Effects of Food Materials on Removal of *Allium*-Specific Volatile Sulfur Compounds

OSAMU NEGISHI,*,† YUKIKO NEGISHI,‡ AND TETSUO OZAWA†

Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan, and Institute of Nutrition Sciences, Kagawa Nutrition University, Sakado, Saitama 350-0288, Japan

Effects of food materials were investigated on removal of several kinds of thiols, sulfides, and disulfides, which arise from vegetables of *Allium* species during food preparation and eating. Methanethiol, propanethiol, and 2-propenethiol were captured by raw foods such as fruits, vegetables, and mushrooms or a mixture of their acetone powders and phenolic compounds. The odor of diallyl disulfide was remarkably reduced by kiwi fruit, spinach, cutting lettuce, parsley, basil, mushrooms, and, particularly, cow's milk, raw egg, boiled rice, and bovine serum albumin (BSA). This suggests that the removal of diallyl disulfide could be caused by a physical and chemical interaction between the disulfide and foods. Furthermore, milk and BSA captured propanethiol, 2-propenethiol, dipropyl sulfide, diallyl sulfide, dimethyl disulfide, and dipropyl disulfide very well. An enzymatic degradation of diallyl disulfide by spinach and asparagus was also observed. These results demonstrate that the deodorization with foods is achieved by multiple actions including physical and chemical interaction between volatile sulfur compounds and foods, enzymatic degradation of disulfides, and addition of thiols to polyphenolic compounds, catalyzed by polyphenol oxidases or peroxidases.

KEYWORDS: Enzymatic deodorization; bad breath; *Allium*; fruit; vegetable; mushroom; acetone powder; polyphenolic compound; polyphenol oxidase; peroxidase; thiol; sulfide; disulfide

INTRODUCTION

We have been working on enzymatic deodorization, which is a novel method involving the use of polyphenolic compounds (PPs) and polyphenol oxidases (PPOs) or peroxidases (PODs) to remove bad odors from the mouth and the environment (*1*−*5*). In this method, methanethiol, which leads to halitosis, was removed by raw fruits, vegetables, and mushrooms or by mixtures of their PPs and their acetone powders, which contain PPOs and PODs (*6*). It was also demonstrated by sensory examination that eating fruits such as apple, pear, and prune significantly reduced bad breath after eating garlic. The mechanism of enzymatic deodorization has been attributed to the addition reaction between thiols and *o*-quinones formed from PPs of the raw foods by the maceration of these foods in the mouth. Aside from methanethiol, 2-propenethiol and propanethiol also have bad odors. **Figure 1** shows the formation of volatile sulfur compounds from *Allium* species (*7*−*9*). Alliin (*S*-allyl-L-cysteine *S*-oxide, R = −CH₂CH=CH₂) is degraded to allylsulfenic acid by alliinase, followed by formation of allicin (allylthiosulfinate, R = R′ = −CH₂CH=CH₂) when the tissues are disintegrated. Furthermore, allicins are converted to diallyl disulfides and diallyl trisulfides, which are degraded to 2-propenethiols after ingestion of garlic. *Allium* species have different
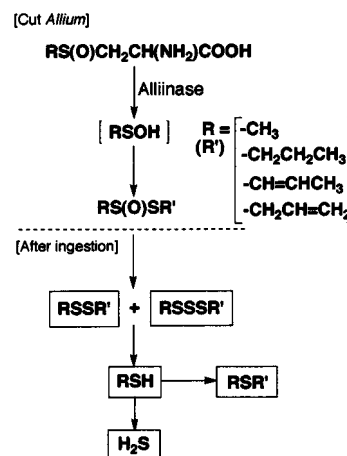
Figure 1. Formation of volatile sulfur compounds from *Allium* species. Several *Allium* species have characteristic *S*-alk(en)yl-L-cysteine *S*-oxides with a predominant substituent (R). For examples: R = −CH₃, Chinese chives (*A. tuberosum*); R = −CH₂CH₂CH₃, scallion (*A. fistulosum*) and chives (*A. schoenoprasum*); R = −CH=CHCH₃, onion (*A. cepa*); R = −CH₂CH=CH₂, garlic (*A. sativum*). In cut *Allium* plants, *S*-alk(en)yl-L-cysteine *S*-oxides [RS(O)CH₂CH(NH₂)COOH, alliin in garlic] are degraded to thiosulfinates [RS(O)SR′, allicin in garlic] via sulfenic acids (RSOH). Furthermore, thiosulfinates are converted to disulfides (RSSR′) and trisulfides (RSSSR′) (and more polysulfides), followed by thiols (RSH), sulfides (RSR′), and hydrogen sulfide (H₂S), after ingestion of *Allium* plants.

