant difference (p>0.05) between the 3 phases. In the *ES* group, average values in the *after* phase were smaller than the average in the phases *before* and *30 min* (Table 6).

**Table 6-** Multiple comparisons of pH in tongue coating between phases

| Phase | Group SC | Group TS | Group ES | Group EO | Group CTRL |
|---|---|---|---|---|---|
| Before | 7.40±0.52[a] | 7.30±0.48[a] | 7.40±0.52[b] | 7.30±0.48[a] | 7.10±0.32[a] |
| After | 7.10±0.32[a] | 7.50±0.53[a] | 5.90±0.74[a] | 7.40±0.52[a] | 7.20±0.42[a] |
| 30min | 7.50±0.53[a] | 7.20±0.42[a] | 7.10±0.32[b] | 7.20±0.42[a] | 7.4.0±0.52[a] |

Phases with same letter at each stage have no statistically significant difference between themselves (Tukey's test, p>0.05).

## DISCUSSION

The reduction in salivary flow during sleep and consequent increase in the number of epithelial cells scaled from oral mucosa lead to tongue coating formation and therefore in halitosis, even in healthy pacients[20]. Several masking and antimicrobial agents have been proposed to control both physiological and pathological halitosis. Their clinical efficacy has often been tested on morning breath[4,11] rather than in real clinical situations for evident ethical reasons. It has been postulated that a decrease in salivation during sleep promotes proliferation of the oral bacteria responsible for the release of the offending gases in morning bad breath[4,11]. Strong evidence that morning breath odor can be used as a model for investigation of other offensive odors is still lacking but universally accepted[18]. This fact justifies the use of oral healthy patients with morning breath odor in this research.

Saliva of individuals with "dry mouth" (common situation after a night of sleep) often presents acidic pH[5,16]. However, there is a tendency that salivary pH becomes more alkaline during the day, by the act of talking or chewing. Salivary pH is slightly acidic before its secretion in oral cavity. It becomes alkaline at the time of gland's secretion due to loss of carbon dioxide ($CO_2$) and increase of saliva's bicarbonate concentration when salivary flow is increased[20]. Hypothetically, body fluid pHs remain relatively constant because of buffering systems, fact that is not different in the saliva, which, many times, keeps its pH constant, even after rising with acidic solutions, for example. In the present study, the most notable changes in pH were observed immediately after rising, but were stabilized after 30 min. Moreover, salivary pH remained slightly acidic in all stages in all groups, possibly due to the effect of mouthrinses or the buffer capacity of saliva.

To discuss these findings of this study, we put in question whether salivary pH, considered acidic after sleeping, would have a direct influence in halitosis or whether morning breath odor is only due to increased mucosa desquamation and formation of tongue coating. According to McNamara, et al.[12] (1972), pH is the major regulating factor in the formation of bad breath and is clearly established that acidity inhibits the production of odors while neutrality and alkalinity favor it[12]. The same context is applied to tongue coating pH, which has alkaline pH due to the production of odorivetores during proteolysis[20]. Among the final products of proteolysis are amines, ammonia and urea, which have alkaline pH, characteristic of physiological or pathological halitosis[20]. It is also questioned whether the use of mouthrinses could change these pH values and thus influence, in the reduction of halitosis after their use. For that reason, it would be logical that mouthrinses provide reduction of saliva and tongue coating pH in order to reduce halitosis.

In the present study, there was no statistically significant difference in salivary pH between the groups, in the phases before and after 30 min of rinsing. In the *after* phase, the *ES* group presented smaller mean values than the *TS* and *EO* groups. In the *CTRL* and *SC* groups, there was no statistically significant difference in salivary pH between the 3 phases. In the *TS* and *EO* groups, the mean pH values in the phase *after* were higher than those recorded before and after 30 min of rinsing. In the *ES* group, the mean pH values before rinsing was higher than immediately after rinsing. According to the present research, rinsing with mouthwashes based on triclosan and essential oils lead to an increase in salivary pH, both lasting for 30 min. This can be explained by the fact that mouthrinses that cause mouth burning (fact reported by the volunteers of the groups *TS* and *EO*) stimulate salivation and thus increase salivary pH[20]. For the *ES* group, there was a pH decrease immediately after its use; however, there was no information in literature about pH decreasing caused by enzymatic solutions.

In the control group, in which the volunteers rinsed with distilled water, there was an increase in salivary pH during the measurements. It can be explained because of the increase in the saliva's bicarbonate concentration when salivary flow is increased, a condition that is common over time: salivary pH increases with the increase of flow[2]. In the study of Suarez, et al.[19] (2000), patients with complete oral health were submitted to measurements of bad breath after any kind of oral hygiene and water only when necessary. The authors observed that the concentrations of each gas tended to decrease in the first hour after

waking. After this period the concentration tended to remain stable or increase in the next 7 h.

In our research, the analysis of tongue coating pH showed no statistically significant difference between the groups, in the phases before and after 30 min of rising. In the *after* phase, the *ES* group presented smaller mean values than all other groups. In the *SC, TS, EO* and *CTRL* groups, there was no significant difference between the three phases. In the *ES* group, the mean values of the *after* phase were lower than those obtained before and after 30 min of rinsing. It is known that tongue coating is the main cause of oral halitosis[5,11,20-22] and that its pH tends to be alkaline. Only risings with enzymatic solution (*ES* group) provided pH decrease immediately after its use; however, the mechanism of action is not clearly described in the literature. The beneficial impact of oral mouthrinses on the bacterial load on the dorsum tongue is clearly demonstrated in the study of Steenberghe, et al.[18] (2001).

This study was conducted in oral healthy patients, which is a factor of extreme importance to avoid the development of chronic halitosis, since bad breath is closely associated to poor oral hygiene. The measurements of saliva and tongue coating pH in healthy patients with physiological halitosis homogenized the method. Patients should be aware that some bacteria are inevitably left behind after mechanical plaque control, even with an optimal technique. If the oral hygiene is not performed properly, gingivitis, caries, periodontitis and eventually halitosis may develop. The dental professional could explain that antiseptic rinsing kills additional bacteria and helps controlling plaque. For absolute clarity, rinsing should be described as an adjunct to an established daily oral-care routine, rather than a substitute for brushing and interdental cleaning[7].

## CONCLUSIONS

According to the methodology applied in this study, it may be concluded that:

-In a situation of physiological halitosis, salivary pH tended to be acidic while tongue coating pH tended to be alkaline, even after the use of mouthrinses;

-Only triclosan and essential oil mouthrinses increased salivary pH immediately after rising;

-The enzymatic solution was able to decrease saliva and tongue coating pH immediately after rising.

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