

# The Relationship Between Volatile Sulfur Compounds and Major Halitosis-Inducing Factors

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**Background:** Although tongue coating and periodontal conditions have been reported to be major halitosis-inducing factors, the relationship between volatile sulfur compounds (VSC) and these 2 major factors is not yet fully understood. The aim of this study was to investigate the relationship of VSC concentrations to tongue coating and periodontal health.

**Methods:** Forty subjects (mean age 33.3 years, range 14 to 64 years) were enrolled in this study. Gas chromatography was performed to analyze each VSC component from the mouth air sampled prior to tongue scraping, after tongue scraping, and after a subsequent prophylaxis on the interdental spaces.

**Results:** CH<sub>3</sub>SH was the most malodorous component among the 3 major VSC from the mouth air. The high CH<sub>3</sub>SH group showed a significantly higher organoleptic rating ( $P < 0.01$ ), gingival index ( $P < 0.01$ ), bleeding index ( $P < 0.01$ ), probing depth ( $P < 0.05$ ), and VSC concentrations prior to tongue scraping ( $P < 0.01$ ), except for the amount of tongue coating, compared to the low CH<sub>3</sub>SH group. All VSC concentrations were vastly reduced by tongue scraping in both groups, and the remaining contents were nearly all removed by the subsequent prophylaxis. The VSC contents produced by the tongue coating played a major role [H<sub>2</sub>S: 76%; CH<sub>3</sub>SH: 52%; (CH<sub>3</sub>)<sub>2</sub>S: 55%] in the low CH<sub>3</sub>SH group. In the high CH<sub>3</sub>SH group which had poor periodontal health, the tongue coating still played a major role [H<sub>2</sub>S: 67%; CH<sub>3</sub>SH: 59%; (CH<sub>3</sub>)<sub>2</sub>S: 48%], but the interdental spaces also contributed to VSC production [H<sub>2</sub>S: 26%; CH<sub>3</sub>SH: 32%; (CH<sub>3</sub>)<sub>2</sub>S: 36%].

**Conclusions:** The tongue coating was demonstrated to be a primary halitosis-inducing factor. Periodontal health was also shown to contribute to VSC production. *J Periodontol* 2003;74:32-37.

## KEY WORDS

Halitosis/etiology; periodontal diseases/adverse effects; sulfur compounds; tongue diseases/adverse effects.

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Halitosis, often called oral malodor or bad breath, is a commonly experienced condition with a variety of etiologic factors.<sup>1</sup> Although numerous non-oral sites and many different causes have been suggested,<sup>2</sup> an estimated 80% to 90% of all bad breath odors originate from the mouth itself.<sup>3</sup> It has been also reported that Gram-negative anaerobic bacteria are directly responsible for most of the offensive gases.<sup>4-6</sup>

The main components of human oral malodor are volatile sulfur compounds (VSC), particularly hydrogen sulfide (H<sub>2</sub>S), methyl mercaptan (CH<sub>3</sub>SH), and dimethyl sulfide [(CH<sub>3</sub>)<sub>2</sub>S].<sup>7,8</sup> These gases originate from the breakdown of amino acids such as cysteine, cystine, methionine, or peptides by microbial putrefaction within the oral cavity,<sup>9-11</sup> mainly on the dorsum of the tongue.<sup>6,12</sup> Besides the dorsum of the tongue, there are numerous microenvironments that harbor bacteria and produce malodor in the oral cavity.<sup>1,13</sup> Halitosis has been reported to be caused by the same microorganisms causing gingivitis and periodontitis.<sup>13,14</sup> These findings may implicate periodontal disease as a causative factor leading to halitosis.

It has been reported that the VSC level and the CH<sub>3</sub>SH/H<sub>2</sub>S ratio in mouth air from patients with periodontal involvement were 8 times higher than those of control subjects.<sup>13</sup> Yaegaki and Sanada<sup>13</sup> also found 4 times more tongue coating in patients with periodontitis than periodontally healthy controls. In another report, deep and inflamed crevicular sites

exhibited a significantly higher  $\text{CH}_3\text{SH}/\text{H}_2\text{S}$  ratio than the corresponding shallow or non-inflamed crevicular sites, and total sulfur in the deep and inflamed sites was significantly higher than in the corresponding shallow and non-inflamed sites.<sup>14</sup> It has been proposed that the existence of active inflammation in periodontal tissue is more important than the mere presence of deeper periodontal pockets for the production of oral malodor.<sup>15</sup>

However, it has been reported that a large proportion of individuals with oral malodor are periodontally healthy and that the mucosal surface of the tongue is a major site of oral malodor production.<sup>16</sup> No significant relationships between oral malodor and periodontal condition were found. Therefore, it is still arguable that periodontal conditions play a major role in the production of oral malodor.

