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Effect of Mouth Rinses with and without Alcohol on Halitosis: Randomized Crossover Controlled Trial Employing Gas Chromatography

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Abstract

Halitosis is a disease that negative limpactsy people's lives. This single centre, randomized, crossover controlled trial compared: Periogard® with alcohol (positive control) (CHXw/a) and without alcohol (CHXn/a); Listerine Total® (EOw/a) and Listerine ZeroTM (EOn/a) arranged into four sequences of use. 21 volunteers with intra-oral halitosis used each product at once, followed by a one-week washout period. The breath was measured by portable gas chromatograph OralChromaTM before rinsing and after 1, 2 and 3 hrs. Compared to the baseline, at the first hour, only EOw/a was able to significantly reduce the breath (H2S $p<0.0001$ and CH3SH $p=0.001$) for both gases and its effect lasted for up to three hours (H2S $p<0.0001$ and CH3SH $p=0.001$). CHXw/a (control) reduced H2S at the first hour ($p=0.001$) and lasted for three hours (H2S $p<0.0001$) without effect on CH3SH. CHXn/a just reduce H2S levels. EOn/a had no effect on breath, which increased with time for both gases. It can be indicated the essential oil based product containing alcohol and zinc chloride more efficiently with the proviso that only essential oils are approved for continuous use. The EOw/a presented the best performance against intra-oral halitosis followed by the control CHXw/a and CHXn/a.

Keywords: Halitosis; Mouth rinses; Gas chromatography; Crossover controlled trial

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Introduction

Halitosis is an unpleasant condition for both the carrier and for the persons with whom it relates to be cause of a social constraint and to possibly precipitate a neurosis. The tongue coating, being a bacterial mass formed by squamous epithelial cells, salivary proteins and protein remains food metabolized by proteolytic bacteria, gives a foul smelling compound called Volatile Sulfur Compounds (VSCs) such as hydrogen sulfide (H_2S), dimethyl sulfide $(CH_3)_2S$ [1] and methyl mercaptan (CH_3SH) [2-5]. Hydrogen sulfide (H_2S) is a colorless, flammable and water soluble characterized by the smell of rotten eggs. Methanethiol or methyl mercaptan is also a mouth breath odorivector with recognition threshold about 1/30 that of H_2S with a much higher odour potential than H_2S, indicating that MM causes odor problems at much lower concentrations than H_2S, and may also be produced by methylation of hydrogen sulfide as a detoxification mechanism by mucosal thiol S-methyltransferase [2-4]. Given the importance of H_2S to the physiological process and whereas values above 112ppb from this gas can be detected by the human sense of smell with an unpleasant odor, as well as methanethiol at 26ppb, it is common sense to attempt the elimination or reduction of these compounds in breath by local routes, using products in the oral cavity, without systemic interference. Strategies for controlling bad breath is related to control of the growth of bacteria, especially proteolytic, and engages the teeth and tongue cleaning in combination with the use of antimicrobials [5]. Therefore, a variety of products has been used in an attempt to inhibit or mask bad breath odor, including tongue sanitizers, agents in chewing gum, toothpaste and mouthrinses. The primary concern leading to the frequent use of mouthrinses is halitosis [6]. According to Loesche in 1999 [7], the first clinical trials with rinses against oral malodor were designed with a cosmetic claim [8-13]. Therefore, recent studies show the importance of comparative trials to determine the true efficacy of the use of mouthwash [14-16]. Healthy individuals who complain of bad breath have used mouthwash containing masking or antimicrobial agents [11,17,18]. Many products with different formulations and mechanisms of action have been proposed in order to help against halitosis, among them we can mention: cetylpyridinium chloride, triclosan, chlorhexidine

Figure 1: Mouthrinses bottles relabeled and on the original covers. Graduated 15 mL-measuring cups with their respective product identification label at the bottom (externally).

