



UvA-DARE (Digital Academic Repository)

Prevention and therapy of periodontal diseases and oral malodour

van der Sluijs, E.

[Link to publication](#)

Citation for published version (APA):

van der Sluijs, E. (2017). Prevention and therapy of periodontal diseases and oral malodour: Brush, rinse and cool DIDES

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <http://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.



5

The effect of water on morning bad breath: a randomized clinical trial

E. van der Sluijs

D.E. Slot

E.W.P. Bakker

G.A. van der Weijden

Abstract

Aim

To assess the effects of water on the parameters of 'morning bad breath' (MBB) and to evaluate whether there is a difference between rinsing with water and drinking a glass of water.

Methods

A total of 50 participants were recruited and were randomly divided into two equal groups. One group rinsed with 15 ml of water for 30 s, and another group drank 200 ml of water within 30 s. Clinical assessments were carried out during one visit between 7:30 am and 12:00 pm. Pre- and post- intervention measures were assessed organoleptically as primary outcome parameters, and a secondary outcome parameter was assessed using both the Halimeter® and OralChroma™ apparatuses to evaluate volatile sulphur compounds (VSCs), hydrogen sulphide (H₂S), methyl mercaptan (CH₃SH) and dimethyl sulphide ((CH₃)₂S). In addition, the presence of tongue coating (discoloration/thickness) and tongue fissures was assessed.

Results

All 50 participants completed the study. In both groups, a significant reduction in the organoleptic score and the OralChroma™ H₂S and CH₃SH readings was obtained after the intervention. Both regimens resulted in a CH₃SH reduction of approximately 60%, whereas the reduction in H₂S was between 30% and 50%. The acceptable change between pre- and post- assessments of the clinical parameters was not significantly different between the drinking and rinsing groups.

Conclusion

Rinsing with water or drinking a glass of water had a statistically significant effect on the MBB parameters. No significant difference was obtained between the two regimens.

Introduction

Halitosis is a general term used to describe an unpleasant or offensive odour emanating from the oral cavity. A precise estimate of the prevalence of halitosis is not possible. In different parts of the world, different assessments and cut-off points are presented in the literature, resulting in a prevalence range of 2–42% (1). Several (non-oral) pathological conditions have been related to oral malodour, including infection of the upper and lower respiratory tracts, infection of the gastrointestinal tract, and some metabolic diseases involving the kidneys or the liver (2). However, clinical surveys have shown that approximately 90% of all bad breath odours originate in the mouth (3).

It is well accepted that the pathogenesis of oral malodour is associated with the bacterial degradation of sulphur-containing amino acids (methionine, cysteine and cystine) into volatile sulphur compounds (VSCs), the principal components of which are hydrogen sulphide (H_2S), methyl mercaptan (CH_3SH) and, to a lesser extent, dimethyl sulphide ($(CH_3)_2S$) (4–9).

Increased public awareness and demand for oral malodour remedies have resulted in a substantial growth of the breath industry and in saturation of the market with breath-improving products such as mints, chewing gum, breath sprays and pills. Although some of these products provide modest breath improvement, the majority have only a short-term 'masking' effect on bad breath and are essentially ineffective (10). Due to the importance of social interactions in contemporary society, many populations in countries around the world are becoming increasingly concerned about halitosis and now pay more attention to this condition. This concern was reflected in the results of a telephone survey in which 60% of American women and 50% of American men reported the use of cosmetic breath-freshening products (11).

After sleeping, malodorous breath upon awakening is a common condition known as 'morning bad breath' (MBB). This problem tends to be transient in nature, in contrast to persistent halitosis. Both halitosis and MBB appear to result largely from excessive quantities of sulphur-containing gases of bacterial origin (6,12,13). Low salivary flow, particularly during the night, creates a favourable environment for bacterial proliferation and putrefaction and results in physiological 'morning breath', which is the most common breath complaint (6,14–17). To reduce MBB, several consumer websites suggest that rinsing with or drinking water upon awakening is effective (18) because MBB can be caused by a dry mouth. The hypothesis is that drinking water helps to stimulate the production of saliva and to saturate the whole mouth (19–21). However, rinsing with water is also suggested as a remedy for dry mouth (22); therefore, it seems to be the obvious first-aid measure to take. This home remedy is, however, not supported by any scientific evidence.

Therefore, considering the absence of scientific substantiation, this study was initiated with the primary aim of evaluating the effects of the use of water on MBB parameters in periodontally and systemically healthy participants. The secondary aim was to compare the effects of rinsing with water with those of drinking a glass of water.

Materials and methods

Ethical procedures

This study adhered to the principles of the Helsinki Declaration, approximating Good Clinical Practice guidelines, and received the approval of the medical ethics committee of Amsterdam Medical Centrum (number 2011_302#C2011821). The study was registered in the Dutch Trial Register under number NTR3241 and was performed at the ACTA Department of Periodontology from October to December 2011. Before enrolment, all participants were given verbal and written instructions as well as descriptions of the aim, the rationale and the duration of study participation. All eligible subjects who agreed to participate signed an informed consent form prior to study enrolment.

Sample size

In the absence of previous studies on this topic, insufficient data were available for *a priori* power/sample size calculations. Furthermore, the guidelines for product testing used in the Management of Oral Malodour (2003) by the American Dental Association (ADA) (23) make no reference to the minimum number of participants that need to be included. An analogous ADA guideline on Floss and Other Interdental Cleaners (24) recommends at least 25 participants per group. Therefore, it was decided to follow this recommendation regarding the sample size, and 50 participants were recruited for this study. To verify this decision, a *post hoc* power analysis was performed.

