SIII-5

FUNCTIONAL BEVERAGE FOR REDUCING BAD BREATH

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Abstract

This study was performed to examine a possible application of the beverage as a bad breath controlling food. To achieve this objective, methods of gas chromatography, electronic nose, sensory analysis and halimeter were used to detect reduction in odor intensities of bad breath caused by the functional beverage as well as its active ingredients. According to results of GC and electronic nose, adding green tea and champignon extracts to bad breath indicators, methylmercaptan and trimethylamine, resulted in significant reduction in headspace concentrations of two indicators. GC results revealed that headspace concentrations of 5 μg/ml of methylmercaptan and 30 μg/ml of trimethylamine added to various concentrations of two extracts were reduced up to 100% after incubating mixtures at 37℃ for 5min. When the functional beverage was properly formulated with green tea extract, champignon extract and α-cyclodextrin and evaluated for its deodorizing effect systematically, it also showed distinctive deodorizing activities against bad breath indicators. Conclusively, results obtained from this study might encourage introduction of a new type of bad breath control food in near future.

Keywords: Bad breath reducing food, Green tea, Champignon, α-Cyclodextrin, GC, Halimeter

INTRODUCTION

As a social activity of human being is emphasized more than ever, we have more chances of contact with other people. Such trend brings forth so-called “etiquette food” or “fashion food”. Reducing bad breath is one of the most attractive applications that etiquette foods can afford to. Bad breath (halitosis) is a common problem but very complicate to deal with. Although there is no way of knowing for sure, most adults probably suffer from bad breath occasionally, with perhaps a quarter suffering on a regular basis. The first and most common cause of bad breath is considered as coming from actions of Gram – microbes on either protein-rich food or turned-over mucosal cells in a mouth. Volatile sulfuric compounds (VSC) such as hydrogen sulfide, methylmercaptan and dimethyl sulfide are the key responsible products. Taking VSC rich foods like onion and garlic also can cause bad breath not only through a mouth but also through a respiratory system. In addition to VSC, volatile nitrogenous compounds such as trimethylamine and ammonia produced from actions of decarboxylase and deaminase on protein sources can play an important role in causing bad breath. Others like extensive dental decay, periodontal (gum) disease, oral infections or abscesses, oral cancers, diabetes and continuous drug abuse are considered to some extend as being involved with bad breath production [1, 2)].

Various efforts to reduce bad breath through taking foodstuffs have been tried [3)]. These efforts are largely categorized into three groups; 1) developing polymers that can absorb volatile VSC or volatile nitrogenous compounds and 2) developing masking flagrant which seems to be most popular and widely applied by now, and finally, 3) developing compounds that can change volatile off-flavors to less volatile ones. Polyphenols and chlorophyll are good examples of the third group and they have been widely applied to candy and chewing gum products [4, 5, 6)]. Because these products contain limited amounts of active ingredients, it would be beneficial to develop a beverage type of bad breath controlling food. This research was focused on evaluating effectiveness of the functional beverage on reducing bad breath intensity through various methods.

MATERIALS AND METHODS

Materials

In this experiment, methylmercaptan sodium salt (15% in water, Tokyo Kasei Kogyo Co.) was used as an indicator for volatile sulfuric compounds and trimethylamine (30% in water, Cica) as an indicator for volatile nitrogenous compounds. These indicators were properly diluted and stored immediately at a freezer. Active ingredients that were examined for their deodorizing effects against bad breath indicators and finally used as main components of the functional beverage were green tea extract (Sunphenon), champignon extract and -cyclodextrin.