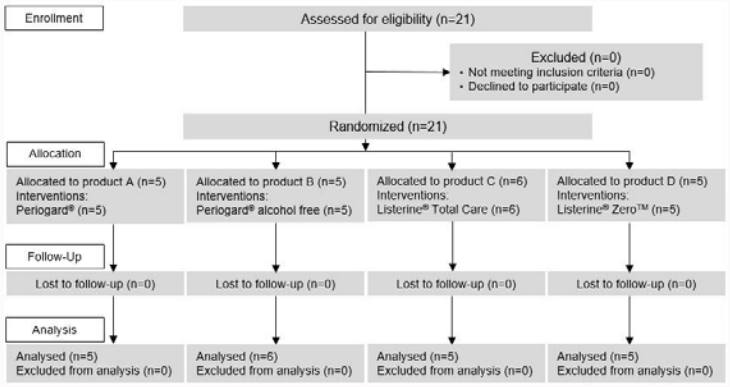

Figure 2: CONSORT Flow Diagram.

gluconate, chlorine dioxide, stannous fluoride, essential oils, lactate, citrate zinc chloride [7,14,19-22]. Some products are used to reduce oral malodor by chemical neutralization of VSC. The active ingredients of these products are often metal ions and oxidizing agents. Metals such as zinc, sodium, tin and magnesium are considered for interacting with sulfur. The interaction forms insoluble sulfides. The proposed mechanism is that the metal ions oxidize thiol groups in the precursors of VSC [23,24]. The oxidizing agents can reduce oral malodor by reducing the necessary conditions for the metabolism of sulfur-containing amino acids [25]. Although there are a large number of studies evaluating the effect of these products on halitosis, there is still much disagreement between the results and their actual effectiveness. A recent Cochrane review on this topic concluded that randomized controlled trials comparing the effectiveness of available mouth rinses are needed [26]. Due to the relatively sparse literature to assess the efficiency/effectiveness of mouthwashes (chemical methods) to fight and/or control of halitosis, premise of this work was an *in vivo* crossover, controlled trial comparing chemical methods for evaluation of volatile sulfur compounds H_2S and CH_3SH against halitosis of oral origin. For positive control was chosen chlorhexidine to be considered the gold standard as an antimicrobial agent and present some evidence of its effectiveness in breath control despite its limited time of effectiveness in studies with halitosis [25-27]. Essential oils were also selected for their nominations for daily use and possibility to compare their versions with and without alcohol, in addition to its known masking breath effect [22-26].

Objectives

The primary objective of this study was to evaluate the over-the-counter mouthrinses effectiveness in reducing oral breath and measure how long the effect was lasting, compared to a control with active compound. As specific objectives we sought to evaluate the efficacy of:

1. Chlorhexidine digluconate (0.12%) - bis-biguanide (with alcohol - control);

2. Chlorhexidine digluconate (0.12%) - bis-biguanide (alcohol free);

3. Essential oils (thymol, menthol, eucalyptol methyl salicylate) with alcohol and zinc chloride;

4. Essential oils (thymol, menthol, methyl salicylate and Eucalyptol) alcohol free.

Material and Methods

This trial consists of a randomized, crossover clinical trial with 4 groups and 4 experimental periods, single-center and masking of patients, the examiner and analysis. All subjects invited to participate were well informed of the study protocol and objectives, given and signed their written consent before participation. The project was approved by the Research Ethics Committee of the Ribeirao Preto Dental School – (FORP – USP), under number CAAE 02122812.8.0000.5419 from Plataforma Brasil – Brazilian Ministry of Health, according to declaration of Helsinki (2008). After OralChroma™ calibration [28], volunteers who were in search of diagnosis and treatment for bad breath or by invitation of researchers for the study were pre-screened with the equipment. The randomization was done with 4 sequences of usage order and the sequence 3 had 6 volunteers instead of 5 [29]. The sample size was defined according to our previous study [30] being considered sufficient n=20. Complete medical and dental history is essential to eliminate confounding effect. The primary focus of the medical history was on drugs and systemic diseases. Oral history and