Recruitment and inclusion

Non-dental students from different universities and colleges in and around Amsterdam who had indicated in a database that they were potentially interested in participating in clinical research were notified by email and flyer about applying for a screening appointment. Participants with self-reported MBB at least five times a week were potentially eligible for inclusion. Potential participants were questioned in the recruitment email and follow-up telephone interview about MBB. Question included: *Do you suffer from MBB as assessed by yourself or as noticed by others? If you suffer from MBB, is this at least five times a week?*

Before enrolment, participants provided their written informed consent, which included their consent to adopt specific lifestyle rules prior to study initiation. The participants were first screened using a questionnaire and were also screened clinically by a dental hygienist. The participants were assessed for the following eligibility criteria: age ≥ 18 years old; classified as systemically healthy, as assessed by the medical questionnaire, and periodontally healthy, as assessed by a Dutch Periodontal Screening Index DPSI score ≤ 3 (25,26); and the presence of at least 5 teeth per quadrant. Respondents who presented with an orthodontic appliance or a removable (partial) denture or who were smokers were excluded. Additional exclusion criteria were the following: caries, any pathological alterations of the oral mucosa, pregnancy, acute sinusitis or severe oral-pharyngeal infections; any medications that can cause malodour; and a reduced salivary flow due to pathological reasons. In addition, respondents who had participated in a clinical study within the previous 30 days were not allowed to participate.

Prior to the experiment, the participants were instructed to adhere to specific lifestyle rules to avoid factors that may influence the oral malodour examination, as suggested in the ADA guidelines (23) and described in detail in Box 1 (27).

Box 1 Details of the lifestyle rules to which the participants were requested to adhere to avoid interference of food constituents on morning bad breath assessments:

Time before MBB assessment	Participants were requested...
>48 h	not to eat spicy food or drink alcohol
>8 h	to abstain from eating and drinking prior to the assessment
>8 h	not to use scented products (shampoo, body lotion, perfume, aftershave, lipstick, make-up)
>8 h	not to use, e.g., chewing gum and peppermint drops
>3 h	not to drink water
Morning of the assessments	to refrain from all oral hygiene practices and come to the clinic while fasting

Design

The study was designed as a randomized clinical trial (RCT). Due to the nature of the interventions, which demanded active contribution by the participants, they could not be blinded. Figure 1 shows a flowchart (according to the CONSORT statement/guidelines) (28) of the study outline and the clinical assessments. Randomization was performed using true random numbers obtained via www.random.org, which was performed by an independent investigator (DES). This process, which refers to ‘atmospheric noise’, generate true random numbers which involves identifying little, unpredictable changes in the data.

Outcome measurements

Assessments were carried out during a single visit in the same room where pre- and post-scores were obtained before and after the allocated intervention, which was performed in another (same) room to ensure that the examiner was blinded to the intervention. The clinical assessments were taken between 7:00 am and 12:00 pm for each participant during the study. Lifestyle rules requested participants to avoid spicy foods, alcohol and the use of any kinds of perfume. In addition to appear with an empty stomach on the morning of the assessments (see Box 1).

Organoleptic score

The primary MBB outcome variable was the organoleptic score, which was determined by one blinded examiner (EvdS) who was a trained and calibrated judge. As a refresher, the judge tested the ability to distinguish odours using the Smell Identification Test® (Sensonics Inc., Haddon Heights, NJ, USA); in addition, the judge tested the ability to detect odours at low concentrations using a series of dilutions of the following substances: skatole, putrescine, isovaleric acid and dimethyl disulphide (29). Each participant was instructed to close his/her mouth for 2 min and then to slowly open his/her mouth at the request of

the examiner. Immediately upon opening the mouth, the judge sniffed the dorsum of the tongue of the participant at a distance of approximately 3–5 cm. Participants were instructed to breathe through the nose through-out this procedure. The judge performed two consecutive organoleptic assessments, and the mean of both scores was used as the individual organoleptic score. The organoleptic scale was defined as follows: 0 = absence of odour, 1 = barely noticeable odour, 2 = slight odour, 3 = moderate odour, 4 = strong odour and 5 = extremely strong odour (6 further modified by 30).

Volatile Sulphur Compound assessments

In addition, VSC assessments were performed using the Halimeter® RH-17 (Interscan Corporation, Simi Valley, CA, USA) and the OralChroma™ CHM-1 using the data management software: ABIMEDICAL for Windows version 3.5.0 (FIS Inc. Itami, Hyogo, Japan). Prior to the study, a calibration of the apparatuses was performed according to the manufacturer's recommendations. The OralChroma™ and Halimeter® were switched on 24 h before each visit to enable them to acclimatize to the ambient air. Before the assessments, each apparatus was calibrated to approximately zero. A second examiner (SCS) was responsible for operating this equipment in the absence of the organoleptic judge to avoid introducing any feedback bias to the organoleptic assessment based on the outcomes of the oral malodour equipment. Before the VSC examinations, each participant was instructed to keep his/her mouth and lips closed, to breathe through the nose for 2 min and not to swallow, which facilitated the build-up of VSCs in the oral cavity.