Measuring Deodorizing Effect

A. Gas Chromatography

After 1.5ml of the active ingredients as well as the beverage was taken into GC vial, 0.5ml of methylmercaptan or trimethylamine was added and tightly sealed. The mixture in the vial was incubated at 37°C for 5min. and 0.5ml of headspace was taken by a gas tight syringe and injected into *GC (Hewlett Packard model 6890, USA) which was equipped with a mass selective detector.* For a control sample, an equal volume of buffer was added instead of active ingredient solutions. Once the relationship between concentrations of bad breath indicators and their peak areas on GC was established, % reductions of bad breath indicators in headspace after incubation with the ingredients or the beverage were expressed as following [7];

$$\text{Deodorizing effect of active ingredients (\%)} = (C-S)/C \times 100$$

C : Concentrations of indicators in headspace after incubation with water(control)

S : Concentrations of indicators in headspace after incubation with active ingredients or the beverage

B. Electronic Nose

Deodorizing effects of the beverage against bad breath indicators were estimated by an AromaScan (model A 32, AromaScan plc., U.K.) installed with 32 conducting polymer sensors. The analysis was run by dynamic headspace sampling at 30℃ with humidity of 40%RH, 5 min. of equilibration time, pump flow of 150ml/min and valve sequence of reference (60sec)-sample (60sec)-wash (240sec)-reference (60sec). The amount of 15ml of the functional beverage was prepared in an AromanScan bottle and 0.5ml of methylmercaptan (1500ug/ml) or trimethylamine (3000ug/ml) was added. All the volatile compounds in headspace were wiped out by air and carried to sensors. Various response patterns as well as intensities of each sensor toward bad breath indicators were measured and compared.

C. Sensory Analysis

To evaluate whether our olfactory senses can differentiate intensities of bad breath odors in water or in the beverage, methylmercaptan (30 or 60ug/ml) and trimethylamine (60 or 120ug/ml) were added to 75ml of the functional beverage or water followed by tightly sealing and incubating at 37℃ for 5min. Then, 5ml of headspace was taken by a gas tight syringe and slowly released with consistent pressure in front of the well-trained panel's nose. The intensity of bad breath indicators in the control as well as the beverage were recorded according to the 9 point scale from 1 for "very weak" to 9 for "very strong".

D. Halimeter

A halimeter is widely used for a clinical purpose to diagnose halitosis. The halimeter in the

Medical Center of Younsei University was used to evaluate bad breath deodorizing effect of the functional beverage. Participants were asked to keep hold 5ml of 6mM cystein solution in their mouths for 30 sec. and spit it. Then they put the tube connected to a halimeter into their mouths and held breath to be measured the highest concentrations (ppb) of volatile sulfur compounds remaining in their mouths (1st reading). Immediately after 1st reading, they asked to drink 75 ml of water as a control or the functional beverage and concentrations of remaining sulfuric compounds in their mouths were measured again (2nd reading). Differences between 1st and 2nd readings represented changes in concentrations of volatile sulfuric compounds in a mouth.

RESULTS AND DISCUSSION

GC Analysis

A. Deodorizing effect of active ingredients on methylmercaptan

Among various food ingredients known as effective in controlling bad breath, three active ingredients, green tea extract, champignon extract and α-cyclodextrin were selected for their easy application for a beverage processing. Those active ingredients were examined for their deodorizing effects on two bad breath indicators, methylmercaptan and trimethylamine. Conclusively, as concentrations of active ingredients added and incubated with two indicators were increased, concentrations of two indicators in headspace were further decreased (Fig. 1). When 1.2, 4.8 and 20mg of green tea extract were added to 5 μg of methylmercaptan, its concentrations in headspace were reduced by 25.6, 61.6 and 77.4%, respectively. Although effectiveness of champignon extract was less significant than that of green tea extract, it still reduced 24.2, 64.4 and 85.0% of methylmercaptan in headspace when it was added at 2.0, 8.0 and 50mg, respectively. However, addition of α-cyclodextrin caused no change in headspace concentrations of methylmercaptan.

Fig. 2 showed changes in headspace concentrations of various amounts of methyl mercaptan added to active ingredients. More than 60% reduction in headspace concentrations occurred when the ratio between methylmercaptan and active ingredients was around 1:1,000 (wt: wt). With an information that higher than 5ppm of methylmercaptan caused strong reluctance from sensory panels, it might be reasonable to narrow down amounts of active ingredients suitable for the beverage formula at the range of 5-15mg/ml.