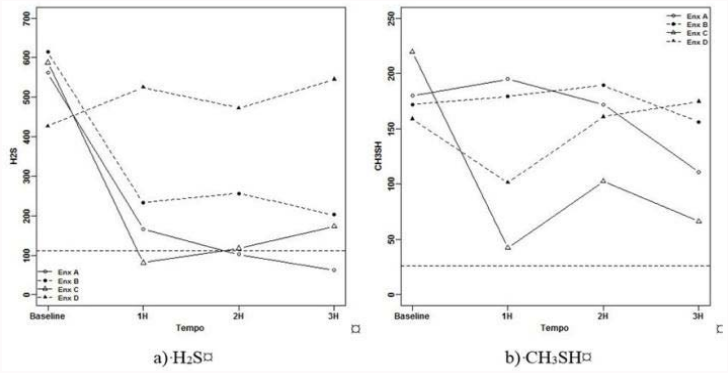

Figure 3: Line graph illustrates the relationship between concentrations (ppb) and time (hours), for each rinse. The dotted horizontal line represents the minimum detectable value to the human sense of smell, at 112ppb for H_2S and 26ppb for CH_3SH.

a specific questionnaire related to halitosis was used, according to the consensus of an international workshop and described by Seeman et al. [31] in 2014 about halitosis management. For inclusion criteria in the study, the volunteers had to meet the following requirements to clinical examination [32-33].

1. Presence of at least 20 natural teeth;

2. Good oral hygiene;

3. Absence of periodontal disease, xerostomia, or any chronic inflammatory process;

4. Patients with intraoral halitosis cause (VSC above 112ppb for H_2S or 26ppb for CH_3SH - detected by OralChroma™).

5. Were excluded smokers, pregnant or lactating volunteers, patients with systemic diseases (liver, kidney, and diabetes), pharyngeal or tonsillar infection, upper or lower respiratory tract inflammation, patients with dentures, fixed prostheses clinically unsatisfactory, dental caries, lesions on the oral mucosa, those using drugs regularly or who had undergone treatment with antibiotics for less than three months. Therefore was avoided the confounding effects, namely it was prevented from a third interference factor between exposure and outcome.

The tests were conducted with undergraduate and graduate students from the campus, staff at the faculty from RibeirãoPreto Dental School, USP. All 21 participants were within the inclusion criteria and had healthy conditions of the mouth, without spontaneous gingival bleeding, absence of periodontal disease (or probing depth more than 3 mm), but some had dental supra and subgingival calculus less than 3 mm depth. Dental prophylaxis was performed in all volunteers prior to the study to maintain periodontal health status. After prophylaxis was provided to the patients an oral hygiene kit for their maintenance of hygiene during the period of study. In the week before the start of the study, participants received instructions for oral hygiene and dental brush set (Toothbrush Oral-B Pro Clinical Protection Flex® soft - Procter & Gamble) and toothpaste (Colgate Toothpaste Maximum Anticaries Protection) provided by the researcher, so as not to make use of any kind of mouthwash, since the week before the first intervention and to remain unused for the entire study period. The elected toothpaste did not contain antiseptic active ingredients other than sodium fluoride. The flossing was not restricted. This period led to the standardization of pre-clinical period, free of possible mouth rinse waste. Before starting the experiments, the volunteers were instructed to remain for 12 hrs without using any type of oral hygiene and were asked to avoid eating foods which decomposition could produce strong odor (such as garlic, onion, egg and cabbage) and the intake of alcoholic beverages in order to register the initial levels of VSCs in the morning of the appointment [34]. It was evaluated the efficacy on reduction of VSC, using the mouth rinses identified by codes at (Table 1) and (Figure 1):

During the pre-trial period all participants had to follow the instructions below [34]:

- Avoid the intake of food and liquids (except water). However, they were allowed to have breakfast two hours before the evaluation period.

