Evaluation of Off-flavor Formation and its Deodorization during 
Functional Food Production from Sweet Potato and Garlic

2008 年 1 月

玉城 和彦
CONTENTS

Introduction 2

Chapter I 11
The Concept of Functional Model Food and Volatile Components of the Model Food Made by Sweet Potato and Garlic

Chapter II 33
Odor Characterization of Steamed Sweet Potato and Apple by Using Sensory Analysis, Commercially Available Odor Sensor and Electroencephalography

Chapter III 52
Measurement of Odor Quality of Model Food Ingredients by Using Newly Developed Odor Sensor

Chapter IV 63
Deodorization of Off-odor of Sweet Potato by Using Physical and Chemical Deodorants

Chapter V 85
Measurement of Odor after In-vitro or In-vivo Ingestion of Raw or Heated Garlic, by Using New-type Odor Sensor, GC and Sensory Analysis

Chapter VI 99
Deodorization of Garlic-Induced Oral Malodor by Mushroom Extract

Chapter VII 123
Colligation and Vision

References 132
Presented paper 147
Acknowledgement 148
Introduction

The food is the most important element in human life. Longer and stable consumption of the food depends on its palatability as well as its quality. With the change of consumer's preference, cereals (i.e. sorghum and Hungarian grass) which used to be a staple diet in many countries, is now mainly utilized as a feed or fermentation materials for a domestic animal. Although the consumption of corn as a staple diet is still high in developing countries, it was substituted by wheat or rice in many developed countries.

Although unprocessed wheat is not edible, rice can be consumed as grain particles after cooking, and also can be applied to many dishes when it is ground, which helps rice consumption steadily rise in developing countries such as those in Africa.

Odor of the food largely influences the preference of the food. Developing new techniques for analytically evaluating flavor of foods and food ingredients, and integrating these information into developing system for improving their flavor or reducing off-flavor contribute to gain the popularity of food ingredients by expanding its use or developing new food applications.

In this study, sweet potato and garlic were chosen as functional ingredients which possess nutritious and health benefits. These ingredients also possess a problem in use as ingredients in processed foods because of their distinct flavor. Widely established throughout the world, the sweet potato is a favorite staple of many cultures. In many developing countries, it is now grown as a substitute for rice or wheat. In Japan, sweet potatoes used to be a staple diet that was the main source of nourishment for Japanese after the World War II. Sweet potato is distributed in the market as an unprocessed vegetable. However, the processing of peeling and cutting of sweet potato is quite time consuming, which becomes the bottle neck for consumers or food services which prefers the product typified by a "ready-to-use" pre-cooked
ingredients or ready-to-eat" pre-cooked ingredients. Although it is known to produce the favorable sweet potato like aroma when it is baked, when boiled potatoes are stored, they develop an off-flavor, which can be described as sweet potato-like odor in Japan. This peculiar property of the aroma production is a serious problem in food service systems or in house cooking, which limits the use of sweet potato in the culinary recipe because of the unique flavor properties which may overwhelm the taste quality of the prepared dishes.

Also, garlic has been reported as a therapeutic food, useful in prevention of the cancer. Numerous studies showed that garlic and its organic allyl sulfur components are effective inhibitors of the cancer process. Especially, strong correlation was found between garlic and prevention of prostate and stomach cancers. Also, NIH describes foods that naturally contain or are enriched with cancer-preventing substances as "designer foods" and its food pyramid place a heavy stress on garlic.

Quality is often defined as the totality of features relevant to the ability of a product to fulfill its requirements. Although the concept of food quality is consisted of a broader basis to account for the different demands of the manufacturer and the consumer, requirements necessary to satisfy the needs and expectations of the consumer must be placed first to consider the food quality of the commercial product. These requirements include safety, sensory good taste and nutritional, functional requirements.

The consumers have become more conscious of information about food products. They are not only interested in the safety of the products, but also in their origin of ingredients, process, storage and handling of foods. Especially, when the food is processed, the authenticity of the original quality and properties of ingredients would be considered. These kinds of qualities could be controlled by ensuring the traceability. The current growing interest of the consumer toward the health improving properties of the food is based on the study results that the regular consumption of the certain food has beneficial effects on health and strengthens the
immunological defense power against a number of chronic diseases. Functional foods are defined as "foods containing significant levels of biologically active components that impart health benefits beyond basic nutrition when consumed in typical or optimal serving sizes" (International Food Information Council, 1995). For functional foods to deliver their potential health benefits, the regulatory process is established to support the health effects or claims for the functional components or the foods containing them. Foods for Specified Health Use (FOSHU) regulatory system is an example for the approval of the specific health claim for the food or the food containing the health beneficial ingredient. As the consumer demand for functional foods has steadily increased, the more new product is created enriched with functional compounds. For instance, the probiotic dairy products are the fast grown functional product, which add the probiotic bacteria to the regular fermentation cultures to provide therapeutic benefits such as modification of the immune system, reduction in cholesterol, alleviation from lactose intolerance (1,2).

These nutritional requirements of the food not only affect to the food quality but also the important factor to influence the food choice. Research reports have shown that nutritional compositions in food and its health benefit are an important criterion for purchase and a parameter of quality for many consumers (3,4,5).

Although health is an important factor in food selection, other factors are known to play a role. The food availability and cultural factors are recognized as a dominant factor in food selection, which leads to the habitual consumption of certain foods and the food preparation, or restriction of certain diets. Stress and negative emotions may influence food selection and consumption (6). Convenience is an important factor in today's time-oriented society and the many ready-prepared type food products which allow consumers to prepare the meal very quickly (7,8). Other factor includes the price. The branding of food products is the most important asset for companies in the food and beverage industry. The consumer links the quality of products to product brands, which ensures the origin of the product and the responsibility of manufacturer
for safety. This influences the consumer's choice of products greatly (9).

Although these nutritional and functional requirements are important factors for the food quality, in the individual level of food choice, the sensory requirement is the most important factor in food quality, which directly reflects consumer's preference (10,11). Numerous researches have shown that taste is a key factor in food choice (12,13,14). Perception of taste, smell, appearance and texture for the product interacts to strongly influence preference and purchase decision.

Among the sensory requirement of the food quality, the flavor largely influences the marketability of the product. The flavor of a food product is the integrated perception of a consumer using both the senses of smell and taste. It is a large problem in the industry for loss or degradation of desirable flavor or sensory characteristics of its aroma during processing or storage. Off-odors is generally generated by changing in chemical or physical properties of ingredients as a result of decomposition due to endogenous enzymes, contaminated microbes, chemical oxidation, or other means of contamination to produce undesirable flavor compounds (15). Off-odor impaired to the product is a serious problem which undermines the consumer preference and confidence in the retail product, and must be controlled by employing effective methods.

This is especially a problem in the development of functional food. In order to maximize the health benefits, the functional food includes many nutritional components.

However, many functional ingredients possess adverse sensory attributes. For instance, incorporation of various vitamins or minerals into final product can lead to an unacceptable flavor. Also, depending on the manufacturing process, functional ingredients are likely to change their physical or chemical properties, such as amino-carbonyl reactions (Maillard reaction), which lead to the undesirable flavor change. Therefore, by understanding the care of behavior for ingredients can be taken to minimize or counteract off-notes caused by ingredients in the final product.
As consumer's interest in health benefits of food is grown, development of the food products with high and balanced nutritional value are desired, but also these products should also be superior in taste, flavor and texture. All these requirements add up to an ideal concept of the food quality, so the food quality should not be evaluated solely on its nutritional and functional value but also on its sensory value the economic success of a product.

Unlike the nutritional and safety requirement, such as microbial contamination, which can be measurable and controlled by meeting the requirements during production and storage, sensory requirements, such as flavor quality is hard to quantify, and is still under intensive research. The consumer acceptance toward the aroma of the product is also hard to predict. It is critical to measure the consumer's acceptance quantitatively for the product to be successful in the market, without solely depending on the subjective opinion of the product development staff. Over the years, many investigators have worked on various techniques to measure the flavor acceptability of food products. However, there is still no convenient technique available for measurement of consumer acceptability besides simple sensory evaluation.

To date, many methods to test the product for off-odor have been developed. The human nose is as sensitive as an instrument and trained sensory panels can detect aroma changes in a sample from subtle taints to major differences. Therefore, sensory evaluation is currently the foremost tool for odor analysis. Also, since it is the method used by end-users in the final assessment of a product prior to consumption, it is the only method used in evaluation of the consumer acceptance. The great advantage of the sensory evaluation is to put a quantitative value on the total aroma perception, and analyze the concentration, potency, and hedonic value of an aroma (16).

Although sensory evaluation would be the most appropriate tool for odor analysis, it is quite time consuming and expensive to repeatedly assemble the sensory panels for the multiple measurements during the development of the product. The sensory
panelists also quickly suffer from fatigue, and to conduct an appropriate evaluation task without bias is a difficult task.

Another problem is the lack of chemical information. Although the sensory evaluation provides a direct link between the flavor profile of the product and consumer acceptance, it does not provide any information about the chemical compounds which are responsible for the aroma in terms of the volatile chemistry. Use of a proper chemical measurement method, such as GC-MS, can identify and quantify the specific odorous compounds which may impact the aroma profile, and provide clues to improve the aroma characteristics of the developing product.

To ensure the flavor quality and that food will conform to consumer expectations, measurement of the flavor have to be taken in terms of a flavor quality management. Evaluation method must work on different levels and, therefore, different analytical procedures have to be established for different steps of production. In the quality control environment, fast and simple analytical methods must be employed. The more sophisticated and elaborated analytical system must be taken in for qualification and quantification of the aroma components for Research and Development process. These different analytical methods suit their purpose at different levels and contribute to enhancing the overall food quality.

Gas chromatography and mass spectroscopy can be used to monitor and identify compounds and their concentrations in an aroma which leads to the potential flavor change during the production process. Consequently, GC or GC-MS analyses are often used in monitoring and inspection at quality control environment. In the product development, by understanding chemistry of volatile components in foods, GC technique can be used to improve flavor characteristics by eliminating or masking off-notes in combination with masking agents, which result in minimizing the flavor change caused by off-odor compounds.

The disadvantage of GC or GC-MS analysis is to find difficulties in identifying compounds which contribute to the recognized aroma or malodor and to what extent in
case of the complex odors. Also the sampling of volatiles may be destructive in injection into the instrument.

In recent years, odor sensor (electronic nose) instrumentation has found expanded use as a complimentary tool to fill gaps in flavor or odor analysis not achieved by use of sensory panels and GC/MS techniques in conjunction. The electronic odor sensor is aimed at simulating and predicting human responses by characterizing the response of the sensor array against the volatile of product. Electronic odor sensors have a unique advantage over GC and MS techniques because it is an analytical technique that samples an entire aroma rather than identifying it by its comprising component (Payne 1998). With use of the multivariate statistical analysis, the odor sensor can discriminate the sample with an aroma defect among the large number of samples. It is a powerful and faster tool for the aroma or malodor analysis in the quality control environment, which can test odors that human sensory panel would not be willing to.

However, despite advancement of these techniques, the human olfaction system is still the most sensitive device available for flavor measurement, which comprises the perception of flavor of the product when assessing the preference or acceptance. Therefore, it is still critical that any odor monitoring methods used in quality control or quality assurance is correlated odors that may be found to be offensive by the human olfactory system. This is the reason that human sensory panels are still the basis of aroma measurements in the food industry. This fact set the electronic odor sensor is limited in use in the norm of monitoring the odor deficiency as a complimentary tool of the sensory evaluation. With a combination of both instrumental and human senses to find the possible correlation between these methods, the electronic odor sensor can achieve the characterization of the chemical and sensorial aroma profile. In an attempt to improve the evaluation of the off-odor, the correlation between the sensor evaluation and the odorous compounds, and the human odor perception still has to be investigated further for application of the electronic odor sensor as a quicker and more reliable tool. Although evaluation of the odor perception in human sense is not really
a favorite subject of the electronic odor sensor, finding a correlation between the
human perception and the sensor response may lead the way to develop a new
electronic odor sensor system which could predict the human acceptance based on the
sensor response to classify the odor if it conformed to the consumer's acceptance. In
the realm of the flavor evaluation, changes in methodology and advance of the new
technique are ongoing. Evaluation of the off-odor using a new electronic odor sensor
system will be contributed to evaluating the sensory characteristics of the product in
terms of the product quality in a cost and time efficient manner.

In view of the above, the objective of this study is to develop an electronic odor
sensor evaluation system, which could detect the odor defect and the predict consumer
acceptability. For this purpose, the study were to chose the functional ingredients,
sweet potato and garlic, which impart the off-odor. The odor quality of the sweet
potato and the garlic in the production of the novel developed functional food were
evaluated. Sweet potato has a unique sweet potato flavor when it is baked.
However, during the thermal processing, it was found to alter the overall aroma by
formation of off-odor, which is highly undesirable to consumers; therefore research is
necessary to identify the odorous compound that cause significant flavor alterations
which has negative impacts on perceived flavor quality. Garlic is also enriched with
the various nutrition and functional compositions for health benefits. However, its
distinct smell and the oral malodor when it is ingested are often problematic.
Therefore, the research first has to identify the odorous compound that cause
significant flavor alterations which has negative impacts on perceived flavor through
the instrumental and sensory analysis of flavor.

Secondly, to investigate electronic odor sensor systems can perform effectively in
discriminating between odor with acceptable and unacceptable volatile levels, the
deodorizing effect was analyzed for the sample which was treated with the deodorizing
agent. Throughout the study, relationships to the electronic odor sensor measurement,
the volatile compounds and the sensory attributes are sought to ensure the
measurement of the off-odor by the electronic nose in the selected ingredient. Mechanism of the off-odor formation and the deodorization were investigated to ensure that the response of the electronic odor sensor is correlated with the volatile chemistry of odor compounds which is perceived as a malodor in human nose.

The following research objectives are addressed for each of the section in the dissertation;

1. To evaluate the headspace volatile components of model food made by sweet potato and garlic.

2. To evaluate the odor of sweet potato by sensory evaluation methods and the relation between sensory evaluation, odor sensor and electroencephalography.

3. To develop the new electronic odor sensor system which could discriminate between the odor with conforming and without conforming to the consumer acceptance.

4. To investigate the deodorization of off-odor of model functional food by using physical and chemical deodorants, and to evaluate the deodorization effect by using the newly developed electronic odor sensor system.

5. To test the ability of the newly developed electronic odor sensor system to accurately classify the sample as having either conforming or non-conforming to acceptable in human sense by measuring the odor of raw or heated garlic when it is graded and ingested.

6. To investigate deodorization of garlic-induced oral malodor by mushroom extract, and to evaluate the deodorization effect, comparatively with the commercial electronic nose systems, which discriminates samples in multiple variable analysis.
Chapter I
The Concept of the Functional Model Food and Volatile Components of the Model Food Made by Sweet Potato and Garlic

1. The concept of the functional model food

In the first chapter, as functional ingredients which contain physiologically active components with nutritious and health benefits, sweet potato and garlic were chosen and identify volatiles to determine the flavor profile of these ingredients through the volatile compounds detected, which may contribute to the off-odor of model food. These ingredients possess a problem in the usage as an ingredient in processed foods because of their distinct flavor. In our study, these ingredients were found to be problematic in development of functional foods with probiotic value etc..

Widely established throughout the world, sweet potato is a favorite staple of many cultures. In many developing countries, it is now grown as a substitute for rice and corn. In Japan, sweet potatoes used to be a staple diet that was the main source of nourishment for Japanese after the World War II.

Sweet potato is marketed as an unprocessed vegetable. However, cooking, peeling and cutting for processing are quite time consuming, which becomes the bottle neck for consumers or food services which prefer the product typified by a “ready-to-use” pre-prepared ingredient or ready-to-eat” pre-cooked ingredient. Although it is known to produce the favorable sweet potato-like aroma when it is baked, when boiled potatoes are stored, they develop an off-flavor, which can be described as sweet potato-like odor (called “Imo-shu”) in Japanese. This peculiar property of off-odor development is a serious problem in food service systems or in house cooking, which limits the use of sweet potato in the culinary recipe because of the unique odor property which may overwhelm the taste quality of the prepared dishes.

Recently, a new variety of sweet potato with improved β-carotene content and
protein quality have been developed by selective propagation, and the nutritive value of the sweet potato gains consumer interest. As a need to carry out value-added activity in agricultural sectors in the sweet potato production area, new value-added specialty food products using sweet potato as an ingredient are developed, and government research institutes also participate in support of the development of value-added food products and micro-enterprises. Also, garlic has been reported as a therapeutic food, useful in prevention of cancer. Numerous studies showed that garlic and its organic allyl sulfur components are effective inhibitors of cancer propagation. Especially, strong correlation was found between garlic and prevention of prostate and stomach cancers (1). Also, NIH describes foods that naturally contain or are enriched with cancer-preventing substances as "designer foods" and its food pyramid place a heavy stress on garlic.

These evidences reflect a view of garlic as a functional ingredient, which provides nutritional attributes and health-enhancing benefits. As a food ingredient, garlic has long been popular in Italian, northern China and Korea. In the culinary recipe of these countries, many dishes include use of raw garlic, and garlic's pungent smell is well accepted as a flavor enhancer. However, cultural aspects or consumption habits influence the acceptability of garlic odor to consumers in non-garlic habitual consumed countries. For instance, in the Japanese cuisine, except certain ethnic dishes such as Chinese or Korean foods, garlic is not a common ingredient.

A smell of garlic has been socially frowned upon in Japan, and strong odor of garlic is either dislike or unfamiliar with Japanese consumer, despite the rise of understanding in its health benefit. In view of above, the author developed a novel yogurt like product utilizing the rich carbohydrate property of sweet potato. Account into the fact that the distinct flavor of sweet potato and garlic limits the development of new product or dishes in commercial market, development of the functional food using these foods as main ingredients could add new value on its use and the local enterprise where demands for use of local specialty foods steadily increase. The developed
product was comprised of sweet potato as a main ingredient, inoculated to form a yogurt derived solely from enzymatic transformation of sweet potato starch. This product is expected to possess probiotic properties and other health beneficial properties of garlic. The challenge encountered in development was to minimize the distinct flavor of garlic and sweet potato in saccharification process.

The aim of the first chapter is to identify and quantify the compounds which are responsible for the characteristic odors of sweet potato and garlic, and then find possible mechanism of off-odor formation for selection of masking agent which will efficiently eliminate the identified odorous compounds.

1) Preparation of model food

The model food is as follows. Sweet potato cultivar "Benihayato" containing high amount of β-carotene is raw material and garlic which is the top on the list of anti-cancer in the American Cancer Association among the vegetables, is auxiliary material. The mixture is fermented by probiotic lactic acid bacteria. The final product possesses functionality promoting our health. The heat-treated sweet potato and garlic are used as a representation of unpleasant odor.

2. Volatile components in the model food made from sweet potato and garlic

1) Volatile components and off-odor components of sweet potato

Sweet potato produces a pleasant aroma when baked. Various studies have been conducted to identify and quantify the volatile organic compounds in baked sweet potato (2,3,4). Some other odor-related compounds have also been identified. Tiu et al. have studied the contribution of some volatile compounds to sweet potato aroma and identified individual sweet potato cultivars on the basis of 27 volatiles by using gas chromatograms of each cultivar, and found the difference between cultivars of good or poor flavor (5). Sun et al. found that maltol is a key component of characteristic aroma of baked sweet potato, and is produced through Maillard reaction,
caramelization, and Strecker degradation, which are the most common mechanisms in the thermally induced synthesis of aroma volatiles (6).

Owing to its unique health benefit and odor property, sweet potato serves as a potential functional food ingredient in various food applications. In this chapter, functional juice using “Benihayato” sweet potato has been studied. Benihayato is a sweet potato cultivar, that resembles carrot in color, odor and β-carotene content (7). β-carotene, independent of its role in the formation of vitamin A, is anti-carcinogenic as evidenced by its effectiveness in the treatment and management of cancer at different sites in several different cancer model systems, using different inducing agents (8) in different animal species. During the development of functional sweet potato juice, roots of Benihayato are steamed by stepwise heating, mashed to homogeneity, and then saccharified with amylase. However, after boiling and saccharification the mashed juice develops a heavy unappetizing odor, unlike the fragrant aroma of baked sweet potato. To suppress this strong boiled odor, mashed sweet potato juice is then supplemented with other ingredients.

Since boiling in sweet potato juice production results in potential off-odor, it can possibly be a bottleneck in the development of an attractive functional food using Benihayato as an ingredient.

Although there are many available reports on baked sweet potato, there are no published reports on the volatile compounds of boiled sweet potato. Since steaming procedures are utilized during the manufacture of sweet potato-based food products, including the preparation of functional sweet potato drinks, it is important to evaluate the nature of volatile compounds responsible for the flavor of sweet potato and to elucidate the mechanisms involved in the formation of these compounds as a result of steaming.

Our main objective in this chapter is to clarify the odor characteristics and the difference of odor between raw and heated-saccharified sweet potatoes. The identity of the volatile components is investigated by gas chromatography (GC) and gas
chromatography-mass spectrometry (GC-MS). In addition to the mechanism of the formation of volatile compounds, the odor impact of each volatile component is discussed by evaluating the influence of each component on total odor characteristic of sweet potato.

2) **Volatile components and off-odor components of garlic**

Garlic (*Allium sativum* L.) is widely used in the various food recipes for its unique flavor and nutrition. However, garlic is also the cause of malodorous-smell after ingestion. Studies have shown that various sulfur substances of garlic are the main cause of malodor (9,10,11,12). The principal components of malodor detected by the human nose are hydrogen sulfide, methyl sulfides, dimethyl sulfide and allyl sulfide compounds. In particular allyl sulfide compounds are detected in breath after garlic ingestion (13). These mercaptan compounds have a strong disagreeable smell in human nose but it is experientially known that heat-treated garlic odor is not as strong as raw garlic odor and breath after eating heat-treated garlic is not as strong as after eating raw garlic. Studies have also shown that there is a difference in the amount of volatile compounds in heat-treated garlic and fresh garlic. In this study, I studied the change in the odor characteristic of garlic and volatiles released from in-vivo and in-vitro samples were identified by GC and GC-MS.

3. **Materials and methods**

1) **Volatile components and off-odor components of sweet potato.**

(1) **Material of sweet potato**

The raw sweet potato cultivar “Benihayato”, cultivated in Kagoshima, Japan, and harvested in October of 2004, was used in this study. The raw tubers of this cultivar contained 8 mg of β-carotene per 100g wet weight.

(2) **Preparation of sweet potato juice**

Raw sweet potato juice was prepared as follows. One kg of sweet potato was
shredded without peeling and added to 1.8 litter of water. This preparation was homogenized for 30 sec by using a macerator. The resulting slurry was placed in a 3 litter flask. For the preparation of heated sweet potato juice, 1 kg of sweet potato was steamed by stepwise heating (at 60-65°C for 3.5 hrs and at 100°C for 1 hr). To this preparation 1.8 litter of water and 5.0 g of α-amylase (Uniase BM-8, with a specific activity of about 80,000u/g) were added, and potato mixture was incubated for 3 hrs under warm conditions (60°C). Then 5.0 g of β-amylase (Uniase L, with a specific activity of about 80,000u/g) was added to partially hydrolyze starch and incubated at 55°C for 1 hr. After the incubation 50 g of glucoamylase (Uniase 30, with a specific activity of about 80,000u/g) was added to the preparation. The mixture was incubated at 55°C for 1 hr. Both samples were cooled to room temperature, separated into smaller beakers and wrapped in aluminum foil. In addition, 100 g each of raw samples and heated juices was used for GC-MS.

(3) Thermal desorption cold trap (TCT) analysis

The volatiles of 2 juices were concentrated and trapped in Tenax TA adsorbent tubes by sparging with nitrogen gas (N₂) at a rate of 80 ml/min and heating at 50°C for 60 min. Each Tenax tube was placed in the heating block of a TCT injector and heated to 220°C to desorb the volatiles. The desorbed volatiles were injected into GC mass spectrometers through a TCT injector, and total ion chromatograms were obtained. The TCT conditions are as follows. Apparatus: CHROMPACK Company, rod temperature; 220°C, desorption/cold trap material; silica mega bore column of φ0.53mm, pre-cool time; 2 min, cold trap temperature; -130°C, desorption oven temperature; 200°C, desorption time; 1 min, injection temperature; 200°C, injection time; 1 min.

(4) GC-MS conditions

The GC-MS conditions are as follows. Apparatus; GC Hewlett-Packard Model 6890, MS Hewlett-Packard Model 5973, column temperature; 40°C for 10 min, and programmed increase from 40°C to 200°C at 5°C/min, column; fused-silica capillary
column (GL Science, TC-WAX) of 0.32 mm×60 m, and 0.25μm film thickness, carrier gas; He (50 KPa), ion source temperature; 230°C, MS transfer line; 220°C in El mode (70 eV), scan range; m/z 35-600.

Volatile were detected in the headspace of the samples. These compounds were measured in the scan mode. The measured mass spectra were compared with a library of commercially available mass spectra and identified (i.e. Wiley 275).

2) Volatile components and off-odor components of garlic

(1) Material of garlic

The raw garlic, cultivated in Aomori, Japan, and harvested in 2004, was used in this study.

(2) GC analysis

a. Analytical conditions

The identities of sulfur-containing gases for in-vivo and in-vitro samples were established by using GC and GC-MS spectrometry. As a standard reagent for GC analysis, the following chemicals were used as standard reagents for the GC analysis: methanethiol, dimethyl sulfide and dimethyl disulfide (Wako Pure Chemical Industries, Osaka, Japan), allylthiol and methyl propyl disulfide (Aldrich Chemical Co. Inc, USA), allyl methyl sulfide and diallyl disulfide (Tokyo Kasei Organic Chemicals, Tokyo, Japan).