Yasuda and Arakawa [7)] suggested that ortho-quinone of (-)epigallocatechin gallate(EGCg), which was one of the most abundant polyphenols in green tea, could form a complex with methyl thio group

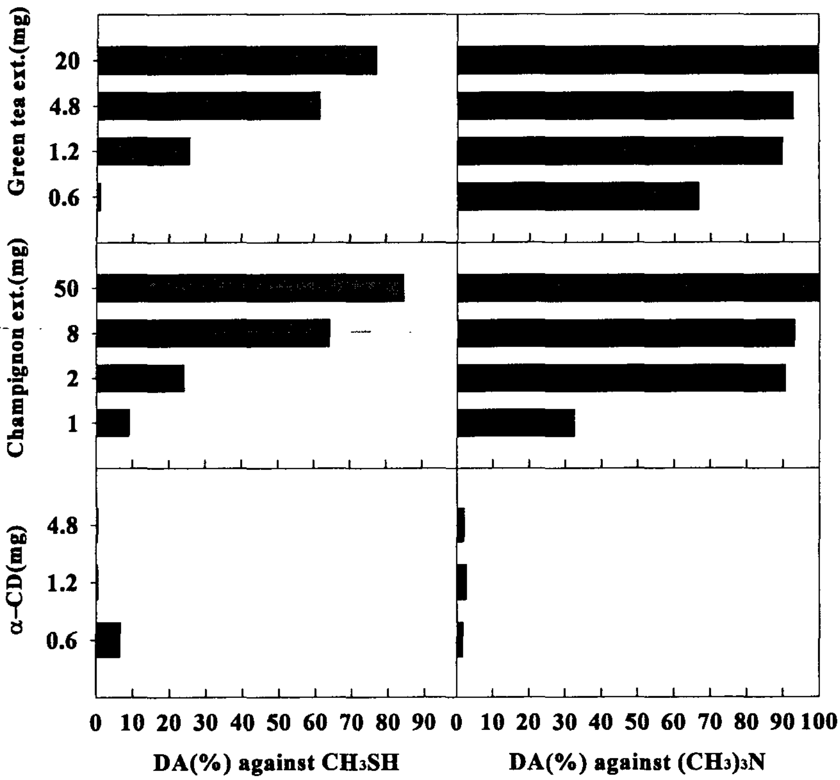

Fig. 1. Deodorizing effects (%) of green tea extract, champignon extract and alpha cyclodextrin. on methylmercaptan(left) and trimethylamine(right) measured by GC.

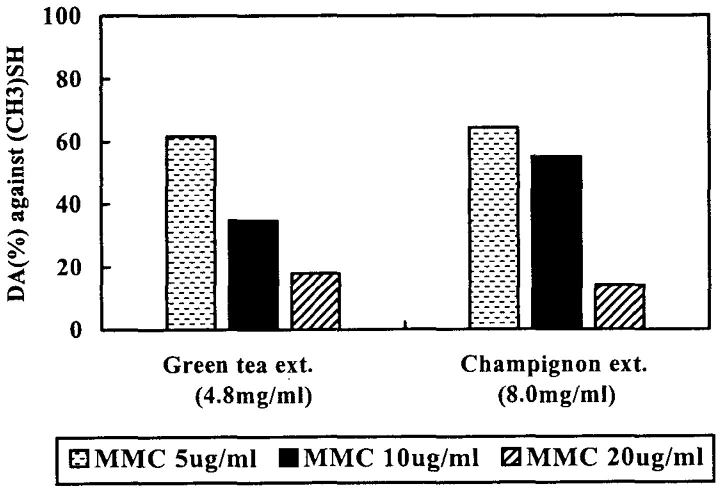

Fig. 2. Deodorizing effect (%) of green tea extract and champignon extract at different concentrations of metylmercaptan (MMC)

of methylmercaptan. This condensation reaction eventually prevents methylmercaptan from being volatilized (Fig 3). Although there has been few attempts to explain chamginon's activity on methylmercaptan, its native organic acids of carboxy and α, β–unsaturated ketone groups might be involved with its deodorizing potential.