- Refrain from any oral hygiene procedures.

- Avoid the use of chewing gum, mints, and candy or breath fresheners. In this crossover study, each of the 21 subjects were randomly allocated into a sequence according to a random of numbers (generated by the software R version 3.2.0 Windows®), so that the first volunteer received the treatment 3, the second received treatment 1 and so on, to one of the 4 sequences for the products (see CONSORT Flow Diagram, Figure 2).

The portable gas chromatograph (OralChroma™, FIS Inc., Itami, Japan), was used to measure the concentration of H_2S, CH_3SH, both intraoral sources. The sample collection occurred by use of a disposable syringe (fully plastic 1 mL) purchased with the unit, which was inserted into the mouth of volunteers. Subjects closed their mouth and "mouth washed" the air for 30 seconds prior to sample collection. The volume of 1 mL mouth air was then injected into the measuring device. After 8 min the process was completed and the concentration of the three gases were displayed in ng/mL or 10 ppbv (nmol/mol) according to the corrections on the chromatogram made by tangerman and colleagues in 2008 [28]. The participants were submitted to analysis by portable gas chromatography for identification of hydrogen sulfide and methyl mercaptan (methanethiol). On the morning of the experiment, the volunteers were allowed to have breakfast without being made any kind of oral hygiene and should not use any cosmetic that would release odor/scent [35]. Each individual used 15 mL of mouth rinses A or B and 20 mL of mouth rinses C or D at the rate of one minute according to the manufacturers recommendations only once during the day of the assay. Therefore, the breath of each volunteer was examined in the following intervals [36-38]:

- time 0 = before using the product (initial data/baseline);

Table 1: Mouthrinses tested for efficacy in reducing the emission of volatile sulfur compounds.

Code	Product	Manufacturer	Active Ingredient	Lot n. and Expiration Date
A	Periogard®	Colgate	Gluconatechlorhexidine 0.12% (control)	4089BR122CH20- BB.03/17
B	Periogard® alcohol free	Colgate	Gluconate chlorhexidine 0.12% (alcohol free)	4138BR122CH14– BB.05/17
C	Listerine® Total Care	Johnson & Johnson do Brasil	Eucalyptol, thymol, methyl salicylate, menthol and zinc chloride (alcohol)	1494B07 – BB.05/16
D	Listerine® Zero™	Johnson & Johnson do Brasil	Eucalyptol, thymol, methyl salicylate, menthol (alcohol free)	1134B09 – BB.04/16

- time 1 = 1 hours after rinsing;
- time 2 = 2 hours after rinsing;
- time 3 = 3 hours after rinsing;

After obtaining the data, the volunteers were instructed to sanitize the mouth, making use of toothbrush and dentifrice only supplied by researchers for a period of over one week (7 days) (washout) to avoid interference with the results of next week (carry over) based on similar studies with CHX [34,39-45]. After this period, the next interventions occurred having their washout intervals until all the products were tested by all individuals at the end of the experiment. The material collected for analysis in chromatograph was injected directly into the device via the specific aperture (hole feeder). The data recorded by OralChroma™ were collected and tabulated in an Excel spreadsheet and in accordance with the codes marked on the bottles and submitted to the following statistical tests described:

It was initially performed an exploratory analysis of data through graphs and central position and dispersion measurements (mean, standard deviation, median, minimum and maximum values). Mean comparisons of variables: H_2S and CH_3SH were made by linear regression mixed models [46]. When using this model, it is necessary that their residues have a normal distribution with zero mean and constant variance. The model fit was done by PROC MIXED of SAS® 9.2 software. It was found that significant differences were noted only when $p<0.05$ (5% significance level).

