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Effect of Green Tea on Volatile Sulfur Compounds in Mouth Air

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Summary Many food products are claimed to be effective in controlling halitosis. Halitosis is caused mainly by volatile sulfur compounds (VSCs) such as H_2S and CH_3SH produced in the oral cavity. Oral microorganisms degrade proteinaceous substrates to cysteine and methionine, which are then converted to VSCs. Most treatments for halitosis focus on controlling the number of microorganisms in the oral cavity. Since tea polyphenols have been shown to have antimicrobial and deodorant effects, we have investigated whether green tea powder reduces VSCs in mouth air, and compared its effectiveness with that of other foods which are claimed to control halitosis. Immediately after administrating the products, green tea showed the largest reduction in concentration of both H_2S and CH_3SH gases, especially CH_3SH which also demonstrated a better correlation with odor strength than H_2S; however, no reduction was observed at 1, 2 and 3 h after administration. Chewing gum, mints and parsley-seed oil product did not reduce the concentration of VSCs in mouth air at any time. Toothpaste, mints and green tea strongly inhibited VSCs production in a saliva-putrefaction system, but chewing gum and parsley-seed oil product could not inhibit saliva putrefaction. Toothpaste and green tea also demonstrated strong deodorant activities in vitro, but no significant deodorant activity of mints, chewing gum or parsley-seed oil product were observed. We concluded that green tea was very effective in reducing oral malodor temporarily because of its disinfectant and deodorant activities, whereas other foods were not effective.

Key Words green tea, chewing gum, mints, parsley-seed oil, halitosis

Halitosis is one of the main concerns about the oral cavity for many people. Among businessmen in Tokyo, about 30% of them complained of their own halitosis (*1*). Breath odor derived from the oral cavity is mainly caused by volatile sulfur compounds (VSCs) such as H_2S and CH_3SH produced through the putrefaction activity of oral microorganisms (*2*). The action of microorganisms degrades proteinaceous substrates originating from exfoliated oral epithelial cells, blood cells, food debris, etc., to amino acids such as cysteine and methionine, ultimately leading to the production of VSCs (*3*, *4*). This putrefactive activity is especially increased in subjects with periodontal diseases (*3*). It has also been shown that VSCs are periodontally pathogenic compounds (5). VSCs increase the degradation of gingival collagen and reduce the synthesis of collagen (*6*, *7*). Furthermore, Ng and Tonzetich (*8*) reported that VSCs increased the permeability of the crevicular epithelial lining, thus exposing the underlying connective tissue to harmful substances. Recently, it has been reported that H_2S involves carcinogenicity (*9*).

Thus, controlling VSC concentration in mouth air may be an effective measure to eliminate oral malodor, and also to reduce the risk of periodontal diseases. Most treatment procedures for oral malodor focus on controlling the number of microorganisms in the oral cavity. Toothpastes or mouthwashes that are claimed to reduce oral malodor mostly contain anti-microbial compounds. Tea polyphenols have been shown to be antimicrobial to oral microorganisms (*10*), especially *Mutans Streptococci*, which is a main cause of dental caries (*11*, *12*), and tea polyphenols are effective in improving periodontal conditions (*13*). Green tea contains tea catechins in which (−)-epigallocatechin gallate (EGCg) is known to dominate (*10*). To explain the deodorant activity of ECGs, Yasuda and Arakawa (*14*) proposed a pathway whereby EGCg reacted with CH_3SH with the hydroxyl groups in the B ring instead of those on the galloyl moiety, increasing the reaction. However, the effect of green tea on oral malodor has not yet been described in an in vivo study.