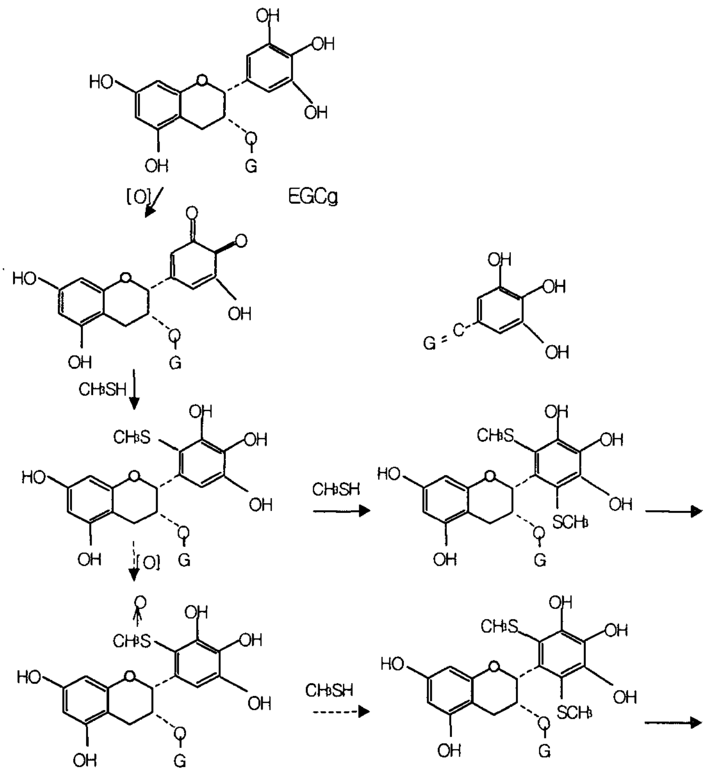

Fig. 3. A suggested reaction pathway occurred between (-) epigallocatechin gallate(EGCg) and CH_3SH [7].

B. Deodorizing effect of active ingredients on trimethylamine

Addition of 1.2mg of green tea extract to 30 μg of trimethylamine followed by incubation suppressed headspace concentrations of trimethylamine by 66.8%. Higher than 90% reduction in headspace was observed by addition of 2.0mg of green tea or champignon extracts (Fig. 2, 4). As in the previous case, however, cyclodextrin didn't induce any reduction in concentrations of trimethylamine in headspace. The minimum amount of green tea and champignon extracts needed to eliminate more than 80% of trimethylamine in headspace was about 3mg/ml.

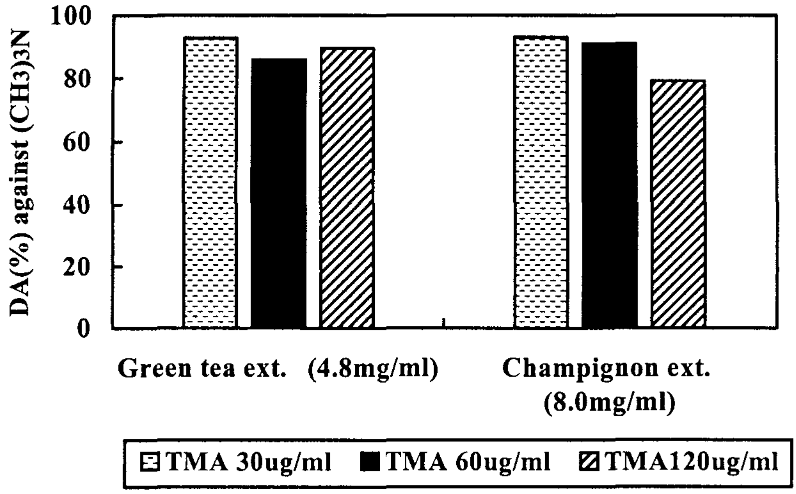

Fig. 4. Deodorizing effect (%) of green tea extract and champignon extract at different concentrations of trimethylamine(TMA) measured by GC.