Results and Discussion

This study was designed as a crossover clinical trial, since its main advantage is to eliminate the existing large variation among individuals in response to a treatment, given that all treatments are assigned to all individuals. Thus, each patient served as his own control, increasing the study efficiency from a statistical point of view, given the necessity of a smaller number of participants and such drawing has been widely used [20,33,35,47-53]. For this to happen, each treatment should be reversible, following a similar course during all periods and there should be no residual effect transfers from one treatment to another. In addition, individuals must be willing to receive all treatment regimens and each treatment period should be of sufficient duration to provide convincing data. This can make a long-term project an impractical crossover [54]. From the 106 pre-screened volunteers, 81 did not fit the inclusion criteria, by smoking habits, systemic diseases or antibiotic use within the previous two weeks. From the 25 pre-selected to the study, 23 reported unaware of being carriers of halitosis. Only 1 patient reported knowledge on having halitosis by aversive social behavior from colleagues. One patient reported feeling bad taste and presence of caseum in the throat with some frequency. Four volunteers dropped out from the trial in the preclinical period (after completion of dental prophylaxis and before the start of the tests). Twenty-one volunteers of both genders were selected, aged between 18 and 50 years with good general health. It has been shown that the confidence intervals better inform readers about the possibility of inadequate sample size than post hoc power calculations [55]. After exploratory data analysis, it was possible to construct a summary table of the measurements. With these values it was possible to construct a graph that allows display the gases behavior for each rinse in each time, according to (Figure 3). When people are allocated into sequences some individual characteristics such as age or sex (i.e.) can bring to the sequence some significantly differences. Groups containing older people may have the biggest breath values. This can occur when the randomization process for allocating the sequences volunteers is not done properly. The comparison of sequences is important to infer if a higher number of people with more elevated levels of breath were allocated to one or another sentence. For Both H_2S and CH_3SH, the values did not differ among the sequences. It can be inferred, then, that the distribution of volunteers in these sequences was valid and therefore it did not cause interference into the results, which can be seen on (Table 2). However, there were significant differences among the products compared with each other, regardless of time, both H_2S and for CH_3SH (Table 3).

Table 2: Comparisons between the products regardless of time for the compounds H_2S and CH_3SH.

Variable	Comparisons	Difference between means estimate	P Value	CI 95%	
				IL	SL
H_2S	(rinse A - B)	-97.74	0.05	-196.76	1.28
	(rinse A - C)	-22.98	0.65	-122.00	76.04
	(rinse A - D)	-271.48	<.0001	-370.50	-
					172,46
	(rinse B - C)	74.76	0.14	-24.26	173,78
	(rinse B - D)	-173.74	0.001	-272.76	-74,72
	(rinse C - D)	-248.50	<.0001	-347.52	-
					149.48
CH_3SH	(rinse A - B)	-10.18	0.68	-58.59	38.24
	(rinse A - C)	54.33	0.03	5.91	102.75
	(rinse A - D)	13.04	0.60	-35.38	61.46
	(rinse B - C)	64.50	0.01	16.08	112.92
	(rinse B - D)	23.22	0.35	-25.20	71.63
	(rinse C - D)	-41.29	0.09	-89.70	7.13

Comparison of baseline values of H_2S and CH_3SH for each product

When mouthrinses baselines were compared, no differences were noted between them. This indicates a washout period sufficient to prevent residual effect (carry-over) for both gases (H_2S and CH_3SH) studied - as shown in Table 3. According to Figure 3, the baseline averages of hydrogen sulfide for all groups were above 400ppb for H_2S, indicating strong halitosis values, and the CH_3SH means were from 150ppb, which are also considered high values (threshold 26ppb), and 5 times larger than the gas threshold perception. The

Table 3: Comparison of VSC baseline periods for each product.