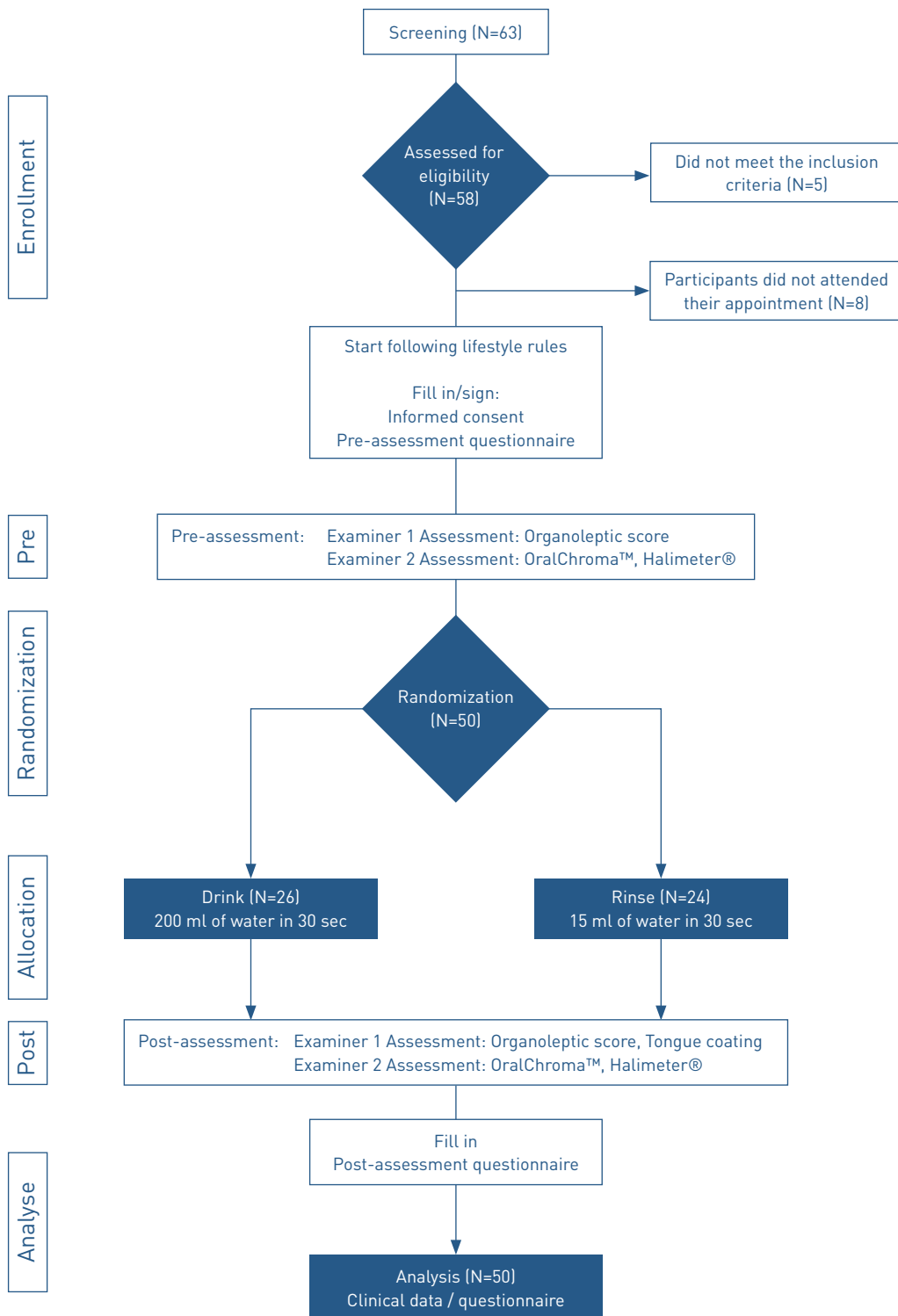
OralChroma™

Upon the request of the examiner, the mouth was slightly opened. A sterile disposable syringe was inserted through this opening into the oral cavity and placed between the front teeth. The participant was instructed to avoid touching the tip of the syringe with the tongue. The piston was subsequently pulled to the very end of the syringe to fill the syringe with a breath sample from the oral cavity. The syringe was then removed from the oral cavity. Any adherent saliva was wiped off the syringe with tissue paper. A gas injection needle was connected to the tip of the syringe, and 0.5 ml of the breath sample was discarded. The remaining 0.5 ml of the breath sample was injected into the OralChroma™ with a single push. The VSC reading of the OralChroma™ provided the concentration values of H₂S, CH₃SH and (CH₃)₂S in (parts per billion) ppb and ng ml⁻¹. These values were recorded separately, and chromatograms were printed for analysis.

Halimeter®

The VSCs were also scored using a portable industrial sulphide monitor (Halimeter®; Interscan Corp., Chatsworth, CA, USA). The unit was zeroed to ambient air before each measurement using the technique established by Rosenberg et al. (6). A disposable straw was placed between the participant's front teeth. The participant placed his/her teeth around the straw and held his/her breath as the instrument drew air from the mouth to the sensing chamber. The operator recorded the peak concentration of VSCs, displayed in ppb. The values were recorded, and the mean of these values was determined in ppb of sulphide equivalents.

Figure 1 Flow chart of the study design and the study population.



Tongue surface readings as possible confounding factor

The tongue coating (thickness and colour) was examined by the first examiner (EvdS) (25). The procedure used to assess tongue coating was a modification of the method described by Miyazaki et al. (8) and further described in detail by Gómez et al. (25). The tongue was assessed from the vallate papillae to the tip, that is the back third, the middle third and the front third (8), as well as from the left to the right, that is the left third, the middle third and the right third. For each of the nine sections, discoloration and coating were visually assessed. The discoloration was scored on a scale from 0 to 4 (0 = pink, 1 = white, 2 = yellow/light brown, 3 = brown and 4 = black), and coating was scored according to thickness on a scale from 0 to 2 (i.e. 0 = no coating, 1 = light-thin coating and 2 = heavy-thick coating). Light-thin coating was scored when the pink colour underneath remained visible through the coating. Heavy-thick coating was scored if no pink colour could be observed under the coating. For each section of the tongue, more than 1/3 had to be covered to obtain a score other than 0. As a potential source of oral bacteria, the presence or absence of fissures on the tongue surface was also recorded.

Questionnaire

The participants were asked to provide details about their daily use of oral hygiene tools and products. In addition, their own perception of their MBB before and after the intervention was assessed using a visual analogue scale (VAS). On a 10-cm- long uncalibrated line, 0 corresponded to 'stale' and 10 corresponded to 'fresh'. The participants indicated their perception by placing a vertical mark along this line.

Allocation, blinding and intervention

An independent supervisor (RSK) used the obtained allocation randomization sequence to assign the participants to the intervention regimen and concealed the allocation from the examiners. The instructions were provided to the participants in sealed opaque envelopes, and the rinse and drink intervention procedures were supervised. Due to the nature of the interventions, the participants were aware of the intervention to which they were assigned, but they were requested not to reveal this information to the examiners. The participants received and read the detailed written and illustrated instructions in a room shielded from the examiners. Based on the randomization sequence, participants were assigned to the drink or rinse group. The drink group drank 200 ml water and were instructed to drink the 200 ml of water calm and gradually within the time frame that was given. The other group rinsed with 15 ml water with moderate power on the cheeks, for 30 s. A stopwatch was used to keep track of the time of either rinsing or drinking. The water used for both regimens was Bar-Le-Duc water [which contained the following compounds: chloride (Cl) 10.3 mg l⁻¹, calcium (Ca) 47 mg l⁻¹, sodium (Na) 10.6 mg l⁻¹, potassium (K) 0.6 mg l⁻¹, magnesium (Mg) 3.4 mg l⁻¹, hydrogen carbonate (HCO₃) 170 mg l⁻¹] within 30 s.