Evaluating Deodorizing Effect of the Functional Beverage by GC

The functional beverage was formulated considering effective concentrations of ingredients to suppress formation of bad breath odor and suit consumer's taste. Amounts of tea extract and champignon extract added to fill up a bottle of 75ml were 60 and 100mg, respectively. Although it didn't show any deodorizing effect, 60 mg of α-cyclodextrin was still added expecting any additional improvement in deodorizing effect [8]. Deodorizing activity of the finally formulated beverage was examined by GC with exactly the same procedure as used for examining active ingredients. As shown in Table 1 and 2, when 5 μg/ml of methylmercaptan or 30 μg/ml of trimethylamine was added to the beverage and incubated, neither of two odors was detected in headspace. Even when the beverage was diluted by fifty times with water, only 37.4% of methylmercaptan and 23.2% of trimethylamine were detected in headspace. This indicated that the beverage exhibited very strong deodorizing activity against two bad breath indicators.

Evaluating Deodorizing Effect of the Functional Beverage by Electronic Nose

An electronic nose is designed to mimic human's olfactory sense. Each sensor is coated by a thin polymer and this determines each sensor's specific pattern in response to volatile compounds. When volatile compounds have contact with sensors, electro-polymerization is formed and this leads to changes in electrical conductance and finally sensed by a detector [9]. When 15ml of the functional

Table. 1. Deodorizing effect (%) of functional beverage against methyl mercaptan determined by GC

	Conc. of CH_3SH (μg/ml)	Deodorizing Effect (%)
Beverage[a]	5	100.0[b]
	50	43.4
	100	22.2
1/10 Diluted Beverage	5	69.0
1/50 Diluted Beverage	5	62.6

[a]greentea extract (0.080%), champignon extract (0.134%), μ-cyclo dextrin (0.080%) were contained
[b]peak of methylmercaptan in headspace wasn't detected

Table. 2. Deodorizing effect (%) of functional beverage against trimethylamine determined by GC

	Conc. of $(CH_3)_3N$ μg/ml)	Deodorizing Effect (%)
Beverage[a]	30	100.0[b]
	100	96.0
	750	97.1
1/10 Diluted Beverage	30	88.0
1/50 Diluted Beverage	30	76.8

[a]greentea extract (0.080%), champignon extract (0.134%), α-cyclo dextrin (0.080%) were contained
[b]peak of trimethylamine in headspace wasn't detected

beverage or water as a control were added to 10 μg/ml of methylmercaptan and 20 g/ml of trimethylamine, each sensor's response intensities in the beverage groups were much lower than those of the control groups although response patterns were very similar (Fig. 5).

To investigate further about differences in response intensity between sample groups, a principal component analysis was done for mapping groups according to their quantitative differences in response intensity. A quality factor (QF) between groups can be calculated by a principal component

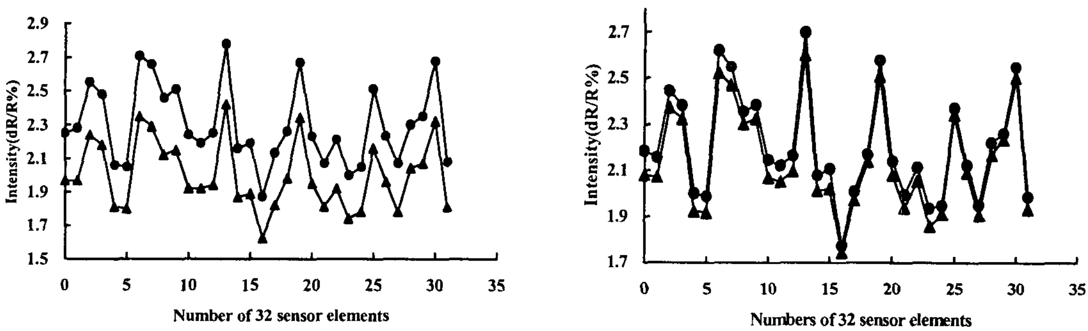

Fig. 5. Effects of the functional beverage on reducing odor intensities detected by electronic nose system. (left : spiked with methylmercaptan, right : spiked with trimethylamine, beverage with(▲) and without(●) active components which were green tea ext. (0.080%), champignon ext. (0.134%), and -cyclodextrin (0.080%).