Variable	Comparisons	Difference between means estimate	P Value	CI 95%	
				IL	SL
H_2S	(rinse A - B) baseline	-41.27	0.68	-239.31	156.77
	(rinse A - C) baseline	-42.72	0.67	-240.76	155.32
	(rinse A - D) baseline	128.04	0.20	-70.00	326.08
	(rinse B - C) baseline	-1.45	0.99	-199.49	196.59
	(rinse B - D) baseline	169.31	0.09	-28.73	367.35

latter compound has more penetrating odor than hydrogen sulfide. Thus, even in lower concentrations their odor can more easily overlap the others [4]. For tangerman and Winkel (2007), the odor index of Methyl Mercaptan (MM) is about three times greater than the H_2S, MM recognition threshold is about 1/30 of the threshold of H_2S and objection capacity about 1/8 of the H_2S. These figures show that MM has a much higher potential than the H_2S odor, indicating discomfort at much lower concentrations than H_2S. These results suggest that MM is the predominant causal factor in oral halitosis, which is in accordance with results of other researchers [56,57]. During the first hour, comparisons were made among mouth rinses. The mean values of H_2S for chlorhexidine with alcohol, chlorhexidine alcohol-free and essential oils with alcohol/zinc chloride were significantly lower than the averages for those who used essential oils alcohol-free. Essential oils containing alcohol/zinc chloride had the best performance, followed by chlorhexidine with alcohol and then chlorhexidine alcohol-free. Clinically there was a lower level from baseline for these three products. The alcohol effect in this case can be of fundamental importance, because zinc chloride without alcohol could not be better than distilled water in a recent study by Kim and colleagues, published [58]. The disadvantage of this product was the intense burning sensation and tearing of the volunteers who resisted with sacrifice to rinse 20 mL of the product for 30 seconds. During the second hour, the average H_2S values were almost identical of those from the first hour, where the average values of H_2S for chlorhexidine with alcohol, chlorhexidine alcohol-free and the essential oils with alcohol/zinc chloride were significantly lower than the averages for those who used essential oils alcohol-free. Chlorhexidine with alcohol had the best performance, followed by essential oils with alcohol/zinc chloride and after the chlorhexidine alcohol-free. At this moment, the effect was similar to the previous, but the CHXw/a had the best performance, perhaps for the continuity of its antimicrobial action, reaching an average below the olfactory threshold (102ppb), namely, the bad breath already was (by average) imperceptible in the second hour. Next were the essential oils with alcohol and zinc chloride and chlorhexidine alcohol-free, in that order, corroborating other results of studies on the effects of neutralization of compounds or antibacterial activity of these agents [20,59]. During the third hour, the mean behavior was similar to the first and second hours. The mean values of H_2S to chlorhexidine with alcohol, chlorhexidine without alcohol and essential oils with alcohol/zinc chloride were significantly lower than the averages for those who used essential oils without alcohol. Chlorhexidine with alcohol had the best performance, followed by essential oils with alcohol/zinc chloride and after the chlorhexidine without alcohol. This can be explained by the inherent antimicrobial activity of chlorhexidine, regardless of the alcohol component, against gram-negative anaerobic micro-organisms H_2S producer [26,60,34]. Listerine Total Care® reached the lowest average (about 80ppb) being below the threshold of perception. In its composition with alcohol solvent in addition to the essential oils, has a penetration enhancer factor, both as bacterial and epithelial cells, and, more, in its composition there is zinc chloride which is readily soluble in ethanol and water, and for being metal ions can oxidize the sulfur compound and turn it into a non-volatile product [58]. Essential oils are regarded as breath masking, in other words, with its high odorous power may overlap the odor of the tested compounds [26].

