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Chemical Constituents of the Volatile Derived from the Black Garlic Processing

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Authors' contributions

This work was carried out in collaboration among all authors. Author JIS designed the study, wrote the protocol and the first draft of the manuscript. Authors HH and YO prepared the black garlic to collect garlic-released gas for study. Author CS performed mainly anti-bacterial analysis of volatiles together with electron analysis. All authors read and approved the final manuscript.

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ABSTRACT

Pungent gas released from the black garlic production was analyzed by Gas Chromatography to clarify compounds included. Three major constituents as ammonia, sulfide-group and aldehyde-group were identified as stimulating agents. Vegetables (plants) released-gas from garlic, onion, horse radish, et al. showed bacteria killing activity against *P. aeruginosa, B. natto*, enterohemorrhagic *E. coli* O157, MRSA and *C. albicans*. Scanning electron micrographs showed that anti-bacteria mechanisms by the volatile seem different depending on species of vegetables (plants).

Keywords: Black garlic volatile; chemical constituents of volatile; garlic processing; antibacterial activity.

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1. INTRODUCTION

Novel type of vegetable black garlic that had been developed in Japan 20 years ago is now rapidly extending its reputation due to a broad range of bioactivity [1,2]. To make the black garlic fresh garlic should be processed by controlling temperature and humidity in a processing room for a month. During processing garlic releases strong pungent gas by chemical reaction, called as *Maillard reaction,* is gradually reducing disliked stimulating odor along with aging times. Further garlic color is also changing from white to deep black and complete processing by creating sweetness and it is directly edible after peeling out-coat.

Whereas both garlic and garlic-release odor (volatile, gas) possess strong bacteria killing potency to be considered as self-defense system to survive in nature [3,4].

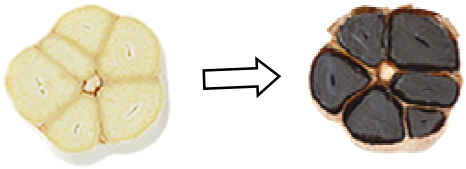
Further garlic is known to contain high amount of sulfur that works both beneficial activity and/or adverse effect for human being. One of representative adverse effects is the pungent gas releasing from injured cells or from the black garlic making, but no data of chemical constituents of the released- gas had not been reported until now.

This report therefore focused on the chemical constituents in volatile that is derived from black garlic processing.

2. MATERIALS AND METHODS

2.1 Pungent Gas (Volatile) Originated During Procession of Black Garlic Production

Fresh white garlic gradually changes its color in a temperature and humidity controlling room along with releasing gas (Photo 1). Pungent gas was offered for chemical analysis by Gas Chromatography (Daiya Bunseki Center, Japan). Final product is deep black in color without pungent smell and edible directly taking off the outer coat (Photo 1).



Before processing **After processing**

(One month later)

Photo 1. Fresh garlic and processed black garlic

Fresh garlic that had been processed under controlling temperature and humidity gradually change color releasing stimulating gas and finally became deep black a month later.

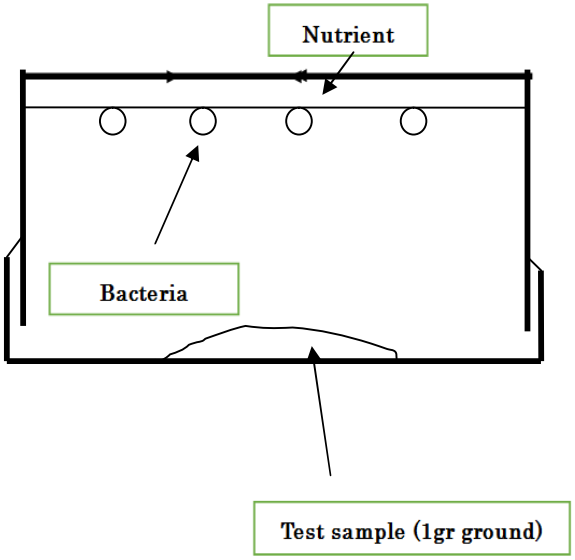


Fig. 1. Petri-dish culture method to examine anti-bacteria activity of volatile released from vegetables (plants)