The gas chromatograph (Shimadzu GC, 14B) conditions were as follows. PPE 5 ring 10%, 3.2mm x 3.1m glass column, temperature programmed from 65°C for 3 min to 170°C at 30°C/min, the detector was an FPD (140°C) and the carrier gas was N₂ (55ml/min).

The GC-MS conditions were as follows. GC-MS (Shimadzu GC-MS QP 1000) had a fused-silica capillary column (Supelco SPB-1), 0.32mm x 30m glass, temperature programmed from 60°C to 170°C at 30°C /min and held at 170°C for 2 min, carrier gas was He (2ml/min), El mode was at 70eV, the source temperature was 180°C
and GC-MS interface temperature was 250°C.

b. **In-vitro test**

Three grams respectively of raw and heated-garlic were crushed by applying pressure with a spoon. The heated-garlic was prepared by heating for 1 min in microwave oven. 0.2g of each sample was transferred into a 125ml vial which was sealed tightly, then kept in a container temperature maintained at 23°C and then 1ml of head space gas was removed from the vial for analysis.

c. **In-vivo test**

One healthy subject (Japanese female, age 19yrs) with no malodor took part in this study. The subject had not ingested any garlic for 24 hrs before the study. The basic study consisted of two treatment periods; on one day the subject chewed and then swallowed 1 g of raw garlic and measurements were carried out over the next 2 hrs. The next day the breath of a subject was measured over 2 hrs period after ingesting 1g of heated-garlic. The breath of a subject was collected in syringes following the method described by Aoki (14). Samples were collected immediately before garlic consumption and then immediately after garlic consumption.

4. **Results and discussion**

1) **Volatile components and off-odor components of sweet potato**

   (1) **Overall aroma difference**

   The representative total ion chromatograms of volatiles from samples in this study are shown in Figure 1. The volatiles identified from raw and heated-saccharified sweet potato juices were tabulated with the relative proportion of each compound (Table 3). The total numbers of odor components detected by GC-MS were 26 in the raw sweet potato juice, and 42 in the heated-saccharified sweet potato juice. It appears that relative levels of the majority of the compounds in the former are also larger than the latter. These suggest that the overall odor intensity for the heated-saccharified juice significantly increased, as a result of the increase in the
levels of all the compounds after treatment.

Among the compounds detected and tentatively identified in this study, the majority of the volatiles that showed an increase in the heated-saccharified sweet potato vapor and associated with its sensory profile are aldehydes and ketones such as acetaldehyde, β-damascenone and β-ionone, terpenoids such as linalool, limonene, α-terpinene, cymene, and β-cyclocitrinal, furans and pyrans such as 2-pentylfuran, furfural and 2-methylbenzofuran. On the other hand, aldehydes of high molecular weight such as 2,3-butanedione, benzaldehyde, 2,4-decadienal, phenylacetaldehyde, 3-ethyl-2-methyl-1,3-hexadiene, 2-octenal, 1-octene-3-ol 2-nonenal and hexanal were identified in higher amounts in raw sweet potato juice.

Figure 1  Total ion chromatograms of raw sweet potato (upper chart) and heated sweet potato juice (lower chart) obtained by GC-MS
Volatile compounds in Table 1 are corresponding to those characterized by GC-MS in Figure 1. The analytical conditions were described in the text. Values are expressed in peak height of total ion chromatograms.

Peak identification numbers correspond to compounds listed in Table 1. These volatile compounds were characterized by GC-MS under the analytical conditions described in the text. Volatiles fractionated using the TC-WAX coated 60m×0.32mm fused-silica capillary are described in the text. The relative concentrations of most of the compounds markedly increased after the treatment.

Table 1 Comparison of the concentrations of individual volatile components in headspace of volatiles from raw and heated sweet potato juices

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Compound</th>
<th>Peak height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Raw</td>
</tr>
<tr>
<td>1</td>
<td>Pentane</td>
<td>52.0</td>
</tr>
<tr>
<td>2</td>
<td>Acetaldehyde</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3</td>
<td>2-Methylpropanal</td>
<td>53.6</td>
</tr>
<tr>
<td>4</td>
<td>Acetone</td>
<td>&lt;5</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl acetate</td>
<td>&lt;5</td>
</tr>
<tr>
<td>6</td>
<td>2-Butanone</td>
<td>&lt;5</td>
</tr>
<tr>
<td>7</td>
<td>2-Methylbutanal</td>
<td>33.2</td>
</tr>
<tr>
<td>8</td>
<td>3-Methylbutanal</td>
<td>37.0</td>
</tr>
<tr>
<td>9</td>
<td>Ethanol</td>
<td>12.6</td>
</tr>
<tr>
<td>10</td>
<td>2,3-Butanedione (Diacetyl)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>11</td>
<td>Toluene</td>
<td>&lt;5</td>
</tr>
<tr>
<td>12</td>
<td>2-Butenal</td>
<td>&lt;5</td>
</tr>
<tr>
<td>13</td>
<td>2-Methyl-3-buten-2-ol</td>
<td>&lt;5</td>
</tr>
<tr>
<td>14</td>
<td>Hexanal</td>
<td>70.4</td>
</tr>
<tr>
<td>15</td>
<td>α-Terpinene</td>
<td>7.0</td>
</tr>
<tr>
<td>16</td>
<td>p-Mentha-1,8-diene (Limonene)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>17</td>
<td>2-Pentylfuran</td>
<td>26.6</td>
</tr>
<tr>
<td>18</td>
<td>Isopropyltoluene (Cymene)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>19</td>
<td>Pentanol</td>
<td>21.1</td>
</tr>
<tr>
<td>20</td>
<td>Cyclohexanone</td>
<td>&lt;5</td>
</tr>
<tr>
<td>21</td>
<td>1-Octen-3-one</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>Chemical Name</td>
<td>%</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------------------</td>
<td>----</td>
</tr>
<tr>
<td>22</td>
<td>2,2,6-Trimethylcyclohexanone</td>
<td>&lt;5</td>
</tr>
<tr>
<td>23</td>
<td>2-Heptenal</td>
<td>42.6</td>
</tr>
<tr>
<td>24</td>
<td>6-Methyl-5-hepten-2-one</td>
<td>24.5</td>
</tr>
<tr>
<td>25</td>
<td>Hexanol</td>
<td>33.8</td>
</tr>
<tr>
<td>26</td>
<td>Nonanal</td>
<td>&lt;5</td>
</tr>
<tr>
<td>27</td>
<td>3,5,5-Trimethyl-2-cyclohexen-1-one</td>
<td>&lt;5</td>
</tr>
<tr>
<td>28</td>
<td>3-Ethyl-2-methyl-1,3-hexadiene</td>
<td>80.2</td>
</tr>
<tr>
<td>29</td>
<td>2-Octenal</td>
<td>99.2</td>
</tr>
<tr>
<td>30</td>
<td>Isopropenyltoluene</td>
<td>&lt;5</td>
</tr>
<tr>
<td>31</td>
<td>1,2,3,4-Tetrahydro-1,1,6-trimethylnaphtalene</td>
<td>&lt;5</td>
</tr>
<tr>
<td>32</td>
<td>1-Octen-3-ol</td>
<td>60.4</td>
</tr>
<tr>
<td>33</td>
<td>2,4-Heptadienal</td>
<td>42.0</td>
</tr>
<tr>
<td>34</td>
<td>Furfural</td>
<td>50.5</td>
</tr>
<tr>
<td>35</td>
<td>2-Ethylhexanol</td>
<td>&lt;5</td>
</tr>
<tr>
<td>36</td>
<td>Decanal</td>
<td>&lt;5</td>
</tr>
<tr>
<td>37</td>
<td>Benzaldehyde</td>
<td>50.0</td>
</tr>
<tr>
<td>38</td>
<td>2-Nonenal</td>
<td>52.0</td>
</tr>
<tr>
<td>39</td>
<td>3,7-Dimethyl-1,6-octadien-3-ol (Linalool)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>40</td>
<td>2-Methylbenzofuran</td>
<td>&lt;5</td>
</tr>
<tr>
<td>41</td>
<td>β-Cyclocitral</td>
<td>43.0</td>
</tr>
<tr>
<td>42</td>
<td>Pheny lacetaldehyde (Benzeneacetaldehyde)</td>
<td>36.0</td>
</tr>
<tr>
<td>43</td>
<td>2,4-Nonadienal</td>
<td>27.9</td>
</tr>
<tr>
<td>44</td>
<td>Naphthalene</td>
<td>&lt;5</td>
</tr>
<tr>
<td>45</td>
<td>2,4-Decadienal</td>
<td>76.5</td>
</tr>
<tr>
<td>46</td>
<td>(1R)-6,6-Dimethylbicyclo[3,1,1]hept-2-ene-2-methanol (Myrtenol)</td>
<td>15.9</td>
</tr>
<tr>
<td>47</td>
<td>2,4-Decadienal</td>
<td>44.8</td>
</tr>
<tr>
<td>48</td>
<td>2,6,6-Trimethyl-1,3-cyclohexadienyl-1-propenylketone (β-Damascenone)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>49</td>
<td>Geranylacetone</td>
<td>&lt;5</td>
</tr>
<tr>
<td>50</td>
<td>2,6-di-tert-butyl-p-cresol (BHT)</td>
<td>&lt;5</td>
</tr>
<tr>
<td>51</td>
<td>β-Ionone</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

(2) Aldehydes and alcohols

Numerically, aldehydes were the main compounds identified in both sweet potato juice samples. In this study, the heated-saccharified sweet potato juice showed a large quantity or a small quantity in the levels of aldehydes depending on their functional type to compare with raw sweet potato. Although the levels of low molecular weight
aldehydes such as acetaldehyde showed a large trend, those of aromatic aldehydes such as benzaldehyde and phenylacetaldehyde, along with C6 aldehydes, such as hexanal and 2-heptanal, and other high molecular weight compounds such as 2,4-decadienal, 2,4-heptadienal, 2-octenal and 2-nonenal, that were present in higher proportions in the raw sweet potato juice, showed a small trend in the heated sweet potato juice.

C6 and high molecular weight aldehydes are derived from fatty acid decomposition and found at higher levels in the homogenates of fruits. Buttery et al. have shown that lipid-derived volatile aldehydes such as cis-3-hexenal, which are present at low levels in intact tomatoes, rapidly increase in level after homogenization (15). This mechanism is proved to involve enzyme (lipoxygenase and hydroperoxy trienoic acid lyase)-catalyzed oxidative processes, which contribute to lipid degradation in fatty acid substrates. Since the lipoxygenase activity in tomato has been reported in both membranous and soluble portions (16), it may lead to enhanced lipoxidation during homogenization of tomato.

Sweet potato contains a higher amount of lipid oxygenation-related enzymes (17). Since the skin was not discarded (during homogenization) while preparing heated-saccharified sweet potato juice, it is likely that the enzymes and polyunsaturated fatty acids present in the skin account for higher levels of aldehyde products in raw sweet potato juice. Thus, it is possible that boiling influenced the time over which the enzymes were activated and subsequently yielded lower levels of lipid degradation products in heated-saccharified sweet potato juice.

Aldehydes are important odor impact compounds. C6 compounds such as hexanal, (E)-2-hexenal and (Z)-3-hexenal, contribute markedly to the green note of the aroma (18,19,20). In our experiment, the amount of hexanal decreased because of boiling. They also showed that significant decrease in the intensity of aroma profile for green note in heated-saccharified sweet potato juice, which may be related to the loss of C6 aldehydes.

A reduction in the level of aroma compounds such as C6 compounds and high molecular weight compounds may result in the loss of green odor in heated sweet
potato juice and contribute to the predominance of other odor compounds in odor profile of heated-saccharified sweet potato juice, which in turn may result in a higher degree of off-odor in heated-saccharified sweet potato juice.

Alcohols were also identified in both samples. Interestingly, note that the level of alcohols with 6 carbon atoms, such as hexanol, decreased in heated-saccharified sweet potato juice. C6 alcohol is also responsible for the herbaceous odor of several fruits (21), and thus such a decrease may contribute to the loss of green aroma of heated-saccharified sweet potato.

(3) Ketone compounds

A distinctive increase in the levels of ketones was observed after boiling. The relatively higher proportions of acetone, β-ionone, β-damascenone, 2-hydroxy-2,6,6-trimethyl-cyclohexanone in heated sweet potato juice can be explained by β-carotene oxidative mechanism, which may contribute to the enhanced sweet aroma of heated-saccharified sweet potato juice.

β-carotene acts as a precursor that forms β-damascenone via enzymatic and nonenzymatic pathways such as thermal degradation (22,23). In other food systems containing β-carotene, the oxidative degradation of β-carotene also forms a mixture of β-ionone, β-damascenone, 2-hydroxy-2,6,6-trimethyl-cyclohexanone (24), β-cyclocitrinal (25) and acetone (26).

"Benihayato" tubers have been found to contain 8mg of β-carotene per 100g (wet basis), which is as much major component as that in carrot, and it is assumed that β-carotene oxidative mechanism results in the production of these compounds. Certain ketones, such as β-ionone, 2,3-butanedione, and geranyl acetone, possess a sweet floral aroma and may add a sweet floral note to the total sweet profile of heated-saccharified potato juice. An increase in the level of these compounds in considerable amounts in heated-saccharified sweet potato juice, may reflects the result of the sensory evaluation of high intensity of sweet aroma. Although β-damascenone possesses a heavy floral aroma and contribute to the floral note, owing to its strong
odor intensity and low threshold (threshold in water: 0.2ng/litter) (27), it has often been considered to contribute to the overall off-flavor (28,29).

Although it is widely known that vegetables containing high β-carotene content emit a distinct carrot-like odor when bruised, the reason behind this remains to be elucidated. Although the association with the smell produced and β-carotene breakdown is not yet known, it is possible that an oxidative carotenoid is involved in the production of aroma compounds. Because such aroma compounds have not been identified in this study, further studies are needed to identify these compounds.

(4) Terpenoids

In this study, the levels of linalool, limonene, α-terpinene, cymene, and β-cyclocitrinal increased after heating (Table 1). From this result for terpenoids, the following scheme of terpenoid synthesis is proposed (Fig 2). From liberated monoterpenes such as geraniol 1 and nerol 3, the corresponding allylic cationic species 2 and 4, respectively, are formed by dehydration, at a boiling temperature of about 100°C and the presence of an acidic phenol (i.e. 2,6-di-tert-butyl-p-cresol, Table 3), leads to the formation of an identical cationic species 5, whose reaction with water furnishes linalool 6. On the other hand, cation 4 with Z-stereochemistry undergoes cyclization, which through different deprotonation pathways is converted to limonene 8 (by elimination of H^b) and α-terpinene 9 (by elimination of H^c after H^b migration, or by elimination of H^b followed by isomerization), the latter of which is further converted to cymene 10 by aromatization with the loss of a H_2 molecule.

In general, monoterpane hydrocarbons and oxygenated monoterpenes tend to give a sweet aroma in the total aroma quality due to their higher volatility and polarity. "Imo-Shochu", a traditional Japanese spirits from fermented sweet potatoes, has a pleasant sweet potato flavor. Ohta et al. reported that geraniol, nerol, linalool, citronerol and terpeneol contribute to the distinctive flavor of sweet potato distillates with a pleasant fruity and floral smell (30). They found that monoterpane alcohols, which are precursor compounds in their glycoside forms in sweet potato, are liberated
by enzymatic hydrolysis when sweet potatoes is blended, and geraniol and nerol are produced during fermentation. Through distillation, these alcohols are further converted to linalool and limonene at high temperature under acidic condition, and contribute to the distinctive aroma profile of sweet potato distilled alcohol. In our sensory evaluation, a higher intensity of the sweet note was suggested for the heated-saccharified sweet potato juice. The characteristics of the standard of terpenoids found by GC-MS are described as follows: benzaldehyde, an aroma like that of bitter almond; linalool, lemon and rose-like; and β-cyclocitral, camphor and cereal-husk-like aroma. Therefore, the high proportion of these compounds found in heated-saccharified sweet potato vapor could be associated with the “sweet” aroma of heated-saccharified sweet potato juice.

Geraniol 1 and nerol 3, the corresponding allylic cationic species 2 and 4, respectively, were formed by dehydration, leading to the identical cationic species 5, whose reaction with water furnished linalool 6. On the other hand, cation 4 with Z-stereochemistry underwent cyclization, which through different deprotonation pathways, is converted to limonene 8 and α-terpinene 9, the latter of which was further converted to cymene 10.
Figure 2  Proposed mechanism for formation of terpenoids via interconversion of terpenes during boiling.

(5) Pyrans and furans

Only three furan and pyran compounds namely, 2-pentylfuran, furfural and 2-methyl benzofuran, were identified in this study. 2-Pentylfuran and furfural tended to increase in the heated-saccharified sweet potato juice.

It has been reported that baked sweet potato contains various furans and pyrans (2), which are formed via Maillard reaction or caramelization (31,32). The basic constituents of sweet potato such as sugars, amino acids and fatty acids act as main
precursors of volatiles, and are later hydrolyzed during baking to form pyrans and furans. Among various furan derivatives in baked sweet potato volatiles, both maltol and furfural are the final compounds produced via amino-carbonyl reactions. In particular, maltol has been reported to be the potent aroma impact compound of baked sweet potato. Sun et al. reported that a sensory panel described the aroma of a baked sweet potato volatile fraction with maltol as a sweet potato-like aroma but fractions without maltol as a caramel-like aroma (23).

In our experiment, the results of the sensory analysis showed a higher intensity for heavily boiled odor and a lower intensity for the sweet aroma in heated-saccharified sweet potato juice, which indicates that off-flavor was more predominant after heating and saccharification processes. Furfural, but not maltol, was detected in the heated-saccharified sweet potato juice. This may explain why the heated-saccharified sweet potato juice was perceived to have an unpleasant odor instead of a sweet potato-like aroma.

The reason furfural but not maltol was formed during condition is linked to the temperature difference between the heated-saccharified sweet potato juice production (wherein temperature normally does not exceed 100°C) and baked sweet potato juice production (wherein temperature normally exceeds 200°C) (Figures 3 and 4). Furfural is formed according to the following scheme: Aldehyde 1, in the form of an acyclic pentose isomer reacts with an amino acid to produce imine 2, which after isomerization forms enamine 3. Enamine 3 after dehydration forms alkenyl imine 4. The hydrolysis of the imine part, and a second dehydration from the resultant intermediate 5 enables the formation of dihydrofuran 6, and then after a third dehydration, the final material, furfural 7 is formed. However, maltol under the heated-saccharified condition is hardly synthesized due to low conversion feasibility of intermediate from 4 to 5. Although the hydrolysis of 4 in furfural formation is considered to readily occur on the basis of generally low stability of imines in under

27
aqueous heating conditions, the C-N bond cleavage of 4 in maltol formation may be disfavored due to high nucleophilicity of amino group that renders the C-N bond less prone to disconnection under boiling conditions at about 100°C (Fig. 4).

Figure 3  Proposed mechanism of furfural formation during boiling.

Aldehyde 1 reacts with an amino acid to produce imine 2, which upon isomerization forms enamine 3, and then after dehydration forms alkenyl imine 4. The resultant intermediate 5, enables the formation of dihydrofuran 6 and the final product, furfural 7.

Figure 4  Proposed mechanism of maltol formation during baking
Condensation with an amino acid 1 followed by the isomerization of 2 leads to the formation of enamine 3. The dehydration of enamine 3 converts it to allylamine 4. Further removal of the amine part and the cyclization of 4 and 5 converts allylamine 4 to the six-membered compound 6, which upon two consecutive isomerizations of 7 and 8 leads to maltol 9 formation.

2) Volatile components and off-odor components of garlic

(1) In-vitro test

The identities of sulfur-containing gases arising from grated raw garlic and heated garlic were analyzed by GC and GC-MS (Figure 5). Eight peaks were found and these were identified as methanethiol, dimethyl sulfide, allylthiol, allyl methyl sulfide, dimethyl disulfide, methyl propyl sulfide, diallyl disulfide and 3-(allylthio) propionic acid, respectively.

Methanethiol, dimethyl sulfide and allylthiol are low-molecular sulfur compounds (LMSC) and were present in a slightly larger quantity than allyl methyl sulfide and dimethyl disulfide, which are relatively high molecular sulfur compounds (HMSC) in both raw garlic and heated garlic.

(2) In-vivo test

Gas chromatograms of raw and heated garlic in in-vivo test are shown in Figure 6. Immediately after ingesting raw garlic, the concentrations of LMSCs were greatest especially methyl sulfide and allyl sulfide. In contrast in the heat-treated garlic eating, lower concentrations of LMSCs (methanethiol, allylthiol and allyl methyl sulfide) were observed. Therefore these results indicate that the mouth normally contains a small concentration of methanethiol and dimethyl sulfide (Figure 4).
Figure 5  Gas chromatograms of the head-space vapors sampled just after grating raw garlic (left) and heat-treated garlic (right).

Peak components were as follows; 1. methanethiol 2. dimethyl sulfide; 3. allylthiol; 4. allyl methyl sulfide; 5. dimethyl disulfide; 6. allyl methyl disulfide; 7. methyl propyl disulfide; 8. diallyl disulfide and 9. 3-(allylthio) propionic acid.
after eating

Figure 6 Gas chromatograms of the breath odor of one subject, (a) before, (b) after eating 1g of grated raw garlic, and (c) after eating 1 g of grated heat-treated garlic. Peak components are described in Fig 5.

5. Conclusion

The research is aimed at developing novel odor evaluation methods for the development of functional food. Although texture and appearance are important constituents for the sensory perception of a food product, flavor is the most important characteristic that signifies consumers' preference in food choice. The heat treated sweet potato and garlic were investigated as a representative model food with an unfavorable odor.

In this chapter, the volatile components in these samples were analyzed with GC-MS analyzer as a generalized method.

Twenty six and forty two volatile components were identified in raw sweet potato and heated-saccharified sweet potato respectively, by GC-MS. Not only the number of volatile components but also the quantity were larger in heat treated sample. The raw sample showed higher levels of aldehydes, and on the other hand heated-saccharified sweet potato yielded a headspace rich in terpenoids, ketones, furans and aromatic aldehydes.

On the other hand, the volatile components of garlic were as follows;

In vitro test; The identities of the sulfur-containing gases arising from grated raw garlic and heat treated garlic were analyzed by GC and GC-MC. The volatile sulfur compounds were identified as methanethiol, dimethyl sulfide, allylthiol, allyl methyl sulfide, dimethyl disulfide, methyl propyl sulfide, diallyl disulfide and 3-(allylthio) propionic acid respectively.

In vivo test; Immediately after ingesting the raw garlic, the concentrations of LMSCs(methanethiol, allylthiol and allyl methyl sulfide) were greatest especially
methyl sulfide and allyl sulfide. In contrast in the heat-treated garlic eating, lower concentrations of LMSCs were observed. Thus, the volatile components of sweet potato and garlic were clarified.
Chapter II
Odor Characterization of Steamed Sweet Potato and Apple by Using Sensory Analysis, Commercially Available Odor Sensor and Electroencephalography

1. Introduction

1) Sensory evaluation

The word “quality” is widely used for evaluation of commodities and with many meanings. Food quality includes characteristics that are external properties; appearance, feel, internal properties; taste, aroma, texture and technical properties; nutrition, safety. Among them, flavor quality is a critical parameter for acceptability of foods and their marketing. Several methods for evaluations of flavor quality have been developed. Instrumental analysis such as gas chromatograph or GC-MS is a useful tool in monitoring the possible aroma detect with its separation and detection capabilities in the analysis of food volatiles. Olfactometric analysis is more direct tool to make sure that off-flavored product doesn't get made in the process as part of a raw material acceptance and in-process QC program. Since flavor is a sensory perception, regardless of the result of instrumental analysis, the last thing important for the aroma defect is whether it falls the consumer expectations. Trained human nose is very sensitive and judgment by trained human nose process should be built in QC program for quick detection of any off-odor formation during processing. Flavor of foods in food industry is often evaluated by graders who grade flavor quality based on their personal experience. Although grading can assess the overall quality of products very rapidly in convenience, it is somewhat subjective in nature and because of its inherited subjectivity, it is hardly quantitative.

2) Discrimination tests and descriptive analysis for flavor research

To compare the sensory difference of products quantitatively, which could make it possible statistical comparison, several different methods of sensory evaluation have been developed. Sensory evaluation methods are separated into analytical methods;
discrimination tests and descriptive analysis, and affective test methods; preference tests and acceptance tests (1).

Discrimination test is a simple but powerful way to evaluate the quality deficiency of foods. Discrimination test is a useful technique when the objective of the test is to determine whether any difference is perceived between two products. By screening panelists to determine their ability to differentiate between several odors and with proper training for the attribute in focus, panelists can distinguish even subtle flavor differences as sensitive as instrument. The most common forms of the discrimination procedure are triangle test. In the test, the judge is asked to choose one sample which is different from the other two.

Alternatively, flavor can be more analytically described by descriptive sensory analysis, which provides a quantitative specification of all the sensory attributes of a food or a product. Descriptive analysis is useful for specifying sensory changes in product development or in quality control due to a function of ingredient or processing variables. For the purpose of creating the specification of all the sensory attributes of a food or a product, the first step in descriptive analysis involved in creating a lexicon for describing a product is to identify a wide number of relevant descriptive terms.