In view of the positive aspects of green tea in reducing oral malodor, we compared its activity against VSCs with four other products in both in vitro and in vivo studies. The aim of this experiment was to determine if green-tea powder reduces VSCs concentrations in mouth air and to compare its effectiveness with other

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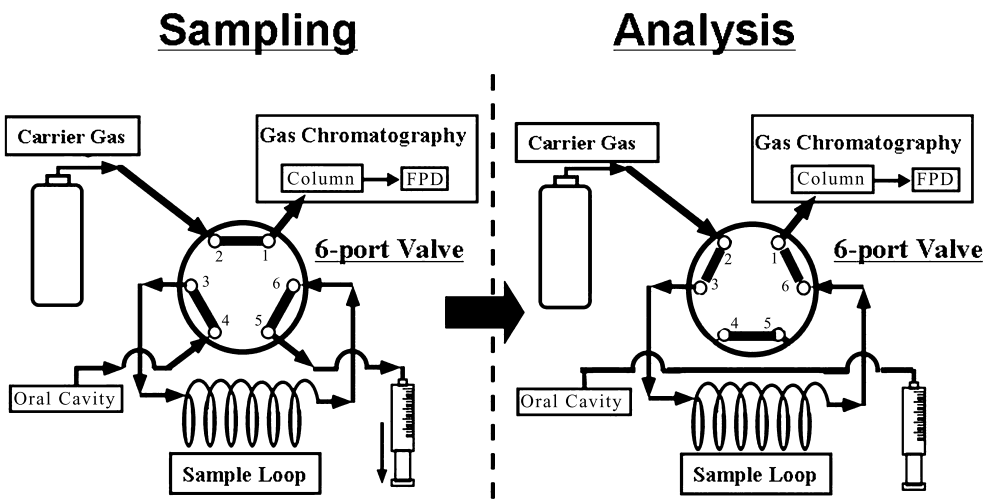

Fig. 1. A gas chromatograph equipped with a flame photometric detector (GC-8APFP, Shimadzu, Kyoto, Japan) was modified to inject exactly 10 mL or 3 mL samples of gas. A 10 mL or 3 mL sample loop was incorporated into the carrier gas line with a 6-port valve. Solid line demonstrates the carrier gas flow line during sampling. Broken line shows the line during injection of sample gas after valve position is changed.

foods which are claimed to reduce malodor.

EXPERIMENTAL

Subjects. Subjects (n=15) producing more than 1.5 ng H_2S/10 mL and/or 0.5 ng CH_3SH/10 mL in mouth air were selected for the study, since these concentrations are recognized as the threshold of the sense of smell (*15*). Female subjects were excluded from this study to avoid the effect of menstruation on VSC production (*16*). All protocols for this study were reviewed and approved by the Clinical Research Ethics Board of the University of British Columbia and by the Research Ethics Committee of Nippon Dental University.

Materials. As all commercial sugarless chewing gums include similar components, one containing xylitol, maltose and flavors was utilized. One chewing-gum tablet was chewed for 2 min and then disposed of. For mints, which are the most popular product in the world-wide market, two tablets were sucked for 2 min and then swallowed if still undissolved. For parsley-seed oil product, which is also a very popular product in the North American market, two capsules were swallowed with no water following the manufacturer's instructions. Green-tea powder was utilized in this study rather than tea extract, since Japanese green-tea tablets or foods containing green-tea compounds are more popular than drinking green-tea extract, and because preliminary studies showed that not only regular green-tea extract but also those tea leaves available on the market were difficult to standardize for this study in order to avoid artifacts (Data are not shown). Hence, a standardized green-tea powder, which is a component of green-tea tablets (Jinseido, Shizuoka, Japan), was obtained directly from the factory for this study. A 670 mg powder sample, equivalent to 2 tablets of the product, was poured onto the back portion of the tongue, dissolved in the saliva on the tongue surface, then naturally swallowed; only in this way could we determine the effect of green tea on VSCs in mouth air under standardized conditions. Crest™ toothpaste (Procter & Gamble, Cincinnati, OH, USA), which is also a most popular product in North America, was utilized as a positive control. The subjects brushed their teeth the way they usually do for 3 min using an Oral B® 40 toothbrush. All products except the green tea were obtained from a local market in Vancouver, BC, Canada.