Although tongue coating and periodontal conditions have been reported to be the major halitosis-inducing factors,<sup>13,15,17</sup> the relationship between VSC and these 2 major halitosis-inducing factors is unclear.

The aim of this study was to investigate the relationship of VSC concentrations to tongue coating and periodontal health. Gas chromatography was performed to analyze each VSC component from the mouth air sampled prior to tongue scraping, after tongue scraping, and after a subsequent prophylaxis on the interdental spaces.

## MATERIALS AND METHODS

### Study Population

Among the patients who visited the oral malodor clinic at the Seoul National University Dental Hospital, 40 subjects (mean age  $33.3 \pm 13.4$  years, range 14 to 64 years; 19 males, 21 females) were recruited for this study. No subjects had a history of periodontal treatment or antibiotic medications for the previous 3 months. They did not report any systemic disorders that could affect oral malodor. They were instructed to abstain from eating, chewing, and any other oral hygiene procedures from the night before to the morning of the test day. The mouth air was sampled 6 times with syringes, 3 times for the organoleptic tests, and 3 times for the gas chromatographic analyses as the baseline data in the morning between 9 a.m. and 11 a.m. Subsequently, tongue scraping was performed. This was followed by mouth air sampling 3 times for gas chromatography. Immediately after these procedures, oral prophylaxis on the interdental spaces was performed and the mouth air was again sampled 3 times for gas chromatography.

### Objectionability of 3 VSC

It is well known that human oral malodor is mainly composed of VSC, particularly  $\text{H}_2\text{S}$ ,  $\text{CH}_3\text{SH}$ , and  $(\text{CH}_3)_2\text{S}$ . Because the 3 main components differ from

one another in their volatility, objectionability, and odor characteristics, the total VSC concentration, which is a simple sum of the 3 VSC, may not represent the actual severity of the oral malodor.

To determine which component is appropriate as a severity indicator of oral malodor instead of the total VSC concentration, 9 healthy volunteers (with no problems in olfactory function) had an organoleptic test performed on a standard sample of the 3 VSC gases with a range of 0 to 15.0 ng/10 ml by a degree of 0.5 ng/10 ml. For the standard VSC gas sample, a gas permeator and standard permeation tubes<sup>†</sup> were used.

### Periodontal Examination

All subjects were assessed for their gingival and periodontal status by probing depth, gingival index,<sup>18</sup> and bleeding index<sup>19</sup> employing 6 Ramfjörð sample teeth on the first day. Probing depth (PD) was measured with a Michigan O probe to the nearest mm marking. The results were scored as a mean of all the surfaces examined.

### Sampling Method

The subjects were instructed to close their mouth for 3 minutes in an upright chair position prior to each sample collection. The "negative pressure method," which entraps mouth air rapidly and minimizes loss of highly volatile components, was used. A syringe<sup>‡</sup> with a 6 cm long and 3 mm diameter Teflon tube was used. The plunger was retracted to the 10 ml portion with the valve closed. The Teflon tube was located in the intraoral area at a distance of 4 cm inward from the mandibular anterior teeth and 1 cm above the tongue dorsal surface. The mouth air was collected into the syringe barrel by opening and then closing the valve. During sample collection, the subjects were instructed to hold their breath to avoid lung air interruption.

### Tongue Scraping and Prophylactic Procedure

Prior to tongue scraping, an air syringe was used to remove saliva. The coating was carefully scraped from the circumvallate papilla to the apex of the tongue with cellulose strips (15 cm  $\times$  1 cm), bent at 2 points in the middle area to remove the tongue coating effectively. The collected tongue coating was weighed, and the mouth air was sampled 3 times in quick succession for gas chromatography. Oral prophylaxis was then performed, which included cleaning the interdental spaces using dental floss and pumice. This procedure was performed to remove the substrates and microorganisms within the interdental spaces. The mouth air again was sampled 3 times immediately after the prophylaxis for gas chromatography.