analysis. Although initial difference in response intensity as represented by QF value was only 0.82 between the beverage and control, the difference turned to be greater, 2.21, after methylmercaptan was added to each group (Fig. 6). Considered that a QF value within beverage groups (with or without methylmercaptan addition) was much lower, 0.57, it was obvious that the beverage caused significant effect on suppressing odor intensity of methylmercaptan. Similar results were observed with trimethylamine. The QF was only 0.57 within the beverage groups of with or without trimethylamine addition otherwise QF was 2.25 between the beverage and control groups (Fig. 7).

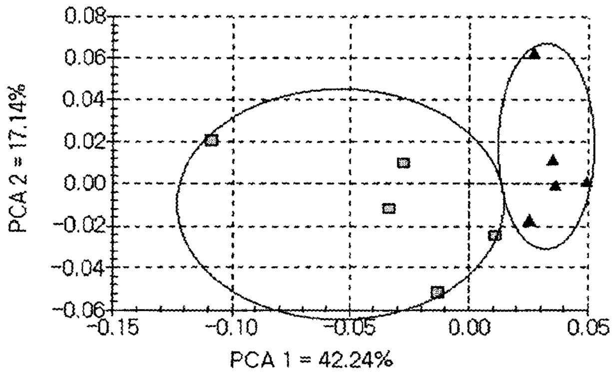

Fig. 6. Discrimination of aroma patterns of electronic nose spiked with methylmercaptan between beverage with (■) and without active components(▲) .

*** Active components are green tea ext. (0.080%), champignon ext. (0.134%), and α - cyclodextrin (0.080%).**

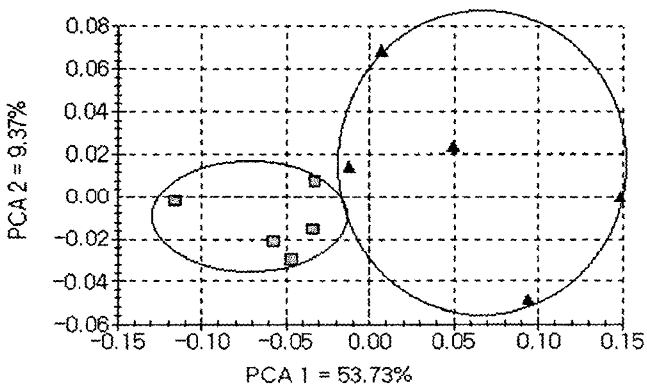

Fig. 7. Discrimination of aroma patterns of electronic nose spiked with trimethylamin between beverage with (■)and without active components(▲) .

*** Active components are green tea ext. (0.080%), champignon ext. (0.134%), and - cyclodextrin (0.080%).**

Evaluating Deodorizing Effect of the Functional Beverage by Sensory Analysis

Fig. 8 showed results of sensory analysis done by well-trained panels in differentiating odor intensities of each group. With the assumption that the odor intensity of 30 μg/ml methyl mercaptan in water was 9, panels estimated the odor intensity of methyl mercaptan in the beverage as 6.1. When even lower amounts of methyl mercaptan (10 μg/ml) was added to water or the beverage, panels still could differentiate odor intensities between a control (5.9) and the beverage (3.9) groups demonstrating powerful deodorizing effects of the beverage Almost similar results were found when deodorizing effect of the beverage on trimethylamine was evaluated. When 120 g/ml of trimethylamine was added to the beverage or water and its odor intensities were compared, panels judged that there was significantly lower intensity (5.7) of trimethylamine in the beverage.