Mouth air analysis. A crossover design was used for this study. Each food was given to the subjects on a different day, and 1 wk as a washout period was allowed between each two foods. On the day of each experiment, the subjects were asked to abstain from eating, drinking and oral-hygiene practices from midnight till the end of the experiment. At time periods of immediately after applying the food products and 1, 2 and 3 h later, two mouth air samples were analyzed, using a gas chromatograph equipped with a flame photometric detector (GC) (GC-8APFP, Shimadzu, Kyoto, Japan). The GC was modified to inject exactly 10 mL or 3 mL samples of gas, i.e., a 10 mL or 3 mL sample loop was incorporated into the carrier gas line with a 6-port valve (Fig. 1) (*17*). If both an injection port of the GC and an injection syringe are utilized for sampling and analyzing, as in ordinary GC analysis, the strong back pressure coming out from an injection port increases the dead volume in the syringe and sometimes causes leaking during injection. Thus, reproducibility of the GC method is not reliable without a sample loop. The GC was calibrated using standards for H_2S and CH_3SH from Dynacalibrator™ (VICI Metroinics, TX, USA) and Dynacal Permeationtubes™ (VICI Metronics). The concentrations of H_2S and CH_3SH, the main components within oral malodorous sulfur compounds, were determined in each sample. Total VSC concentration was obtained as the aggregate of H_2S and CH_3SH. The concentrations of total VSCs, H_2S and CH_3SH were expressed as ng/10 mL as previously reported (*15*).

Deodorant Activity. To determine the deodorant activity of the products, H_2S at 8 ng/10 mL air flowing from the Dynaclibrator™ (VICI Metronics) mentioned above was used to fill each test tube, which was then sealed so as to be airtight. H_2S at 8 ng/10 mL air is considered a moderate strength of oral malodor. Thirty milligrams of one of the products had been added to each test tube beforehand, while control test tubes had been left blank (n=10). The test and control tubes were incubated for 5 min at 37°C. A 3 mL sample of the headspace air was used for analysis by a GC.

Saliva putrefaction system. Paraffin-stimulated saliva from the subjects was used, and each subject's saliva after filtration with a cheese cloth was analyzed using each of the products. Aliquots of 1 mL saliva were added to 18 mL test tubes with airtight Teflon™ seals as reported previously (*18*), along with 30 mg of the product being tested which had been added previously, and the test tubes were incubated at 37°C to induce putrefaction. After 24 h of incubation, a 3 mL gas sample was analyzed by a GC.

Statistics. Statistical analysis was performed using analysis of variance (ANOVA), and by a t test. Statistical significance was accepted at a p value less than 0.05.

RESULTS

Clinical studies

Immediately after administration, chewing gum, mints and parsley-seed oil product produced no reduction in the concentration of total VSCs in mouth air (Fig. 2). In contrast, immediately after administration, green tea showed the largest potential found for reduction of VSCs in mouth air (Fig. 2).

We followed these effects for 3 h after administering the products. The changes in H_2S or CH_3SH concentration (ng/10 mL mouth air) are shown in Tables 1 and 2. CH_3SH concentration immediately after taking green tea was significantly reduced compared to baseline (p<0.0005), whereas H_2S was not reduced. At 1, 2 or 3 h after taking tea, neither of VSCs fell significantly. None of products demonstrated significant reduction of H_2S or CH_3SH concentrations at 1, 2, or 3 h after administration.

Saliva putrefaction study

The differences observed among reduction (%) of VSC concentration in headspace air by the products were statistically significant (p<0.0005, ANOVA) (Fig. 3). Toothpaste, mints and green tea strongly inhibited VSCs production compared to the control in the saliva putrefaction system (n=15, p<0.05) (Table 3). However, chewing gum and parsley-oil product could not inhibit VSC production in the saliva putrefaction sys-