* Author to whom correspondence should be addressed (telephone +81-298-53-4682; fax +81-298-53-4605; e-mail negishi@sakura.cc.tsukuba.ac.jp).
† University of Tsukuba.
‡ Kagawa Nutrition University.

Removal of *Allium*-Specific Volatile Sulfur Compounds

J. Agric. Food Chem., Vol. 50, No. 13, 2002 **3857**

kinds of *S*-alk(en)yl-L-cysteine *S*-oxides with different substituents (R) in **Figure 1**. Disulfides and thiols having methyl groups arise mainly from Chinese chives (*A. tuberosum*), propyl groups from scallion (*A. fistulosum*) and chives (*A. schoenoprasum*), 1-propenyl groups from onion (*A. cepa*), and 2-propenyl (allyl) groups from garlic (*A. sativum*). A number of groups have investigated *Allium* breath volatiles (*10–14*). It is well-known that malodorous breath (halitosis) can originate in the mouth as well as in the gut, particularly in the case of sulfur compounds produced after the ingestion of *Allium* species. Furthermore, it has been demonstrated that most of the allyl methyl sulfide (AMS) gas originates from the gut (rather than the mouth), and this gas is likely to account for the well-known persistence of malodorous breath long after garlic ingestion (*10*). These volatile sulfur compounds, particularly 2-propenethiol and propanethiol, cause an intense bad breath after the consumption of vegetables of these *Allium* species. This paper deals with the removal of *Allium*-specific volatile sulfur compounds by food materials.

MATERIALS AND METHODS

Food Materials. Fruits and vegetables were purchased from supermarkets. Mushrooms were collected from fields and mountains in Yamanashi, Fukushima, Nagano, Ibaraki, and Saitama prefectures, Japan. Some were obtained from the markets. The mushrooms were lyophilized, milled, and stored at −20 °C.

Chemicals. A 15% sodium methanethiolate aqueous solution, propanethiol, 2-propenethiol, dimethyl sulfide, dipropyl sulfide, diallyl sulfide, dimethyl disulfide, dipropyl disulfide, diallyl disulfide, and chlorogenic acid were purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. Tyrosine and L-DOPA were obtained from Wako Pure Chemical Industry, Osaka, Japan. (−)-Epicatechin, variegatic acid, and γ-L-glutaminyl-4-hydroxybenzene were prepared from green tea (*15*), *Boletus subvelutipes* (*4*), and *Agaricus bisporus* (*5*), respectively.

Detector tubes, which were used for measuring the amounts of thiols, were products of Gastec Corp., Kanagawa, Japan (*2, 5*).

Preparation of Acetone Powder. Raw food material (100 g) was homogenized with 400 mL of cold acetone (−20 °C) in a Waring blender. The homogenate was filtered, and the residue was washed three times with 80% acetone (500 mL) at 4 °C. After removal of the acetone from the residue, it was lyophilized. The acetone powders, which contain enzymes, were stored at −20 °C.

Measurements of the Thiol-, Sulfide-, and Disulfide-Capturing Activities of Food Materials. *Reactions with Thiols (Method I).* The reaction was done by crushing food materials with a mill (total volume of chamber = 300 mL, SCM-40A, Sibata Co., Ltd., Tokyo, Japan) (*2*). Five grams of sliced raw food material, 45 g of water, and 200 μL of a 0.1% thiol aqueous suspension were put into a chamber of the mill maintained at ∼35 °C. The materials were crushed and mixed at 7000 rpm for 5 s at intervals of 25 s for 5 min. After that, an aliquot volume of the headspace gas (7–18 mL) in the chamber was pulled out by passing through a detector tube with a needle from the hole of the chamber cap to measure the amount of remaining thiol.

Reactions with Thiols (Method II). In another method (*5*), to a mixture of lyophilized mushroom powder (100 mg) and 4.9 mL of 0.1 M acetate buffer (pH 5.0) in a 30-mL borosilicate glass vial with an open-top screw cap and Teflon/silicon disk (Pierce) was added 100 μL of a 0.1% thiol aqueous suspension, and the vial was shaken by hand at the rate of 2 strokes/s at 25 °C. After 5 min, an aliquot volume of the headspace gas (3–8 mL) was passed through a detector tube. The effect of acetone powder and PP was measured by mixing 20 mg of an acetone powder, 1.2 mL of a 0.1 M acetate buffer (pH 5.0), 0.2 mL of a 7.5 mM PP solution, and 100 μL of a 0.1% thiol aqueous suspension.