† Gastec Co., Ayase, Japan.

‡ SampleLock syringe, 10 mL vol., Hamilton Co., Reno, NV.

### Organoleptic Method

A disposable paper cup was used to perform the organoleptic test. A small hole was made at the base of the cup to connect a 6 cm plastic tube into which the Teflon tube of the syringe was inserted. A thin wax film was used to seal the connection. For a more precise organoleptic test, one examiner placed the cup over his nose, and another examiner expelled the sample through the plastic tube into the cup.

The organoleptic ratings (OR) were estimated on a scale of 0 to 5 as follows: 0 = no odor; 1 = barely noticeable odor; 2 = slight but clearly noticeable odor; 3 = moderate odor; 4 = strong odor; 5 = extremely foul odor.<sup>20</sup>

### Gas Chromatographic Analysis

A gas chromatography system<sup>§</sup> equipped with a flame photometric detector specific for sulfur compounds and 60/80 mesh size column<sup>||</sup> was used.<sup>21,22</sup>

After the injection of the sample, the oven temperature was held at 40°C for 2 minutes, then increased by 8°C/minute to 100°C for 10 minutes. The carrier gas was nitrogen at a flow rate of 20 ml/minute. The detector output was monitored on an integrator.<sup>¶</sup>

### Data Analysis

The mean values for the OR, GI, BI, PD, the amount of tongue coating, VSC concentrations, and the CH<sub>3</sub>SH/H<sub>2</sub>S ratio in the low and high CH<sub>3</sub>SH groups were compared using an independent samples *t* test. The concentrations of each VSC in the 2 groups were compared between steps by a paired *t* test.

### RESULTS

The objectionability test of the 3 VSC gases is shown in Table 1. An H<sub>2</sub>S concentration of 15 ng/10 ml was recorded as grade 3. An organoleptic rating of 6 ng/10 ml CH<sub>3</sub>SH was grade 4. The highest organoleptic rating for (CH<sub>3</sub>)<sub>2</sub>S was grade 1. These results showed that CH<sub>3</sub>SH was the most malodorous component and was relatively well matched with the organoleptic ratings. The concentration range of CH<sub>3</sub>SH in which most of the volunteers could clearly detect the odor was 1.5 to 2.5 ng/10 ml (grade 2). Each detected the odor clearly over 2.0 ng/10 ml of CH<sub>3</sub>SH. Based on these results, the subjects were divided into the high (19) and low (21) halitosis groups according to whether they scored above or below 2.0 ng/10 ml CH<sub>3</sub>SH, respectively.

Subjects were evaluated based on clinical periodontal scores and tongue coating. Except for tongue coating, there was a significant difference between the 2 groups (Table 2). In the low CH<sub>3</sub>SH group, 8 of 21 subjects (38.1%) had one or more periodontal pock-

**Table 1.**  
Objectionability Test for Standard VSC Concentrations

VSC	Organoleptic Rating					
	0	1	2	3	4	5
H <sub>2</sub> S (ng/10 ml)	0.5	1.0-2.5	3.0-10.5	11.0-15.0	—	—
CH <sub>3</sub> SH (ng/10 ml)	—	0.5-1.0	1.5-2.5	3.0-5.5	6.0-15.0	—
(CH <sub>3</sub> ) <sub>2</sub> S (ng/10 ml)	0.5-2.5	3.0-15.0	—	—	—	—

ets >3 mm. In the high CH<sub>3</sub>SH group, 14 of 19 subjects (73.7%) had one or more periodontal pockets deeper than 4 mm.

The concentration of each VSC was investigated prior to tongue scraping, after tongue scraping, and after prophylaxis (Table 3). The VSC concentrations of the high CH<sub>3</sub>SH group were higher than those of the low CH<sub>3</sub>SH group prior to tongue scraping. The concentrations of all the VSC were much lower after tongue scraping in both groups. However, some VSC remained. These remaining contents were nearly all removed following prophylaxis.

The CH<sub>3</sub>SH/H<sub>2</sub>S ratios of the high and low CH<sub>3</sub>SH groups prior to tongue scraping were 0.52 and 0.44, respectively. These ratios increased to 0.68 and 0.72 after tongue scraping in both groups (Table 3).