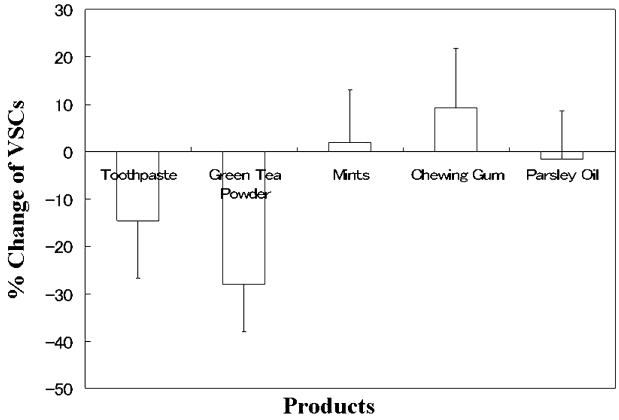

Fig. 2. Change (%) in total VSC level immediately after taking the products. Chewing gum, mints and parsley-seed oil product produced no reduction in the concentration of VSCs in mouth air. Green tea showed a reduction in VSCs found in mouth air, but a significant difference was not observed (n=15, mean±SE).

Table 1. Changes in oral hydrogen sulfide level during the 3 h after taking the products.

	Baseline	Immediately after	1 h	2 h	3 h
Toothpaste	3.9±0.4	3.4±0.6	3.1±0.4	3.1±0.3	3.4±0.5
Green tea	3.6±0.4	3.2±0.3	3.5±0.4	3.6±0.4	3.8±0.4
Mints	3.9±0.5	4.0±0.5	3.7±0.4	3.8±0.4	3.9±0.4
Chewing gum	3.9±0.5	4.2±0.5	3.9±0.5	3.9±0.5	4.4±0.5
Parsley oil	4.1±0.5	4.2±0.5	4.1±0.4	4.2±0.4	4.6±0.6

Mean±SE (ng/10 mL).

Table 2. Changes in oral methyl mercaptan level during the 3 h after taking the products.

	Baseline	Immediately after	1 h	2 h	3 h
Toothpaste	2.0±0.2	1.7±0.3	1.5±0.2	1.4±0.2	1.6±0.3
Green tea	1.8±0.1*	0.8±0.2*	1.4±0.2	1.7±0.2	1.8±0.2
Mints	2.1±0.3	2.2±0.3	2.0±0.3	2.3±0.5	2.4±0.4
Chewing gum	1.9±0.2	2.0±0.2	1.7±0.3	1.9±0.3	2.3±0.3
Parsley oil	2.0±0.2	1.9±0.2	2.0±0.1	2.1±0.2	2.2±0.3

*p<0.05. Mean±SE (ng/10 mL).

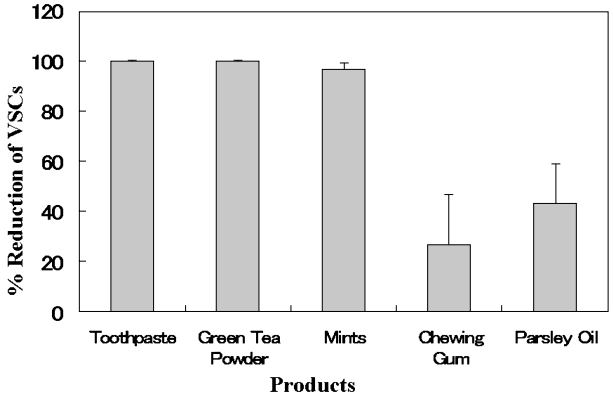

Fig. 3. A 1 mL sample of saliva along with 30 mg of the product being tested was added to 18 mL test tubes with airtight seals. After 24 h of incubation, a 3 mL gas sample was aspirated through the airtight Teflon™ seal using an airtight syringe, then analyzed by a GC. The differences observed among the products in suppression of VSCs were statistically significant (n=15, mean±SE, p<0.0005, ANOVA). Toothpaste, mints and green tea significantly inhibit VSC production compared to control (data are not shown as % reduction was demonstrated in the figure) (p<0.05).

Table 3. Effect of the products on VSC production in putrefied saliva (n=20).

	Hydrogen sulfide	Methyl mercaptan
Saliva control	11.3±1.7	10.6±1.2
Mints	0.8±0.3*	0.6±0.2*
Chewing gum	12.3±1.5	8.2±1.2
Toothpaste	0.4±0.2 *	1.2±0.1*
Parsley oil	12.3±2.4	10.1±1.8
Green tea	2.0±0.8*	0.2±0.1*

*p<0.05. Mean±SE (ng/10 mL).

tem.