Reactions with Sulfides and Disulfides. The reactions were carried out as described for thiols. In method I using sulfides and disulfides, the reaction was carried out with 20 g of foods, 35 g of water, and 2

Table 1. Thiol-Capturing Activities of Raw Fruits and Vegetables

food	capturing activity[a] (%)		
	MeSH	PrSH	AllSH
cooked rice	0	6	5
apple (cv. Ourin)	45	47	35
prune	100	100	100
blueberry	37	53	41
kiwi fruit	15	57	35
burdock	100	100	100
basil	100	100	100
eggplant	100	100	100
mushroom	100	100	100

[a] For the measurement of the thiol-capturing activity, method I in the text was applied. The thiols used were methanethiol (MeSH), propanethiol (PrSH), and 2-propenethiol (AllSH).

μL of sulfide or disulfide. The residual sulfur compounds, however, were measured with a gas chromatograph. Five milliliters of headspace gas was analyzed by a GC-14B (Shimadzu Corp., Kyoto, Japan) equipped with an FID detector (250 °C) and a glass column (PPE 5ring 10%, 3.2 mm × 3.1 m). Column temperature was held at 60 °C for 3 min, raised from 60 to 150 °C at 30 °C/min, and then held at 150 °C for 14 min. Helium gas (60 mL/min) was used as a carrier gas.

Control reaction was carried out without a food material or an acetone powder. The thiol-, sulfide-, and disulfide-capturing activity of each food material was measured in duplicates. Capturing activity (percent) was expressed as $(C − P)/C × 100$, where C is the amount of sulfur compound in the control reaction and P is the amount of sulfur compound in the reaction with a food material or an acetone powder.

Analysis of Conjugates between Chlorogenic Acid and Thiols. *Reaction with a Burdock Acetone Powder.* The reaction was done by mixing a burdock acetone powder (40 mg), 0.4 mL of a 15 mM chlorogenic acid solution, 1.1 mL of a 0.1 M acetate buffer (pH 5.0), and 4 μL of a thiol solution in a 30-mL borosilicate glass vial, which was shaken by hand at the rate of 2 strokes/s at 25 °C. After 10 min, the reaction was stopped by the addition of 0.15 mL of 2 N HCl.

HPLC Analysis. Reaction mixtures were analyzed by HPLC (equipped with a DP-8020 pump and a UV-8020 spectrophotometer, Tosoh Corp., Tokyo, Japan) with an Inertsil prep-ODS column (6.0 × 250 mm, 10 μm, GL Sciences, Tokyo, Japan) at 30 °C. Elution was performed with MeOH/2% AcOH (40:60) at a flow rate of 1.0 mL/min. Eluates were monitored at 320 nm, and their spectra were measured using a photodiode array UV–vis detector, SPD-6MA (Shimadzu Corp.).

LC-MS Analysis. LC-MS analysis was carried out using a Waters LC-MS system (Waters Corp., Milford, MA) equipped with a Waters ZQ mass detector, a Waters 2690 separations module, a Waters 996 PDA detector, and MassLynx software version 3.5. Column and chromatographic conditions were the same as those used for the above HPLC analysis. A postcolumn split was 4:1. Electrospray ionization (ESI) mass spectrometry was used for the detection of the conjugates. Following are the MS parameters: ionization mode, ES⁺; scan range, m/z 50–800; scan rate, 0.5 s; capillary voltage, 3.4 kV; cone voltage, 15 V; source block temperature, 120 °C; and desolvation temperature, 400 °C. Nitrogen was used as a desolvation and cone gas at flows of 400 and 50 L/h, respectively.

RESULTS AND DISCUSSION

Capture of Thiols by Fruits, Vegetables, and Mushrooms. Effects of fruits, vegetables, and mushrooms on removal of three kinds of thiols are shown in **Tables 1** and **2**. Previously, we have reported the capture of methanethiol by the raw foods (*2, 5*). Methanethiol, propanethiol, and 2-propenethiol were eliminated with raw fruits and vegetables such as prune, burdock, basil, and eggplant (**Table 1**). Although the thiol-capturing activity of apple is lower than those of others, the effect could be increased by prolonging the reaction time or increasing the

3858 *J. Agric. Food Chem.,* Vol. 50, No. 13, 2002

Negishi et al.