The step usually involves the group session and training session where a minimum of 7 panelists requires several hours for creating sensory lexicons of products, initial training and then maintenance training thereafter (2). There are different procedures to create an appropriate lexicon, of which the most usual is the unguided free selection technique. Each panelist assesses the product and writes down the attributes that characterize a product. After that, from this list, a lexicon is created that describes the qualitative characteristics of the sensory attributes of product; taste, flavor, texture after taste, which panelist are asked to agree as a common vocabulary. These sensory lexicons must be more specific than a consumer would use to describe a product so the attributes would be more analytical. Once they establish a carefully defined set of descriptors acceptable and usable by the panelists, panelists are individually trained to make judgments about the perceived
intensity of all the defined attributes of the product on an intensity scale. Then, repeatability of each panelist's judgment is tested and compared with other panelist's performance. The training is repeated if it is necessary for certain panelist.

One of the good features of this technique is amenable to experimental design and statistical analysis. Evaluation by each panelist for a sample is usually repeated to ensure the test reliability. The panelists are usually trained to perform similarly, but usually differences between their scores are observed due to either different use of the scale or different sensitivity to some of the attributes. Analysis of variance repeated measures could be applied to partition judge effects from product differences. Result is denoted as significance for the existence of product differences.

In contrast to the analytical method, a consumer test is to measure their personal responses; preference, degree of liking, and/or acceptance, by potential or existing customers of a product, a product idea, or specific product attributes (3). It is an affective tool in predicting success in the market and product functionality based on consumer's feedback. Preference test is widely used as a standard method to determine the consumers' subjective responses to product samples and their reasons for selecting their choice (4).

Acceptance or hedonic tests is another method to measure the degree of acceptance of the product being tested (4). It is designed to measure specific consumer responses to particular sensory attributes; appearance, color, flavor, texture, etc., of a product. Consumer is asked to rate the degree of preference for the presented product on the hedonic scales. The major advantage of acceptance tests is that they provide significant insight into a consumer's preferences and dislikes of a particular food product or a sample and provides numerical values which can be subjected to statistical analysis.

In flavor research, sensory evaluation is essential. Especially, descriptive analysis is useful in its ability to describe sensory properties and correlate them with instrumental data helps in understanding the sense of flavor. With association of the sensory profile and the volatile chemistry profile, the specific sensory attribute which is contributed to the off
flavor will be clarified.

3) Relationship between sensory evaluation and physiological activity in brain

(1) Electroencephalography analysis

There is significant interest in understanding how consumer acceptance of food is constructed in the cognitive level. The cognitive perception of the odor has for years been the focus of research, utilizing psychophysiological, electrophysiological and neuroimaging techniques and clinical studies to investigate theories of physiological organization of preference.

In the physiological level, olfaction begins with the olfactory epithelium in nasal cavities where 50 million specialized receptors react to numerous scents. The information signals are sent through the axon and the olfactory bulb where the information yielded by the odor is distributed to the proper limbic structures such as the prepyriform cortex, amygdala, hypothalamus. Information is further sent to the terminal points of the medial dorsal thalamus, orbitofrontal cortex, and lateral posterior orbitofrontal cortex. These physiological findings indicate the odor's directly connection to the limbic system, which may be associated with its strong affective and emotional connections (5). This also indicates perception of the odor is associated with liking or disliking (pleasantness or unpleasantness) (6, 7).

The studies have been focused on measuring the neurological activity of brain structures is through the usage of electroencephalography (EEG). EEG measures distinct brain wave patterns that result from being in a wake state with closed eyes. N original signal data obtained from an electrode are transformed into N transformed numbers (Af, δ) by Fourier transformation. Then the result for each subject is denoted by total power and relative power (= absolute power / total power in diverse frequency bands in each set totaled 100%). Relative % power is normally obtained for the frequency bins of interest z, delta = 2-5 Hz, theta = 5-8 Hz, alpha = 8-13 Hz and beta > 13 Hz (8). This framework of EEG analysis has provided an observation in brain activity responses associated with odor.
stimuli with pleasant and unpleasant. EEG results indicate that olfactory stimulation does influence the physiological response of central nervous system (9, 10).

Researches have also shown that exposure to odor of food or beverage alters the state of brain activity. Particularly, alpha waves, which are associated with relaxation, have been observed as increasing in the presence of odors that are assumed to be relaxants, such as lavender (11). Furthermore, association with preferences or disliking of the odor has been indicated with increase or decrease of alpha wave and alpha wave power. The ability of odors to influence judgment of preference suggests the potential for using the EEG measurement in research into possible prediction of consumer's acceptance of odor in terms of physiological response (12, 13).

4) Purpose of the study

The aims of the present study were to evaluate the odor of heated sweet potato in regards to the sensorial and cognitive analysis to investigate association between sensory and instrumental data. To aim this, quantitative descriptive analysis and EEG analysis were applied to compare the odor properties of heated sweet potato.

Owing to its unique health benefit and odor property, sweet potato serves as a potential functional food ingredient in various food applications. During the development of functional sweet potato juice, a heavy unappetizing off-odor was produced after heating and saccharification of sweet potato. The previous section identified compounds which may be responsible for the type of off-flavor that heated potato develops after saccharification.

In this study, first, raw and heated-saccharified potato was sensorily evaluated using descriptive analysis to determine which sensory attributes are contributed to the off-odor. Then, association between the identified volatile detected by GC-MS in the chapter I with sensory attributes was discussed. In combination with chemical measurements underlying sensory and chemical relationships will be more clarified.

Secondly, to test the cognitive response to the off-odor of sweet potato, using an
EEG measurement. To test the hypothesis that steamed sweet potato off-odor can elicit distinctive changes in the electrical activity of the cognitive perception. EEG measurement was taken with exposure to the odor of apple juice and steamed sweet potato. Subjective odor liking responses (preference test result) were correlated with the objective physiological (EEG) responses to investigate differences in perceptual responses between changes in physiological behavior (EEG) and preference response of odors, results were compared with the degree of preference data using a consumer preference test for these stimulant.

The odor measurement system used in this study serves the purpose of further understanding the validity of the sensory evaluation at the chemical and cognitive level by investigating differences in responses between the sensorial measurement (descriptive analysis and preference test) and the physiological response (EEG measurement).

2. Materials and methods

1) Sweet potato

The raw sweet potato cultivar “Benihayato”, cultivated in Kagoshima, Japan, and harvested in October 2004, was used in this study. The raw tubers of this cultivar contained 8mg of \( \beta \)-carotene per 100g wet weight.

2) Odor characteristic of raw and boiled-saccharified sweet potato

(1) Preparation of sweet potato juice

Raw sweet potato juice was prepared as follows. One kg of sweet potato was shredded without peeling and added to 1.8 litter of water. This preparation was homogenized for 30 sec by using a macerator. The resulting slurry was placed in a 3 litter flask. For the preparation of boiled sweet potato juice, 1 kg of sweet potato was steamed by stepwise heating at 60-65\(^\circ\)C for 3.5hrs and at 100\(^\circ\)C for 1 hr. To this preparation 1.8 litter of water and 5.0g of \( \alpha \)-amylase (Uniase BM-8, with a specific activity of about 80,000u/g) were added, and the potato mixture was incubated for 3
hrs under warm condition (60°C). Then 5.0g of β-amylase (Uniasi L, with a specific activity of about 80,000u/g) was added to partially hydrolyze starch and incubated at 55°C for 1 hr. After the incubation 50g of glucoamylase (Uniasi 30, with a specific activity of about 80,000u/g) was added to the preparation. The mixture was incubated at 55°C for 1 hr. Both samples were cooled to room temperature, separated into smaller beakers and wrapped with aluminum foil. In addition, 100g each of raw samples and boiled juices were used for GC-MS analysis and 100ml each of the samples were used for sensory evaluation.

(2) Descriptive analysis of model sample

To determine the difference between odors of raw and heated-saccharified sweet potato juices, descriptive analysis was conducted. Thirteen assessors who were Japanese females, aged 19~25 years old, were recruited for the sensory evaluation of raw and heated sweet potato juices. The panelists were selected by using the difference test with following compounds at indicated concentrations. β-phenylethyl alcohol $10^{-4.0}\%$ (w/w), methyl cyclopentenolone $10^{-4.5}\%$ (w/w), isovaleric acid $10^{-5.0}\%$ (w/w), γ-undecalactone $10^{-4.5}\%$ (w/w) and skatole $10^{-5.0}\%$ (w/w). All chemicals are produced by Daiichi Yakuhin Kogyo Co., Ltd., Japan.

Sensory training and sensory descriptor development were carried out prior to the sensory evaluation in a round-table discussion. Both raw and heated-saccharified sweet potato juices were served in a 150ml glass wrapped with aluminum foil prior to the sensory evaluation by the assessors. Table 1 shows the descriptors used by trained panels for raw and heated sweet potato juices and the preparation of the reference standard for each descriptor. The following sensory evaluation was performed on an individual basis. Two samples on a tray were presented to each panelist. Raw and heated-saccharified sweet potato samples were presented in a glass and panelists were asked to score the attribute according to the appropriateness of the reference using a 7-point scale anchored on the left by the term 'none' and on the right by the term 'extreme' for nine odor sensory descriptors; overall intensity, grassy, sweet, sour, carrot, tomato, powdery, caramel and heavily boiled.
T-test was performed to examine the significance of difference (SPSS ver.11.0). The panelists were also asked to describe their odor impression of chemical standards found in volatiles of raw and boiled sweet potato juice by GC-MS.

Table 2  Sensory descriptors used in the descriptive analysis and the standard reference prepared for each sensory descriptor

<table>
<thead>
<tr>
<th>Grassy odor</th>
<th>100ml of pressed juice of boiled spinach, without roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet odor</td>
<td>100ml of maple juice produced in LB Maple Treat Inc. Canada, diluted to 10% with pure water</td>
</tr>
<tr>
<td>Sour odor</td>
<td>Sunkist 100% lemon produced by Mitsukan Co. Japan</td>
</tr>
<tr>
<td>Caramel odor</td>
<td>Sucrose heated up to about 170°C</td>
</tr>
<tr>
<td>Carrot odor</td>
<td>Pressed carrot juice after blanching</td>
</tr>
<tr>
<td>Tomato odor</td>
<td>Heated juice after pressing tomato</td>
</tr>
<tr>
<td>Powdery odor</td>
<td>30g of corn starch dissolved in 100ml of pure water</td>
</tr>
<tr>
<td>Heavily boiled odor</td>
<td>100g of sweet potato, 50g of noodles and 0.3g of miso were dissolved in 250ml of pure water with heating</td>
</tr>
</tbody>
</table>

3) Odor characteristic of steamed sweet potato compared with apple juice aroma

(1) Preparation of sweet potato juice

One kg of sweet potato was heated at 60-65°C for 3.5hrs and then steamed at 100°C for 1 hr with boiling water for use in this study. Steamed sweet potatoes which were kept at 30-35°C and apple juice (Dole, produced by Yukijirushi Co., Ltd.) were used for the experiment.

(2) Sensory evaluation

Seven subjects (Japanese, female, aged 20～35 years) were selected by using a difference test with the following compounds at indicated concentrations; β-phenylethyl alcohol 10⁻⁴.₀% (w/w), methyl cyclopentenolone 10⁻⁴.₅% (w/w), isovaleric acid 10⁻⁵.₀% (w/w), γ-undecalactone, 10⁻⁴.₅% (w/w) and skatole 10⁻₅.₀% (w/w). All chemicals are produced by Daiichi Yakuhin Kogyo Co. Ltd., Japan. The 7 subjects were asked to evaluate their preference for steamed sweet potato, apple juice, and distilled water based on the 7-point scale, where 1 indicates very unpleasant odor and 7, very pleasant odor. The subjects were asked to have at least 1-min rest period between evaluations of samples, and the evaluation was conducted twice each day for 2
Next, using the semantic differential (SD) method (14), odor quality was evaluated in terms of "heavy to light", "heated to fresh", and "weak to strong". After the subjects had familiarized themselves with the samples, they were instructed to rate each sample in terms of heavy to light odor on the 7 points scale, where -3 is very heavy odor, -2 is fairly heavy odor, -1 is moderately heavy odor, 0 is intermediate odor, +1 is moderately light odor, +2 is fairly light odor and +3 is very light odor. Then the subjects were instructed to rate each sample in terms of heated to fresh odor on the 7-point scale, where -3 is very heated odor, -2 is fairly heated odor, -1 is moderately heated odor, 0 is intermediate odor, +1 is moderately fresh odor, +2 is fairly fresh odor and +3 is very fresh odor. Lastly, they were instructed to rate each sample in terms of weak to strong odor on the 7 points scale, where -3 is very weak odor, -2 is fairly weak odor, -1 is moderately weak odor, 0 is intermediate, +1 is moderately strong odor, +2 is fairly strong odor and +3 is very strong odor. They had at least 1-min rest period between stimuli and evaluations were made twice at different days. All tests were conducted for two days.

(3) Odor sensor research

A Fox 4000 electronic sensor equipped with an ACU 500 humidifier was used. This instrument is equipped with 18 metal oxide sensors inside 3 chambers. Each chamber contains 6 metal oxide sensors (p, flat-plate sensor; T, tubular sensor; SY, non-doped tin oxide sensor; a temperature sensor; and a relative humidity sensor). To provide constant flux of vector gas (humidified synthetic air) through the electronic sensors, a humidifier (air conditioning unit, model 1997, Alpha-MOS) was used. The analytical parameters (sample quantity, headspace generation time, temperature, flow rate, and injection time) were determined. The sensors were carefully monitored to make sure that they returned to the baseline during the preliminary trials, and the optimum time was found to be 5 min after sample injection. Data collection time was 120sec. Carrier gas flow was set to 150 ml/min and relative humidity was regulated at 20% RH.
Although the electronic sensor is equipped with 18 sensors, only the results of a limited number of metal oxide sensors were chosen for further analysis, after eliminating sensors with high multicollinearity by stepwise regression analysis. SPSS version 11.0 was used for data processing.

(4) EEG analysis

Subjects recruited for EEG measurement were Japanese females, aged 20~35 years old. Each subject was asked to sit on a chair, relax, and close her eyes while the EEG apparatus with a ten-twenty electrode system was set. A laboratory assistant held the first sample approximately 5cm from the subject’s nose and the subject was given 2 min to smell the sample. Next, the subject was given distilled water to smell for 1 min and thereafter asked to remain seated for 10-15 sec. Then, the subject was asked to smell the second sample for 2 min. These procedures were repeated twice each day for two days. The samples were presented at random order to the subjects. The electroencephalogram was recorded with a Neurofax EEG-1518 (NIHON KOHDEN). EEG was reviewed with a time constant of 0.03 sec and a Hi Cut Filter of 60 Hz. EEG of frequency 5.3~60 Hz was analyzed with 512-point FFT and power value was measured. Peak frequency, $\alpha_1$ wave power (8~10 Hz), and $\alpha_2$ wave power (10~13 Hz) were measured for 5 sec each after the subject was shown the sample, with an EEG analysis program (NIHON KOHDEN QP-220A) after eliminating artifacts. Distilled water was used as control and t-test of the results was calculated.

3. Results and Discussion

1) Odor characteristic of raw and boiled-saccharified sweet potato

(1) Sensory evaluation

Table 2 shows the descriptive profiles of all the samples in terms of the nine descriptors. According to the majority of the profiles, the heated-saccharified sweet potato
juice has higher odor intensity than the raw sweet potato juice. Significant differences were observed with respect to 5 of the 9 descriptors. Heated saccharified sweet potato generated more intensive odors of "heavily boiled" and "sweet" than the raw sweet potato juice, with the exception of the "carrot" and "grassy" odors. There was also no difference in terms of the, "caramel", "sour", "tomato" or "powdery" descriptor between the two juices. Interestingly, note that the intensity of off-odor component "heavily boiled" markedly increased for the heated sweet potato juice. The intensity of the odor component "sweet" also greatly increased. From this result, the odor impression of heated sweet potato juice can be both sweet and heavily boiled.

Table 2 Significant differences in sensory descriptors between raw and heated sweet potato juices

<table>
<thead>
<tr>
<th>Sensory descriptor</th>
<th>raw</th>
<th>heated</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassy odor</td>
<td>3.2±0.7</td>
<td>2.1±0.5</td>
<td>p&lt;0.001***</td>
</tr>
<tr>
<td>Sweet odor</td>
<td>3.1±0.8</td>
<td>5.0±0.7</td>
<td>p&lt;0.001***</td>
</tr>
<tr>
<td>Sour odor</td>
<td>3.1±0.8</td>
<td>2.5±0.5</td>
<td></td>
</tr>
<tr>
<td>Heavily boiled odor</td>
<td>3.5±0.5</td>
<td>6.1±0.6</td>
<td>p&lt;0.001***</td>
</tr>
<tr>
<td>Carrot odor</td>
<td>3.8±0.7</td>
<td>2.9±0.6</td>
<td>p&lt;0.05 *</td>
</tr>
<tr>
<td>Tomato odor</td>
<td>2.9±0.5</td>
<td>2.9±0.8</td>
<td></td>
</tr>
<tr>
<td>Powdery odor</td>
<td>3.5±0.5</td>
<td>3.0±0.8</td>
<td></td>
</tr>
<tr>
<td>Caramel odor</td>
<td>1.8±0.6</td>
<td>2.1±0.5</td>
<td></td>
</tr>
<tr>
<td>Odor intensity</td>
<td>3.6±0.7</td>
<td>4.8±0.4</td>
<td>p&lt;0.001***</td>
</tr>
</tbody>
</table>

Values indicate the mean ± SD given by 13 panelists. Significant differences within rows indicated by asterisk; *** significant at 0.1% level, ** significant at 1% level, * significant at 5% level

(2) Relationship between volatile components and sensory value

The sensory evaluation showed that the intensity of the overall odor for heated-
saccharified sweet potato juice was significantly higher than that for raw sweet potato juice. These results suggest that the overall odor intensity for heated-saccharified juice significantly increased, as a result of the increase in the levels of all the compounds after the treatment. Also, the sensory test showed that the intensities of two sensory parameters (sweet and heavily boiled odors) significantly increased after the treatment. This result suggests that the formation of some volatiles and the loss of some compounds lead to a sensory change in the characteristic odor of sweet potato.

Although it is not easy to determine the relationship between the sensory descriptor’s results of the sensory evaluation and the individual chemical compounds identified, it may be assumed that some of the compounds contributed to the sensory components. The sensory impact of some important compounds is discussed below for the sensory parameters with a significant result.

Certain aldehydes such as acetaldehyde, benzaldehyde and benzeneacetaldehyde, possess a distinctive sweet floral aroma. Sensory evaluation results showed that the heated saccharified sweet potato juice possesses a significant sweet odor (Table 2), and an increase in the levels of these compounds may contribute to the sweet aroma profile of the heated-saccharified sweet potato juice.

Certain ketones, such as β-ionone, 2, 3-butanedione, and geranyl acetone, possess a sweet floral aroma and may add a sweet floral note to the total sweet profile of heated-saccharified potato juice. An increase in the level of these compounds in considerable amounts in heated-saccharified sweet potato juice, may reflect the result of the sensory evaluation of high intensity of sweet odor. Although β-damascenone possesses a heavy floral odor and contribute to the floral note, owing to its strong odor intensity and low threshold (threshold in water: 0.2 ng /litter) (15), it has often been considered to contribute to the overall off-odor (16, 17). Sensory evaluation results showed a higher intensity of the heavily boiled odor in heated-saccharified sweet potato juice, suggesting that the higher proportion of β-damascenone found after boiling may contribute to not only the sweet note, but also the off-odor note.
In our sensory evaluation, a higher intensity of the sweet note was suggested for heated-saccharified sweet potato juice. The characteristics of the standard of terpenoids found by GC-MS are described as follows: benzaldehyde, an aroma like that of bitter almond; linalool, lemon and rose-like; and β-cyclocitrall, camphor and cereal husk-like odor. Therefore, the high proportion of these compounds found in heated-saccharified sweet potato vapor could be associated with the “sweet” odor of heated-saccharified sweet potato juice.

In our experiment, the results of the sensory analysis showed a higher intensity for heavily boiled odor and lower intensity for the sweet odor in heated-saccharified sweet potato juice, which indicates that off-odor was more predominant after heating and saccharification processes. Furfural, but not maltol, was detected in heated-saccharified sweet potato juice. This may explain why the heated-saccharified sweet potato juice was perceived to have an unpleasant odor instead of a sweet potato-like odor.

2) Odor characteristic of steamed sweet potato compared with apple juice aroma

(1) Sensory evaluation

Figure 1 shows the results of evaluation of steamed sweet potato and apple juice by using the SD method. The t-test was calculated between evaluated values of steamed sweet potato and those of apple juice. A significant difference for the factor “heavy to light” was observed at 5% level. Next, a significant difference for the factor “heated to fresh” was observed at 1% level. From these results, it is thought that steamed sweet potato has heavy and heated (cooked) odor, while apple juice has light and fresh odor. The standard deviation, which is an indication of variation among subjects, was small. In addition, a significant difference for the factor “weak to strong” was observed at 1% level. From this result, it is thought that steamed sweet potato odor is weak, while apple juice aroma is strong. The standard deviation, which is an indication of variation among subjects, was also small.

The result of pleasantness test is shown also in Figure 1. A significant difference for
the factor "unpleasant to pleasant" was observed at 5% level. Standard deviation was likewise small. From this result, it was concluded that apple juice aroma was more pleasant than steamed sweet potato odor.

Apple juice aroma mainly originates from a mixture of esters, aldehydes, and alcohols. Among ester compounds, ethyl acetate, butyl acetate, and hexyl acetate are present in large amounts. (E)-2-hexenal, an aldehyde, is present in large amount as well (18). Such alcohols as butanol and hexanol are also present in large amounts (18). These aroma compounds are responsible for fruity and fragrant aroma. On the other hand, the heavy odor of steamed sweet potato is due to terpenes, ionone, β-damascenone, benzaldehyde, and phenyl acetaldehyde (19-20). When Satsuma mandarin is heated, an off-flavor which resembles the heavy odor of steamed sweet potato and is called "Imo-shu" (sweet potato off-odor) is generated. Araki and Sakakibara reported that this heavy aroma is due to the large amount of β-damascenone (17). It is clear that aroma compounds in apple juice are different from those in steamed sweet potato.

![Figure 1](image_url)

**Figure 1** Odor characteristics of steamed sweet potato and apple juice measured by SD method

**(2) Odor sensor analysis**

Figure 2 shows the response patterns of 18 sensors to volatile organic compounds in
steamed sweet potato and apple juice. The response rates of the most sensors to steamed sweet potato were clearly lower than those to apple juice. There is an exponential linear relationship between sensor response rate and concentration of volatile organic compounds. However, there is no direct relationship between sensor response rate and sensory odor strength. When the odor qualities among samples are the same and only concentrations of volatile organic compounds in those samples are different, there is a fixed correlation between sensory odor strength and sensor response rate. When the odor qualities among samples are different, there is no relationship between sensory odor strength and sensor response rate. Consequently, from the fact that the sensory odor strength of steamed sweet potato is less than that of apple juice, it cannot be concluded that the sensor response rate of the former is lower than that of the latter. However, it is clear that there is a remarkable difference in the amounts of volatiles between steamed sweet potato and apple juice.

Figure 2  Response patterns of 18 sensors to volatile organic compounds in steamed sweet potato and apple juice

(3) EEG analysis

Organoletic values and changes in the electroencephalogram for the 7 subjects are shown in Table 1. The upper panel shows the results of sensory evaluation and the lower
one shows the results of $\alpha_1$ and $\alpha_2$ wave power measurements. A cross (†) represents significance at 10% level and an asterisk (*) represents significance at 5% level. The results demonstrate that apple juice aroma was pleasant while steamed sweet potato odor was unpleasant, and distilled water without odor was intermediate between pleasant and unpleasant. There were some differences in organoleptic values among the subjects.

When apple juice was presented to the subjects, no significant change in $\alpha_1$ wave power was observed. On the other hand, an increase in $\alpha_2$ wave power was observed for 1 subject, a decrease in $\alpha_2$ wave power was observed for 2 subjects, and no change in $\alpha_2$ wave power was observed for 4 subjects. Further analysis was conducted for the subjects whose $\alpha_2$ wave power was increased. When the subjects smelled control sample, $\alpha$ wave power barely appeared. From that, it was surmised that stress was relieved by smelling apple juice, which effectively increased $\alpha$ wave power. In two subjects whose $\alpha_2$ wave powers were decreased by smelling apple juice, no significant change in $\alpha_1$ wave power was observed. Thus, it seems that the appearance of the fast component of $\alpha$ wave was suppressed.

From these, it was hypothesized that 3 subjects described above became relaxed by smelling apple juice. Although apple juice aroma was evaluated to be pleasant by all subjects in the sensory evaluation, the same tendency did not appear on the electroencephalograms of all subjects.

When steamed sweet potato odor was presented to the subjects, an increase in $\alpha_1$ wave power was observed for 3 subjects, a decrease in $\alpha_1$ wave power was observed for 3 subjects, and no change in $\alpha_1$ wave power was observed for 1 subject (Table 1). When apple juice was presented, no change in $\alpha_1$ wave power was observed. In contrast, when steamed sweet potato was presented, changes in $\alpha_1$ wave power were observed. This is the key difference between apple juice aroma and steamed sweet potato odor. From the finding that $\alpha_1$ wave power increased significantly for 3 subjects, whose $\alpha_1$ wave frequency did not change significantly, it was thought that $\alpha_1$ wave power itself increased for 3 subjects.
In general, the increase in $\alpha_1$ wave power means an increase of consciousness level or relaxation. When a person is in a state of relaxation, $\alpha$ wave frequency decreases. As no change in $\alpha$ wave frequency was observed in this experiment, it appears that not relaxation but consciousness level was increased. For one of the 3 subjects whose $\alpha_1$ wave power was decreased after smelling steamed sweet potato, $\alpha_2$ wave power was also decreased. From that, it was clear that $\alpha$ wave power was decreased totally and the sense of tension was increased. Although the change in $\alpha$ wave power depends on each subject, it seems that steamed sweet potato odor induced a sense of tension. EEG analysis revealed that the tendency for remarkable change was more clearly observed for unpleasant odor than for pleasant odor.