Deodorant activity

Deodorant activity by each product was shown as reduction (%) of H_2S. The differences observed among the products were statistically significant (p<0.0005, ANOVA). Toothpaste and green tea demonstrated significant reduction compared to the control (data are not shown, as % reduction was demonstrated) (p<0.01, respectively). However, mints, chewing gum and parsley-oil product demonstrated very low activities, as shown in Fig. 4.

DISCUSSION

Deodorant products and air fresheners are very popular in daily living; body smell and oral malodor are among people's main concerns. At the same time, people's interest in health foods is growing. Green tea is one such food attracting attention in western society. Green tea products such as tablets or foods containing green tea, rather than tea extract, are becoming very popular.

Significant reduction was found only in CH_3SH concentration in mouth air immediately after taking green

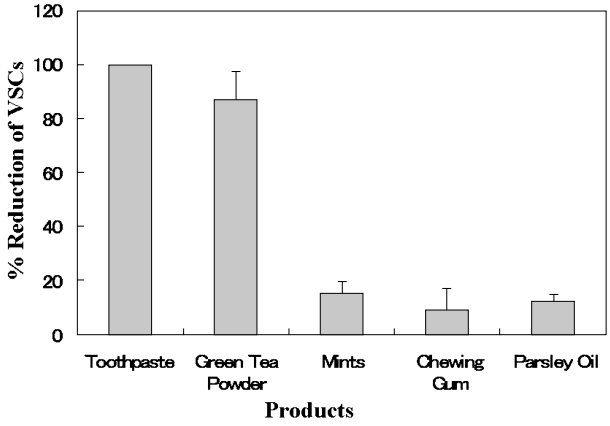

Fig. 4. Deodorant activity of each product against H_2S. Thirty milligrams of the products were added to the test tube beforehand and control test tubes were left blank, then filled with H_2S at 8 ng/10 mL air. After being sealed with an airtight cap, the tubes were incubated for 5 min. The differences observed among the products in reduction of VSCs were statistically significant (n=10, mean±SE, p<0.0005, ANOVA). Toothpaste and green tea demonstrated significant reduction compared to the control (data are not shown, as % reduction was demonstrated) (p<0.01, respectively). However, mints, chewing gum and parsley-oil product demonstrated very low activities.

tea (p<0.0005). However, for other products, a significant difference was not found in H_2S reduction (Tables 1 and 2). CH_3SH shows a better correlation with oral malodor strength than H_2S (*19*), therefore the effect of green tea in reducing oral malodor was expected. Although, its effect did not last longer than 1 h, the in vivo study in this paper yielded promising results for green tea. It was the most efficient inhibitor of VSCs among the products tested, but the rest of the products yielded a positive percentage after administration.

Increment of VSCs by a sugarless chewing gum has been reported previously (*20*). These data allowed us to be confident of the inefficacy of these products in reducing VSCs, other than the masking effect that the flavor might have immediately after applying the products, as shown in organoleptic studies (*20*). However, it has been found that chewing gum with sugar produces a certain amount of reduction of VSCs, due to the acidic change of the oral environment caused by sugar metabolism by microorganisms (*21, 22*). VSCs are generated only at neutral or alkaline pH, and oral microbiota produce organic acids (*22*). Thus, foods containing sugar may reduce oral malodor.

Saliva-incubation tests showed that chewing gum and parsley-oil product were virtually ineffective in reducing VSCs in the saliva-putrefaction system, whereas other products interfered with VSC production in saliva putrefaction. The particular chewing gum and parsley-oil product employed in this study may not have disinfectant properties and thus may not inhibit the VSCs-producing cascade; their efficacy is uncertain. These observations suggest that the mint flavoring contained in the chewing gum might have only a small dis-

infectant effect, as mints demonstrated almost 100% inhibition of VSC production.