Table 2. Thiol-Capturing Activities of Raw Mushrooms

	capturing activity[a] (%)		
mushroom	MeSH	PrSH	AllSH
Agaricus bisporus	100	100	100
Boletus subvelutipes	100	100	100
Gyrodon lividus	100	100	100
Hypholoma sublaterium	100	100	100
Lentinus edodes	43	35	36
Russula nigricans	100	100	100
Suillus grevillei	100	100	100

[a] For the measurement of the thiol-capturing activity, method II in the text was applied.

Table 3. Thiol-Capturing Activities of Phenolic Compound–Acetone Powder Reaction Systems

	capturing activity[a] (%)		
phenolic compound–acetone powder[b]	MeSH	PrSH	AllSH
CG–burdock	100	100	100
EC–pear	100	100	100
VA–*Boletus subvelutipes*	100	100	100
Tyr–*Agaricus bisporus*	61	72	61
L-DOPA–*Agaricus bisporus*	89	88	100
GHB–*Agaricus bisporus*	100	100	100

[a] For the measurement of the thiol-capturing activity, method II in the text was applied. [b] Acetone powders, which contain PPO, were prepared from each food material.

amount of apple (*2*). Mushrooms, which have a high thiol-capturing activity against methanethiol (*4, 5*), also showed high activity against propanethiol and 2-propenethiol (**Table 2**). Particularly, three *Boletus* species, *Russula nigricans*, *Hypholoma sublateritium*, and *Agaricus bisporus*, are good food materials for deodorization. These results confirmed the removal of unpleasant odors formed from the vegetables of *Allium* species by the consumption of raw fruits, vegetables, and mushrooms.

Phenolic Compound–PPO Reactions and Their Addition Products with Thiols. Fruits, vegetables, and mushrooms contain many phenolic compounds and PPOs (*2−6, 16, 17*). The results of reactions between thiols and phenolic compounds, catalyzed by acetone powders prepared from raw foods, are shown in **Table 3**. All phenolic compound−PPO (acetone powder) reaction systems showed high capturing activity against methanethiol, propanethiol, and 2-propenethiol, suggesting that phenolic compounds and PPO in the foods are closely involved in deodorization by the foods. Furthermore, the reaction products in deodorization were analyzed by HPLC (**Figure 2**). The reactions were carried out between chlorogenic acid and methanethiol, propanethiol, and 2-propenethiol with a burdock acetone powder. **Figure 2(1)** shows the HPLC elution profile and UV spectra of the already known conjugates between chlorogenic acid and methanethiol. M-1 and M-2 are 2-methylthiochlorogenic acid and 2,5-bis(methylthio)chlorogenic acid, respectively (*1*). Analyses of the reaction products (P-1 and A-1) between chlorogenic acid and propanethiol and 2-propenethiol [**Figure 2(2),(3)**] revealed that their spectra were about the same as that of M-1. These facts demonstrate that the products were the respective 2-adducts. Furthermore, ESI mass spectra of M-1, P-1, and A-1 were measured. In the 2-methylthiochlorogenic acid (M-1), $[M + H]^+$ at m/z 401, $[M + Na]^+$ at m/z 423, and the characteristic fragment of the methylthiocaffeic acid residue at m/z 209 were detected. P-1 and A-1 showed their respective ESI mass spectra similar to that of 2-methylthiochlorogenic acid
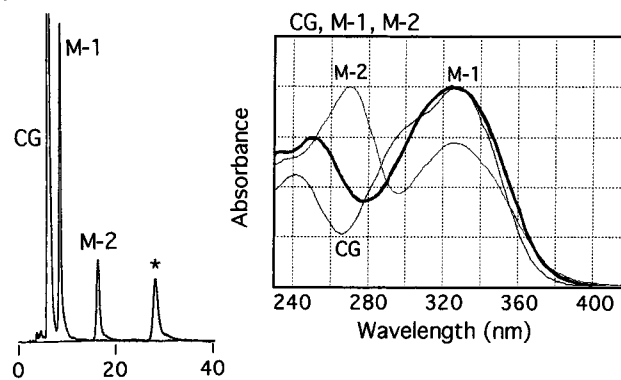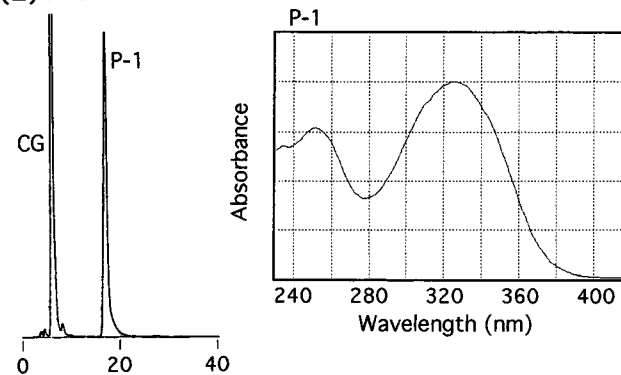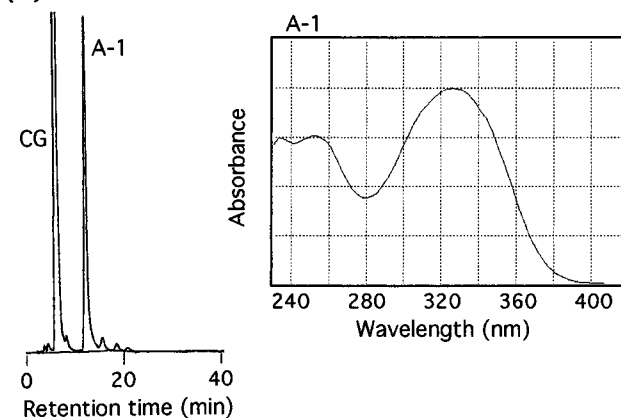
Figure 2. HPLC elution profiles and UV spectra of the reaction products between chlorogenic acid (CG) and thiols with burdock acetone powder in **Table 3**. From reaction 1 between CG and methanethiol (MeSH), three product peaks were obtained. M-1 (thick line) and M-2 were identified as 2-methylthiochlorogenic acid and 2,5-bis(methylthio)chlorogenic acid, respectively, from their spectra (*1*). Peak ∗ was an unknown peak. From reaction 2 between CG and propanethiol (PrSH) and reaction 3 between CG and 2-propenethiol (AllSH), product peaks P-1 and A-1 were obtained, respectively. The spectra of P-1 and A-1 were about the same as that of M-1.