The effects of apple juice aroma and steamed sweet potato odor on mental activity were studied from the changes in the electroencephalogram. It was clarified that apple juice aroma had less impact on the electroencephalogram than steamed sweet potato odor. In general, EEG may react more sensitively to unpleasant odor than to pleasant odor.

From the above results, steamed sweet potato odor was confirmed to be less pleasant than apple juice aroma.
Table 3 Results of sensory evaluation and changes in $\alpha$-wave power spectrum

<table>
<thead>
<tr>
<th>Evaluation subject A</th>
<th>subject B</th>
<th>subject C</th>
<th>subject D</th>
<th>subject E</th>
<th>subject F</th>
<th>subject G</th>
</tr>
</thead>
<tbody>
<tr>
<td>pleasant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>a</td>
<td>w</td>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>s</td>
<td>s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intermediate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>w</td>
<td>a</td>
<td>w</td>
<td>w</td>
<td>a</td>
<td>w</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>s</td>
<td>s</td>
<td>s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>s</td>
</tr>
<tr>
<td>unpleasant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| increase              |           |           |           |           |           |           |
|                       | a         | s         | a         | s         | a         | s         |
|                       | $\alpha$ 1 | $\alpha$ 1 | $\alpha$ 2 | $\alpha$ 2 |           |           |
|                       |           |           |           |           |           |           |
| no change             |           |           |           |           |           |           |
|                       | $\alpha$ 1 | $\alpha$ 2 | $\alpha$ 1 | $\alpha$ 2 | $\alpha$ 1 | $\alpha$ 1 |
|                       | $\alpha$ 2 | $\alpha$ 1 | $\alpha$ 1 |           | $\alpha$ 2 |           |
|                       |           |           |           |           |           |           |
| decrease              |           |           |           |           |           |           |
|                       |           |           |           |           |           |           |
|                       |           |           |           |           |           |           |
|                       | $\alpha$ 2 | $\alpha$ 1 | $\alpha$ 2 | $\alpha$ 1 |           |           |
|                       |           |           |           |           |           |           |

a, s and w indicate apple juice, steamed sweet potato, and distilled water samples, respectively.
* : significant at 5% level (control=H$_2$O)
† : significant at 10% level (control=H$_2$O)
The electroencephalogram indicates change in P3.
The upper panel shows the results of sensory evaluation and the lower panel shows the changes in $\alpha$1 and $\alpha$2 wave powers.

4. Conclusion

The volatile components of sweet potato were clarified in chapter I and the sensory characteristic of sweet potato was studied in this chapter. The differences in odor profile between the heated-saccharified sweet potato juice and raw sweet potato juice were studied by descriptive sensory evaluation method. The sensory attributes of heated-saccharified sweet potato juice showed a high rating for “sweet” odor but also a high rating for “heavily boiled” odor as an off-odor note, whereas raw sweet potato sample possessed enhanced grassy and carrot odor. The evaluation of GC/MS and sensory data suggested that terpenoids, ketones, furans and aromatic aldehydes are the potent odor compounds contributing to the change in odor profile after heating and saccharification. No maltol, which is an important aroma contributor in baked sweet potato, was found in heated-saccharified sweet potato. Boiling may not favor the formation of maltol, which is formed
during baking. Although it is not easy to identify the odor compounds of heated-saccharified sweet potato, the results obtained in this study may show some association between the identified volatiles in the heated-saccharified sweet potato and the characteristic odor attributes of sweet potato, which reflect the descriptions in the sensory evaluation given by the panelists. Further study is planned to elucidate the aroma impact compound by measuring the relative contribution of each identified compound to the sensory attributes.

The study has started from volatile components and developed to sensory field. Furthermore, the study has developed the relationship between sensory evaluation and electroencephalographic analysis. The aroma characteristics of steamed sweet potato were compared with those of apple juice and the difference was clarified by sensory evaluation and odor sensor and electro-encephalographic analyses. Although all 7 subjects evaluated apple juice aroma to be pleasant, the same tendency was not observed for all subjects on the electroencephalogram. Three subjects were relaxed by apple juice aroma while no changes in $\alpha_1$ and $\alpha_2$ wave power were observed for the remaining 4 subjects. It was concluded that apple juice aroma did not elicit an unpleasant mood. On the other hand, by smelling steamed sweet potato odor, $\alpha$ wave power was markedly decreased and the sense of tension was increased. A significant difference between apple juice aroma and steamed sweet potato odor was observed on the basis of the changes in $\alpha$ wave power. It became clear that steamed sweet potato odor was different from apple juice aroma, and was not as pleasant as apple juice aroma. This result indicates that the odor can be evaluated in terms of the cognitive aspect. From these findings, association of the sensory measurement with the chemical and the cognitive measurement was made. Furthermore, possible indication of association of the cognitive response against the odor with the preference of the odor was suggested.
Chapter III
Measurement of Odor Quality of Model Food Ingredients by Using Newly Developed Odor Sensor

1. Introduction

Food quality has traditionally been defined in terms of product properties. More recently quality is being viewed more towards fulfilling requirements of consumers and consumers' acceptance. In the last chapters, the multiple dimension of the odor quality was clarified in terms of the chemical and the cognitive analysis of the off-odor.

With use of an instrumental measurement (GC-MS and EEG analysis), it was found that perception of the odor can be analyzed in chemical and physiological perspective. GC-MS analysis identified the specific compounds, which elicit the various sensorial responses. Specific patterns of brain electrical activity also reveal association of the odor perception with brain activity, which is also related with subject's preference of the odor.

Although these results suggest that the instrumental evaluation of the odor could be used as objective tools to predict the subject's hedonic responses to the odor, they are time-consuming and its relationship with the degree of the subject's preference is still not conclusive. Therefore, sensory evaluation is currently the foremost tool for odor analysis due to its sensitivity and selectivity. In addition, since it is evaluated by end-users, consumer's preference or acceptance can be directly reflected in the final assessment of a product.

Recently, as the mechanism of the human sense of smell has been clarified, chemical sensor technology has become highlighted, which can perceive odors in a similar way to a human nose. Depending on what types of elements are applied, there have been many sensors developed; calorimetric sensors, metal oxide semiconductors (1) and quartz sensors (2, 3). Many odor sensors (electronic noses) are also manufactured by commercial companies and can be applied in a wide range of fields. These companies include UMA Airsense (Germany), Alpha MOS (France) and Cyrano Sciences Inc. (USA).
However, the focus of the odor sensor has been on differentiation of the product based on detection and classification of volatile vapors. It is useful as a fast screening tool to ensure the human sensory panel results in terms of discrimination, but it does not provide a direct measurement to express quality or acceptability in terms of a liking or disliking of the odor. Therefore, to fulfill the gap of the sensory and the sensor assessment, new odor sensor, which can measures the odor quality and its acceptability, is intriguing to pursue. Using the technology previously developed (4), Ehara has developed a new odor sensor. New developed sensor is a semiconductor based odor sensor which incorporated multiple metal oxide elements that can respond to different odor molecules with a wide range of sensitivity (5). Metal oxide semiconductors have an advantage over other sensors in terms of the following properties; easily constructed and sensitive to lower vapor concentration, resistance to change in humidity and corrosive acid vapors (6). Metal oxide sensors also have a wide range of selectivity because of the abundance of electrons on the surface of the semiconductor.

By combining different semiconductor materials, the developed odor sensor can respond sensitively to a wide range of odor molecules (7, 8). This sensor is comprised of 6 different types of semiconductors, doped with catalytic metals, with different conductibility. Patterns of resistance change can be generated to create a specific profile for volatile compounds in sample. In comparison with existing odor sensors the materials in this new sensor have a higher sensitivity to a broad range of compounds, and so the odor sensor is able to record odor compounds at very low ppb concentrations, without an extra pretreatment of the sample.

As already looked into previously, the quality of the odor has a multiple dimension. For instance, ammonia is perceived as an unfavorable odor. However, when it is diluted, it is often confused with a perfume of jasmine. Thus, quality of odor, especially liking or disliking of the odor, cannot be determined by only chemical structural formula but has to depend on the consumer's perception. The odor of food is the important characteristic that signifies consumers’ interest in food choice and it would be very useful that the odor
sensor measures the odor quality in terms of liking or disliking in human perception. Several brief studies have been conducted using this new odor sensor to measure the organic vapors of several food products (9, 10). Preliminary results have suggested that the new developed sensor may have a capability of measuring the odor strength but also the odor quality in view of consumer perception.

In this chapter, the developed sensor and the application to the evaluation of odor quality is described. Method of measuring the odor quality in terms of the consumer's liking was established using the new odor sensor.

2. The characteristic of new odor sensor

1) Odor sensor preparation

Two different types of elements (sintered metal oxide and thin-film oxide) were combined. The former sensor element reacts sensitively to odorant compounds with low molecular weights, perceived as light odorants, while the latter sensor type is more sensitive to odor compounds with relatively large molecular weights, such as an unsaturated aromatic hydrocarbons group compound like toluene or methanethiol, perceived as heavier odorants. The production of the sensor and its properties has been previously described (4). A total of 6 semiconductor sensors were combined in this odor sensor to cover a broad range of chemical properties. Each sensor's material name and properties are shown in Table 1.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Material</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH-1</td>
<td>SnO₂, Sintered-metal oxide</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>CH-2</td>
<td>SnO₂, thin-film metal oxide</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>CH-3</td>
<td>SnO₂, thin-film metal oxide</td>
<td>Sulfur</td>
</tr>
<tr>
<td>CH-4</td>
<td>SnO₂, thin-film metal oxide</td>
<td>Hydrocarbon</td>
</tr>
<tr>
<td>CH-5</td>
<td>SnO₂, Sintered-metal oxide</td>
<td>Alcohol, Aromatic</td>
</tr>
<tr>
<td>CH-6</td>
<td>ZnO, thin-film metal oxide</td>
<td>Sulfur</td>
</tr>
</tbody>
</table>
The sensing principles of the metal oxide semiconductor are not still clear but it can be explained as follows; metal oxide is normally produced in a slightly reduced state, but oxygen in air is adsorbed on the surface and forms an electron depletion layer due to a high electron affinity of oxygen. This increases resistance of the semiconductor. With the existence of reducing gas, an oxidation reaction proceeds against the adsorbed oxygen and electrons captured by oxygen are released. As a result, the resistance of the semiconductor decreases and voltage in the circuit drops. The reaction details also change depending on the condition of the coil heating temperature and sensing materials. Figure 1 shows the structure of the sensor circuit.

The coil and the semiconductor compose a parallel resistance circuit (Rs and Rh). Rs is the sensing element whose resistance varies according to injected gas composition and heater temperature, which is controlled by the bridge voltage that is imposed across the sensing element. Changes in combined resistance are measured when an odor gas is adsorbed onto the surface of the sensing element, which causes electron conductivity changes to decrease the Rs. Changes in R can be measured as a change in voltage since a sensing element is incorporated into the bridge circuit.

\[
\text{(Sensor parallel resistance circuit) } \quad \text{(Bridge circuit)}
\]

\[
\begin{align*}
R_{\text{combined}} &= \frac{R_h R_s}{R_h + R_s} \\
\end{align*}
\]

Figure 1 Simple diagram of the electric circuit; the sensor element consists of a parallel resistance circuit of the semiconductor and heater coil. Combined resistance (R) is converted into a voltage in the bridge circuit, which is connected to the counter.
A simple diagram of the odor sensor design is shown in Figure 2. The sensor was mounted inside a 30ml gas chamber, in which a sample plate was mounted to hold known amounts of the tested compounds. The chamber has an open window, which can be sealed tightly after a sample was placed on the plate. Output from the bridge circuit was connected to a data collector to measure changes in resistance. The data collector was linked to an external computer which controlled data management and analysis. Changes in voltage transmitted through the data collector were displayed on a CRT display at regular time intervals and the data was processed and spider graphs were generated to display the sensor response at any given time.

2) The sensitivity of the sensor

The signals from multi-sensors are analyzed with a computer and the odor strength and the quality of odors were measured. This apparatus is capable to evaluate the odor
strength and the quality in this apparatus. The adopted sensors are high sensitivity and the concentration of ppb order is measured. The sensitivity characteristic is indicated in Figure 3.

![Sensitivity characteristic of sensors](image)

**Figure 3**  The sensitivity characteristic of the sensors of new odor sensor

3) **Data collection procedure**

Data collection for each sample was conducted as follows. The platinum coil wire upon which sintered-oxide and tin-film oxide metal was mounted was heated up to 300°C. After odor compounds were released inside the chamber, the odor detector started collecting odor signal and output value was plotted against time. When the output value reached saturation level after 1 min, a spider graph was also generated to see the instant response pattern of the 6 sensors. The data collection process lasted for about 5 min.

3. **Materials and methods**

1) **Samples for measurement**

The odor of multiple food products and sox was investigated to determine the sensor's response to real food products with different odors. These are wasabi, lemon
grass, coffee, lavender, rose, pine needle, lemon, funazushi, oral malodor, deairated beer, blue cheese, dirty sox, natto and kusaya. These were purchased from commercial markets to represent pleasant and unpleasant odors.

2) Sensory evaluation

Seven subjects (Japanese, female, aged 20 ~ 35 years) were selected using a difference test with the following compounds at the indicated concentrations: β-phenylethyl alcohol 10^{-4.0}\% (w/w), methyl cyclopentenolone 10^{-4.5}\% (w/w), isovaleric acid 10^{-5.0}\% (w/w), γ-undecalactone 10^{-4.5}\% (w/w), and skatole 10^{-5.0}\% (w/w), (all from Daiichi Yakuhin Kogyo Co., Ltd., Japan). The seven assessors evaluated their preference for wasabi, lemon grass, coffee, lavender, rose, pine needle, lemon, funazushi, oral malodor, deairated beer, blue cheese, dirty sox, natto and kusaya based on the 7-point scale, where 1 indicates very unpleasant odor and 7, very pleasant aroma. The assessors had at least a 3-min rest period between evaluations of samples.

3) Odor sensor analysis

Fifty grams of each sample were sliced and placed in turn on the glass plate for analysis. On the other hand, oral malodor is the breath air after ingesting 50g of noodle and it was collected in a commercial plastic bag which was manufactured by OMI ODOAIR SERVICE Co. Ltd.(Tokyo, Japan).

4. Results and discussion

1) Sensory evaluation

The average values for each sample was as follows: wasabi 5.5, lemon grass 6.1, coffee 6.5, lavender 6.5, rose 6.3, pine needle 6.3, lemon 6.4, funazushi 4.5, oral malodor 1.2, deairated beer 2.7, blue cheese 1.6, dirty sox 1.0, natto 3.1 and kusaya 1.3. The significant difference was observed between the former samples, pleasant odor and the latter samples, unpleasant odor.
2) Odor sensor analysis

(1) Measurement of pleasant and unpleasant odor samples

I tested some kinds of foods and the result was shown in figure 4. Resistance values from the 6 sensors were fed into a processor and analyzed by computer program to produce responses displayed in spider graphs. I found that responses from sensors 2 and 4 were small, and those from sensors 3 and 6 were greatly enlarged for pleasant smells. On the other hand, for unpleasant smelling substances the radar graph shape was very different. For instance, natto\(^1\) and kusaya\(^2\) are known for their unpleasant smells, in comparison to the fruity citrus smell of lemon. In contrast to the display for lemon, the responses for natto and kusaya had a similar irregular shape due to the small response of CH 6 and CH 3 (Figure 4). Other examples of pleasant spear-shaped response included coffee and lavender, and unpleasant odors such as dirty sox and blue cheese had irregular shapes as shown in Figure 4.

(2) Definition of F-value and S-value

Odor is messed up beyond all recognition. Ammonia is said to be representative malodor. However the repeated dilution finally results in a perfume close to that of jasmine. It parallels between odor and sense and, further its pleasant or unpleasant odor differ greatly in individuals. Whether an odor is pleasant or unpleasant, it cannot be determined from its chemical structure alone, and the presence of difference between individual measuring conditions make it more difficult to reach a definite determination. However, from a few cases of actual application regarding sensors for evaluating odors, it is thought that pleasant or unpleasant odor is predicted from the data analyzed by these semiconductor sensors.

\(^1\) Natto is a popular food in Japan made by fermentation of cooked soybeans with Bucillus subtilis (natto), and has a characteristic aroma and stickiness. It contains many nutrients originating from both soybeans as well as from intact bacterial cells and metabolites of B. subtilis.

\(^2\) Kusaya is a specially brined and dried red meat sea fish produced in Izu islands located in the south of Tokyo. It is famous for its malodor and it is often the subject of taste controversies, much like British Marmite and French blue cheese. It is popular among people lived in Tokyo Japan for its unique flavor and for long shelf life.
Figure 4  Spider graphs of 50g samples of pleasant and unpleasant odor showing the resistance values (mv) from the 6 sensors outputs (CH1-CH6) in the odor sensor, F-values and S-values

From these analytical values described above, the quality of the odor can be defined by the F-value, which is defined as CH3+CH6 / CH2 +CH4. The strength of the odor can be defined by the S-value, which is defined as an integration of the area under the spider graph. This formula is contextualized for odor substances distinguished clearly to be pleasant or unpleasant odor, but it cannot be contextualized for all odor substances.

As mentioned above, the significant difference between pleasant odor groups such as lemon and unpleasant odor groups such as kusaya was recognized by sensory evaluation. The significant difference was also recognized by odor sensor measurement.

5. Conclusion

Food aroma or odor is a mixture of many different types of chemical compounds and these compounds are correlated to create a unique sensation. For instance, ethyl alcohol odor is not itself very pleasant, but people enjoy its smell in alcoholic drinks, mixed with odor of other volatiles created during fermentation. Thus, it is challenging to develop the method to evaluate odor quality of foods conveniently.

The newly developed odor sensor consists of a semiconductor of combined sintered and thin film metal oxides. The former sensor type reacts sensitively to light odorants (compounds with low molecular weights), while the latter sensor type is more sensitive to heavier odorants (compounds with relatively large molecular weights) such as unsaturated aromatic hydrocarbon group compounds like toluene or methanethiol, perceived as heavier odorants. In this chapter, the development of a sensor analysis method to measure a few kinds of odor substances was described. By using the electronic nose, it was verified that an electronic nose could define the characteristics and strength of the odor in terms of F-value and S-value for these odor substances. These are the substances distinguished clearly to be pleasant or unpleasant odor in the range of livelihood.
This study proves that the developed odor sensor has a feature for evaluating product odor intensity and quality in terms of pleasantness. In the rapid process of food product manufacturing, there are many attributes to be monitored, but the most important criteria are to keep product quality favorable for consumers. Usually both sensory and GC analysis have been combined to measure the quality of odors. Thus, this new odor sensor analysis has the advantage of being a convenient method. The S- and F-values are useful concepts of odor for these odor substances, and the formula is derived experimentally, so it is applicable to odors of these kinds of food products. Also, other than pleasantness and unpleasantness, it could also be possible to express odor characteristics by multiple criteria. Thus, in further experiments, the odor of various food products should be investigated to find how sensor output and the results of analysis for odor compounds are related.
Chapter IV

Deodorization of Off-odor of Sweet Potato by Using Physical and Chemical Deodorants

1. Introduction

Based on the identified odor impact compounds information identified in the chapter I, various deodorants were selected, and its efficacy was evaluated using the new odor sensor system. "Benihayato" one of the sweet potato cultivars, that is being developed as a functional juice resembles a carrot in color, odor and higher content of β-carotene (1). β-carotene, independent of its role in the formation of vitamin A, is anti-cancerous as evidenced by its effectiveness in different animal species, at different cancer sites, and in several different cancer model systems, by using different inducing agents(2,3).

It is known that baked sweet potato generates a pleasant aroma. Studies have been reported on the aroma compounds which may contribute to the total delightful aroma of the baked sweet potato (4,5,6,7). Sun et al. found that maltol was a critical component of the characteristic aroma of the baked sweet potato and was produced through the Maillard reaction, caramelization, and Strecker degradation, the most common mechanisms in the thermally induced synthesis of characteristic aroma volatiles (8).

Although sweet potato has a great potential as an ingredient for the functional food, it exhibits potential off-odor in the boiling process. In our study, roots of the sweet potato cultivar were steamed by stepwise heating and mashed to homogenous mixtures and saccharified with amylase for the development of the functional food. However, it was noted that the heating and saccharification processes imparted not only the characteristic sweet potato odor but also a heavy unappetizing odor in the mashed sweet potato juice.
In this chapter we evaluated the deodorization efficacy of the three different deodorants which reduced the heavy odor generated during the heating process of the sweet potato juice. The first deodorant used was an activated carbon (AC) with a porous structure creating a broad inner surface on which the odor components are adsorbed by Van der Waals force. The second deodorant was maltosyl cyclodextrin (MCD), which is a mixture of maltosyl α-cyclodextrin and maltosyl β-cyclodextrin, which was enzymatically produced by combining maltose with cyclodextrin to enhance the water solubility. MCD encapsulates the odor components into its cavities, and subsequently deodorizes the unpleasant odor or solubilizes the insoluble substances in water. The third deodorant was apple polyphenol, which is oxidized and changed into a quinone type structure of which phenyl hydroxyl groups react with compounds containing SH or NH and eliminates the foul smell. This type of the reaction is called a chemical deodorization.

To show quantitatively the degree of reduction in the heavy odor, the samples of the sweet potato juice treated with three different deodorants were evaluated, by using the olfactory meter, the sensory analysis and GC-MS. Since the mechanism of deodorization for each deodorant differs depending on the odor components, the deodorization scheme of each deodorant for the identified odor compounds mechanism was also explained.

2. Materials and methods

1) Preparation of sweet potato juice

After steaming 10kg of sweet potato by stepwise heating (at 60-65°C for 2.5 hrs and at 100°C for 1hr), 18 liters of water and 50g α-amylase (Uniase BM-8; with a specific activity of 80,000units/g) were added and the mixture was incubated for 3hrs at 80°C. Then 50g β-amylase (Uniase L, with a specific activity of 80,000units/g) was added to the partially hydrolyzed starch of saccharified sweet potato and incubated at 55°C for 1hr, and then 50g of glucoamylase was added and the
mixture was incubated at 55°C for a further period of 1 hr.

2) Deodorants

Two kinds of physical deodorants (AC Japan Enviro Chemicals, Ltd and MCD from Bio Research Corporation of Yokohama, Japan) and a chemical-based deodorant (AP from Asahi Breweries Ltd. Japan) were assessed. The AC had a BET specific surface area of 1077m²/g, specific pore volume of 0.639ml/g and an average pore diameter of 2.37nm. Two percent of each deodorant was added to juice samples for analysis.

3) Evaluation of deodorization efficiency

(1) Electronic nose analysis

New developed sensor was used for the assessment of the odor strength. Two different types of sensor elements, one (sintered metal oxide) that is highly sensitive to low molecular weight odor compounds (generally perceived as light odors by human senses), and the other (thin-film oxide) that is sensitive to relatively larger molecular weight odor compounds (such as the unsaturated aromatic hydrocarbons like toluene, or volatile sulfur compounds such as methanethiol; generally perceived as heavy odors by human senses) were chosen for the electronic nose analysis. The production and properties of the sensors have been reported previously (9, 10). This electronic sensor consisted of 6 semiconductor sensors combined to cover a broad range of chemical properties. The material and characteristics of each sensor are described in Table 1.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Material</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH-1</td>
<td>SnO₂, Sintered-metal oxide</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>CH-2</td>
<td>SnO₂, Thin-film metal oxide</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>CH-3</td>
<td>SnO₂, Thin-film metal oxide</td>
<td>Sulfur</td>
</tr>
<tr>
<td>CH-4</td>
<td>SnO₂, Thin-film metal oxide</td>
<td>Hydrocarbon</td>
</tr>
<tr>
<td>CH-5</td>
<td>SnO₂, Sintered-metal oxide</td>
<td>Alcohol, Aromatic</td>
</tr>
<tr>
<td>CH-6</td>
<td>ZnO, Thin-film metal oxide</td>
<td>Sulfur</td>
</tr>
</tbody>
</table>
As shown in the schematic diagram of the electronic sensor design in Figure 1, the 6 sensors were mounted in a 30 ml capacity gas chamber, in which a heat resistant cup with 3 ml of the sample was placed. The chamber contained an open window, which could be sealed tightly after placing the sample on the plate. Output from the bridge circuit was connected to the data collector for the measurement of change in resistance. The data collector was linked to an external computer equipped with the software to handle the data processing and analysis. Voltage changes transmitted through the data collector were displayed on a CRT display at specific time intervals and processed. A radar chart was also generated to show the sensor response at any given time point.

Data collection for all the samples was conducted in the following manner; a platinum coil wire, upon which sintered-oxide and tin-film oxide metals were mounted, was heated up to 300°C. Soon after the odor compounds were released inside the chamber, the odor detector started collecting odor signals, and the
output values were plotted against time. The instant response pattern of the 6 sensors was evident on a radar chart generated immediately after the output value reached a saturation level in 1 min of measurement. The data collection process lasted for about 5 min. Strength of the odor was calculated and defined as S-value, which integrates the area under the radar chart.