Statistical analysis

First, the population demographics were determined for each group with point estimates and variability measures. Oral hygiene practices and tongue surface characteristics were

Table 1 Subjects' demographic and oral hygiene practices according to the assigned group regimen. In addition, the appearance of the dorsum of the tongue according to the assigned regimen (tongue coating and fissures) is presented.

		Drink N=26	Rinse N=24	
Population N=50	Age Mean (SD) Range Median Min max	23.1 (2.64) 19-30 years 22.5 19-30	21.9 (2.30) 18-26 years 21.0 18-26	
	♀ Female	17/26 (65.4%)	14/24 (58.3%)	
Hygiene devices	Power toothbrush	12/26 (48%)	5/24 (20.8%)	
	Manual toothbrush	20/26 (76.9%)	18/24 (75%)	
	Floss	10/26 (38.5%)	5/24 (20.8%)	
	Woodsticks	9/26 (34.6%)	7/24 (29.2%)	
	Tongue cleaning	15/26 (57.7%)	13/24 (54.2%)	
	Fluoride rinsing	13/26 (50%)	8/24 (33.3%)	
Tongue surfaces	Tongue discoloration ♦	Score 0 Score 1 Score 2 Score 3 Mean score (SD)	16% 36% 47% 0.5% 11.92 (3.68)	11% 32% 55% 3% 13.50 (2.45)
	Tongue coating thickness ◊	Score 0 Score 1 Score 2 Mean score (SD)	31% 24% 40% 9.77 (3.72)	24% 27% 50% 11.33 (2.37)
	Tongue fissure		4%	6%

♦ = On a scale of 0-3; mean percentages of sites (out of 9) for each discoloration score.
 ◊ = On a scale of 0-2; mean percentages of sites (out of 9) for each coating thickness score.

indicated by absolute numbers and frequencies. For all parameters (organoleptic and VSC), baseline and end data were summarized by the mean and SD first by individual and, subsequently, by group. Changes within groups and differences between groups were calculated.

Organoleptic scores (range 0–5) were analysed using the Wilcoxon signed- rank test to assess differences within groups and using the Mann–Whitney U-test to assess differences between groups. Because the VSC data from the bad breath apparatus were continuous, they were tested for normality using the Kolmogorov–Smirnov test. In cases of non-normal data distribution, nonparametric tests were used. P-values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS software (Statistical Package for Social Sciences, version 20.0 for Windows). Correlations between organoleptic scores and tongue coating thickness/discoloration were analysed using Spearman's rank test.

Results

Participants

A total of 63 participants were screened for this clinical trial. Thirteen participants were excluded, namely six participants who did not attend. Four participants were excluded who were not systemically healthy and were using antibiotics. One was excluded because he did not consider himself as suffering from MBB. Six participants did not show up for their appointment and two cancelled their appointment because of illness. A total of 50 participants completed the single visit. Figure 1 provides a flowchart of the study design and the number of participants involved. The demographics of the participants are presented by group in Table 1, which includes a summary of oral hygiene practices.

Tongue coating (thickness/colour)

A mean score for each of the nine sections of the tongue surface was calculated (see Fig. 2/3). The analysis showed no significant difference in total tongue surface discoloration scores ($P = 0.264$) or tongue coating thickness scores ($P = 0.158$) between the groups. Table 1 presents the distribution of scores, with the score corresponding to the highest score recorded for one or more of the nine sections of the tongue. The appearance of the dorsum of the tongue is presented in relation to the assigned regimen. A discoloration score of 3 was seldom observed; the highest percentage was observed for discoloration score 2. Regarding tongue coating thickness, the highest percentage was found for score 2 in both groups. With both regimens, tongue discoloration most frequently received a score of 2 (yellow/light brown) in the posterior- and mid-dorsal regions of the tongue. Tongue coating thickness most frequently received a score of 2 (heavy-thick) in the posterior dorsal sections of the tongue. Both tongue discoloration and tongue coating mostly received scores of 0 in the anterior sections (pink and no coating, respectively). The prevalence of tongue fissures was low in both groups (4–6%).

Primary outcome

Table 2 provides a summary of the outcomes of the organoleptic scores (scale 0–5) for the rinsing and drinking regimens. Both regimens yielded a significant decrease in the organoleptic score. The score reduction was 0.46 (0.51; $P = 0.01$) in the drinking group and 0.33 (0.48; $P = 0.05$) in the rinsing group. There was no significant difference between the regimens at any time point, nor was the incremental change following the regimen different between the groups ($P = 0.360$). The correlation coefficients between tongue discoloration and organoleptic scores ($R = 0.248$, $P = 0.083$), as well as between tongue coating thickness and organoleptic scores ($R = 0.175$, $P = 0.224$), were small and not significant.

Secondary outcome

Most of the VSC outcome data measured by the Halimeter® and OralChroma™ showed a non-normal distribution. Table 2 shows that before each regimen, no significant differences between groups were observed. Following the two regimens, the Halimeter® outcome showed a reduction between the pre- and post-intervention results of 11.12 in the drinking

group and 14.17 in the rinsing group, neither of which was significant ($P = 0.884$). Regarding the VSC levels as assessed by the OralChroma™ apparatus, there was a significant decrease in the levels of the two different gases, H_2S and CH_3SH , in both regimens. $(CH_3)_2S$ showed a reverse trend, namely a non-significant increase of 10.77 in the drinking group and 23.14 in the rinsing group was found. None of the OralChroma™ outcomes related to the three gases showed a significant difference between the groups with respect to the incremental changes between pre- and post-assessments.