(M-1). $[M + H]^+$ at m/z 429, $[M + Na]^+$ at m/z 451, and the fragment of propylthiocaffeic acid residue at m/z 237 in P-1 and $[M + H]^+$ at m/z 427, $[M + Na]^+$ at m/z 449, and the fragment of the allylthiocaffeic acid residue at m/z 235 in A-1 were detected. These mass spectral data confirmed that the capture of propanethiol and 2-propenethiol by chlorogenic acid with a burdock acetone powder was achieved.

Interaction between Disulfides (or Sulfides) and Foods. We have investigated the removal of sulfides and disulfides,

Removal of *Allium*-Specific Volatile Sulfur Compounds

J. Agric. Food Chem., Vol. 50, No. 13, 2002 **3859**

Table 4. Diallyl Disulfide- and Diallyl Sulfide-Capturing Activities of Foods

food	capturing activity[a] (%)	
	All–S–S–All	All–S–All
apple (cv. Ourin)	14	3
prune	38	8
kiwi fruit	85	18
banana	58	11
blueberry	57	21
persimmon	12	0
burdock	33	27
eggplant	27	10
spinach	70	6
cutting lettuce	70	21
parsley	70	33
basil	73	24
asparagus	38	11
yam	41	19
cooked rice	70	22
cow's milk[b]	95	91
egg	84	88

[a] For the measurement of the diallyl disulfide- and diallyl sulfide-capturing activity, method I using these compounds in the text was applied. [b] The fat content of the milk was 3.5%.

Table 5. Diallyl Disulfide- and Diallyl Sulfide-Capturing Activities of Raw Mushrooms

mushroom	capturing activity[a] (%)	
	All–S–S–All	All–S–All
Agaricus bisporus	58 (63)[b]	5 (4)[b]
Boletus subvelutipes	63 (67)	18 (25)
Gyrodon lividus	72 (69)	19 (20)
Hypholoma sublaterium	59 (64)	21 (22)
Lentinus edodes	60 (60)	4 (5)
Russula nigricans	63 (64)	81 (29)
Suillus grevillei	71 (72)	16 (16)

[a] For the measurement of the diallyl disulfide- and diallyl sulfide-capturing activity, method II using these compounds in the text was applied. [b] Values in parentheses were obtained with the heat-treated mushrooms by a microwave oven.