(2) GC-MS analysis

a. Preparation of the sample

Volatile were concentrated on porous polymer (Tenax TA) precolumns by bubbling with N₂ at a rate of 80 ml/min for 60 min at 50°C. The Tenax tube was placed in the heating block of the TCT (Thermal desorption Cold Trap) injector and heated at 200°C to desorb the volatiles. Trapped volatiles were injected into GC-MS by using the TCT injector, and the total ion chromatograms were obtained.

b. Conditions for the GC-MS analysis of sweet potato odor

Apparatus, GC Hewlett-Packard Model 6890, MS Hewlett-Packard Model 5973 ; column temperature was held at 40°C for 10 min, programmed to rise from 40°C to 200°C at 5°C/min ; fused-silica capillary column (GL Science, TC-WAX) 0.25mm×60m, 0.25μm film thickness ; carrier gas, He (100kPa) ; El mode, at 70 eV ; ion source temperature, 230°C; GC-MS interface temperature, 230°C. A library search was carried out using the Wiley GC-MS library.

c. Reaction assessment between the characteristic compounds of the sweet potato and AP

The reaction between the characteristic compounds of the sweet potato and AP was studied using the following standard reagents: Benzaldehyde and linalool (Wako Pure Chemical Industries, Osaka, Japan).

d. Sample preparation

Standard gas was prepared as follows; 0.1g of benzaldehyde and 0.1g of linalool were placed in a tightly sealed 20ml glass vial and after incubation for 1hr at 25°C, 2ml of the standard gas was mixed with 0.2g of AP in the same vial and
incubated for 15 min at 25°C. The headspace gas was then injected into the GC-MS with a headspace sampler.

f. GC-MS condition

Column, fused-silica capillary column (GL Science, TC-WAX) 0.25 mm×60m, 0.25μm film thickness; column temperature, programmed to rise from 100°C to 200°C at 5°C/min; carrier gas, He (100kPa); El mode, at 70eV; ion source temperature, 230°C; GC-MS interface temperature, 230°C. Motoring ions; m/z 106,77 (benzaldehyde); m/z 93, 71(linalool)

g. Head-space sampler

Apparatus, 7694 (Agilent Technologies, Inc.); oven temperature, 60°C; vial heating time, 0.1min; loop temperature, 190°C; transfer-temperature, 220°C; vial pressurization time, 0.3min

(3) Sensory evaluation

Assessors consisting of 20 students of Tokyo Bunka college were trained in evaluating the degree of off-odor in boiled sweet potatoes at the preliminary session. Raw sweet potato juice was diluted to different concentrations in water and served in small containers. The containers were kept sealed until the judge was asked to sniff them. A mark was assigned for the intensity of off-odor on a scale ranging between 0-5, where zero was considered the weakest and 5 was the strongest. In order to calibrate the panel, each panelist received and scored the sweet potato juice samples at different concentration levels. In the following discussion, a consensus for scoring was reached to evaluate the odor defect in the subsequent sensory evaluation according to the consensus scores assigned. Once the panelists were trained, the effect of off-odor reduction by the AC, the MCD and the AP treatment was evaluated. Thirty ml of the sweet potato juice sample was served in 100 ml glasses as an original sample. Sweet potato juice was mixed with AC, MCD and AP to make 2% of each sample preparation, respectively. The four individual samples were randomized over judges so that all possible serving
combinations occurred and presented to judges in equal number of times. Four samples were presented on the tray and evaluated for the intensity of off-odor in the order from left to right. Session was repeated for 3 days until all the combinations were evaluated. Two-way analysis of variance using the deodorants, the judges and their interaction as variable factors was conducted to calculate the significance of the results obtained from the 6-point scale.

3. Results and discussion

During the development and processing of sweet potato as a functional food, it has been reported that the boiling process causes an unfavorable heavy odor. The deodorant which most effectively reduces the unappetizing odor of heated-saccharified sweet potato was identified by using the 3 different analyses, the electronic sensor analysis, the sensory analysis and GC-MS. Subsequently, the odor reduction mechanism of each deodorant for the identified flavor compounds was explained.

1) Results of deodorization by 3 methods of evaluation

(1) Electronic nose measurement

The deodorization efficiency was measured by electronic nose, which is represented by the S-value. The smaller the S-value is, the weaker the odor strength is.
Figure 2  Deodorization of sweet potato odor by 3 kinds of deodorants
Radar chart from the 6 sensors outputs (CH1-CH6) of the electronic sensor measurement of the 6 ml sweet potato juice samples
Broken, thin solid, dot chain and dotted line indicate sweet potato juice, juice with 2% AC and juice with 2% MCD and juice with 2% apple polyphenol respectively, with the sensor output(mV) of each sensor, F-value and S-value. Sweet potato juice ; F=2.700, S=17222  AC ; F=1.772, S=10630 cyclodextrin ; F=2.2179, S=11833 AP ; F=1.739, S=17390

AC treated sweet potato juice showed the smallest S-value of 10630 and AP treated sweet potato juice had the largest S-value of 17390 (Figure 2). These results confirmed that among the 3 deodorants used AC had the highest deodorization efficiency.

(2) Sensory evaluation

Results on the multiple comparison sensory assessments of the deodorant are presented in Table 2. There was a significant difference between the score of the off-odor strength for three deodorant treated sweet potato juice (ANOVA, p<0.01). The score from the lowest to the highest score was in the order of the sweet potato juice sample treated with AC, with MCD, with AP and no treatment. There was no significant interaction between judges and treatments, which means that judges showed a similar trend in attributing the highest score to AC and the lowest to untreated sample. A Tukey multiple comparison was conducted to evaluate the significance of the odor intensity among the AC treated, the MCD treated, the AP treated samples and the no treated sample. The AC treated and the MCD treated sample showed a significant difference but no significance was found between the AP treated sample and the no treated sample (Tukey HSD, p<0.01, P>0.05). From these results, it was clear that while the AC treatment had the strongest deodorization effect, the AP treatment showed almost no effect on reducing the off-odor of the sweet potato juice.
Table 2 mean values for sensory evaluation scores of the off-odor intensity for each deodorant treatment1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AC</th>
<th>MCD</th>
<th>AC</th>
<th>No treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>1.75(^a)</td>
<td>2.95(^b)</td>
<td>3.60(^c)</td>
<td>3.75(^c)</td>
</tr>
<tr>
<td>(Std dev)</td>
<td>(0.44)</td>
<td>(0.22)</td>
<td>(0.50)</td>
<td>(0.44)</td>
</tr>
</tbody>
</table>

1AC=Activated carbon treated sweet potato juice. MCD=maltosyl cyclodextrin treated sweet potato juice. AP=apple polyphenol treated sweet potato juice.

a,b,c mean without a common letter differ (P < 0.01) according to Tukey HSD

(3) GC-MS analysis

The volatiles in the headspace were measured by GC-MS and 20 main components were identified (Table 3). Ion chromatograms of headspace volatiles in the 3 treatments are shown in Figures 3(a), (b) and (c). The deodorization efficiency of the 3 kinds of deodorants was as follows.

AC ; The intensity of all the peaks was reduced by AC, and the extent of reduction was the largest in comparison to the other deodorants. The peak intensities of 13 of the 20 main odor components were lowered to less than peak height 5, which was the minimum limit of detection.

MCD ; In sharp contrast to AC, only one of the 20 main odor components was reduced to less than peak height 5. Since the maximum vacancy size of \(\beta\)-cyclodextrin was 0.70 nm, larger than that for \(\alpha\)-cyclodextrin, none of the compounds could enter this vacancy, as this depends on the orientation. Therefore, it is likely that only a small amount was enveloped by MCD in comparison with the amount adsorbed by AC.

AP ; The intensities of almost all the odor components were the highest in the AP treatment, indicating a very low deodorization efficiency of the compounds listed in Table 3. However, since AP has been shown to rapidly deodorize thiol compounds (11, 12), it is thought that the odor components of sweet potato did not react with the AP due to low
nucleophilicity. The poor deodorization efficiency of AP for sweet potato odor was also confirmed by electronic sensor measurement and sensory experiment.

Figure 3  Total ion chromatogram of sweet potato juice samples
deodorized by AC (a), MCD (b) and AP (c)

Peak identification numbers correspond to compounds listed in Table 3. These volatile compounds were characterized by GC-MS under the analytical conditions described in the text. Values were expressed in μV • s ×10. Volatiles using TC-WAX coated with 60m×0.25mm fused-silica capillary are described in the text.

Thus, considering the results obtained it may be inferred that AC had the highest deodorization efficiency, followed by MCD, and then lastly AP with minimal deodorization efficiency.

Comparison of the 3 deodorants; While a comparison between the AC and the AP treated samples revealed large differences in the odor intensities of benzaldehyde, β-cyclocitral and β-ionone, only a small difference in odor intensities was noted for acetone, ethyl acetate and diacetyl. BHT (Butyl hydroxyl toluene) which is not an odor component but rather an antioxidant, may be a contamination from the polypropylene vessels used for present experiment. Although the intensity of BHT in the MCD treatment was 20% of that observed in the AP treatment, in the case of AC treatment it reduced even further below the minimum limit of detection.

While AC is unable to adsorb odor components selectively, it adsorbs even functional components. On the other hand, MCD exhibits some advantages such as in making a complex with and stabilizing the β-carotene which is usually unstable independently in the sweet potato juice, and furthermore, MCD is also functional in decreasing the value of cholesterol (13). The deodorization efficiency of some plant polyphenols has earlier been investigated. For instance the tea catechine reacts with methanethiol, the main component of oral malodor, and deodorizes the oral malodor (14, 15).

2) Adsorption of odor components with AC
(1) Adsorption with AC

The differential pore diameter distribution curve of the AC used in this study is shown in Figure 4. The average pore diameter was about 2.4 nm, and the diameter of most pores ranged between 1.5-6 nm. Since the largest known molecule of the odor components in the sweet potato is geranyl acetone, with a diameter of 1.45 nm on the major axis, it is expected that all the odor component molecules are small enough to enter the micropores of the AC. However, the AC differed in its adsorption removal ability of the individual odor components. It is thought that the odor removal ability depends on the difference of physical and/or chemical interactions between the AC surface and the odor components.

Since, several kinds of coexisting odor components in water were removed by the AC adsorption, it is difficult to evaluate the potential physical and/or chemical interactions between the AC surface and the respective odor components. Therefore, although the index did not reflect all of the potential interactions mentioned above, the Hydrophile-Lipophile Balance, HLB (16, 17) utilized shows a quantitative balance of hydrophilicity and hydrophobicity.

![Figure 4](image.png)

Figure 4 Distribution of pore diameter for AC

(2) Relationship between peak intensity and HLB value

Table 3 shows the HLB values for the 15 main odor components identified in the sweet potato juice samples. The peak intensities of each compound after
Deodorant treatments are also shown. The peak intensity for acetone (Peak No. 1) was 141 and acetone was not removed completely even after the treatment with AC. In this regard we speculate that the relatively high hydrophilicity for acetone whose HLB value was 10.8, prevented much adsorption on the AC.

Similar was the case with MCD, which removed only a small amount of acetone. Although acetone being small in size could be enveloped easily in its vacancy, the inner hydrophobic environment prevented the hydrophilic acetone from being adsorbed. Ethyl acetate with a relatively high hydrophobicity showed higher HLB value (Peak No. 2) and the peak intensity remained higher after the AC treatment. When treated with the AP deodorant, the peak intensity for ethyl acetate was approximately 4 times larger than that for acetone. On the contrary, the peak intensity for acetone was slightly larger than that for ethyl acetate during AC treatment. In comparison to the AP treatment a conspicuous reduction in the peak intensity was noted in the AC treatment. The most probable reason for such observations was the hydrophilic and hydrophobic nature of acetone and ethyl acetate, respectively. In tune with the above results diacetyl (Peak No. 3), which was the most hydrophilic among all the other odor components showed a HLB value of 16.3. Although the strong hydrophilic nature of diacetyl made it difficult to be adsorbed, its peak intensity was small after the AP treatment. This indicated that the concentration of diacetyl originally present in the sweet potato was low. Similarly, in comparison to the acetone and ethyl acetate, which showed lower hydrophilicity than diacetyl, a greater reduction in the peak intensity of diacetyl was noted after treatment with AC. With regard to the other odor components such as toluene (Peak No.5), 2-methyl-2-butenal (Peak No.7), 4-methyl-3-penten-2-one (Peak No.8), 2,2,6-trimethylcyclohexanone (Peak No.11), 3,5,5-trimethyl-2-cyclohexene-1-one (Peak No.12), 2-ethyl-4,5-dimethylphenol (Peak No.13), cycloisosativene (cycloisosative) (Peak No.14), β-cyclocitra1 (Peak No.17) and 2,6-di-tert-butyl-p-hydroxy toluene (BHT) (Peak No.19), all of them were adsorbed by the AC up to lower than peak height 5. The HLB value of these compounds ranged between 1.07-
7.44, indicating a relatively higher hydrophobicity and lower hydrophilicity. This aspect suggests that the AC could adsorb these compounds more efficiently due to its strong hydrophobic surface. In addition to the above components, three unknown components (U.K.; Peak No.6, 9 and 10) were also strongly adsorbed by the AC, which suggested that they also were strongly hydrophobic in nature. Unexpectedly, even though the HLB values of benzaldehyde (Peak No.15), geranyl acetone (6,10-dimethylundeca-5,9-dien-2-one, Peak No.18) and β-ionone (Peak No.20) were highly hydrophobic, they were not removed completely after the AC treatment. Judging from the residual amounts of these components detected after the MCD and the AP treatments, it is thought that the initial concentrations of these odor components were very high. Generally, adsorption in multi-components system is influenced by the AC dose, the concentration of the adsorbate, the other kinds of coexisting substances, and the time of contact. If the concentration of the adsorbate to the amount of AC is low, the adsorption ratio of the adsorbate increases and consequently the quantity of the adsorbate in the solution system decreases. Conversely, if the concentration of the adsorbate to the amount of AC is high, then the adsorption ratio of the adsorbate is lowered and as a result the quantity of the adsorbate in the solution system does not decrease significantly.
Table 3  Size and HLB value of the main compounds in sweet potato odor and the quantities of each compound after deodorant treatments

<table>
<thead>
<tr>
<th>PK No.</th>
<th>Compound</th>
<th>Axis [nm]</th>
<th>HLB</th>
<th>Deodorant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Major</td>
<td>Minor</td>
<td>AC</td>
</tr>
<tr>
<td>1</td>
<td>Acetone</td>
<td>0.67</td>
<td>0.42</td>
<td>10.8</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate</td>
<td>0.89</td>
<td>0.42</td>
<td>7.50</td>
</tr>
<tr>
<td>3</td>
<td>2,3-Butanedione (Diacetyl)</td>
<td>0.80</td>
<td>0.42</td>
<td>16.3</td>
</tr>
<tr>
<td>4</td>
<td>U.K.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Toluene</td>
<td>0.83</td>
<td>0.42</td>
<td>1.07</td>
</tr>
<tr>
<td>6</td>
<td>U.K.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2-Methyl-2-butanal</td>
<td>0.82</td>
<td>0.42</td>
<td>7.44</td>
</tr>
<tr>
<td>8</td>
<td>4-Methyl-3-penten-2-one</td>
<td>0.89</td>
<td>0.42</td>
<td>6.70</td>
</tr>
<tr>
<td>9</td>
<td>U.K.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>U.K.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2,2,6-Trimethylcyclohexanone</td>
<td>0.93</td>
<td>0.67</td>
<td>4.17</td>
</tr>
<tr>
<td>12</td>
<td>3,5,5-Trimethyl-2-cyclohexene-1-one</td>
<td>0.93</td>
<td>0.67</td>
<td>4.28</td>
</tr>
<tr>
<td>13</td>
<td>2-Ethyl-4,5-dimethylphenol</td>
<td>1.07</td>
<td>0.55</td>
<td>5.75</td>
</tr>
<tr>
<td>14</td>
<td>Cyclodextrinative(Cyclodextrative)</td>
<td>1.06</td>
<td>0.83</td>
<td>1.39</td>
</tr>
<tr>
<td>15</td>
<td>Benzaldehyde</td>
<td>0.88</td>
<td>0.38</td>
<td>5.71</td>
</tr>
<tr>
<td>16</td>
<td>U.K.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>β-Cyclocitrinal</td>
<td>0.90</td>
<td>0.67</td>
<td>3.75</td>
</tr>
<tr>
<td>18</td>
<td>6,10-Dimethylundeca-5,9-dien-2-one</td>
<td>1.45</td>
<td>0.63</td>
<td>2.88</td>
</tr>
<tr>
<td>19</td>
<td>(Geranylacetone)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>β-Ionone</td>
<td>1.21</td>
<td>0.67</td>
<td>3.29</td>
</tr>
</tbody>
</table>

These volatile compounds were characterized and quantified by GC-MS. The analytical conditions described in the text. Values are expressed in peak height.

3) Envelopment of the odor components with MCD

(1) Peak No.1, 2, 3 and 5

As mentioned earlier, since, cyclodextrin has the capability of enveloping
various kinds of compounds into its cavities by forming complexes with them, it was observed that MCD showed lower GC-MS peak intensities in comparison to AP, indicating its relatively higher deodorization efficiency (Table 3).

Both acetone (Peak No.1) and diacetyl (Peak No.3) possess the ketone group and comparatively higher hydrophilicity. A significant difference was not observed when the peak intensity of MCD and that of AP were compared. From the peak intensity data of ethyl acetate (Peak No.2), there was no difference at all between the MCD and the AP deodorants which indicated that these deodorants did not efficiently adsorb ethyl acetate.

While the size of toluene is just adequate to enter into the cavities of α-cyclodextrin, it also can be enveloped by β-cyclodextrin in a loose manner with in the γ-cyclodextrin cylinder (18) (Szejtli, 1981). As shown in Table 2, while the intensity values for toluene upon treatment with MCD and AP were quite similar, a higher intensity value was observed with the AC treatment. Although the exact mechanism or reason for the above result remains unclear, we speculate that this may be due to the influence of the coexisting odor components during the the formation of the CD complex.

(2) Peak No.7 and 8

The chemical structure of 2-methyl-2-butenal (Peak No.7) is follows;

\[
\text{CH}_3
\begin{array}{c}
\text{CH}_3 \\
\text{CHO}
\end{array}
\]

and that of 4-methyl-3-pentene-2-one (Peak No.8) is follows;

\[
\text{H}_3\text{C}
\begin{array}{c}
\text{CH}_3 \\
\text{CHO}
\end{array}
\]

Both have a similar chemical structure, and their peak intensities were also reduced almost equally by MCD, without any steric hindrance.

(3) Peak No.11 and 12

78
The chemical structure of 2,2,6-trimethyl cyclohexanone (Peak No.11) is follows;

\[ \text{H}_3\text{C} \quad \text{O} \quad \text{CH}_3 \]

\[ \text{CH}_3 \]

and 3,5,5-trimethyl-2-cyclohexene-1-one (Peak No.12) is follows;

\[ \text{O} \]

\[ \text{H}_3\text{C} \]

\[ \text{H}_3\text{C} \]

\[ \text{CH}_3 \]

Again, both the compounds have a similar chemical structure, and MCD reduced them by a similar amount relative to AP treatment peak intensities. However, the reduction was much lower than for 2-methyl-2-butenal (Peak No.7) and 4-methyl-3-pentene-2-one (Peak No.8), probably because the cyclohexanone and the cyclohexene compounds possess several methyl groups around the benzene ring.

(4) Peak No.13, 14 and 15

The peak intensities of 2-ethyl-4,5-dimethylphenol (Peak No.13) and cycloisosativene (Peak No.14) after MCD treatment were only slightly lower than that for AP. The reason for the observed absence of any significant difference between the 2 compounds is attributed to their high molecular weight and the occurrence of several side-chain groups. In particular, 2-ethyl-4,5-dimethylphenol possesses one ethyl and two methyl groups and its peak intensity was reduced less than that of cycloisosativene.

It was expected that benzaldehyde (Peak No.15) would be strongly enveloped by MCD, based on the crystal structure study of the complex between benzaldehyde and cyclodextrin by Harata et al.(19). As shown in Table 3, with regards to benzaldehyde the peak intensity of MCD was much lower than that of
(5) Peak No.17, 18, 19 and 20

Szente et al. studied the terpenoid and cyclodextrin complex (20), and reported that α-cyclodextrin has an appropriate cavity for hemi- and monoterpenes but not for higher isoprenoids. β-cyclodextrin was reported to form stable inclusion complexes with both mono- and sesquiterpenes, while γ-cyclodextrin has the most adequate cavity for sesqui and diterpenes. On the other hand, Ajisaka et al. (21) studied the formation of inclusion complexes between various kinds of terpenoids and MCD. In the present study, the peak intensity of the terpenoid, β-cyclocitral (Peak No.17) treated with MCD was slightly smaller than with AP treatment. Hence, it is thought that the occurrence of several methyl groups in β-cyclocitral makes it difficult to form CD complexes, in accordance with previous report on the envelopment of terpene compounds by Ajisaka et al. (21).

On the other hand, the peak intensity of geranyl acetone (Peak No.18) was considerably reduced by MCD. Ionone is formed as a result of the reaction between citral and acetone, and possesses a violet-like odor. As shown in Table 3, the peak intensity of β-ionone (Peak No.20) with MCD was smaller than that with AP. The chemical structure of β-ionone is follows;

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{CH}_3 \\
& \quad \text{CH}_3 \\
& \quad \text{CH}_3 \\
& \quad \text{CH}_3
\end{align*}
\]

It can form CD complexes on either side, with its benzene ring and ketone group, and therefore MCD could reduce its peak intensity to a greater extent than that for β-cyclocitral;
Although MCD can envelope the comparatively small and simple structure molecular compounds and make them soluble, it is more difficult to form CD complexes with the molecules having ringed structure or branched-chains. Therefore, the adsorbing efficiency of MCD was less effective with larger complex compounds, and was not as efficient as AC. As shown in Table 3, the chemical structures of the majority of the detected compounds were quite complex, and therefore it was difficult for MCD to form inclusion complexes due to the steric hindrance or the large molecular size.

4) Reaction of odor components with AP

(1) Properties of AP

Unripe apples contain condensed tannins that are mainly composed of various polymerized catechins. AP is prepared from unripe apples and its main components are oligomeric procyanidins, chrologenic acid, catechin, epicatechin and phloretin glycosides (22). Proantocyanidins (catechin oligomers) possess varying degrees of polymerization and cause bitterness, astringency and browning of apple products.

The possible mechanism of deodorization by AP could be as follows. First, it is well understood that phenols and polyphenols are oxidized into the corresponding quinones by air or oxidizing enzymes. Methanethiol and allylthiol, known as good nucleophiles for 1,4-addition reactions (23), are likely to rapidly attack the generated quinones, leading to the production of the corresponding Michael adducts. This type of reaction results in an effective and quick decrease in the amount of thiols.

(2) Reaction of odor components with AP

Based on their characteristic odors, benzaldehyde, linalool, phenylacetaldehyde, β-damascenone and β-ionone are considered as the important constituents of sweet potato odor. However, their structural features reveal that
just as the thiols described above they do not react with AP by the Michael reaction. Our experimental results with AP indicated that AP did not greatly reduce the amounts of either benzaldehyde or linalool (Figure 5), which supported this theory. Thus, it may be inferred that the characteristic odor of sweet potato was not deodorized by AP.

![Graph](image)

**Figure 5** The reaction between AP and benzaldehyde (a) or linalool (b)

**4. Conclusion**

The present investigation was undertaken to resolve the problem of heavy odor of sweet potato generated during the process of functional juice production. The deodorizing mechanism of heavy odor with three deodorants was clarified. The new developed odor sensor successfully could evaluate the difference of
efficacy by three deodorants. These results show that the new developed odor sensory system could be an alternative method of evaluating the efficacy of elimination of off-odor by various deodorants. Among the three deodorants studied, AC was the most efficient at deodorizing the heavy odor of saccharified sweet potato juice. Although MCD did not deodorize the odor as much as AC, MCD is effective at stabilizing and solubilizing the functional compounds such as β-carotene. MCD may serve as an effective deodorant against the heavy odor of the intermediate substances during the production of the functional sweet potato juice.

Note: HLB value

The Hydrophile-Lipophile Balance, HLB value is derived from the molecular structure of an organic compound by evaluating the strength of polarity and non-polarity around the intermolecular force between molecules with no chemical bonds. The extent of non-polarity and polarity for a molecule is shown as an organic value (OV) and an inorganic value (IV), respectively.

The OV of one-carbon atom (actually, -CH2-) is defined as 20 and the total OV of an organic compound is calculated by multiplying the number of carbons in the compound. When there is branching in the carbon chain, which could alter the boiling point, the OV of the compound is calculated by adding a value assigned to the partial structure to the total OV mentioned above.

The extent of the IV in an intramolecule is determined by comparing the boiling point of an organic compound with that of the hydrocarbon with the same carbon number. The IV describes the effect of fractional or functional groups such as ring structure. When partial structures are repeated, the physical property is also changed, and also, because the change is regular, the IV of the compound is the total of the IV of each partial structure.