Figure 1 shows the reduction rate of each VSC component after tongue scraping in both groups. The low CH<sub>3</sub>SH group showed a reduction rate of 76% for H<sub>2</sub>S, 52% for CH<sub>3</sub>SH, and 54% for (CH<sub>3</sub>)<sub>2</sub>S. The high CH<sub>3</sub>SH group showed a reduction rate of 67% for H<sub>2</sub>S, 58% for CH<sub>3</sub>SH, and 48% for (CH<sub>3</sub>)<sub>2</sub>S. Figure 2 shows the reduction rate of each VSC component after prophylaxis. The low CH<sub>3</sub>SH group showed a reduction rate of 12% for H<sub>2</sub>S, 25% for CH<sub>3</sub>SH, and 13% for (CH<sub>3</sub>)<sub>2</sub>S. The high CH<sub>3</sub>SH group showed a reduction rate of 26% for H<sub>2</sub>S, 32% for CH<sub>3</sub>SH, and 36% for (CH<sub>3</sub>)<sub>2</sub>S.

### DISCUSSION

It should be noted that patient selection by probing depth only might mix 2 different patient groups: one with deeper pockets with active inflammation, and the other with deeper pockets without active inflammation. Therefore, in this study, the subjects were divided not by probing depth but by CH<sub>3</sub>SH level (above or below 2 ng/10 ml). Our results revealed that CH<sub>3</sub>SH was the most malodorous component, which is consistent with Tonzetich and Ng,<sup>23</sup> who reported that CH<sub>3</sub>SH was about 3 times more objectionable than H<sub>2</sub>S. The concentration of CH<sub>3</sub>SH was matched relatively well with the organoleptic ratings, and 2 ng/10 ml was recorded

§ HP5890, Hewlett-Packard Co., Avondale, PA.

|| Chromosil 330, Supelco Co., Bellefonte, PA.

¶ HP3394A, Hewlett-Packard Co.

**Table 2.**  
Intraoral Conditions of the Low and High CH<sub>3</sub>SH Groups (mean ± SD)

Group	OR	PD (deepest)	GI	BI	Tongue Coating (mg)
Low CH <sub>3</sub> SH (n = 21)	2.38 ± 0.80	3.40 ± 0.92	0.40 ± 0.39	0.42 ± 0.38	178.7 ± 64.8
High CH <sub>3</sub> SH (n = 19)	3.4 ± 0.80*	4.30 ± 1.24†	0.99 ± 0.52*	1.08 ± 0.45*	216 ± 120‡

\* Significantly higher than the low CH<sub>3</sub>SH group (P < 0.01).  
† Significantly higher than the low CH<sub>3</sub>SH group (P < 0.05).  
‡ Not significant.

**Table 3.**  
VSC Concentrations Prior to Tongue Scraping (A), After Tongue Scraping (B), and After Prophylaxis (C) in the Low and High CH<sub>3</sub>SH Groups (mean ± SD)

Group	VSC (ng/10 ml)	A	B	C	Significance Between Steps
Low CH <sub>3</sub> SH (n = 21)	H <sub>2</sub> S	3.09 ± 1.17	0.73 ± 0.38	0.36 ± 0.20	(I, II)* (I, III)‡ (II, III)‡
	CH <sub>3</sub> SH	0.98 ± 0.38	0.47 ± 0.28	0.23 ± 0.23	(I, II)‡ (I, III)‡ (II, III)‡
	(CH <sub>3</sub> ) <sub>2</sub> S	0.57 ± 0.51	0.26 ± 0.19	0.18 ± 0.18	(I, II)* (I, III)*
	CH <sub>3</sub> SH/H <sub>2</sub> S	0.44 ± 0.15	0.72 ± 0.52	0.66 ± 0.49	(I, II)* (I, III)*
High CH <sub>3</sub> SH (n = 19)	H <sub>2</sub> S	10.05 ± 5.24§	3.31 ± 2.22§	0.66 ± 0.58¶	(I, II)‡ (I, III)‡ (II, III)‡
	CH <sub>3</sub> SH	4.86 ± 3.19§	2.02 ± 1.34§	0.45 ± 0.30¶	(I, II)‡ (I, III)‡ (II, III)‡
	(CH <sub>3</sub> ) <sub>2</sub> S	1.6 ± 0.95§	0.83 ± 0.60§	0.25 ± 0.16¶	(I, II)‡ (I, III)‡ (II, III)‡
	CH <sub>3</sub> SH/H <sub>2</sub> S	0.52 ± 0.24¶	0.68 ± 0.35¶	0.95 ± 1.02¶	(I, II)* (I, III)*