Questionnaire

The mean scores and standard deviations of the subjective perception of the participants related to their MBB before and after the assigned regimen were not significantly different between the two groups, as demonstrated in Table 3. The change in the VAS score in the drinking group was 0.59 (2.00), which was not significant ($P = 0.146$). The rinsing group perceived a change of 1.00 on the VAS scale, which proved to be statistically significant ($P = 0.001$).

Power and sample size calculation

For the *post hoc* power analysis for the within-group changes in this study (primary aim), the pooled standard deviation of the organoleptic scores (0.495) and the average difference between the baseline and end scores in both groups (0.395) were used. If the Type I error probability associated with the null hypothesis was set at 0.05, the probability (power) of rejecting the null hypothesis that the population means at baseline and at the end of the experiment were equal was 0.789. For the secondary aim of this study, the *post hoc* power analysis between groups showed that the present study, with a pooled SD of 0.495, a Type I error of 0.05, and 25 participants per group, was able to detect a true difference in the mean response of 0.4 with a probability of 0.8 (power). The observed difference between groups in the organoleptic scores (0.13), with a Type I error of 0.05 and 25 participants per group, had a power of 23%.

Discussion

Bad breath in healthy subjects is a cosmetic problem that is analogous to body malodour (31). In healthy people, MBB is caused by a proliferation of oral bacteria during sleep, which release offending gases. Several papers (12,16,32–35) agreed that the MBB model is suitable alternative for testing the clinical efficacy of different treatments for oral halitosis. A regimen of drinking or rinsing with water upon awakening has been promoted in the layman's press (see the Introduction) to reduce MBB. However, to the best of our knowledge, this home remedy is not supported by any scientific evidence. Therefore, the aims of this study were to evaluate the effects of the use of water on MBB and to compare the effects of drinking a glass of water with those of rinsing with water in periodontally and systematically healthy participants. The primary MBB outcome variable was the organoleptic score (6, further modified in 30). The organoleptic score showed, on average, a baseline score >2 in both

Figure 2 Percentages of subjects with a score of 2 as the highest discoloration score (on a scale of 0-3), according to section of the tongue, for the drinking group (D) (N=26) and the rinsing group (R) (N=24).

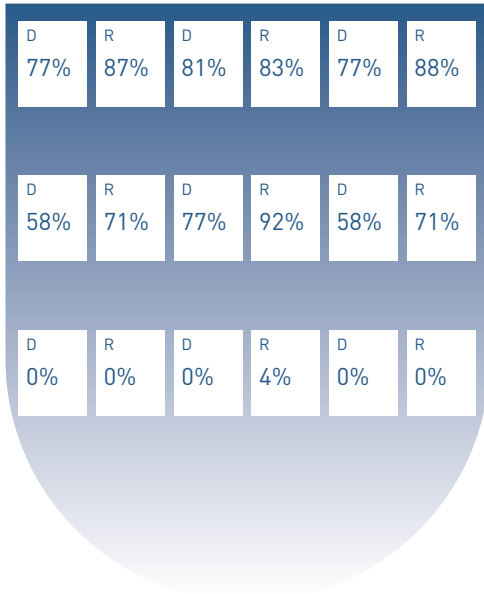
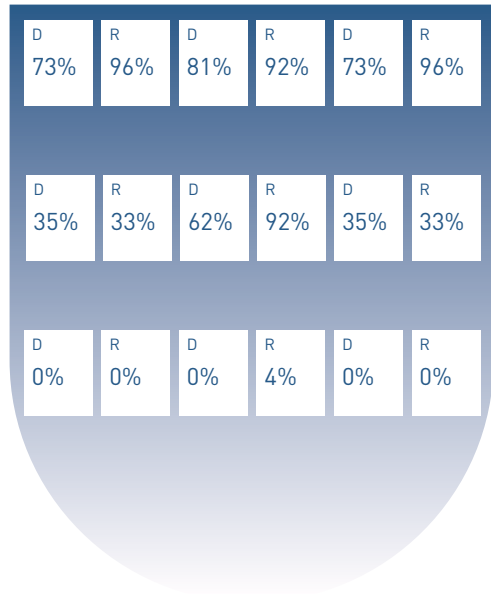


Figure 3 Percentages of subjects with a score of 2 as the highest tongue coating thickness score (on a scale of 0-2), according to section of the tongue, for the drinking group (D) (N=26) and the rinsing group (R) (N=24).



groups. Based on these values, the participants were considered suitable for the present investigation of MBB treatment. The results demonstrated that both regimens resulted in a significant reduction of the organoleptic scores following the intervention; however, there was no difference between drinking and rinsing. When researching oral malodour, it is essential to impose lifestyle rules on the participants with regard to eating habits, body hygiene, alcohol consumption and abstaining from eating or drinking on the morning of the assessment because all of these factors have an impact on MBB. Because the present clinical trial was designed as a single visit, issues such as lack of patient compliance with these rules were not considered to play a major role. A practical implication of the present study is that prior to an MBB examination, the use of water should be considered as a variable that will significantly affect the various outcomes of the assessment. In general, the use of water is not considered to have an impact on the outcome parameters. Some authors have even suggested that water can be consumed up to 3 h before the assessment (36).

Tongue coating

The level of tongue coating discoloration and thickness was established according to the method proposed by Gómez et al. (25). Tongue coating discoloration was especially present on the back part of the tongue in the middle section. A score of 2 (heavy-thick) was most frequently given for tongue coating thickness in the three posterior dorsal sections of the tongue. This result was in agreement with those of Tonzetich et al. (12), who reported that tongue cleaning is twice as effective as tooth brushing for reducing oral malodour. Therefore, they advised that tongue cleaning should be a part of daily home oral hygiene

Table 2 Mean [standard deviation] of all clinical parameters from organoleptic and oral malodor apparatus assessments (Halimeter®, OralChroma™) during the study for both treatment modalities (rinsing and drinking). In addition, the outcome of the statistical analysis is presented.