Values of OV and IV for partial structures related to the compounds detected
as odorous components of sweet potato are shown in Table 4. HLB is defined by the following equation using those values

\[
HLB = 10 \times \frac{\sum \text{(Inorganic value)}}{\sum \text{(Organic value)}},
\]

Table 4  Organic and inorganic values for fractional group in organic compound

<table>
<thead>
<tr>
<th>fractional group</th>
<th>Organic value</th>
<th>Inorganic value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>iso ramification</td>
<td>-10</td>
<td>0</td>
</tr>
<tr>
<td>tert ramification</td>
<td>-20</td>
<td>0</td>
</tr>
<tr>
<td>C= C (duble bond)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>C≡ C (triple bond)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ring (non-aromatic single ring)</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Benzen nucleus (aromatic single ring)</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>-CO─O─</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>&gt;C=O</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>-OH</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>-COOH</td>
<td>0</td>
<td>150</td>
</tr>
</tbody>
</table>

Ex. For 2-Methyl-2-butenal,

\[
HLB = 10 \times \frac{(65 \times 1 + 2 \times 1)}{(20 \times 5 - 10 \times 1)} = 7.44
\]
Chapter V

Measurement of Odor after In-vitro or In-vivo Ingestion of Raw or Heated Garlic, by Using New-type Odor Sensor, GC and Sensory Analysis

1. Introduction

The characteristic of the garlic-induced odor was evaluated by the new developed odor sensor. Despite its notorious value, the use of the garlic to the food application is still limited due to oral malodor after garlic ingestion. Garlic (*Allium sativum* L.) is widely used in the various Chinese, Korean or ethnic food recipes for its unique flavor and nutrition. However, garlic is used in few Japanese food recipes because of its malodorous-smell after ingestion. Studies have shown that various sulfur substances in garlic are the main cause of the malodor (1,2,3,4). The principal components of the malodor detected by the human nose are hydrogen sulfide, methyl sulfides, and dimethyl sulfide and allyl sulfide compounds. In particular allyl sulfide compounds are detected in breath after garlic ingestion (5). These mercaptan compounds have a strong disagreeable smell in the human nose but it is experientially known that heat-treated garlic odor is not as strong as raw garlic odor and breath after eating heat-treated garlic is not as strong as after eating raw garlic. Studies have also shown that there is a difference in the amount of volatile compounds in heat-treated garlic and fresh garlic. By clarifying the mechanism what causes the odor differences, to eliminate the malodor problem can help expansion of the garlic use to the various food categories. Prior to determination of the possible elimination mechanism, odor quality of the raw and heated garlic, and breath after the garlic ingestion were evaluated using the new developed sensor, sensory evaluation and GC-MS to determine the difference of each odor quality and identify the volatile chemicals which may result in the potential odor change.

Recently, as the mechanism of the human sense of smell has been clarified, chemical sensor technology has been developed which can perceive odors in a similar way to a
Depending on what types of sensor elements are applied, there have been many sensors developed; calorimetric sensors, metal oxide semiconductors (6) and quartz sensors (7,8). Many odor sensors are also manufactured by private companies and can be applied in a wide range of fields. These companies include UMA Airsense (Germany), Alpha MOS (France) and Cyrano Sciences Inc. (USA).

Using the technology previously developed by Ehara (9, 10) we started to develop a new odor sensor. We developed a semiconductor based odor sensor which incorporated multiple metal oxide elements that can respond to different odor molecules with a wide range of sensitivity (11). Metal oxide semiconductors have an advantage over other sensors in terms of the following properties; easily constructed and sensitive to a low vapor concentration, resistance to change in humidity and corrosive acid vapors (12). Metal oxide sensors also have a wide range of selectivity because of the abundance of electrons on the surface of the semiconductor. By combining the different semiconductor materials, the developed odor sensor can respond sensitively to a wide range of odor molecules (9, 13). This sensor is comprised of 6 different types of semiconductors, doped with catalytic metals, with different conductibility. Patterns of resistance change can be generated to create a specific profile for each group of volatile compounds in the sample. In comparison with existing odor sensor, the materials in this new sensor have a higher sensitivity to a broad range of compounds, and so the odor sensor is able to record odor compounds at very low ppb concentrations, without the extra concentration step that has been previously required. This is advantageous in capturing the same volatile compounds emitted from the samples that are detected by the human nose. This sensor property allowed us to construct a portable sensor and measure the sample odor in a very convenient form, which closely reflected the ability of the human nose to catch volatile compounds, and had the same profile as other more expensive commercial sensors. Although several brief studies have been conducted using this new odor sensor to measure the organic vapors of several food products (14,15), no studies have investigated how the

86
different response patterns can be observed on testing the odor of garlic products or human breath. Thus, in comparison with the other instrumental and psychophysical methods, proper measurement of the strength of the garlic causing odor must be investigated.

In this chapter, first samples of raw garlic and heat-treated garlic for in-vivo and in-vitro studies were evaluated by using the discrimination method by human subjects. Next, volatiles released from the in-vivo and in-vitro samples were identified by GC and GC-MS with time-release analysis. Then, using the newly developed sensor, which consists of six arrays of metal conductors, the character and the strength of the garlic odor were analyzed for the in-vivo and in-vitro samples. The correlation between the sensory evaluation, GC analysis and sensor analysis was assessed. The results showed that the sensor could successfully evaluate the odor character and strength, and the possibility of applying the odor sensor to the field of the food processing was indicated.

2. Materials and methods

1) Sensory evaluation

(1) Assessors

Thirty five assessors (all Japanese females, age ranged from 19 to 25yrs) took part in the experiment. Assessors were required to demonstrate normal odor sensitivity by first distinguishing the odors of β-phenylethyl alcohol, methyl cyclopentenolone 30ppm, isovaleric acid 10ppm, γ-undecalactone 30ppm and skatole 10ppm. All subjects signed a written consent form.

(2) In-vitro test

Two grams of raw garlic was grated, while another 2 g of garlic was heated for 1 min in a microwave oven and then grated. The grated garlic was transferred into a Petri dish, and then sealed with the plastic lid until the experiment began. A pair comparison test was chosen as the sensory method. This method was used as the most sensitive method to determine the difference between two samples for the specific product attributes. Before the experiment, each of the thirty five assessors was asked to smell the odor of raw grated
garlic, as a warm up, and told to use this smell as a standard. The assessors were blindfolded to prevent them seeing the appearance of the samples presented on a tray, and then asked to choose the sample with the stronger garlic odor. The sample order was randomized and counterbalanced across all the assessors. After the experiment, they were asked to comment on any special attribute they noticed when they chose their sample.

(3) In-vivo test

One gram of raw garlic was grated, while another 2 g of garlic was heated for 1 min in a microwave oven and then grated. One gram of grated garlic from each sample was transferred into a plastic glass, and then the garlic was ingested by chewing for 30 sec. One female (age 18yrs) ingested a whole raw garlic, and the next day the same subject ingested a whole heated garlic. Breath samples were collected in a commercial plastic bag which was manufactured by OMI ODOAIR SERVICE Co. Ltd.(Tokyo Japan). The subject held her breath for 10 sec, and then exhaled in to a bag sealed tightly around her mouth. As described in the procedure for the in-vivo test, 35 assessors were then presented with pairs of the plastic bags, one with raw garlic rinsed breath and another with heated garlic rinsed breath. The assessors were again blindfolded and asked to choose the stronger odor between two bags. Sample order was randomized and counterbalanced across assessors. After the experiment, they were asked to comment on any special attributes they noticed when they chose the sample.

2) GC analysis.

(1) Analytical conditions

The identities of the sulfur-containing gases for in-vivo and in-vitro samples were established by using GC and GC-MS spectrometry. As a standard reagent for GC analysis, the following chemicals were used as standard reagents for the GC analysis: methanethiol, dimethyl sulfide and dimethyl disulfide (Wako Pure Chemical Industries, Osaka, Japan), allylthiol and methyl propyl disulfide (Aldrich Chemical Co. Inc, USA), allyl methyl sulfide and diallyl disulfide (Tokyo Kasei Organic Chemicals, Tokyo, Japan).
Gas chromatograph (Shimadzu GC, 14B) conditions were as follows; PPE 5 ring 10%, 3.2 mm x 3.1m glass column, temperature programmed from 65°C for 3 min to 170°C at 30°C /min, the detector was an FPD (140°C) and the carrier gas was N₂ (55ml/min).

The GC-MS conditions were as follows; the GC-MS (Shimadzu GC-MS QP 1000) had a fused-silica capillary column (Supelco SPB-1), 0.32mm x 30m glass, temperature programmed from 60°C to 170°C at 30°C/min and held at 170°C for 2 min, the carrier gas was He (2ml/min), the EI mode was at 70eV, the source temperature was 180°C and the GC-MS interface temperature was 250°C.

(2) In-vitro test

Three grams respectively of raw and heated-garlic were crushed by applying pressure with a spoon. The heated-garlic was prepared by the same process as described above. 0.2g of each sample was transferred into a 125ml vial which was sealed tightly, then kept in a container temperature maintained at 23°C and then 1ml of head-space gas was removed from the vial for analysis at 0, 30, 60, 90 and 120 min.

(3) In-vivo test

One healthy subject (Japanese female, age 19yrs) with no malodor took part in this study. The subject had not ingested any garlic for 24 hrs before the study. The basic study consisted of two treatment periods; on one day the subject chewed and then swallowed 1 g of raw garlic and measurements were carried out over the next 2hrs. The next day the subject’s breath was measured over a 2 hrs period after ingesting 1g of heated-garlic. The subject’s breath was collected in syringes following the method described by Aoki (16). Samples were collected immediately before garlic consumption and then after 0, 30, 60, 90, 120 min.
3) Odor sensor analysis

(1) Odor sensor preparation
   Same as chapter 3

(2) In-vitro test
   The garlic sample measurements were conducted, using the same as in the sensory experiment.

(3) In-vivo study
   The subject’s odor was measured using the same sample obtained for the sensory experiment.

3. Results and discussion

1) Sensory evaluation of the garlic odor

   (1) In-vitro and in-vivo tests
   A significant majority of the judges indicated that raw garlic had a stronger odor (Binomial test, 33/35, p< 0.001) and also that it produced a stronger breath after garlic ingestion (Binomial test, 27/35, p<0.001). These results indicated that the odor of raw garlic odor is stronger than that of heated-garlic, for both in-vitro and in-vivo samples. Interestingly, the result of the in-vivo study showed a less significant difference than for the in-vitro study. The assessors commented that the garlic odor of the in-vivo study was clearly different from that of the in-vitro study and also very unpleasant. They also commented that the odor of heat-treated garlic and the breath rinsed with heated-garlic became weaker. Considering these results, it can be concluded that judges perceived clear differences in the odors of these four samples from the in-vitro and in-vivo experiments.

2) GC analysis of garlic

   (1) In-vitro test
   The amount of each compound was calculated at different time intervals, except for allyl methyl sulfide and 3-(allylthio) propionic acid (Table 1). Methanethiol, dimethyl
sulfide and allylthiol are low-molecular sulfur compounds (LMSCs) and were present in a slightly larger quantity than allyl methyl sulfide and dimethyl disulfide, which are relatively high-molecular sulfur compounds (HMSCs) in both raw garlic and heated garlic sample.

Although there was little difference in the amount of LMSC between the raw and the heated garlic sample, there was a clear difference in HMSC. Methyl propyl disulfide, and diallyl disulfide which is the main decomposition product from allicin, were found in both the raw and heated garlic, but the concentrations were higher in the raw garlic. The reason may be because the formation of these sulfur compounds would be stopped by thermal inactivation of the enzyme alliinase. This assumption was supported by the fact that these compounds could not be detected when the heating time was prolonged to enhance thermal inactivation (data not shown). GC data for the change in the amount of sulfur compounds followed the typical enzyme alliinase reaction passage. In raw garlic alliinase is activated by grating or cutting of garlic. Allylcystein sulfoxide or alliin is rapidly converted into allicin by its enzymatic reaction. Allicin is further converted into higher molecular disulfide compounds, which are attributed to the unique pungent odor of garlic. In the GC data, allylthiol was detected at 0 min but soon began to decrease. This shows that grating induced a rapid conversion of allicin to allylthiol but the conversion decreased significantly as the reaction progressed. The amount of the other LMSCs showed little change, although there was a slight increase in dimethyl sulfide after 60 min. For HMSCs, diallyl disulfide increased continuously to reach a maximum after 60 min. This fact confirmed that when raw garlic was grated the change of alliin into allicin occurred immediately, followed by conversion into HMSCs as the enzymatic reaction proceeded.

Table 1  Time course of volatile sulfide component formation in raw and heat-treated garlic (in the in-vitro test)
These volatile compounds were characterized and quantified by GC-MS under the analytical conditions described in the text. Values are expressed in ppm; tr represents ‘trace’.

The heated garlic contained higher concentrations of LMSCs such as methanethiol, dimethyl sulfide and allylthiol than the raw garlic. The amounts of methanethiol and allylthiol from the heated garlic increased over time. Also the rate of increase of the thiol compound was considerably greater for heated garlic than for raw garlic. This fact suggests that the production of allylthiol can be accelerated more by physical conditions such as heating or the accompanying reactions, rather than by the enzymatic reaction itself. For HMSCs, allyl methyl disulfide and methyl propyl disulfide increased for 30min and then decreased gradually, although other HMSC components were not detected significantly.

(2) In-vivo test

Table 3 shows the changes in the amounts of the volatile sulfide components over time in the in-vivo test. Immediately after ingesting the raw garlic, the concentrations of

<table>
<thead>
<tr>
<th>Compound</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw garlic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl mercaptan</td>
<td>0.0280</td>
<td>0.0280</td>
<td>0.0270</td>
<td>0.0270</td>
<td>0.0260</td>
</tr>
<tr>
<td>Dimethyl sulfide</td>
<td>0.0200</td>
<td>0.0190</td>
<td>0.0174</td>
<td>0.0275</td>
<td>0.0311</td>
</tr>
<tr>
<td>Allyl mercaptan</td>
<td>0.0467</td>
<td>0.0359</td>
<td>0.0281</td>
<td>0.0232</td>
<td>0.0209</td>
</tr>
<tr>
<td>Allyl methyl sulfide</td>
<td>0.0040</td>
<td>0.0030</td>
<td>0.0040</td>
<td>0.0056</td>
<td>0.0048</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>0.0091</td>
<td>0.0112</td>
<td>0.0118</td>
<td>0.0122</td>
<td>0.0126</td>
</tr>
<tr>
<td>Methyl propyl disulfide</td>
<td>0.0845</td>
<td>0.1140</td>
<td>0.1120</td>
<td>0.0930</td>
<td>0.0830</td>
</tr>
<tr>
<td>Diallyl disulfide</td>
<td>0.6390</td>
<td>1.1900</td>
<td>1.3800</td>
<td>1.1750</td>
<td>0.9200</td>
</tr>
<tr>
<td><strong>Heat treated garlic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl mercaptan</td>
<td>0.0420</td>
<td>0.0755</td>
<td>0.0862</td>
<td>0.0957</td>
<td>0.1068</td>
</tr>
<tr>
<td>Dimethyl sulfide</td>
<td>0.0451</td>
<td>0.0800</td>
<td>0.0767</td>
<td>0.0764</td>
<td>0.0737</td>
</tr>
<tr>
<td>Allyl mercaptan</td>
<td>0.0597</td>
<td>0.1311</td>
<td>0.1565</td>
<td>0.1667</td>
<td>0.1764</td>
</tr>
<tr>
<td>Allyl methyl sulfide</td>
<td>0.0080</td>
<td>0.0064</td>
<td>0.0064</td>
<td>0.0064</td>
<td>0.0064</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>0.0079</td>
<td>0.0070</td>
<td>0.0071</td>
<td>0.0066</td>
<td>0.0050</td>
</tr>
<tr>
<td>Methyl propyl disulfide</td>
<td>tr.</td>
<td>tr.</td>
<td>tr.</td>
<td>tr.</td>
<td>tr.</td>
</tr>
<tr>
<td>Diallyl disulfide</td>
<td>0.0758</td>
<td>0.1290</td>
<td>0.1060</td>
<td>0.0920</td>
<td>0.0658</td>
</tr>
</tbody>
</table>
LMSCs were highest especially methyl sulfide and allyl sulfide, but they soon started decreasing. In contrast in the heat-treated garlic eating, lower concentrations of LMSCs (methanethiol, allylthiol and allyl methyl sulfide) were observed with little detected after 30 min. Therefore these results indicate that the mouth normally contains a small concentration of methanethiol and dimethyl sulfide (Figure 2) but immediately after garlic eating, higher concentrations of methanethiol and allylthiol and lower concentrations of allyl methyl sulfide, allyl methyl disulfide, and diallyl disulfide are produced. These higher concentrations of LMSCs, especially allyl compounds, were uniquely observed in the breath after garlic eating, which is different from pathological breath (17). Thus, the malodorous smell after eating garlic is thought to be attributed to LMSCs. The data also showed that the LMSC concentration in the breath was higher after ingesting the raw garlic solution than the heat-treated garlic. This supports the results of the sensory analysis where the assessors indicated a difference in odor quality difference for the breath after eating heat-treated garlic compared with raw garlic. GC results also showed that no HMSCs were detected after either raw or heat-treated garlic ingestion, except for diallyl disulfide which was found immediately after eating raw garlic. The small amounts of HMSCs suggest that the alliinase enzyme reaction and the following reaction could be affected by the biochemical conditions in the mouth and that HMSCs would not be produced.

Table 2  Time course of volatile sulfide component formation in raw garlic and heat-treated garlic (in-vivo test)
These volatile compounds were characterized and quantified by GC-MS under the analytical conditions described in the text. Values are expressed in ppm; nd represents 'not detected'.

### 3) Odor sensor analysis

#### (1) Garlic odor measurement

Garlic odor is different from lemon (described in chapter 3) in terms of the quality of the odor. The garlic odor in the in-vitro and in-vivo studies was measured with the developed apparatus.

**a. In-vitro test**

When raw garlic is cut or grated, it smells of specific pungent odor. However, when heat-treated garlic is cut or grated, it has a very weakly pungent odor which is different from that of raw garlic. In the in-vitro test the S-value of the raw garlic (S=39995) was much higher than for the heat-treated garlic (S=16889) (Figure 6), although the graph does not have a spear shape. The F-value of the raw garlic was 1.955 compared with 4.302 for the heat-treated garlic. These results are comparable to the results of the sensory analysis showing that heating moderated the garlic’s pungent odor of garlic.
b. In-vivo test

Typically human people's breath after ingesting raw garlic is much more malodorous and unpleasant compared with after ingesting heat-treated garlic. Figure 7 shows the results of the in-vivo experiment, after ingesting garlic. The intensity of odor after raw garlic was ingested was stronger ($S=35819$) than for the heat-treated garlic ($S=15230$). Both values were much higher than that of the breath odor before any garlic was ingested ($S=1463$). The F-value for the heat-treated garlic ($0.9805$) was higher than for the raw garlic ($0.8640$), indicating that the heat-treated garlic had a better quality odor. However, the shape of the spider graph results for the raw garlic and heat-treated garlic in the in-vivo study were fairly similar, compared to the different shapes produced in the in-vitro study. The difference between the S-values for raw garlic eating and heat-treated garlic eating in the in-vivo test ($s=20589$) was smaller than in the in-vitro test ($s=23106$). Also the difference between the F-values was lower in the in-vivo test ($F=0.1165$) than in the in-vitro test ($F=2.347$). This indicates biochemical interactions in the in-vivo test reduced the difference in garlic odor between the raw garlic and the heated garlic.

In the sensory test the assessors mentioned that mouth odor after eating garlic was fairly unpleasant but breath odor after eating heat-treated garlic was not so unpleasant. These comments agreed with the results of the sensor analysis.

Responses from sensors 2 and 4 were small, and those from sensors 3 and 6 were greatly enlarged for pleasant smells, and so the radar graph displays the shape of a spear. On the other hand, for unpleasant smelling substances, the radar graph shape is a similar irregular shape due to the small response of CH 6 and CH 3. The formula calculating F-value was derived from some experiments. It was not applied to all food odors and new formulae must be derived for different types of odor. A two-dimensional map consisting of F- and S-values is used to derive the comfortable zones of different odors, and some mixed odors. Furthermore, the pleasantness or unpleasantness of odor is decided by human senses and this odor is then clarified by this apparatus.
Figure 5  Spider graphs showing the resistance values (mV) from the six sensors outputs (CH1-CH6) of the electronic nose analysis of the in-vitro study samples of 0.2 g/ml grated raw garlic (—) and heat-treated garlic solution (---); the F-values and S-values are also shown.

Figure 6  Spider graphs showing resistance values (mV) from the 6 sensors outputs (CH1-CH6) of the electronic nose analysis of in-vivo study samples of a subject’s breath.
after rinsing with 1ml of pure water (- - -), 1g/ml grated raw garlic solution (-----) and grated heat-treated garlic solution (-----); F-values and S-values are also shown. The values of 6 sensors outputs are shown in the table.

4. Conclusions

In this chapter, we developed a sensor analysis method to measure the changes in odor quality caused by different treatments of garlic. The possibility of characterizing the garlic odor in-vitro and in-vivo was demonstrated. By using the electronic sensor, we verified that an electronic sensor could define the characteristics and strength of the odor in terms of F-value and S-value. The electronic sensor’s F and S values showed that garlic odor characteristics and strength were different in in-vitro and in-vivo. For the in-vitro study, F value was lower and S value was higher for raw garlic than for heated garlic and this result matched the GC and sensory analysis. In the in-vivo study, S-value and F-values of both breath samples were lower than those of the in-vitro study. This suggests that raw garlic has strong odor and its odor changes into unpleasant odor in in-vivo. On the other hand, garlic odor became moderate with heating and so when heated garlic was ingested, it did not produce a strong unpleasant odor.

Again this was supported by sensory and GC analyses. The sensory results showed that the strength of garlic odor was stronger for raw garlic than for heat-treated garlic for both the in-vivo and in-vitro studies. The GC analysis showed higher values of HMSC in raw garlic immediately after grating, and little difference in LMSC between raw and heated garlic. HMSCs such as allyl methyl disulfide, methyl propyl disulfide and diallyl disulfide largely contributed to the specific odor and sharp taste of the garlic, thus the GC result confirmed the sensory result. On the other hand, in the in-vivo test, LMSC values were higher after eating raw garlic, but HMSCs were hardly detectable for either raw garlic or heat-treated garlic eating samples. When garlic is ingested, various LMSC increase and these LMSCs are considered to be the major cause of malodorous breath after garlic ingestion, which was detected in the sensory analysis.
This study proves that the developed odor sensor has a feature for evaluating product odor intensity and quality in terms of pleasantness. In the rapid process of food product manufacturing, there are many attributes to be monitored, but the most important criteria is to keep the product quality favorable for consumers. Usually both sensory and GC analysis have been combined to measure the quality of odors. Thus, this new odor sensor analysis has the advantage of being a convenient method. The S- and F-values are useful concepts, and the formula is derived experimentally so it is applicable to odors of some kinds of food products. It could also be possible to express odor characteristics by multiple criteria. Thus, in future experiments, the odor of the various food products should be investigated to find how odor sensor output and the results of the analysis of odor compounds are related.
Chapter VI
Deodorization of Garlic-Induced Oral Malodor by Mushroom Extract

1. Introduction

In this chapter, based on the information of odorous compounds obtained in the previous chapters, appropriate deodorants were selected and their efficacy against oral malodor were evaluated. In this study, the commercial odor sensor (FOX 4000, Alpha-MOS Co., Ltd) was chosen to evaluate the degree of deodorization for subjects' breath. Although this instrument does not provide specific information of qualitative differences between samples, it is extensively used as an alternative method of human nose for discrimination of multiple samples due to its combined powerful multivariate statistical discrimination analysis. Since the evaluation of oral malodor is the least type of the sample which human assessor wants to evaluate, application of odor sensor should be developed in correlation with sensory results or to specific data of volatile compounds.

Halitosis is a common health problem that can affect one's social interactions. Oral malodor originating from oral cavity is the major cause of halitosis (1). Despite its unique flavor and nutritious bioactive components with antioxidant properties, garlic is one of the causes for oral malodor. Allicin in garlic clove leads to the production of volatile sulfur compounds (VSCs) such as methanethiol, allylthiol, allyl methyl sulfide and allyl disulfide in small amount (2,3,4,5). While various compounds, such as hydrogen sulfide, methanethiol, dimethyl sulfide, n-dodecanol, n-tetradecanol, phenol, indole, diphenylamine, pyridine and others are the causes of oral malodor (6, 7), malodor is mainly attributed to volatile sulfur compounds (VSCs), such as hydrogen sulfide, methanethiol and dimethyl sulfide.

Studies show that some bacteria inside oral cavity metabolize food constituents such as sulfur amino acids, and produce VSCs that contribute to unpleasant smell (8). The odor intensity is significantly associated with the amount of VSC level in oral cavity (9, 10). Previous chapter showed the association of VSCs induced by garlic ingestion with
the cause of oral malodor.

Since VSCs are the major causes of malodor, suppression in production of VSCs is an effective strategy to prevent oral malodor. Researchers have demonstrated that some plant extracts containing a lot of polyphenols and phenolic derivatives have efficient deodorizing activity against malodor (11, 12). Tea catechins have been shown to have a significant effect on depressing methanethiol production (13). The mushroom extract, also known to contain a large amount of polyphenol has been demonstrated to suppress malodor from mouth and environment. In addition, its deodorization mechanism has been attributed to polyphenol oxidase enzymatic reaction involving o-quinone derived from polyphenolic compounds and methanethiol (14, 15).

In the present study, we monitored the oral malodor difference detected in the breath air of individual subjects after garlic and mushroom extract ingestion over time. To evaluate odor-associated chemical compounds, and quantify the odor strength that decreases at rates depending on its reaction with polyphenols and natural dispersion, a combination of electronic sensor, sensory evaluation and gas chromatography (GC) analyses were employed. Although GC analysis can quantitatively measure the level of VSCs, the subjective organoleptic examination (16) is also necessary to evaluate the magnitude of oral malodor directly. The emerging electronic sensor technology that is based on changes in resistance of multiple sensors due to interaction with volatile odorant molecules, is a convenient procedure to quantify the magnitude of odor, and has been applied for evaluation of food product quality (17,18,19). The combined pattern of resistance change from multiple sensors is processed and statistically analyzed by Principal Component Analysis (PCA) to discriminate and identify the odor pattern. A combination of these 3 methods provided reproducible and accurate evaluation of odor intensity and its associated compounds in human exhaled breath. Furthermore, we present the ab initio calculations that suggest an addition reaction mechanism between thiol and polyphenolic compounds. This mechanism successfully explains the degradation speed of difference between methanethiol and allylthiol.
2. Materials and methods

1) Breath sampling

(1) Subject

One subject (Japanese female, 24 years old) participated in measurement of malodor strength for single subject. Eight subjects (8 Japanese females, 18 years old) participated in measurement of malodor strength for multiple subjects. Subjects were chosen on the basis that they did not have halitosis. They did not ingest food for 4 hrs prior to study and avoid onions and garlic for 24 hrs before study. All subjects signed a written consent form.