which have also bad odors. **Table 4** shows the effects of many kinds of foods on the removal of diallyl disulfide and diallyl sulfide. Although apple, persimmon, burdock, and eggplant had high capturing activity against thiols (*2*; **Table 1**), their effects against two sulfur compounds were low. On the other hand, the diallyl disulfide-capturing activities of kiwi fruit, banana, blueberry, spinach, cutting lettuce, parsley, and basil were high. Furthermore, 70% of the disulfide was captured by cooked rice, whereas ~90% of the odor was eliminated by milk and egg. The effects of foods on diallyl disulfide and diallyl sulfide seem to be caused by a physical and chemical interaction between these sulfur compounds and the chemical components of foods such as chlorophylls, proteins, or lipids. Other reactions may be possible, because in the cases of kiwi fruit, spinach, and asparagus, a small amount of thiol gas was detected by gas chromatography.

The effect of mushrooms on the removal of diallyl disulfide and diallyl sulfide was also measured (**Table 5**). Their capturing activities against diallyl disulfide were 60–70%, whereas those against diallyl sulfide were low except for *Russula nigricans*, which captured 81% of the diallyl sulfide. The removal of diallyl disulfide and diallyl sulfide also seems to be caused by a physical and chemical interaction between these sulfur compounds and cell components of mushrooms. However, ~50% removal of diallyl sulfide by *R. nigricans* is demonstrated to

Table 6. Effects of Milk and BSA on Removal of Thiols, Sulfides, and Disulfides

sulfur compound	capturing activity[a] (%)		
	cow's milk[b]	BSA	
		0.1%	1%
MeSH	0	0	0
PrSH	71	6	13
AllSH	46	7	15
Me–S–Me	17	6	15
Pr–S–Pr	91	30	57
All–S–All	90	2	29
Me–S–S–Me	72	13	22
Pr–S–S–Pr	97	41	84
All–S–S–All	96	59	75

[a] For the measurement of the capturing activity, method II in the text was applied. 5 mL of milk (or BSA solution) and 0.2 mL of a 0.1% aqueous suspension of sulfur compound were mixed. [b] The fat content of the milk was 3.5%.
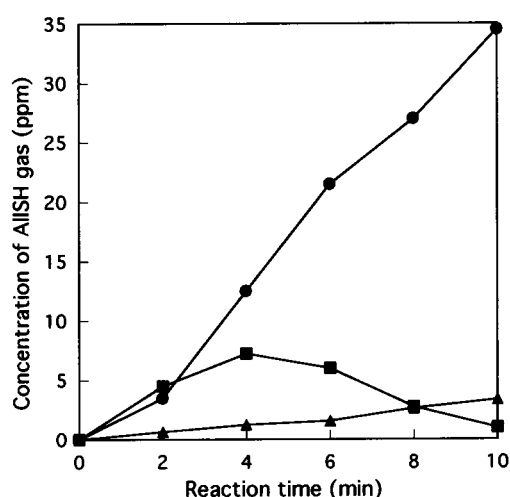

Figure 3. Release of 2-propenethiol (AllSH) from diallyl disulfide by vegetables: ●, asparagus; ■, spinach; ▲, taro. Reactions were carried out with method I using 20 g of vegetable, 35 g of water, and 2 μL of diallyl disulfide. The amount of released thiol gas was measured with a detector tube. No thiol gas from these vegetables in the reactions without diallyl disulfide and from diallyl disulfide in the reactions with heat-treated vegetables by a microwave oven was detected. 1.5% of diallyl disulfide was degraded in 10 min by asparagus.

be due to an enzyme reaction, because the sulfide-capturing activity was 29% with the heat-inactivated mushroom powder. In addition, the fruit body of *R. nigricans* contains ~9500 mg of tyrosine/100 g of dry weight (*5*). In the early stage of the reaction, the solution became as red as blood. It is assumed that a radical addition reaction might occur after radical decomposition of the sulfide, although we could not confirm the reaction products as thiol−PP addition products.

Furthermore, **Table 6** shows the effects of milk and bovine serum albumin (BSA) on the removal of several kinds of thiols, sulfides, and disulfides. High capturing activities by milk against them, except methanethiol, were observed. Furthermore, BSA captured dipropyl and diallyl disulfides preferentially. It is presumed that these effects are caused by interaction between sulfur compounds and molecules of proteins and lipids and that they are based on an affinity among them (hydrophobic property).