Behavior of each mouth rinse over the 3 hour

Chlorhexidine with alcohol (A): There was a significant reduction in average throughout the entire time when compared to initial values. But comparing the first, second and third hours the reduction was not significant among them. There was a significant reduction in VSC levels throughout the time when compared to initial ones. But between the first, second and third hours the reduction was not significant among them, or, there was a considerable drop in the first hour and levels were maintained throughout the experimental period. Considered as a positive control in this study and considered as the gold standard of antiplaque agent and until then for halitosis [61], this product confirmed its validity. The big problems of chlorhexidine are the side effects of dental pigmentation, taste alteration that preclude its daily use. The purpose of this study was to verify the existence of an alternative as or more effective in controlling the breath as this product. This way will be considered the one with best properties to control the volatile sulfur compounds of intraoral origin and fewer adverse effects associated with its continued use. The search for a superior product with possibility of continued use is based on the fact that high concentrations of volatile sulfur compounds should be addressed locally (oral cavity), since both gases (H_2S and CH_3SH) are produced endogenously and contribute to human homeostasis and are not only toxic or unpleasant product of bacterial metabolism. The halitosis diagnosis usually takes some time to be determined in patients seeking treatment due to several factors that may be associated and are causing repulsion of people. Products resulting from tissue necrosis or systemic factors were excluded from this study so that this does not interfere with the behavior of the studied gases, since such problems require other approaches [62,63]. Even healthy patients and apparently without oral problems may be suffering from halitosis by protein degradation from the tongue coating or present in the crevicular fluid. Studies have shown an association of H_2S to bacterial sub-products on the tongue surface and the CH_3SH formation in periodontal conditions of disease [64,65]. The oral hygiene by mechanical removal of tongue biofilm is not always possible within the deepest niches and only mouth rinses would be able to penetrate deeper and have a longer lasting action.

Chlorhexidine alcohol-free (B): There was a significant reduction in average throughout the whole time when compared to initial values. But compared to the first, second and third hours the reduction was not significant among them. With a similar action to the version with

alcohol, its performance over time was not statistically different from the control, although it was a bit lower. It could be a valid option for patients with restrictions on the use of alcohol, unless they have high levels of sulfides (severe halitosis) and require antimicrobial control for a short period, and post-operative treatments or adequacy of oral environment, usually two weeks [82]. Clinically, patients finished the test with weak bad breath, noticeable only less than 10 cm away.

Essential oils with alcohol and zinc chloride (C): There was a significant reduction in average throughout the entire time when compared to initial values. But compared to the first, second and third hours the reduction was not significant among them. With better performance among other products to control the H_2S levels, was effective within the first hour with values under the olfactory threshold and seventh magnitude reductions, in other words, after one hour the average values were 7 times smaller than the initial and at the end of three hours. It was inferior only to the control, consequently a valid alternative for severe intraoral halitosis for unrestricted alcohol at longer periods than chlorhexidine.

Essential oils alcohol-free (D): There was a significant reduction in average throughout the entire time when compared to initial values. Negative values represent the difference between the mean of the breath with the increase over time, demonstrating that mouth rinse was not effective in reducing breath. From first to third there was an increase in values but not significant. The lack of efficacy throughout the study period (although being by the manufacturer as a call from the undesirable effects of alcohol) the product cannot be used in order to reduce the breath volatile sulfur compounds. Worse than that, led to an increase, without significance, baseline levels. Additional studies should be conducted to evaluate its effects on other compounds (such as volatile organic, for example) of putrefactive origin.

CH_3SH analysis

Considering the different values between the CH_3SH levels among mouth rinses regardless of the time (Table 3), paired comparisons were made, noting that the mean values of CH_3SH for essential oils with alcohol/zinc chloride differed from:

- chlorhexidine with alcohol
- chlorhexidine alcohol-free

So we proceeded to perform the analyses among mouthrinses at each time and for each one over time.