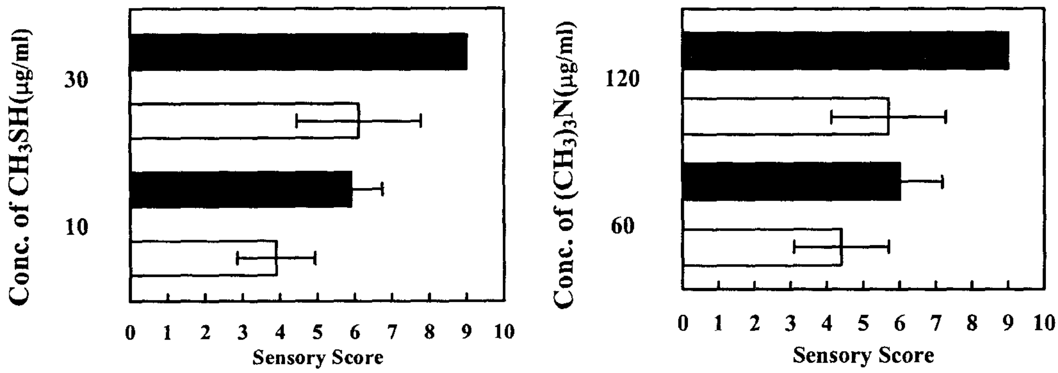

Fig. 8. Effects of functional beverage with(□) and without (■) active components* reducing odor intensities examined by sensory evaluation (left : spiked with methylmercaptan (10, 30 μg/ml), right : spiked with trimethylamine(60, 120 μg/ml).**

*** Active components are green tea ext. (0.080%), champignon ext. (0.134%), and α-cyclodextrin (0.080%).**

**** Based on a scale of 9 (very strong) to 1 (very week) *** Group means are significantly different ($P<0.05$).**

Evaluating Deodorizing Effect of the Functional Beverage by Halimeter

A mercury sensor in halimeter can determine concentrations of sulfuric compounds at ppb level. Concentrations of sulfuric compounds are easily increased to 1,000 ppb when panels keep hold 6mM of cystein for 30 sec. in their mouths and spit. When panels drank 75ml of water immediately after spitting cystein solution, 17.03% reduction in concentrations of sulfuric compounds in mouths was detected. (Fig 9). To some panels, concentrations of sulfuric compounds were even increased after drinking water. When the beverage was taken instead of water, however, concentrations of sulfuric compounds were decreased up to 65.45%. Deoderizing effect of functional beverage was significantly higher than that of control (n=24, P<0.0001, Student's *t*-test for paired data, one-tailed). This result suggested that drinking the beverage could change volatile sulfuric compounds in a mouth into undetectable less volatile ones.

In conclusion, deodorizing activity of the functional beverage against bad breath odors were examined by various methods. Active ingredients effectively reduced intensities of bad breath odors. Once the functional beverage was formulated according to effective concentrations of active ingredients, it also showed excellent deodorizing effects which make us anticipate introduction of a beverage type bad breath control food near future.

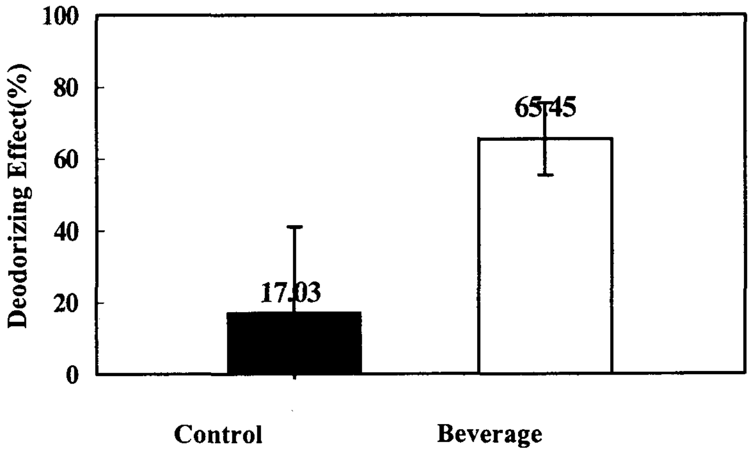

Fig. 9. Deodorizing Effect (%) of functional beverage determined by Halimeter
*** Green tea ext. (0.080%), champignon ext (0.134%), and α-cyclodextrin (0.080%) as active components were contained in the functional beverage.**

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