(2) Preparation of garlic solution

Commercially available raw garlic was diced and crushed by applying pressure with a spoon. The garlic solution was prepared by dissolving 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0g into 20ml of distilled water, respectively.

(3) Preparation of mushroom extract

Mushroom extract was prepared as follows; 400g of mushrooms (Agaricus bisporus) were added to 500ml of boiling water. After boiling for 3 hrs, the extract was cooled, filtered and dried. Then 21% (W/W) aqueous solution of mushroom extract was prepared as a stock solution. Zero, 2, 5, 8 and 12ml of stock solution was diluted with distilled water to make up to 100ml of each concentration of mushroom extract solution, respectively.

(4) Breath sampling

In the experiment 1, breath sample for sensor analysis was collected as follows; subject rinsed each concentration of the garlic solution for 1 min. Breath samples were collected by using commercially available plastic bags (OMI ODOAIR SERVICE Co. Ltd. Tokyo Japan), which were polypropylene tubes (1.4 × 8 cm) connected via stopcock. Subject breathed into the tube, and the tube was closed tightly. Before rinsing with garlic solution, the subject’s breath was also collected as a control. Collection process was
repeated for 4 days.

In the experiment 2, the same subject rinsed each concentration of mushroom extract solution, after rinsed with 1 g of garlic in 20ml of distilled water each time. On each occasion, breath samples were then collected using the above procedure after rinsing and at 10min later.

In the experiment 3, the breath sample from 8 subjects was collected in the same manner before, after and at 10min, but only rinsed with the concentration of 1g of garlic in 20ml of distilled water and 8ml of 21% mushroom extract diluted with water to 100ml.

In the experiment 4, the breath samples of 5 subjects were collected in the same manner as above for sensory evaluation before and after rinsing. The subject rinsed the garlic solution (with concentration of 0.06g of garlic in 20ml distilled water) and 100ml of the mushroom extract solution (with the concentration of 2ml of the 21% mushroom extract stock solution diluted to 100ml with distilled water). The breath sample after rinsed with the garlic solution and water was also collected as a control.

In the experiment 5, breath sample was collected from same 5 subjects for GC analysis by using Aoki’s method (20) at before, 10, 40 and 70min. Each subject rinsed her mouth with 2.0g of grated raw garlic dissolved into 20ml of pure water and 200mg of 21% mushroom extract stock solution dissolved in 50ml of distilled water. Exhaled air was collected respectively, and submitted for GC analysis.

2) GC analysis procedure for the thiol compounds change under in-vitro condition

In the experiment 6, measurement of the reaction rate between thiol compound and mushroom extract was carried out under in-vitro condition. The test solution for GC analysis was prepared as follows.

Methanethiol (or allylthiol) 1 mg was dissolved in 1 ml benzene and it was prepared to 6ppm (methanethiol) or 2ppm (allylthiol) in a 125ml of vial bottle. Six ml of 21% mushroom extract stock solution was used as a mushroom extract solution. Each solution was mixed respectively and subjected to GC analysis to measure the change in
concentration over time for methanethiol or allylthiol. The control was also prepared by mixing thiol solution with 6 ml pure water in a 125ml of vial bottle and subjected to GC analysis. Both the control and the test solution were adjusted to pH 7.2 with sodium hydroxide.

The following chemicals were used as an internal standard; methanethiol, dimethyl sulfide and dimethyl disulfide (Wako Pure Chemical Industries, Osaka, Japan), allylthiol (Aldrich Chemical Co. Inc, USA), allyl methyl sulfide (Tokyo Kasei Organic Chemicals, Tokyo, Japan)

3) Sensory evaluation procedure

Twelve assessors participated in the study. After assessors were trained to score the intensity of garlic odors at various concentrations, they were given one set of plastic bags filled with breath samples on a tray. They evaluated 4 sets of breath samples: (a) breath sample before rinsing, (b) breath sample after garlic solution rinsing, (c) breath sample after garlic solution and mushroom extract rinsing, (d) breath sample after garlic solution and water rinsing. The breath sample bags were coded with randomly generated 3-digit codes and presented in random order. Assessors were instructed to rate odor intensity of each breath sample in the order starting from the left, on the 5 point scale – 0 is no odor; 1 barely noticeable odor; 2 slight but clearly noticeable odor; 3 moderate odor; 4 strong odor; 5 extremely foul odor (21). The assessors waited at least 1 min between stimuli, and each session lasted a total of 5 - 10min.

4) Analytical condition

(1) Odor sensor analysis

A Fox 4000 with ACU 500 humidifier was used. This instrument is equipped with 18 metal oxide sensors, inside 3 chambers. Each chamber contains 6 metal oxide sensors (p, flat-plate sensor; T, tubular sensor; SY, non doped tin oxide sensor, a temperature sensor and a relative humidity sensor). To provide a constant flux of vector gas
(humidified synthetic air) through electronic sensors, a humidifier (air conditioning unit, model 1997, Alpha-MOS) was used. The analytical parameters such as breath sample quantity, headspace generation time, temperature, flow rate and injection time were determined. Sensors were carefully monitored to make sure that they returned to baseline during preliminary trials, and the optimum time was found to be 5 min after the previous breath sample injection. The data collection time lasted 120 sec. The carrier gas flow was set to 150ml/min and relative humidity was regulated at 20%.

Although the FOX electronic odor sensor is equipped with 18 sensors, only the results of a limited number of metal oxide sensors were chosen for further analysis, after eliminating sensors with higher multi-colinary by using stepwise regression analysis. SPSS version 11.0 was used to perform the data processing.

(2) GC analysis

Compounds were identified using GC and GC mass spectroscopy. The gas chromatography (Shimadzu GC, 14B) condition was as follows; PPE 5 ring 10%, 3.2 mm x 3.1 m glass column, temperature programmed from 65°C for 3 min to 170°C at 30°C/min. Detector was FPD (140°C) and carrier gas was N2 (55 ml/min). GC-MS conditions were as follows; GC-MS (Shimadzu GC-MS QP 1000) with fused-silica capillary column (Supelco SPB-1), 0.32 mm x 30 m glass, temperature programmed from 60°C to 170°C at 30°C/min and held at 170°C for 2 min. Carrier gas, He (2 ml/min) with a fused silica capillary column (Supelco, SPB-1) temperature maintained at 60°C/min and 170°C for 3 min. EI mode at 70eV. Source temperature, 180°C, GC-MS interface temperature, 250°C.

3. Results

1) Electronic odor sensor analysis

(1) Experiment 1

In the experiment 1, the sensitivity of the odor sensor against odor intensity after rinsed with different concentrations of garlic solution was studied for a single subject.
Principal component analysis (PCA), which can reduce the complexity of the multiple datasets to a few dimensions, was used to evaluate the data of odor sensor that is based on relative resistance changes (\%Δ R/R) from the multiple metal oxide sensors. Data of 18 sets of sensors were reduced to that of 2 sensors by step-wise regression analysis. PCA was performed on the covariance matrix. Principal component score plot, PC 1 and 2 was shown in Figure 1. PC 1 and 2 were chosen for this representation since the importance of the two principal components was 98.92% and 0.61% and the first two principal components explained most of the variance among breath samples. According to the great importance of PC 1, the data distribution is nearly one-dimensional. In Figure 1, breath samples formed two big clouds. The higher the garlic concentration, the more to the right of the axis the breath sample is located. Controls (breath samples before garlic
rinsing) were grouped together more tightly on the left side of the x-axis. A breath sample, rinsed with a concentration of 0.5g dissolved in 20ml of distilled water, was measured as an outlier since the concentration was so small that the sensors could not detect it well. From the tendency of the PCA graph, it can be concluded that PC 1 represents a concentration of VSC compounds in the breath samples.

To compare the breath samples distance, a similarity index formula was used to measure the relative difference between the breath samples (22). The similarity index is a compilation of all Euclidean distances between two samples. The greater the variation between samples is, the bigger the similarity index becomes. Although the similarity index does not show any statistical significance, by using Euclidean distances, it gives a scale with which it is easy to interpret the relative similarity between individual samples, taking into account the variances among samples. Ordinary Euclidean distance was calculated on the raw data of the sensor response for each concentration.

Comparisons of similarity indices indicate that the all breath samples after garlic rinsing yielded obvious difference from its control. Breath samples with garlic concentration of 1.0g in 20ml distilled water yielded a similarity index of 5.5, which is much bigger than that for garlic concentration of 0.5g in 20ml distilled water and values became smaller as garlic concentration becomes higher from 2.0g to 4.0g.

These results suggest that odor intensity becomes higher as the garlic concentration increases but the intensity may reach a plateau as the VSC concentration in the mouth is saturated. From this result, garlic concentration was fixed at 1.0g in 20ml distilled water for the following experiments.

(2) **Experiment 2**

Deodorization effect of mushroom extract with different concentrations was investigated for the same subject. The quantity of the garlic solution was fixed to 1.0g in 20ml distilled water, while mushroom extract concentrations were varied with zero, 2, 5, 8 and 12ml of stock solution diluted with distilled water to make it to 100ml of each test solution respectively. PCA models were calculated and plots were presented in Figure 2.
N, N' and N'": Breath samples obtained before, after and at 10min rinsing with the garlic solution (1 g of garlic dissolved in 20ml of distilled water).

2, 5, 8 and 12: Breath samples obtained before rinsed with the garlic solution (1 g of garlic dissolved in 20ml of distilled water) and mushroom extract of 2, 5, 8 and 12ml of 21% stock solution diluted with distilled water to 100ml respectively.

2', 5', 8' and 12': Breath samples collected after rinsing with the garlic solution and mushroom extract of 2, 5, 8 and 12ml respectively.

2", 5", 8" and 12": Breath samples collected at 10min after rinsing with the garlic solution and mushroom extract of 2, 5, 8 and 12ml respectively.

Again PC 1 and 2 were chosen for this representation since the importance of the two principal components was 86.43% and 10.82%. PCA result clearly separated the breath samples into 3 different groups. Breath samples rinsed with the garlic solution were found at the far right side of the x-axis. On the other hand, the controls were grouped on the far left side of PC 1. The breath samples after rinsed with the garlic solution and mushroom extract were located around the y-axis through the zero origin. Thus, PC 1 can...
be also interpreted as representing the VSC concentrations in the breath samples.

From Figure 2, it is obvious that the VSC concentrations in the breath samples reached a peak after garlic rinsing and didn’t decrease much after 10 min, but the VSC concentration for the breath sample after mushroom extract rinsing decreased drastically. No large difference of the deodorizing effect among various concentrations of mushroom extract was observed. Therefore, the concentration of 8 ml of the stock solution diluted with distilled water to make it to 100ml aqueous solution was chosen for the following experiment for convenience.

(3) Experiment 3

Measurement was conducted to demonstrate the deodorant ability of mushroom extract for multiple subjects. Results of eight subjects are shown in Figure 3 for breath samples collected before, after and at 10 min.

Figure 3  Principal component score plot for breath odor of eight subjects rinsed with the garlic solution (1 g of garlic dissolved in 20ml of distilled water) and mushroom extract (8ml of 21% stock solution diluted with distilled water to 100ml) as obtained with electronic sensors used.

W1: Group of breath samples for 8 subjects collected before rinsing with the garlic solution.
G0: Group of breath samples for 8 subjects collected after rinsing with the garlic solution.

G10: Group of breath samples for 8 subjects collected at 10min after rinsing with the garlic solution.

W2: Group of breath samples for 8 subjects collected before rinsing with the garlic solution and mushroom extract.

M0: Group of breath samples of 8 subjects collected after rinsing with the garlic solution and mushroom extract.

M10: Group of breath samples of 8 subjects collected at 10min after rinsing with the garlic solution and mushroom extract.

PC 1 and 2 were chosen for this representation since the importance of the two principal components was 76.50% and 19.46%, which explained most of the variance among the breath samples. In this PCA map, breath samples can be sorted into three different clouds; the group in which samples were collected after garlic rinsing (G0), the group before the mushroom rinsing and after the garlic rinsing at 10min (W2 and G10) and the group before the garlic rinsing, and after mushroom rinsing and at 10min (W1, M0 and M10). Although plot shows the strong deodorizing effect of mushroom extract toward multiple subjects, the fact that each subject within a group was not closely spaced in the PCA map suggests that there was considerable variance in the output of the sensor values as a result of the inherent variability of VSC quantities in each subject’s breath. Thus, it is important to account for physiological difference of each subject and identify the outlier.
Figure 4  $T^2$ chart of breath odor samples of eight subjects at $\alpha=0.01$, after rinsing with the garlic solution (1 g of garlic dissolved in 20 ml of distilled water) as obtained with instrument sensors used.

NBE S1~NBE S8: Breath samples of eight subjects collected before rinsing with the garlic solution.

NOM S1~NOM S8: Breath samples of eight subjects collected after rinsing with the garlic solution.

N10 S1~N10 S8: Breath samples of eight subjects collected at 10min. after rinsing with the garlic solution.

Figure 5  $T^2$ chart of breath odor samples of eight subjects at $\alpha=0.01$, after
rinsing with the garlic solution (1 g of garlic dissolved in 20ml of distilled water) and mushroom extract ((8ml of 21% stock solution diluted with distilled water to 100ml) as obtained with instrument sensors used.

PBE S1∼PBE S8: Breath samples of 8 subjects collected before rinsing with the garlic solution and mushroom extract.

P0M S1∼P0M S8: Breath samples of 8 subjects collected after rinsing with the garlic solution and mushroom extract.

P10 S1∼P10 S8: Breath samples of 8 subjects collected at 10min after rinsing with the garlic solution and mushroom extract.

$T^2$ charts (Figure 4 and 5) provide a clear overview of the magnitude by which each subject is deviated from the desired mean at the 5% level of confidence (23, 24, 25). If variables do not exceed the desired mean, the $T^2$ control chart is in control, and the process is supposed to be set on a stable variability. The first control chart in Fig 4 shows the data from 8 subjects after rinsed with the garlic solution. $T^2$ value, which is conveniently labeled as an odor unit, accounts for deviations of each 8 subject’s average. Upper control limit represents $2\sigma$ and $T^2$ value higher than upper control limit shows the subject deviated from the target value. For $\alpha=0.01$, computed $T^2$ values of all 8 subjects exceeded the control limit, while values at 10 min fell under the control limit.

These results show that corresponding means of sensor outputs for the breath odor after garlic solution rinsing were significantly different from the means of the control, but the intensity level of the breath odor returned to the control level after 10min ($T^2>\text{UCL}$).

Figure 5 shows a $T^2$ chart at $\alpha=0.01$ breath samples after rinsed with mushroom extract before, after and at 10 min. Although there is one outlier, it is worth noting that the breath samples of the subjects who rinsed with mushroom extract fell under the desired limit, regardless of the collection time. Thus, by using a $T^2$ control chart, we could determine whether the majority of subjects fell under the desired criteria, allowing for
individual variances and proved the strong deodorant effect of mushroom extract.

2) Sensory evaluation

(1) Experiment 4

Breath samples of 5 subjects after rinsed with the garlic solution and mushroom extract were also analyzed by using the sensory evaluation. ANOVA with LSD post testing compared the differences in increase of the intensity between the breath samples over time for different subjects. ANOVA [4 main factors: 3 treatments (without and with mushroom extract and water) x 2 sampling times (before and after) x 5 subjects x 12 assessors] was used and their interactions were calculated. The result revealed that significant effect among treatments, times, assessors but no significant difference among subjects (P<0.001, P<0.001, P<0.001 and P>0.05, Table 1).

The post hoc comparisons using LSD test was calculated to compare the difference among treatments. The result revealed that the breath odor after mushroom extract rinsing was rated as significantly weaker than the breath samples with water rinsing (P<0.001).

Since main effect of “sampling time” was significant (P<0.001), it may lead us to speculate that there is no significant difference of odor intensity after mushroom extract rinsing. However, interaction between “time” x “treatment” was also significant (P<0.001). By examining the average value of each subject after mushroom extract rinsing, it appears that increase of the intensity score after mushroom extract rinsing is much smaller than that of without mushroom extract rinsing. This concludes that mushroom extract rinsing could effectively suppress the intensity of the breath odor after the garlic solution rinsing.

3) Correlation between electronic odor sensor and sensory analysis data

The next problem is whether the result of sensors is correlated with that of the odor intensity derived from sensory measurements. The partial least squares (PLS) method
determines the correlation between multiple breath samples of odor intensity scores from sensory evaluation and the output sensor values from instrument sensors (26). If there is high correlation between the two outputs, sensor values can predict the odor intensity of unknown breath samples estimated by the appropriate correlation model.

Figure 6 shows the PLS model derived from the output value versus the average intensity scores of sensory evaluation. The x-axis represents the average odor intensity obtained by sensory evaluation and the y-axis represents the predicted output value of the sensor. This PLS model resulted in a correlation of 0.85 between the two measurements. And its equation of the model was \( Y_1 = 0.596 + 0.725X_1 \).

This figure shows that the sensor assessment accurately predicted the odor intensity scores of different odor breath samples as assessed by human evaluation.

![Figure 6](image)

**Figure 6**  PLS model derived from the results output value from instrument sensors for 8 subjects versus the averaged intensity scores of 12 assessors from sensory evaluation

4) GC analysis

(1) Experiment 5

The GC analysis was conducted for the determination of change in the VSC
concentration after mushroom extract rinsing in human breath by using Aoki's method (20). Breath sample of 5 subjects without and with mushroom extract rinsing was analyzed. Typical gas chromatograms of breath sample of subjects after rinsing with garlic solution under without (left) or with (right) mushroom extract rinsing condition are shown in Figure 7.

![Gas Chromatogram](image)

Figure 7  Gas chromatogram analysis of the expirations of the subjects assessed after 10 min of ingesting garlic under rinsing without (left) or with (right) mushroom extract condition

Analytical conditions are described in the text. Peak components were as follows: 1. methanethiol; 2. dimethyl sulfide; 3. allylthiol; 4. allyl methyl sulfide; 5. dimethyl disulfide.

Five peaks were identified by GC-MS with the standard reagent, corresponded to methanethiol, dimethyl sulfide, allylthiol, allyl methyl sulfide and dimethyl disulfide,
respectively (Figure 7). The molecular ion (M⁺) values of each substances identified by GC-MS were as follows: methanethiol M⁺ = m/z 48; dimethyl sulfide M⁺ = m/z 62; allylthiol M⁺ = m/z 74; allyl methyl sulfide M⁺ = m/z 88; dimethyl disulfide M⁺ = m/z 94. Values of 5 identified compounds for 5 subjects were analyzed by separate ANOVA (3 factors: 2 treatments x 3 collected times x 5 subjects) for each compound (Table 2).

Table 1  Intensity scores of 12 assessors for 5 subjects’ breath with and without mushroom extract rinsing, and water rinsing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No mushroom rinsing*</th>
<th>Water**</th>
<th>Mushroom rinsing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Subject 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.333</td>
<td>3.083</td>
<td>1.500</td>
</tr>
<tr>
<td></td>
<td>(0.1304)</td>
<td>(0.1304)</td>
<td>(0.1304)</td>
</tr>
<tr>
<td>Subject 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.500</td>
<td>3.167</td>
<td>1.583</td>
</tr>
<tr>
<td></td>
<td>(0.1304)</td>
<td>(0.1304)</td>
<td>(0.130)</td>
</tr>
<tr>
<td>Subject 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.333</td>
<td>2.917</td>
<td>1.417</td>
</tr>
<tr>
<td></td>
<td>(0.130)</td>
<td>(0.130)</td>
<td>(0.130)</td>
</tr>
<tr>
<td>Subject 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.417</td>
<td>3.083</td>
<td>1.500</td>
</tr>
<tr>
<td></td>
<td>(0.1304)</td>
<td>(0.1304)</td>
<td>(0.1304)</td>
</tr>
<tr>
<td>Subject 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.417</td>
<td>3.083</td>
<td>1.417</td>
</tr>
<tr>
<td></td>
<td>(0.1304)</td>
<td>(0.1304)</td>
<td>(0.1304)</td>
</tr>
</tbody>
</table>

Scores are assessed in the scale of 1 to 5 ± standard error of the mean of five subjects.

Scores are assessed in the scale of 1 to 5 ± standard error of the mean of five subjects.

*p<0.05: Significant difference of the odor intensity between the water rinsing condition and no rinsing condition by LSD.

**p<0.001: Significant difference of the odor intensity between the mushroom extract rinsing and the water rinsing condition by LSD.
Table 2  Concentrations of thiol and sulfide compounds with and without mushroom extract rinsing by GC measurement.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Methanethiol Without extract</th>
<th>Methanethiol With extract*</th>
<th>Dimethyl sulphide Without extract</th>
<th>Dimethyl sulphide With extract*</th>
<th>Allythiol Without extract</th>
<th>Allythiol With extract**</th>
<th>Allyl methyl sulphide Without extract</th>
<th>Allyl methyl sulphide With extract**</th>
<th>Dimethyl disulphide Without extract</th>
<th>Dimethyl disulphide With extract**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>0.014</td>
<td>0.014</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>(0.031)</td>
<td>(0.031)</td>
<td>(0.036)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>(0.000)</td>
</tr>
<tr>
<td>10 min</td>
<td>5.922***</td>
<td>2.562***</td>
<td>0.024</td>
<td>0.012</td>
<td>8.278***</td>
<td>4.982***</td>
<td>0.328</td>
<td>0.294</td>
<td>0.008</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>(3.072)</td>
<td>(2.253)</td>
<td>(0.018)</td>
<td>(0.027)</td>
<td>(2.727)</td>
<td>(4.021)</td>
<td>(0.096)</td>
<td>(0.348)</td>
<td>(0.000)</td>
<td>(0.011)</td>
</tr>
<tr>
<td>30 min</td>
<td>0.452</td>
<td>0.560</td>
<td>0.008</td>
<td>0.000</td>
<td>1.264</td>
<td>1.526</td>
<td>0.188</td>
<td>0.296</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>(0.273)</td>
<td>(0.593)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>(0.566)</td>
<td>(1.568)</td>
<td>(0.099)</td>
<td>(0.238)</td>
<td>(0.000)</td>
<td>(0.000)</td>
</tr>
<tr>
<td>40 min</td>
<td>0.068</td>
<td>0.120</td>
<td>0.000</td>
<td>0.000</td>
<td>0.132</td>
<td>0.240</td>
<td>0.026</td>
<td>0.116</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>(0.109)</td>
<td>(0.112)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>(0.189)</td>
<td>(0.159)</td>
<td>(0.036)</td>
<td>(0.128)</td>
<td>(0.000)</td>
<td>(0.000)</td>
</tr>
</tbody>
</table>

Concentrations are expressed in ppm ± standard error of the mean of five subjects.

*p<0.01 (reduction of the concentration for mushroom extract rinsing condition by analysis of variance)

**p<0.05 (reduction of the concentration for mushroom extract rinsing condition by analysis of variance)

***p<0.01 (reduction of the concentration over time by analysis of variance)

Although there was a physiological variation found among subjects (P<0.001), there is a significant reduction of the amount of methanethiol and allylthiol compounds after mushroom extract rinsing as indicated by a significant main effect of the treatment (P<0.01, P<0.05). In contrast, values of sulfide compounds showed no significant difference between with treatment and without treatment (P>0.05). Amount of the concentration of thiol and other sulfide compounds significantly decreased over time (P<0.001). Since interaction between the rinsing condition and the time was also significant for thiol compounds (P<0.001), subsequent analysis of time difference in each condition was conducted. Although amount of the reduction of thiol compounds over time was
significant in both conditions (P<0.01), more reduction of the concentration over time was observed with treatment. Measurement of methanethiol and allylthiol made at 10min showed more reduction of the concentration with treatment than without treatment. These analyses show that mushroom extract can effectively suppress the production of methanethiol and allylthiol quickly.

(2) Experiment 6

The reducing activity of mushroom extract against methanethiol and allylthiol was measured in in-vitro environment to investigate under the different reaction rate.

![Graphs showing deodorant activity of mushroom extract against methanethiol (left) and allylthiol (right) solution in the in-vitro condition.](image)

Figure 8  Deodorant activity of mushroom extract against methanethiol (left) and allylthiol (right) solution in the in-vitro condition.

- : Change in the head space gas concentration of thiol compound dissolved into benzene,

- : Change in the head space gas concentration of each thiol compound dissolved in pure water

- : Change in the head space gas concentration of each thiol compound solution mixed with mushroom extract.

Figure 8 shows the GC measurement of the amount of the residual thiol compound over time in mixing with polyphenol compounds. Clearly, over time of the reaction,
methanethiol content rapidly decreased and reached zero in 30min after mixing. The same tendency was found for the amount of allylthiol monitored. As a control, change in the headspace gas concentration of thiol compound dissolved in distilled water was measured. Little amount of thiol compounds decreased over time. This result indicated that deodorization of the breath odor was significant in the results of the all above experiments mentioned, not because the odorous compounds were dissolved into the aqueous solution, but because mushroom extract had a significant impact on eliminating odorous compounds.

