

Volatile sulphur compounds in morning breath of human volunteers

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ABSTRACT

Objective: Morning breath contains elevated concentrations of volatile sulphur components (VSCs). Therefore, morning breath is recognised as a surrogate target for interventions on breath quality. Nevertheless, factors influencing morning breath are poorly understood. Our aim was to evaluate concentrations of VSC at the time of awakening.

Methods: A procedure was developed to collect breath samples at home. Intra- and interperson variations were determined in two small studies based on measurements of hydrogen sulphide, methyl mercaptan and dimethyl sulphide in healthy volunteers.

Results: Highest levels of VSC were found directly after waking up, followed by a significant decline afterward. Considerable day-to-day variation was found, but could not be linked to dietary intake. A significantly higher concentration of H₂S and CH₃SH was observed in the group of female subjects compared to males.

Conclusions: When morning breath is used as a target for interventions, breath collected at the time of or shortly after waking up is preferred over breath collected later during the morning. Gender plays an important role in VSC levels, and should be taken into account. \bigcirc 2010 Elsevier Ltd. All rights reserved.

Breath malodor or halitosis is an important negative factor in social communication and therefore a problem to many individuals. Although most adults suffer from breath malodor only occasionally, an estimated 10–30% has problems more regularly.^{1–3} A good correlation between organoleptic measurements and monitoring of volatile sulphur compounds (VSCs) was found in studies where breath malodor was evaluated by both techniques (reviewed by [1]). Therefore, VSCs are considered to be the most important contributors to breath malodor, and thereby provide a suitable biomarker for breath quality evaluation.⁴

The tongue is the major site for production of VSCs.^{3,5,6} VSC production on the tongue is dependent on microbial activity by mostly Gram-negative proteolytic bacteria. This activity results in degradation of organic substrates from either endogenous or dietary origin. Degradation of the sulphur-containing amino acids cysteine and methionine greatly contributes to the production of VSCs, generating the end-products hydrogen sulphide (H_2S), methyl mercaptan (CH₃SH) and dimethyl sulphide ((CH₃)₂S), which are renowned contributors to the malodor perceived.^{2,4,7,8} Most breath clinics make use of the Halimeter to measure the VSCs and to detect oral malodor. However, the Halimeter cannot differentiate between the three VSCs. Moreover, it is more sensitive to H_2S than to CH₃SH and it is almost insensitive to (CH₃)₂S.⁹ The OralChromaTM forms a good

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alternative that enables differentiation between the three major VSCs. $^{\rm 10}$

Determination of VSCs in morning breath is generally recognised as a surrogate measure to evaluate the efficacy of therapeutic interventions on breath malodor. Several studies have examined the impact of various treatments on the quality of morning breath.^{11–13} Nevertheless, to the best of our knowledge, systematic studies describing the VSC levels in morning breath in comparison to other times of day are limited to one study with 8 healthy adults.¹⁴ Except for this study, morning breath has been determined not directly after awakening but by inviting subjects at a fixed time point in the morning to the laboratory.

We hypothesised that VSCs were the highest directly after awakening. To test this hypothesis, a method was applied to collect breath samples immediately after awakening for later analysis in the laboratory. This method was used in a small study to evaluate morning breath composition at different key moments during the morning. In addition, we were interested to determine whether variation in morning breath after awakening could be correlated with factors such as age, gender, or diet. Therefore, this newly developed method was applied in a study in which morning breath was determined in 50 volunteers on three subsequent days.

1. Materials and methods

1.1. Breath composition during the morning (Study I)

The study was approved by the Medical Ethical Committee of Wageningen University. Twelve subjects of mixed gender were recruited through the staff of NIZO Food Research on the basis of their willingness to participate only. They were instructed to collect three morning breath samples: (1) directly after waking up while still in bed, (2) shortly before breakfast, and (3) directly after breakfast. This gives insight in the effect of light physical activity and food consumption. Morning breath samples were collected according to a standard procedure, and all brought to the laboratory before 9 a.m. In short, all volunteers were provided with three 10 ml type Injekt syringes (B. Braun Medical BV, Oss, The Netherlands) since these syringes gave a good recovery of VSCs.¹⁵ Subjects were carefully instructed to place the syringe between their closed lips and maintain the syringe in position for 1 min. During that time, they could breathe normally through their nose. After exactly 1 min, using the syringe-plunger, they collected 10 ml air from their oral cavity and locked the syringe as fast as possible with a 3-way combilock (Codan BV, Deventer, The Netherlands) to allow analysis of the sample without directly opening the syringe. They were further instructed to restrain from oral hygiene procedures like tooth-brushing until they had collected all three samples. There were no restrictions with respect to diet, neither on the days before sampling or during breakfast. Samples were brought to the research facility and analysed within 3 h.

1.2. Breath composition at awakening (Study II)

The study protocol was approved by the Medical Ethical Committee of Wageningen University. Twenty-five male and twenty-five female volunteers were recruited from the staff of NIZO Food Research, and their families, friends and neighbours. Inclusion was based on a short questionnaire in which they indicated to be without dentures, braces, piercings in the oral cavity. In addition, they stated that they were not under treatment of oral diseases or disorders such as periodontitis, and had no history of major surgery in the oral cavity. Volunteers were not using mouth-rinse solutions, antibiotics, probiotic products, or medication which may interfere with breath quality. All gave their informed consent.

The volunteers were instructed not to use tongue scrapers, and restrain from yoghurt products or garliccontaining foods during the entire study period. For a period of the three consecutive days of the study, they were asked to register their consumed food products in a diary, followed by collection of a morning breath sample the next morning. Directly after waking up in the morning, while still in bed, they were asked to collect morning breath according to a standard procedure as described in Study I.

1.3. Sample analysis

All breath samples from Study I and Study II were analysed for three volatile sulphur compounds, hydrogen sulphide, methyl mercaptan and dimethyl sulphide, using a portable gas chromatography device (OralChroma TM , Abilit Corporation, Japan) as indicated by the manufacturer. A 1 ml syringe was connected to the 3-way combi-lock associated with the syringe containing the breath sample. One ml of the breath sample was transferred to the syringe and thereafter directly injected into the gas chromatograph. One ml was used instead of a halve ml as recommended by the manufacturer, because of a higher sensitivity.10 After 8 min, the chromatogram was checked. Data were calculated from the display values since a double amount of gas was used. These display values were subsequently corrected by comparison with values obtained by injected standards, since the OralChromaTM cannot be calibrated manually.¹⁰ Detection limits were 4 ppm for each of the three VSCs.¹⁰

1.4. Data analysis

Data from samples of Study I were averaged, and time points were compared. For Study II, the average VSC concentration of each subject was taken for further analysis. The parameters gender and age were analysed for their influence on breath composition at awakening. For many other parameters (e.g. smoking or intake of specific dietary components) the number of subjects within the study population was not sufficient to draw solid conclusions. Data were analysed using either the Student's t-test, or the Mann–Whitney U test if parameters were not normally distributed, using the StatisticaTM software package. P-values < 0.05 were considered as statistically significant. Pearson product moment correlation coefficients were determined using the statistical software package StatisticaTM. Download English Version:

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