\* Significant difference between steps (P < 0.05).  
† Significant difference between steps (P < 0.01).  
‡ Significant difference between steps (P < 0.001).  
§ Significant difference between the low and high CH<sub>3</sub>SH Groups (P < 0.01).  
¶ Not significant.

as grade 2 without exception. Therefore, this CH<sub>3</sub>SH concentration can be considered to be a severity indicator of oral malodor.

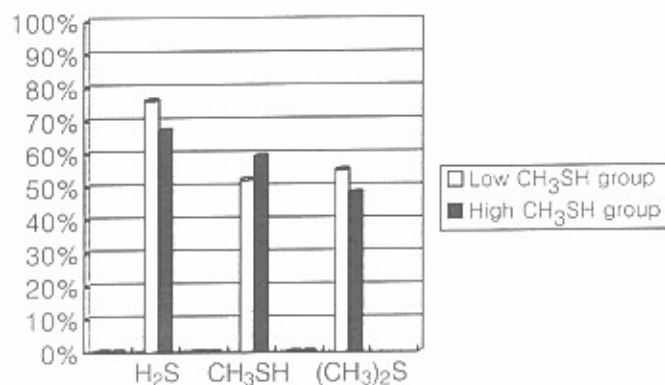
The subjects were examined for their intraoral conditions such as organoleptic ratings, periodontal status, and the amount of tongue coating in the high and low CH<sub>3</sub>SH groups. The 2 groups were significantly different in all variables except for the amount of tongue coating. There were significant differences in PD, GI, and BI between the 2 groups, although these were relatively low.

There are many reports<sup>13,15,16</sup> showing that the amount of tongue coating was closely correlated with oral malodor. However, in this study, the amount of the tongue coating was not significantly different. This suggests that the composition of the tongue coating, including Gram-negative anaerobic bacteria and sulfur-containing substrates, is more critical to the production of oral malodor than the amount of tongue coating itself.

The VSC concentrations from the mouth air sampled prior to tongue scraping, after tongue scraping, and after the prophylaxis were examined in the 2 groups. The

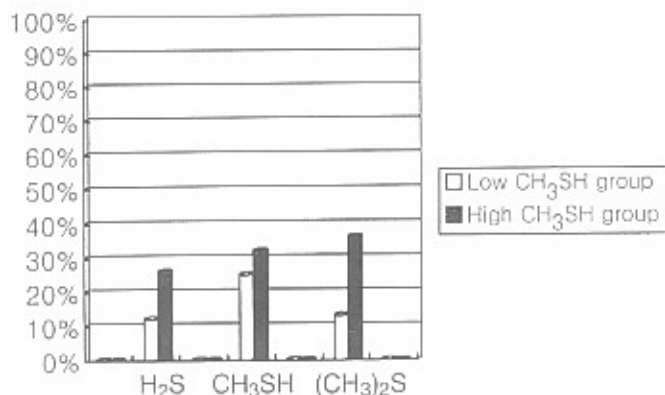
concentrations of all VSC detected prior to tongue scraping in the high CH<sub>3</sub>SH group were higher than those in the low CH<sub>3</sub>SH group (Table 3). The CH<sub>3</sub>SH/H<sub>2</sub>S ratios of the high and low CH<sub>3</sub>SH groups prior to tongue scraping were 0.52 and 0.44, respectively. These results agree with those of Coil and Tonzetich,<sup>14</sup> who reported that the CH<sub>3</sub>SH/H<sub>2</sub>S ratio was 0.37 in the crevicular air of control subjects, 0.71 in the crevicular air of deep gingival sites, and 0.95 in the crevicular air of inflamed gingival sites. The CH<sub>3</sub>SH/H<sub>2</sub>S ratios of the high and low CH<sub>3</sub>SH groups after tongue scraping increased to 0.68 and 0.72, respectively. The difference in the CH<sub>3</sub>SH/H<sub>2</sub>S ratio in the high group was smaller than that of the low group. These results can be explained by the fact that tongue scraping in the high group reduced higher percentages of CH<sub>3</sub>SH or lower percentages of H<sub>2</sub>S than in the low group.