4. Discussion

VSC content in a mouth consists essentially of hydrogen sulfide, methanethiol and dimethyl sulfide, which has very low threshold, with a distinct unpleasant odor (8). In the above experiments, halitosis was induced by mouth rinsing with garlic solution. Rinsing results in the generation of a malodor and its VSC content is similar to those arising from the oral malodor breath (5). Negishi et al. proved the chemical mechanism of deodorization of polyphenol compounds in mushroom extract against methanethiol (15, 27). Thus, with this mechanism as a hypothesis, the deodorization effect of the mushroom extract against the garlic induced breath odor was demonstrated by way of the electronic odor sensor, the sensory evaluation and the GC analysis with in-vivo and in-vitro setting. In the experiment 1 to 4, measurement by the odor sensor and the sensory analysis demonstrated that oral malodor was correlated with the VSC concentration in mouth and mushroom extract significantly reduced the malodor. The GC analysis can identify and quantify individual components in the breath sample, even when there is a masking effect by flavored components, which makes it harder to detect the odor by the sensory evaluation. In the experiment 5 and 6, the GC result showed that malodor breath after rinsing with the garlic solution is caused by volatile compounds, particularly by thiol compounds. Although the individual difference among the subjects was observed, the concentration of thiol compounds was significantly reduced upon rinsing
with mushroom extract. Therefore it can conclude that results obtained from three different analyses were all correlated. Although quantity of garlic used for sensory evaluation is smaller than that for electronic odor sensor and GC, it is due to the sensitivity difference for each analysis that was found in the preliminary experiment.

Although what mechanism might underlie the reduction of volatile sulfur compounds must be investigated further, the main deodorization mechanism may be attributed to the addition reaction of thiol compounds to polyphenol compounds in the mushroom extract, which results in suppression of volatile sulfur compound production. It is widely known that monophenolic compounds in plants and fruits & vegetable are hydroxylated to o-diphenols in the presence of oxygen and polyphenol oxidase, and are oxidized to o-quinones (28, 29, 30). These quinones are subsequently polymerized with other phenolic compounds to cause browning reactions (31).

Numbers of the studies have been demonstrated that conjugating thiol compounds to the o-quinones to form addition compound can prevent further polymerization reaction and browning reaction (27, 32, 33, 34). This mechanism has been applied further to study the deodorizing activity of plants and fruit & vegetable extracts. In green tea, production of methanethiol was found to be suppressed by the methylthio group addition to the o-quinone generated from (-)-epigallocatechin gallate in the presence of the oxygen. Mushroom contains a large amount of polyhydric phenols, mainly γ-L-glutaminyl-4-hydroxybenzene (GHB) in the gill tissue and tyrosine. L-3-(3,4-dihydroxyphenyl) alanine (DOPA) and guanosine 5'-diphosphate also present in small amount (34, 35).

GHB is known to be involved in melanogenesis in Agaricus bisporus (36), catalyzed by tyrosinase or phenolase. Tyrosinase is the principal enzyme involved in browning reactions in Agaricus bisporus (37), which catalyzes hydroxylation of monophenols to form o-diphenols and oxidation of diphenols to form o-diquinones in the presence of oxygen. It has been reported that Agaricus bisporus carried a high deodorizing activity toward methanethiol (15). Conjugated substance 2,5-bis(methylthio)-DOPA was found
as a result of the addition reaction of methanethiol to the o-quinone structure produced by oxidation of tyrosine by tyrosinase. Since it is known that tyrosinase hydroxylates GHB into γ-L-glutaminy1-3,4-dihydroxybenzene (GDHB) and then to oxidize GDHB into γ-L-glutaminy1-3,4-benzoquinone during the melanization process of sporogenesis (38, 37), same addition reaction as tyrosine can also be speculated for GHB.

For further clarification of the elimination mechanism, the reaction rate formula was calculated for each compound from the result of the experiment 6. The reaction rate formula of methanethiol or allylthiol with polyphenol compounds was as follows, respectively; \( \ln[\text{CH}_3\text{SH}] = -0.6299t + 4.2921 \), \( \ln[\text{C}_3\text{H}_6\text{SH}] = -0.5723t + 2.0280 \)

Value of the reaction rate for methanethiol was smaller than that for allylthiol. That means the reaction of the former proceeds somewhat faster than that of the latter.

For obtaining information affecting this reaction rate difference, we decided to perform \textit{ab initio} calculation of conjugate additions of methane- and allylthiol to o-quinone as the simplest model at the B3LYP/6-31+G* level of theory. First of all, HOMO energy levels of these two thiols were obtained as -6.582 and -6.649 eV, respectively, which at least qualitatively supported the better reactivity of methanethiol as the Michael donor on the basis of the frontier orbital theory. Computation of the transition states was further carried out for methanethiol addition to the activated protonated o-quinone leading to the formation of intermediates Int-A, Int-B, and Int-C by the attack at its 1, 3, and 5 positions. Mechanism of the reaction and its formation of the intermediates are illustrated in Figure 9. It was clarified that attack at the 5 position of the activated carbonyl group was the plausible route because only this process furnished the energetically more stable intermediate Int-C by 0.58 kcal/mol after passing the very low energy barrier of 4.41 kcal/mol. Attack at the 1 and 3 positions, with the activation energy of 3.61 and 12.8 kcal/mol, furnished the products 3.13 and 2.24 kcal/mol less stable than the substrates, respectively. In the case of allylthiol, reaction at the same site was found to require the higher activation energy by 1.28 kcal/mol. These results at least qualitatively demonstrated the faster reaction of methanethiol rather than allylthiol with mushroom
extract, and on the basis of this computation, nucleophilic addition of thiols is expected to occur at the 5 position preferentially while we have not analyzed these adducts in detail after isolation.

![Chemical structures](image)

Figure 9  Formation of intermediates by methanethiol addition to the activated protonated o-quinone.

Therefore, calculation can deduce that thiol compounds are eliminated by the mechanism of the addition reaction with polyphenols. In the in-vivo experiment, the average concentration of allylthiol was still higher than that of methanethiol for the measurement made at 30min (Table 2). The result of the calculation could explain why methanethiol decreased faster than allylthiol.

Although further experimental work is needed to determine the precise reaction speed and its pathway, the current studies provided a strong reason to provide the evidence that mushroom extract has a faster and more effective deodorization effect against thiol compounds. Since mushroom extract contains a mixture of different chemical compounds, it can be assumed that multiple actions such as physical and chemical interaction between volatile sulfur compounds and other chemical compounds, other than addition reaction, should be involved in elimination of the malodor after garlic ingestion. However, above calculation suggests that addition reaction of methanethiol and allylthiol to o-quinones, produced from polyphenolic compounds by polyphenol oxidase, can be attributed to the main cause in the elimination mechanism.
5. Conclusion

In this chapter, the deodorizing effect of mushroom extract on the malodor produced after garlic consumption was investigated. Comparative gas chromatography analysis revealed that the quantity of methanethiol and allylthiol that were usually found after garlic solution rinse, significantly fell after mushroom extract rinsing. Furthermore, in-vitro analysis (mixing the garlic solution and mushroom extract) showed that methanethiol reaction with mushroom extract proceeded faster than that of allylthiol. *Ab initio* calculations implicated an addition reaction as a possible mechanism between thiol compounds and polyphenols. In comparison to methanethiol, the higher activation energy required by allylthiol for a feasible reaction pass way with the model acceptor, *o*-quinone, is expected to contribute to the difference in the rate of the reaction.

Those findings were shown to be well correlated with results obtained from commercial odor sensors. The odor sensor system could be an alternative method of evaluating the efficacy of elimination by deodorants for garlic caused malodor. It is especially useful in evaluation of a large amount of malodor samples with subtle difference.
Chapter VII
Colligation and Vision

Longer and stable consumption of the food depends on its palatability as well as its quality preservation property. Consumption of miscellaneous cereals (i.e. sorghum, Hungarian grass, German millet, Japanese millet), which used to be a staple diet in many countries, declines sharply in recent several decades in the developed country because of its palatability. Although texture and appearance are important constituents for the sensory perception of the food, aroma or odor is the most important characteristic that signifies consumers' interest in food choice.

Thus, it is critical problem to develop new techniques for analytically assessing flavor of foods and integrate the information into developing methods of improving the flavor or reducing the off-flavor, which will contribute to gaining the popularity of food ingredients and expanding their use to new food applications.

This is especially critical in development of functional foods. In order to maximize the health benefits, it is often necessary to include many nutritional components. However, many functional ingredients possess adverse sensory attributes. For instance, incorporation of various vitamins or minerals into final products can often lead to an unacceptable flavor. Also, depending on the manufacturing process, functional ingredients are likely to changes in their physical or chemical properties, caused by amino-carbonyl reaction (Maillard reaction) and oxidation, which lead to the production of off-flavors.

For evaluation of off-odor, sensory evaluation is a prevalent and basic method. However, due to the limitations of the sensory evaluation which is imperatively subjective and requires large numbers of sensory panels that can test a limited number of samples each time, improvement of the evaluation methods must be attempted.

This research was aimed at developing novel odor evaluation methods regarding the instrumental analysis, in the development of sweet potato and garlic based functional product as a model for the flavor evaluation. By integrating these methods, the
evaluation system of improving flavor or reducing off-flavor was developed. Results in the studies can be contributed to expansion of use of functional ingredients into new food applications.

In the chapter I, the analysis was intended to identify and quantify volatile compounds which are responsible for the characteristic odors of sweet potato and garlic. The possible mechanism of the off-odor formation was discussed. As a prerequisite for developing the novel odor assessment, the flavor chemistry of the off-odor formation of sweet potato and garlic must be understood. Off-odor extract of heated-saccharified sweet potato were obtained by using a purge and trap system and analyzed by GC-MS. Head space vapor of grated garlic (in-vitro sample) and human breath after garlic ingestion (in-vivo sample) were also analyzed by GC-MS. Sweet potato was shredded with water and homogenized. Then, the resulting slurry was steamed and saccharified with amylases as a sample preparation. GC-MS analysis revealed that approximately 42 flavor compounds were identified in heated-saccharified sample, compared with 26 compounds in raw sweet potato sample.

Higher proportions aldehydes (2,4-decadienal, 2,4-heptadienal, 2-octenal and 2-nonenal), aromatic aldehydes (benzaldehyde and phenyl acetaldehyde), C6 aldehydes (hexanal, 2-heptanal) were present in raw sweet potato juice. This was assumed to involve enzyme lipoxygenase for lipid degradation in fatty acid substrates. Higher proportions of Ketones (β-ionone, β-damascenone, hydroxy-trimethyl-cyclohexanone) and terpenoids (linalool, limonene, α-terpinene, cymene, β-cyclocitrail) were identified in heated-saccharified sweet potato sample. These were characteristic impact compounds which may comprise the basic heated-saccharified sweet potato odor. Formation of terpenoids were assumed to be formed by enzymatic hydrolysis of liberated monoterpenes when sweet potato is blended under high temperature (about 100°C) with presence of acidic phenols and also undergoes cyclization, which is further converted to the final terpenoids by elimination of proton.

On the other hand, only 3 furan and pyrans were identified (2-pentyl furan, furfural
and 2-methyl benzofuran). Relatively lower temperature condition of roasting (about 100°C) may cause lower amounts of furan and pyrans and no maltol, which is less prone to produce under boiling conditions at about 100 °C.

Secondly in GC analysis of garlic vapor and garlic ingested breath, presence of high molecular weight sulfide compounds (HMSC) and low molecular weight sulfide compounds were confirmed, respectively. Higher proportions of HMSC such as allyl methyl disulfide, methyl propyl disulfide and diallyl disulfide in raw garlic were found immediately after grating, but little difference in LMSC such as methane sulfide, allyl sulfide were found between raw and heated garlic. On the other hand, in the breath sample after garlic ingestion, LMSC values were higher for breath after eating raw garlic, but HMSC values were hardly detectable for eating breath of either raw garlic or heat-treated garlic.

In the chapter II, the study was to develop an analytical procedure (sensory evaluation + cognitive recognition analysis) for off-odor assessment by using heated-saccharified sweet potato. Due to its sensitivity and selectivity, sensory evaluation is the most appropriate tool often used in the evaluation of consumer's acceptance and detection of any aroma defect. Descriptive analysis provides a quantitative specification of all the sensory attributes of food or products.

One further complicating factor of human odor perception is the cognitive level of perception. Odor experience is naturally hedonistic; the initial response to odor by consumer is often defined as preferable or disliking, with the most salient attribute of odors being pleasantness or unpleasantness. Therefore, measurement of off-odor should be also taken into account at the cognitive level to elucidate physiological changes associated with exposure to pleasant and unpleasant odors.

Firstly, to identify the critical sensorial components responsible for off-odor, heated-saccharified sweet potato samples were analyzed for its flavor by using descriptive analysis. Application of a descriptive analysis makes it possible to specify which sensory attribute contributed to the off-flavor formation. Result of the analysis generated total of 8 aroma
or odor attributes (grassy aroma, sweet aroma, sour aroma, caramel aroma, carrot odor, tomato odor, powdery odor and heavily boiled odor).

Although sensory evaluation would be to determine the consumer acceptance of the aroma profile for final food products, it is due to aroma volatiles. Significant differences in "sweet", "heavily boiled" sensory components were found in heated-saccharified sweet potato juice, while "grassy", "carrot" aroma were found to be significantly higher in raw sweet potato juice when two samples were evaluated. From evaluation of the characteristics of the standard found by GC-MS, the terpenoids, ketones were sweet and flowery like, while certain aldehydes, which were higher in raw sweet potato, were described as "greeny" or "grassy". Although it is hard to assume what compounds are contributed to "heavily boiled" odor, these ketones and terpenoids comprise "sweet" odor, and the presence of high quantity of these aroma impact compounds in the heated-saccharified sweet potato may contribute to the "heavily boiled " off-odor.

Secondly, to evaluate the cognitive aspect of the off-odor of sweet potato, electroencephalography (EEG) measurement was conducted with exposure of off-odor of steamed sweet potato. To test the hypothesis that steamed sweet potato off-odor can elicit distinctive changes in the electrical activity of the cognitive perception, apple juice aroma was chosen as a positive comparison. A significant difference between apple juice aroma and steamed sweet potato odor was observed on the basis of changes in α wave power. Although apple juice aroma was evaluated to be pleasant by all 7 subjects, the same tendency was not observed for all the subjects on the electroencephalogram. Three subjects were relaxed by apple juice aroma, however no changes in α1 and α2 wave powers were observed for the remaining 4 subjects. It was concluded that apple juice aroma elicits no unpleasant mood. With exposure of steamed sweet potato odor, α wave power was markedly decreased in half of the subjects. Interpretation of α wave changes in α1 and α2 power showed an increase of tension in mental state with an exposure of heated sweet potato odor, while no changes in α1 and α2 wave power were observed for subjects in general when they were exposed to apple juice. These results were correlated with the
result of the preference grading, which showed that odor of apple juice were prefer over heated sweet potato. This result showed that off-odor of heated-saccharified sweet potato influences the physiological state of the subject.

With use of instrumental measurements (GC-MS and EEG analysis), it was found that perception of odor can be analyzed in the chemical and physiological perspective, of which results were associated with the odor preference of the subject. Identification by GC-MS analysis found the specific compounds elicit various sensorial responses. Specific patterns of brain electrical activity also reveal association of odor with brain activity.

In the chapter III, development of the new odor sensor, which evaluates the odor quality in terms of the subject's preference, was attempted. In the chapter II, the ability of odors to produce pleasant or unpleasant experiences was evaluated by measuring the physiological state of brain activity. Because EEG measurement is inconvient to use, it is desirable to develop a handy method of measuring the odor pleasantness. The odor sensor is a new technology which could partly substitute human nose in quality control environment. The odor sensor is based on non-specific conductive metals which measure aroma components as a whole. Since they measure undefined total volatiles, it can not detect the character impact component of the odor, and cannot give any indication of the hedonic component of the odor evaluated. Although it is difficult to take into account the cognitive aspect of the odor, it would be very intriguing to develop the odor sensor which could separate the odor in terms of pleasantness and unpleasantness.

The odor sensor developed in this study consists of semiconductors of combined sintered and thin film metal oxides. The former sensor type reacts sensitively to light odorants (compounds with low molecular weights), while the latter sensor type is more sensitive to heavier odorants (compounds with relatively large molecular weights) such as unsaturated aromatic hydrocarbon group compounds like toluene or methanethiol. A total of 6 semiconductor sensors were combined in this odor sensor to cover a broad range of chemical properties.

Evaluation of odor for various food products was found that responses from sensors 2
and 4 were small and those from sensors 3 and 6 were greatly enlarged for pleasant smells. On the other hand, for unpleasant smelling substances the radar graph shape was very different. From these results, the quality of the odor in terms of the degree of preference was defined as an F-value, which is calculated as “sensor responses of CH3+CH6 / CH2 +CH4”. The strength of odor was defined as an S-value, which is integration of the area under the spider graph. This formula is contextualized for odor substances distinguished clearly to be good or bad odor, but cannot be contextualized for all odor substances. Although the data shown in this study is still premature to be conclusive, the new developed sensor may suggest the possibility of evaluating the degree of pleasantness in the odor quality in a measure from the data analyzed by these semiconductor sensors.

In the chapter IV, based on the identified odor impact compounds information found in the chapter I, various deodorants, activated carbon (AC), maltosyl cyclodextrin (MCD) and apple polyphenol (AP), were selected. The efficacy of the deodorants in reducing the "heavily boiled odor" of heated-saccharified sweet potato juice were evaluated by using the new odor evaluation system, using a new odor sensor, sensory evaluation and GC-MS. The new developed odor sensor found the highest deodorizing efficiency for AC, followed by MCD and AP, of which result was also confirmed by the sensory analysis.

Furthermore, flavor compounds in the deodorized sample were identified by the GC-MS analysis. While the AC reduced the peak intensities of 13 compounds in raw sweet potato juice to less than peak height 5 after deodorization, MCD did not reduce the peak intensities to a similar extent. To clarify the mechanism of adsorption with AC and envelopment with MCD for the identified odor components of the sweet potato juice, the result was analyzed with hydrophilic lipophilic balance value conception. Lower HLB value compounds such as β-cyclocitrail, 2-methyl-2-butenal, which indicates the relatively high hydrophobicity, were adsorbed in AC effectively, while higher HLB value compounds such as acetone, diacetyl, were hardly adsorbed. This proved that AC's inner hydrophobic environment prevented the hydrophilic compounds from being adsorbed, while hydrophobic compounds could be enveloped easily in its vacancy. Although MCD
follows the same tendency, some of the lower HLB value compounds were found not to be adsorbed effectively. This may be due to steric hindrance of the structure. The compounds with several side-chain groups such as (2-ethyl-4,5-dimethylphenol, cycloisosativene) or compounds with ringed structure such as β-cyclocitrall after MCD treatment were hardly adsorbed. These results conclude that deodorant's efficacy can be evaluated by the new odor sensor system. This result was found to be attributed to the degree of interaction between the odorous compounds and the deodorant.

In the chapter V, the characteristic of the garlic-induced odor was evaluated by the new developed odor sensor system. Despite its notorious value, the use of the garlic to the food application is still limited due to oral malodor after garlic ingestion. Elimination of malodor problem can help expansion of garlic use to the various food categories. Prior to determination of possible elimination mechanism, odor quality of raw and heated garlic, and breath after garlic ingestion were evaluated by using the new developed odor sensor, sensory evaluation and GC-MS to determine the difference of each odor quality and identify volatile chemicals which may result in the potential odor change.

The F and S values of the new odor sensor showed that garlic odor characteristics and strength were different in-vitro sample and in-vivo samples. For the in-vitro sample, the F value was lower and S value was higher for raw garlic than for heated garlic and this result matched the data of GC and sensory analysis. In the in-vivo sample, S-value and F-values of both breath samples were lower than those of the in-vitro sample. This suggests that raw garlic has a strong odor and its odor changes into unpleasant odor in in-vivo. On the other hand, garlic odor became moderate with heating and so when heated garlic was ingested, it did not produce a strong unpleasant odor.

This was supported by sensory and GC analyses. The sensory results showed that the strength of garlic odor was stronger for raw garlic than for heat-treated garlic for both the in-vivo and in-vitro samples. From the GC analysis, HMSCs such as allyl methyl disulfide, methyl propyl disulfide and diallyl disulfide, were found to be largely contributed to the specific odor of garlic, thus the GC result confirmed the sensory result. On the other hand,
in the in-vivo sample, LMSC values were higher after eating raw garlic and these were considered to be the major cause of malodorous breath after garlic ingestion. These results showed that odor sensor data could evaluate the quality difference of each garlic odor and were well correlated with sensory and GC-MS analysis.

In the chapter VI, based on the odorous compounds information obtained in the previous chapters, the appropriate deodorant was selected and its efficacy against oral malodor was evaluated. In this study, the commercial odor sensor (FOX 4000, Alpha-MOS Co., Ltd) was chosen to evaluate the degree of deodorization for the breath of the subjects.

Although the instrument does not provide specific information of the qualitative differences between samples, it is extensively used as an alternative method for discrimination of multiple samples due to its combined powerful multivariate statistical discrimination analysis. Since evaluation of the oral malodor is the least type of the sample which human assessor wants to evaluate, application of the odor sensor should be developed in correlation with sensory results or to specific instrumental data. The results demonstrated that the odor sensor could select the optimum dose of mushroom extract for elimination of off-odor and could evaluate its deodorant effect of mushroom extract for the malodor after ingesting garlic in multiple subjects. Correlation between electronic odor sensor and sensory analysis data was significantly high, and the sensor assessment proved to accurately predict the odor intensity scores of different odor samples as assessed by human evaluation.

GC analysis showed significant decrease in the amounts of methane- and allylthiols both of which were found in the expirations of 5 subjects after ingesting garlic. Subsequently, it was shown that the reaction proceeded faster for methanethiol than allylthiol. *Ab initio* calculation was made using the simplest model at the B3LYP/6-31+G* level of theory, and proved that under thiol compounds existence, addition reaction between thiol compounds and o-quinones occurs and its reaction proceeds quickly. The novel odor evaluation system showed that mushroom extract could effectively suppress the
production of methanethiol and allylthiol quickly, and its effect was proved to attribute to Michael addition mechanism.

In summery of all, an advance has been made in understanding the molecular and cognitive mechanisms involved in off-odor perception and its deodorization of heated-saccharified sweet potato and garlic. These kinds of information were integrated into developing the new odor sensor which could potentially evaluate the odor quality in terms of consumer's degree of preference, and multiple odor evaluation system combining the sensory evaluation, odor sensor and GC-MS analysis. Although further researches must be conducted for elucidation, this study is a beginning and provides some pointers for the next step in developing odor sensor systems which could evaluate the quality aspect of the odor and aroma of foods and foods ingredients.

The present research showed that the off-odor of heated-saccharified sweet potato and garlic-induced oral malodor could be effectively eliminated by using the multiple instrumental evaluation system, and their elimination mechanisms were also elucidated. These instrumental analyses are viable alternatives for sensory evaluation in evaluation of off-odor.

The developed methods in this study not only contribute to the increase in consumption of the sweet potato and garlic, which leads to revitalize for production of sweet potato products and local industries, but also contribute as a new system to the progress of aroma or odor evaluation in the product development and quality control of all foods and food ingredients in food industry.
List of references

Introduction


**Chapter I**


(2000)

Chapter II


(20) Tamaki K., Ehara K., Tamaki T. and Yamazaki T.: Determination of aroma changes in sweet potato (Ipomoea batatas (L.) Lam) during sweet potato juice production, Food Preservation Science 33, 51-61 (2007)

Chapter III


(7) Ehara K.: Development of odor sensor apparatus corresponding to sensory evaluation,
Function and materials, 13, 14-19 (1993)


Chapter IV


Chapter V


**Chapter VI**


(1991b)


(1994)


Presented paper

1. Determination of Aroma Changes in Sweet Potato (Ipomoea batatas (L.) Lam) during Sweet Potato Juice Production
   Kazuhiko Tamaki, Katsuo Ehara, Takeshi Tamaki and Takashi Yamazaki
   *Food Preservation Science, 33* (2), 51-61 (2007)

   Kazuhiko Tamaki, Takeshi Tamaki and Takashi Yamazaki

3. Deodorisation of Off-odour during Sweet Potato Juice Production by Employing Physical and Chemical Deodorants
   Kazuhiko Tamaki, Takeshi Tamaki and Yoshitake Suzuki

4. Measurement of Odour after In-vitro or In-vivo Ingestion of Raw or Heated Garlic, Using Electronic Nose, Gas Chromatography and Sensory Analysis
   Kazuhiko Tamaki, Shigenori Sonoki, Takeshi Tamaki and Katsuo Ehara

5. Aroma Characteristics of Steamed Sweet Potato
   - Comparison with apple juice aroma characteristics –
   Kazuhiko Tamaki, Takeshi Tamaki and Yuko Matsuo
   *Food Preservation Science, 34*(2) (2008)
Acknowledgement

I would like to thank my advisor Dr. Takaaki Fujii who is a professor of Chiba University, for his guidance and concern on completion of the dissertation.

I would like to thank Dr. Takasuke Ishitani who is the president of Japan food packaging Research Association, Dr. Yoshitake Suzuki of Meiji University, Dr. Takashi Yamazaki of Tokyo University of Agriculture and Technology and Dr. Yuko Matsuo of Kanagawa University of Human Services, Kanagawa for all of their help and guidance in completion of this research.

I would like to thank Mr. Hiroaki Shiozawa of New Cosmos Electric Co. Ltd, Mr. Hitoshi Iwasaki of NIHON KODEN CORPORATION and Mr. Koichi Yoshida of Primetech Corporation for lending the expertise and facilities that made this project possible.

Dr. Michael O’hamony who is a professor of University of California Davis, has given me a special teaching about sensory evaluation and I would like to thank him too.

I would like to acknowledge the support provided by Hayashibara Co., Ltd. and HAYASHIBARA international Inc.

Lastly, I express my appreciation and deepest sympathy to Dr. Katsuo Ehara who belonged to Tokyo Institute of Technology, for his dedicated advices of the sensor development and aroma analysis of throughout our research period, who helped me to guide into the flavor research and unfortunately passed away halfway of his researches.