(N=50)		Drink (N=26)			Rinse (N=24)			Statistical analysis between groups**
		Pre	Post	Difference	Pre	Post	Difference	
Organoleptic score		2.23 [0.71]	1.77 [0.59]	-0.46 [0.51]*	2.25 [0.61]	1.92 [0.58]	-0.33 [0.48]*	0.360
Halimeter®		129.08 [62.45]	117.96 [52.16]	-11.12 [40.02]	152.54 [104.56]	138.38 [81.12]	-14.17 [76.68]	0.884
OralChroma™	H ₂ S	400.08 [394.03]	199.65 [185.46]	- 200.42 [388.32]*	350.67 [272.84]	216.79 [182.58]	-133.88 [101.39]*	0.923
	CH ₃ SH	154.46 [226.06]	42.38 [50.90]	-112.08 [241.75]*	93.50 [96.71]	32.63 [46.92]	- 60.88 [101.39]*	0.930
	(CH ₃) ₂ S	86.73 [61.56]	97.50 [106.13]	+10.77 [116.82]	60.46 [54.86]	83.58 [95.91]	+ 23.13 [100.37]	0.676

* Wilcoxon signed-rank test for significant differences within groups (P<0.05)

** Mann-Whitney U test for significant differences between groups (P<0.05)

procedures (37). The tongue coating and discoloration results showed that the tip of the tongue had the lowest scores. This finding is agreement with those of Gómez et al. (25), whose study of the geographic prevalence of tongue coating showed that most discoloration is found in the dorsal 2/3 of the tongue. In the present population, tongue coating was predominantly found in the posterior sections, especially in the middle section. An analysis of the correlation between tongue coating thickness/discoloration and organoleptic scores revealed a small and non-significant correlation coefficient. Furthermore, tongue fissures were a rare phenomenon in this study population, implying that the oral malodour data were not confounded by tongue surface appearance.

Halimeter®

According to Iwanicka-Grzegorek et al. (38), the average level of VSCs considered to correspond to 'no halitosis', as assessed with the Halimeter®, is defined as an average VSC concentration of <75 ppb. Physiological halitosis is defined as an average VSC level ≥75 ppb. In the present study, the average Halimeter® scores at baseline were 129.08 and 152.54 in the rinsing and drinking groups, respectively, which were both well above the level of 75 ppb, indicating that oral malodour was present. According to Brunner et al. (39), the use of a combination of Halimeter® and organoleptic scores is recommended because they can be obtained easily, reliably and quickly. These authors showed that Halimeter® outcomes ≤50 ppb indicated 100% 'no halitosis', as confirmed by the organoleptic scores. For Halimeter® levels in the range of 100–150, the authors observed a 45% agreement with score 1 of the organoleptic assessment. The outcome of the present study is within this Halimeter® level range; however, the observed organoleptic scores were higher (2.23 and 2.25 for the rinsing and drinking groups, respectively). This result may have occurred due to the different

Table 3 Mean (standard deviation) of the Visual Analogue Scale score of the participants' perception of their morning bad breath as assessed by a questionnaire before and after each regimen (rinsing or drinking).

Extreme	(N=50)	Pre	Post	Difference	P-value **
Stale - Fresh (0 - 10)	Drink (N=26)	2.60 (1.96)	3.19 (1.52)	0.59 (2.00)	0.146
	Rinse (N=24)	1.66 (1.76)	2.66 (1.66)	1.00 (1.36)	0.001
	Difference	0.94 (0.53)	0.52 (0.45)	-0.42 (0.49)	
	P-value*	0.612	0.252	0.399	

* Independent samples T-test.

** Paired samples T-test. Significant difference within the groups, pre- vs. post-assessment (P>0.05)

manner in which the organoleptic assessment was performed. The present study judge sniffed the tongue surface at a close distance (3–5 cm) to the dorsum of the tongue of the participant (6,30), whereas the Brunner et al. (39) examiners sniffed at distances of 10 cm and 1.5 m. The results of the present study showed that although a reduction was observed, participants still had an organoleptic score close to 2 and a Halimeter® score >75 ppb, which can still be considered ‘unpleasant’.

OralChroma™

In addition to the organoleptic score, mouth air was analysed using gas chromatography (OralChroma™). H₂S is a major component of physiological halitosis, whereas CH₃S is a cause of pathological oral halitosis (40). In the present study, the greatest reduction from baseline was found for H₂S (-200 ppb and -133 ppb for the rinsing and drinking groups, respectively (Table 2)). Dimethyl sulphide correlates with oral malodour strength (40–42). The present results showed a small, non-significant increase in dimethyl sulphide. The explanation for the absence of a significant effect or even of a trend towards an increase may be that dimethyl sulphide levels are reported to be more related to extra-oral causes (43). It is therefore not surprising that in the present study, no relationship between dimethyl sulphide levels and an oral intervention (rinsing or drinking) was observed.

Effect size

In the absence of studies with comparable interventions, this study lacked a proper *a priori* power calculation. The lack of significant differences between groups may lead to the assumption that this study was underpowered. With respect to the primary aim of this study, which was to evaluate the effects of the use of water on MBB, the *post hoc* power analysis revealed that the within-group change had a power of 0.795. The comparison between groups was, however, underpowered to reject a false null hypothesis. The incremental difference in organoleptic scores between groups should have been 0.4 instead of the observed 0.13 to have been significantly different with a power of 0.8. The effect size of the rinsing regimen on the organoleptic score was 0.33, which was larger than that suggested in a recent systematic review on the effect of mouthrinses on oral malodour (10). This systematic review included twelve papers, and it was concluded that nearly all mouthwashes

with active ingredients had beneficial effects with regard to reducing oral malodour in both the short-term and long-term studies. The review of the short-term studies demonstrated a small reduction of organoleptic scores resulting from the control mouthwash interventions (0.14–0.20), the magnitude of which was approximately half the effect size of the single-use intervention observed in the present study.