Figure 1 shows the reduction rate of each VSC component after tongue scraping in both groups, i.e., the contribution of the tongue dorsum to each VSC component. The contribution of the tongue dorsum to H<sub>2</sub>S production was dominant in the low CH<sub>3</sub>SH group. The tongue dorsum contribution to H<sub>2</sub>S and (CH<sub>3</sub>)<sub>2</sub>S pro-



**Figure 1.**

Reduction rates of VSC components after tongue scraping in the low and high CH<sub>3</sub>SH groups.



**Figure 2.**

Reduction rates of VSC components after prophylaxis in the low and high CH<sub>3</sub>SH groups.

duction was higher in the low CH<sub>3</sub>SH group than in the high CH<sub>3</sub>SH group. However, the contribution to CH<sub>3</sub>SH production was lower than in the high CH<sub>3</sub>SH group, which had poorer periodontal health. Therefore, the high CH<sub>3</sub>SH group showed a lower contribution of the tongue dorsum in terms of both H<sub>2</sub>S and (CH<sub>3</sub>)<sub>2</sub>S production, and a higher contribution in terms of CH<sub>3</sub>SH production than the low CH<sub>3</sub>SH group. These results suggest that the tongue coating in the high CH<sub>3</sub>SH group contributed a higher proportion to CH<sub>3</sub>SH production than that in the low CH<sub>3</sub>SH group. These findings indicate that the composition of the tongue coating in the high CH<sub>3</sub>SH group included more CH<sub>3</sub>SH-producing bacteria such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Bacteroides forsythus*, *Treponema denticola*, etc. and their substrates. These results concur with a previous report<sup>12</sup> showing that the tongue scraping of subjects with a high organoleptic score yielded more BANA (+) bacteria such as *P. gingivalis*, *B. forsythus*, and *T. denticola*.<sup>12,24</sup>

Figure 2 shows the reduction rate of each VSC component after prophylaxis in both groups, i.e., the contribution of the interdental spaces to each VSC component. The contribution to H<sub>2</sub>S production in the low CH<sub>3</sub>SH group was approximately half as much as that in the high CH<sub>3</sub>SH group (poorer periodontal health). This means that little H<sub>2</sub>S was produced by the interdental spaces in the low CH<sub>3</sub>SH group. The contribution to CH<sub>3</sub>SH and (CH<sub>3</sub>)<sub>2</sub>S production was also lower in the low CH<sub>3</sub>SH group than in the high CH<sub>3</sub>SH group. These results suggest that the interdental spaces in poor periodontal health contribute a higher proportion of all 3 VSC productions than those with healthy periodontium. Despite this, a lower contribution of the interdental spaces compared to the tongue dorsum in the high CH<sub>3</sub>SH group was thought to be due to the smaller area than the tongue dorsal surface.

In summary, the high CH<sub>3</sub>SH group showed significantly higher OR, GI, BI, PD, and higher concentrations of all 3 VSC prior to tongue scraping compared to the low CH<sub>3</sub>SH group. The reduced proportions of all 3 VSC by tongue scraping were higher than those by the subsequent prophylaxis in both groups. Compared to the amounts and proportions of reduced VSC in the low CH<sub>3</sub>SH group, the high CH<sub>3</sub>SH group showed a higher contribution of the interdental spaces to all 3 VSC components and a higher contribution of the tongue dorsum to CH<sub>3</sub>SH production.

In the low CH<sub>3</sub>SH group, which had close to normal periodontal health, the VSC produced by the tongue coating played a major role in the severity of oral malodor. In contrast, in the high CH<sub>3</sub>SH group, with poorer periodontal health, the tongue coating still played a major role in VSC production, but the role of the interdental spaces should not be disregarded. The interdental spaces of the subjects with poorer periodontal health produced more of the 3 VSC components and made the tongue dorsal surface more likely to produce VSC, particularly CH<sub>3</sub>SH.

In conclusion, tongue coating was demonstrated to be a primary halitosis-inducing factor. Periodontal health was also shown to contribute to VSC production, both directly and indirectly.

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