Degradation of Diallyl Disulfide. The amounts of 2-propenethiol gas released from diallyl disulfide by vegetables were measured (**Figure 3**). One and a half percent of diallyl disulfide

3860 *J. Agric. Food Chem.,* Vol. 50, No. 13, 2002

Negishi et al.
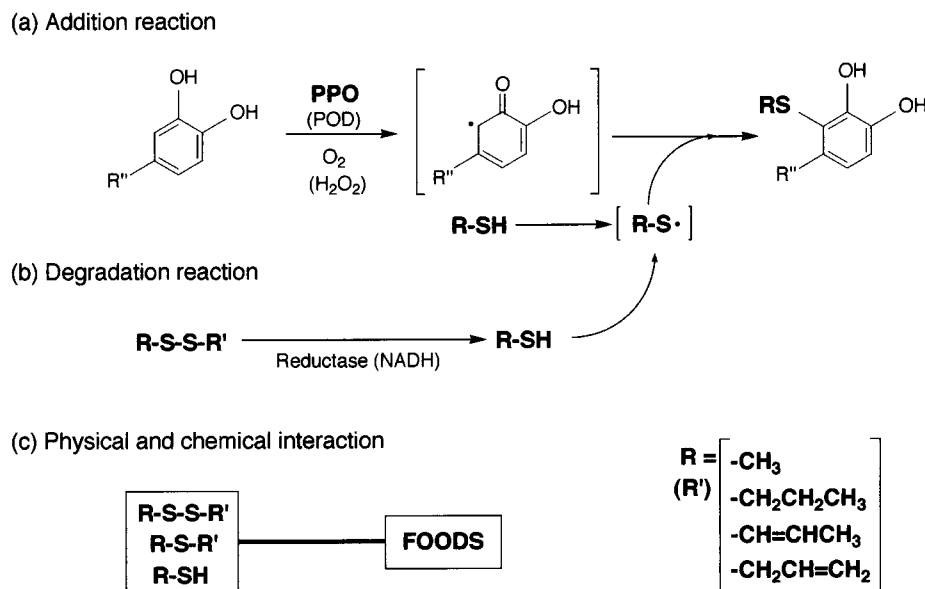
Figure 4. Mechanism of deodorization by food materials. Bad ordors were removed by multiple actions including the physical and chemical interaction between volatile sulfur compounds and foods, the enzymatic degradation of disulfides, and the addition of thiols to polyphenolic compounds, catalyzed by PPO or POD.

was degraded in 10 min by asparagus, whereas in the case of spinach, the amount of 2-propenethiol decreased after 4 min of reaction. It is assumed that the thiol gas is bound to PP contained in spinach and that this reaction is catalyzed by PPO. In taro, a small amount of thiol was also detected. The degradation of diallyl disulfide by microorganisms was also reported (*18*). These degradation reactions seem to be caused by the action of a reductase as seen in the degradation of asparagusate by asparagusate reductase, using NADH as a cofactor (*19*).

Mechanism of Deodorization by Food Materials. Previously we demonstrated that bad breath was removed by the consumption of raw apple, pear, and prune (*2*). Our investigations suggest that bad odors such as halitosis are eliminated by foods due to following three actions (**Figure 4**).

Addition Reaction. Thiols are eliminated by binding to *o*-quinone compounds formed from PP. This reaction rate is enhanced by PPO or POD contained in raw foods. This is called "enzymatic deodorization" (*1, 2*).

Degradation Reaction. Disulfides are degraded by heat to a very limited extent unless high temperatures are involved (*14, 20*), whereas degradation by enzymes such as reductases is suggested by our results. The degradation products, which are thiols, could be removed by an addition reaction to *o*-quinone (the above reaction).

Physical and Chemical Interaction. This is a physical and chemical interaction between volatile sulfur compounds and foods by an affinity to molecules (hydrophobicity) or by a trapping to porous polymers contained in foods.

Consequently, it is demonstrated that unpleasant odors generated in our lives could be eliminated or reduced by the multiple actions of foods given above. If we eat garlic with raw foods having deodorizing activities, much of the disulfides and thiols formed in the mouth and the gut is removed and, furthermore, AMS, which is formed in the gut and causes the persistence of malodorous breath (*10*), may be reduced.

ABBREVIATIONS USED

AMS, allyl methyl sulfide; CG, chlorogenic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; EC, (−)-epicatechin; ESI, elec-

trospray ionization; GHB, γ-L-glutaminyl-4-hydroxybenzene; POD, peroxidase; PP, polyphenolic compound; PPO, polyphenol oxidase; Tyr, L-4-hydroxyphenylalanine; VA, variegatic acid.

LITERATURE CITED

(1) Negishi, O.; Ozawa, T. Effect of polyphenol oxidase on deodorization. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 2080−2084.