Questionnaire

Table 3 shows the participants' perception of their MBB. The participants reported an increased feeling of freshness with respect to their own MBB after both regimens. This result is in line with the organoleptic observation. However, this change in perception was only significant in the rinsing group, which indicated that moving the fluid vigorously through the mouth most likely had a greater impact on the feeling of freshness than drinking a glass of water.

Limitations

No information was gathered with respect to the hours the participants were awake. Although appearing on an empty stomach, the change in salivary flow rate could influence baseline levels. As spontaneous fluctuation in breath samples has been demonstrated by several studies (40,43). This may explain the large differences in OralChroma™ and Halimeter® readings, although the ORG readings were rather low. The results could have been strengthened if for both apparatus, three breath samples had been taken at each time point and averaged these values.

Implications for further research

Seemann et al. 2014 (44) published a paper which summarizes the results of a consensus workshop about how to assess and diagnose patients' breath odour. This paper gives professionals a general guideline for the treatment of halitosis.

The outcome of MBB studies or of any study on oral malodour is influenced by eating habits and by the use of cosmetics, soaps and deodorants (23,27). Therefore, studies of breath odour are usually preceded by a 2-day period during which specific lifestyle rules must be followed (Box 1). The outcome of the present study indicates that prior to an MBB assessment, the use of water (rinsing or drinking) will affect the outcomes of such studies. Consequently, water use should be considered as part of the lifestyle rules so that it does not affect MBB parameters.

Directions for further research

In the present study, the judge assessed the organoleptic score by sniffing the dorsum of the tongue of each participant at a distance of 3–5 cm. Several other methods and distances are described in the literature. For instance, Vandekerckhove et al. (45) assessed nasal breath (when the subjects exhaled through the nose while keeping the mouth closed), Evirgen et al. (46) assessed air at a distance of 10–20 cm from the nose of the clinician and the volunteer in the study by Peruzzo et al. (31) was instructed to slowly release air from the mouth at a 10-cm distance from the examiner's nose. A study to determine the most sui-

table method for obtaining organoleptic scores should be performed to help standardize organoleptic assessments.

Furthermore, salivary flow rate diminishes during sleep which is considered one factor responsible for morning breath odour. It would be interesting to see what odour reduction levels one simply gets by virtue of being awake, with salivary flow restored to normal function. Eventually, in further research, specific pathologies in relation to MBB could be investigated.

Conclusion

Rinsing with 15 ml water or drinking a glass of 200 ml water had statistically significant effect on the MBB parameters. No significant difference was obtained between the two regimens.

Acknowledgements

The authors thank the Stichting Nederlandse Vereniging voor Parodontologie (NVvP) for providing financial support for this study. This paper was prepared as part of the obligation of the first author to fulfil the requirements of the 'Master of Evidence-Based Practice' of Amsterdam Medical Center. The first author thanks the employability fund of ACTA for financially supporting this additional education. The authors fully acknowledge Sam Supranoto as the second examiner, who helped conduct the study and who was responsible for operating the Halimeter® and OralChroma™ apparatuses. In addition, Rosalien Keukenmeester is gratefully acknowledged for her help in explaining the instructions for the rinsing and drinking procedures to the study participants.

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest. The department of Periodontology from ACTA department received financial support from the Stichting NVvP, which is the Foundation Dutch Society for Periodontology for this project.

Authors contributions

Conception or design of the study: EVDS, DES, GAW

Analysis and/or interpretation of the data: EVDS, DES, EWPB, GAW

Drafted the manuscript: EVDS

Critically revised the manuscript: DES, EWPB, GAW

All authors gave their final approval and agreed to be accountable for all aspects of the work

References

1. Rösing CK, Loesche W. Halitosis: an overview of epidemiology, etiology and clinical management. *Braz Oral Res* 2011; 25: 466–471.
2. Tangerman A, Winkel EG. Intra- and extra-oral halitosis: finding of a new form of extra-oral blood-borne halitosis caused by dimethyl Sulphide. *J Clin Periodontol* 2007; 34: 748–755.
3. Delanghe G, Ghyselen J, van Steenberghe D, Feenstra L. Multi-disciplinary breath-odor clinic. *Lancet* 1997; 350: 187.
4. Tonzetich J, Carpenter PA. Production of volatile sulphur compounds from cysteine, cystine and methionine by human dental plaque. *Arch Oral Biol* 1971; 6: 599–607.
5. Persson S, Edlund MB, Claesson R, Carlsson J. The formation of hydrogen sulfide and methyl mercaptan by oral bacteria. *Oral Microbiol Immunol* 1990; 5: 195–201.
6. Rosenberg M, Septon I, Eli I et al. Halitosis measurement by an industrial sulphide monitor. *J Periodontol* 1991; 62: 487–489.
7. Kleinberg I, Westbay G. Salivary and metabolic factors involved in oral malodor formation. *J Periodontol* 1992; 63: 768–775.
8. Miyazaki H, Sakao S, Katoh Y, Takehara T. Correlation between volatile sulphur compounds and certain oral health measurements in the general population. *J Periodontol* 1995; 66: 679–684.
9. Waler SM. On the transformation of sulfur-containing amino acids and peptides to volatile sulfur compounds (VSC) in the human mouth. *Eur. J Oral Sci* 1997; 105: 534–537.
10. Blom T, Slot DE, Quirynen M, Van der Weijden GA. The effect of mouthrinses on oral malodor: a systematic review. *Int J Dent Hyg* 2012; 10: 209–222.
11. Tessier JF, Kulkarni GV. Bad breath: etiology, diagnosis and treatment. *Oral Health* 1991; 81: 19–22, 24.
12. Tonzetich J, Ng SK. Reduction of malodor by oral cleansing procedures. *Oral Surg Oral Med Oral Pathol* 1976; 42: 172–181.
13. De Boever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodour. *J Am Dent Assoc* 1995; 126: 1384–1393.
14. Rosenberg M, McCulloch CA. Measurement of oral malodor: current methods and future prospects. *J Periodontol* 1992; 63: 776–782.
15. Rosenberg M. Clinical assessment of bad breath: current concepts. *J Am Dent Assoc* 1996; 127: 475–482.
16. Suarez FL, Furne JK, Springfield J, Levitt MD. Morning breath odor: influence of treatments on sulfur gases. *J Dent Res* 2000; 79: 1773–1777.
17. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. *J Periodontol* 1977; 48: 13–20.
18. Webdocument. Available at: <http://www.gezondheidsweb.eu/dieet/meer-water-drinken-kan-probleem-van-slechte-stinkende-adem-helpen-oplossen> (accessed 21 April 2015).
19. Webdocument. Available at: http://sochicken.nl/hoe_verhelp_je_een_slechte_adem_10_tips (accessed 21 April 2015).
20. Webdocument. Available at: <http://www.urbanlotus.nl/slechte-adem.html> (accessed 21 April 2015).
21. Webdocument. Available at: <https://www.tandheelkundigcentrumburen.nl/> (accessed 21 April 2015).
22. Webdocument. Available at: <http://mens-en-gezondheid.infonu.nl/aandoeningen/84847-wat-zijn-natuurlijke-middelen-tegen-slechte-adem.html> (accessed 21 April 2015).
23. American Dental Association. Acceptance Program Guidelines. Products Used in the Management of Oral Malodor. American Dental Association, November 2003. Available at: http://www.ada.org/~media/ADA/Science%20and%20Research/Files/guide_oral_malodar.ashx (accessed 21 April 2015).
24. American Dental Association. Acceptance Program Guidelines. Floss and Other Interdental Cleaners. American Dental Association, 2011. Available at: http://www.ada.org/~media/ADA/Science%20and%20Research/Files/guide_floss.ashx (accessed 21 April 2015).