Consequently, the saliva-putrefaction system has shown that green tea, mints and toothpaste exhibited encouraging values that dispose one to accept these products as efficient VSC inhibitors. A saliva-putrefaction system has been employed in other studies to determine the efficacy of products in reducing oral malodor (*23–25*). However, when a treatment is carried out on saliva itself, as in this study, in vitro results do not always conform to in vivo results. On the other hand, Quirynen et al. (*26*, *27*) have shown that when saliva was sampled from the treated oral cavity, in vitro results did conform to those in the in vivo study, possibly because the number of microbiota in vitro was similar to that in vivo. For in vitro study, the improvement or modification of the protocols or measures might be required.

In addition to deodorant activity, tea polyphenols showed inhibitory activity against the growth of not only *Streptococcus mutans* (*10*), but also that of other pathogenic strains. Hence, tea catechin was expected to inhibit VSC production because of catechins' strong disinfectant activity, and the data show a potential in green tea for inhibition of saliva putrefaction.

For testing deodorant activity, organoleptic or instrumental analysis is utilized (*28–30*). Since we focused on H_2S as an odorant, GC analysis was employed in this study. Mints, chewing gum and parsley-oil product showed poor deodorant activity against H_2S, with a peak percent reduction of about 34, 16 and 32% respectively. Green tea, however, showed about 88% reduction over control samples. A factor of much importance while testing deodorant activity might be the surface area of the product in contact with the environment inside the test tube. The green tea was provided in powdered form to begin with and thus had a large surface area; therefore, the reaction of CH_3SH with the hydroxyl groups in the B ring of EGCg could proceed quickly (*14*). On the other hand, the toothpaste was in a semi-solid form but demonstrated complete deodorant activity against H_2S; vaporized mint oil or similar compounds in toothpaste might react with H_2S very quickly. Another factor that did not affect this experiment but could be an area of further investigation is the possible evidence of deterioration in the tea compound. Green tea a few months after opening the seal did not yield such favorable results as it had previously (data are not shown). This might be because of oxidation of tea catechins, producing as its final product tannin or other products. Green tea is heated after harvesting to stop this reaction; otherwise green teas would be easily oxidized and become fermented teas such as an ordinary tea or black tea. The B ring of EGCg is perhaps necessary for reaction with VSCs, more so than the galloyl moiety (*14*). Therefore, if this ring undergoes a dehydration reaction with another molecule, such as tannin, or if the B ring is altered, it can no longer form the transition state and thus may not react with VSCs to form a stable benzene ring via resonance.

The effect of parsley-seed oil product or mints on oral malodor has not yet been reported. The results in this study showed no effect of these products in reducing oral malodor. Although mints showed strong reduction of VSCs in a salivary incubation system, their disinfectant activity or the inhibitory activity of VSC production might not be enough in vivo. Only a masking effect is expected as the effect of mints on oral malodor. Parsley-seed oil products also demonstrated no effect on oral malodor. Following the manufacturer's instructions, two capsules were swallowed with no water in this study. If the products were chewed in the oral cavity, some effects would be expected. Parsley extract was reported strongly to reduce the odor caused by one VSC, namely diallyl disulfide, in an in vitro study measuring head space air (*31*). As shown in this study, the compounds involving strong deodorant activity markedly reduced the concentration of VSCs in vivo; parsley-seed oil products may affect oral malodor when the product is chewed. On the other hand, the relationship between parsley-seed oil products and the concentration of allyl compounds, which are the reason for garlic breath, has not yet been determined in vivo. Therefore, the effect of the products on oral malodor caused by allyl compounds is still obscure.

There are some reports to suggest the effect of green tea or tea catechins on oral malodor (*14*, *32*). A clinical study has shown a large reduction of oral malodor by tea catechin (*32*). In the report, a portable sulfide monitor was used instead of a GC. Furthermore, the subjects abstained from eating, drinking and oral-hygiene practices only for 2 h before measurements, although the effect of eating lasts longer than 3 h (*33*). To obtain reliable results, it is recommend to refrain from these activities from midnight until the end of the experiment.

CONCLUSION

Green tea was very effective in reducing oral malodor because of its disinfectant and deodorant activities, but temporarily. It was also found that other foods, such as sugarless chewing gum or mints may not be effective in reducing VSCs, even though a great effect is generally expected from them.

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