(2) Negishi, O.; Negishi, Y. Enzymatic deodorization with raw fruits, vegetables and mushrooms. *Food Sci. Technol. Res.* **1999**, *5*, 176−180.

(3) Negishi, O.; Ozawa, T. Inhibition of enzymatic browning and protection of sulfhydryl enzyme by thiol compounds. *Phytochemistry* **2000**, *54*, 481−487.

(4) Negishi, O.; Negishi, Y.; Ozawa, T. Enzymatic deodorization with variegatic acid from *Boletus subvelutipes* and its mechanism. *Food Sci. Technol. Res.* **2000**, *6*, 186−191.

(5) Negishi, O.; Negishi, Y.; Aoyagi, Y.; Sugahara, T.; Ozawa, T. Mercaptan-capturing properties of mushrooms. *J. Agric. Food Chem.* **2001**, *49*, 5509−5514.

(6) Vamos-Vigyazo, L. Polyphenoloxidase and peroxidase in fruits and vegetables. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 49−127.

(7) Block, E. The organosulfur chemistry of the genus *Allium*−Implications for the organic chemistry of sulfur. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1135−1178.

(8) Block, E.; Naganathan, S.; Putman, D.; Zhao, S.-H. *Allium* chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (greatheaded) garlic, chive, and Chinese chive. Uniquely high allyl-to-methyl ratios in some garlic samples. *J. Agric. Food Chem.* **1992**, *40*, 2418−2430.

(9) Block, E.; Putman, D.; Zhao, S.-H. *Allium* chemistry: GC-MS analysis of thiosulfinates and related compounds from onion, leek, scallion, shallot, and Chinese chive. *J. Agric. Food Chem.* **1992**, *40*, 2431−2438.

(10) Suarez, F.; Springfield, J.; Furne, J.; Levitt, M. Differentiation of mouth versus gut as site of origin of odoriferous breath gases after garlic ingestion. *Am. J. Physiol.* **1992**, *276* (2, Part 1), G425−G430.

(11) Laakso, I.; Seppänen-Laakso, T.; Hiltunen, R.; Müller, B.; Jansen, H.; Knobloch, K. Volatile garlic odor components: gas phases and adsorbed exhaled air analysed by headspace gas chromatography−mass spectrometry. *Planta Med.* **1989**, *55*, 257−261.

(12) Cai, X.-J.; Block, E.; Uden, P. C.; Quimby, B. D.; Sullivan, J. J. *Allium* chemistry: Identification of natural abundance organoselenium compounds in human breath after ingestion of garlic using gas chromatography with atomic emission detection. *J. Agric. Food Chem.* **1995**, *43*, 1751−1753.

(13) Taucher, J.; Hansel, A.; Jordan, A.; Lindinger, W. Analysis of compounds in human breath after ingestion of garlic using proton-transfer-reaction mass spectrometry. *J. Agric. Food Chem.* **1996**, *44*, 3778−3782.

(14) Tamaki, T.; Sonoki, S. Volatile sulfur compounds in human expiration after eating raw or heat-treated garlic. *J. Nutr. Sci. Vitaminol.* **1999**, *45*, 213−222.

(15) Takino, Y.; Imagawa, H. Studies on the mechanism of the oxidation of tea leaf catechins. Part I. On the enzymic oxidation of catechins. *Nippon Nogeikagaku Kaishi* **1963**, *37*, 417−422 (in Japanese).

(16) Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990.

(17) Gill, M.; Steglich, W. Pigments of fungi (Macromycetes). In *Progress in the Chemistry of Organic Natural Products 51*; Herz, W., Grisebach, H., Kirby, G. W., Tamm, Ch., Eds.; Springer-Verlag: New York, 1987; pp 1−317.

(18) Kanagawa, T.; Mikami, E. Removal of methanethiol, dimethyl sulfide, dimethyl disulfide, and hydrogen sulfide from contaminated air by *Thiobacillus thioparus* TK-m. *Appl. Environ. Microbiol.* **1989**, *55*, 555−558.

(19) Yanagawa, H.; Egami, F. Asparagusate dehydrogenases and lipoyl dehydrogenase from asparagus mitochondria. *J. Biol. Chem.* **1975**, *251*, 3637−3644.

(20) Yu, T.-H.; Wu, C.-M.; Ho, C.-T. Volatile compounds of deep-oil fried, microwave-heated, and oven-baked garlic slices. *J. Agric. Food Chem.* **1993**, *41*, 800−805.