25. Gómez SM, Danser MM, Sipos PM, Rowshani B, van der Weijden GA. Tongue coating and salivary bacterial counts in healthy/gingivitis subjects and periodontitis patients. *J Clin Periodontol* 2001; 28: 970–978.
26. Van der Velden U. The Dutch periodontal screening index validation and its application in the Netherlands. *J Clin Periodontol* 2009; 36: 1018–1024.
27. Greenman J, Lenton P, Seemann R, Nachnani S. Organoleptic assessment of halitosis for dental professionals—general recommendations. *J Breath Res* 2014; 8: 017102.
28. CONSORT Group. The CONSORT statement 2010. Available at: <http://www.consort-statement.org/> (accessed 21 April 2015).
29. Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L. Smell identification ability: changes with age. *Science* 1984; 226: 1441–1443.
30. Greenman J, Duffield J, Spencer P et al. Study on the Organoleptic intensity scale for measuring oral malodour. *J Dent Res* 2004; 83: 81–85.
31. Peruzzo DC, Salvador SL, Sallum AW, Nogueira-Filho Gda R. Flavoring agents present in a dentifrice can modify volatile sulphur compounds (VSCs) formation in morning bad breath. *Braz Oral Res* 2008; 22: 252–257.
32. Shinada K, Ueno M, Konishi C, Takehara S, Yokoyama S, Zaitsu T. Effects of a mouthwash with chlorine dioxide on oral malodour and salivary bacteria: a randomized placebocontrolled 7-day trial. *Trials* 2010; 12: 11–14.
33. Quirynen M, Avontrodt P, Soers C et al. The efficacy of amine fluoride/stannous fluoride in the suppression of morning breath odour. *J Clin Periodontol* 2002; 29: 944–954.
34. Hoshi K, Van Steenberghe D. The effect of tongue brushing or toothpaste application on oral malodour reduction. In: van Steenberghe D, Rosenberg M, eds. *Bad Breath: A Multidisciplinary Approach*. Leuven University press ISBN 9061867797, 1996, chapter 23, pp. 255–264.
35. Van Steenberghe D, Avontrodt P, Peeters W et al. Effect of different mouthrinses on morning breath. *J Periodontol* 2001; 72: 1183–1191.
36. Faveri M, Hayacibara MF, Pupio GC, Cury JA, Tsuzuki CO, Hayacibara RM. A cross-over study on the effect of various therapeutic approaches to morning breath odour. *J Clin Periodontol* 2006; 33: 555–560.
37. Erovic Ademovski S, Lingström P, Winkel E, Tangerman A, Persson GR, Renvert S. Comparison of different treatment modalities for oral halitosis. *Acta Odontol Scand* 2012; 70: 224–233.
38. Iwanicka-Grzegorek E, Michalik J, Kepa J, Wierzbicka M, Aleksinski M, Pierzynowska E. Subjective patients' opinion and evaluation of halitosis using Halimeter® and organoleptic scores. *Oral Dis* 2005; 11: 86–88.
39. Brunner F, Kurmann M, Filippi A. The correlation of organoleptic and instrumental halitosis measurements. *Schweiz Monatsschr Zahnmed* 2010; 120: 402–408.
40. Yaegaki K, Brunette DM, Tangerman A et al. Standardization of clinical protocols in oral malodour research. *J Breath Res* 2012; 60: 17101.
41. Van den Velde S, van Steenberghe D, Van Hee P, Quirynen M. Detection of odorous compounds in breath. *J Dent Res* 2009; 88: 285–289.
42. Engen T, Pfaffmann C. Absolute judgments of odor intensity. *J Exp Psychol* 1959; 58: 23–26.
43. Tangerman A, Winkel EG. The portable gas chromatograph Oral-Chroma™: a method of choice to detect oral and extra-oral halitosis. *J Breath Res* 2008; 2: 017101.
44. Seemann R, Conceicao MD, Filippi A et al. Halitosis management by the general dental practitioner—results of an international consensus workshop. *J Breath Res* 2014; 8: 017101.
45. Vandekerckhove B, Van den Velde S, De Smit M et al. Clinical reliability of non-organoleptic oral malodour measurements. *J Clin Periodontol* 2009; 36: 964–969.
46. Evirgen S, Kamburovlu K, Gulsahi A. Effect of clinician's experience, age, gender and calibration on the assessment of halitosis. *Oral Health Prev Dent* 2013; 11: 17–22.