Whereas there were no differences between baselines among all mouthrinses, it was observed that:

Analysis of time:

First hour: During the first hour, comparisons were made between mouth rinses. The mean values of CH_3SH for essential oils with alcohol/zinc chloride were significantly lower than the averages for those who used chlorhexidine with alcohol and chlorhexidine without alcohol. Essential oils with alcohol/zinc chloride had the best performance, followed by a version without alcohol. Meanwhile, the versions of chlorhexidine had no effect on this odorivector. The Listerine® containing alcohol and zinc chloride was the most effective in reducing this compound followed by its alcohol-free version. The biguanide had no influence on this compound, in agreement with the predilection of periodontists by essential oils to combat periodontal bacteria [66]. It seems to exist one direct relationship between the essential oil activity and methanethiol generation. It must also be explored in future studies, the possibility of alcohol penetration and zinc chloride participation as this gas oxidant. Another factor to consider is the possibility to have occurred methanethiol demethylation into hydrogen sulfide using essential oils without alcohol.

Second hour: During the second hour, CH_3SH the mean values did not differ for each of the mouth rinses.

Third hour: During the third hour, the mean values of CH_3SH for essential oils with alcohol/zinc chloride were significantly lower than the averages for the essential oils without alcohol. Essential oils with alcohol/zinc chloride had the best performance. There was no difference among the other mouthrinses.

Behavior of each mouth rinse over the third hour:

Chlorhexidine with alcohol (A): It was not able to reduce the mean values compared to baseline or to other times.

Chlorhexidine alcohol-free (B): It was not able to reduce the mean values compared to baseline or to other times. It can be concluded that the presence of alcohol was unable to influence the performance of this ingredient on methanethiol.

Essential oils with alcohol and zinc chloride (C): It was the only mouth rinse that declined in the other three times. There was a significant reduction in average throughout the entire time when compared to initial values. Among the first, second and third time, there were no significant differences. It was the only mouth rinse that showed efficacy at all times compared to baseline and the effect lasted for up to 3 hrs. Analysis of the chemical reaction between alcohol, zinc chloride, the VSC and with association of proteolytic bacteria are required to elucidate these mechanisms.

Essential oils alcohol-free (D): It was not able to reduce the mean values compared to baseline or to other times. Compared to baseline values increased at the end of the third hour, however insignificant In summary, the mouth rinse based on essential oils with alcohol and zinc chloride was the only one capable of significantly reducing both values of H_2S and CH_3SH at the first hour and its effect lasted for up to three hours below the baseline values (significantly). Clinically appeared as weak values or imperceptible to the human sense of smell after the 1st, 2nd and 3rd hrs. Just 10 cm away could notice a faint breath, when it was possible. For H_2S, the mouth rinse with chlorhexidine with and without alcohol also obtained satisfactory results for reducing the breath. Only the product based on essential oils without addition of alcohol was not able to reduce the breath and enabled to increase the H_2S values initially recorded. For CH_3SH, none mouth rinse was able to reduce the breath from the initial values than the one based on essential oils with alcohol and zinc chloride in the formulation. This information can be best seen in the graphs a) and b) of (Figure 3). Products with alcohol in the composition achieved the best results for reducing H_2S but for CH_3SH chlorhexidine alcohol was not able to significantly reduce of breath, whereas compound with alcohol and zinc chloride (essential oils) was unsurpassed. The alcohol-free mouth rinses presented the worst results, and essential oils being able to increase the initial values for both gases after 3 hrs.

Conclusions

At the end of the clinical phase, with the agreement and abidance of 21 volunteers throughout the study, in light of the results and within the limitations explored in the discussion of the work, we may

conclude that:

- The mouth rinse based on essential oils, alcohol and zinc chloride was the only one able to efficiently reduce the breath by controlling the volatile sulfur compounds (odorivectors) from intra-oral source for up to three hours after single use;
- The elimination of alcohol and the absence of zinc chloride in the new Listerine "zero" version had a negative effect on the efficacy of the product against halitosis of oral origin;
- The control product had the second best performance, being effective only against the odorivectors sulfur hydrates. The alcohol-free version can also be an option in cases where alcohol prohibition is imperative. The elimination of alcohol does not significantly alter the performance of chlorhexidine.

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