2.2 Anti-bacteria Activity of Vegetable-originated Volatile

Anti-bacteria activity of vegetable-originated volatile (garlic, onion, horse radish, dokudami (*Houttuynia cordata*), rubbed sage, whole clove, and cinnamon were tested on *Petri-dish culture method* (ø 3 mm, Fig. 1) against bacteria of *P. aeruginosa*, *B. natto*, MRSA, O157 and *C. albicans*. Bacteria killing potency of volatile was evaluated after 24 hrs incubation.

2.3 Scanning Electron Micrograph Inspection

Electron microscopic observation (Shimazu product, Japan) was made to examine cell surface structure of bacteria exposed to volatile (gas).

3. RESULTS AND DISCUSSION

3.1 Chemical Constituents of Gas Released from Black Garlic Processing

Ten compounds out of 22 items examined by Gas Chromatography were detected as summarized in Table 1. Ammonia (0.4 ppm), hydrogen sulfide (0.21), methyl merecaptan (0.03), methyl sulfide (0.03), methyl disulfide (0.14), acetaldehyde (0.47), propionaldehyde (0.57), isobutylaldehyde (0.12), isovaleraldehyde (0.38), normalvaleraldehyde (0.025) and others were below detection limit. Ammonia amount in gas was at 17%, sulfide group at 17% and aldehyde group at 66% respectively. These three compounds groups occupied at 99% in the exhausted gas amount obtained from garlic processing. Most stimulating gas released from garlic processing might be played by aldehyde group gas.

3.2 Bacteria Killing Activity by Vegetables (Plants) Originated Gas

Anti-bacteria activity of plant-produced volatile (gas) had been first reported from our laboratory [5,6] and suggested that gas (smell, odor, volatile) from vegetables (plants) is generalized phenomenon for plants to survive in the plant world. Because they do not have moving means to escape from attacking natural enemies like bacteria, insects, animals.

Garlic was a prominent representative vegetable to produce the strongest bacteria killing gas, and we did confirm its activity against *P. aeruginosa*, *B. natto*, MRSA, O157 as indicated in Photo 2 and Table 2.

Ground test sample (1.0 gr.) was placed in the lid and incubated for 24 hrs (Fig. 1).

Bacteria cultured: From left; *P. Aeruginaosa (P)*, *B. Natto (B)*, O157 (O), and MRSA (M). No growth of any bacteria in the right culture plate incubated with garlic volatile.

3.3 Scanning Electron Microscopic View

Bacteria killing mechanisms of volatile are not well understood but protein degeneration is mostly conceivable way of thinking. As aldehyde group gas is major component included in black garlic released gas. Aldehyde is very powerful disinfectant in a medical field due to protein degeneration of bacteria.

Table 1. Composition of gas released from black garlic processing

Measurement item	Measured value (ppm)	Measuring method
Ammonia	0.4	Absorptiometry
Hydrogen sulfide	0.21	Gas chromatographic
Methyl mercaptan	0.03	〃
Methyl sulfide	0.03	〃
Methyl disulfide	0.14	〃
Trimethylamine	0.0006	〃
Acetaldehyde	0.47	〃
Propionaldehyde	0.57	〃
Isobutyraldehyde	0.12	〃
Normal butyraldehyde	<0.0009	〃

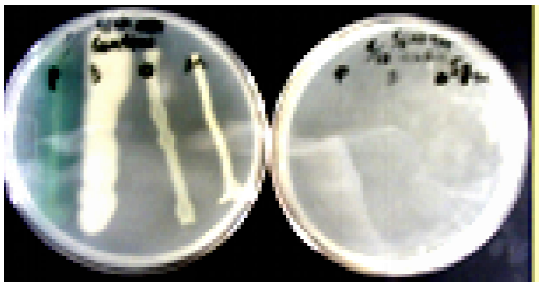
Measurement item	Measured value (ppm)	Measuring method
Isovaleraldehyde	0.38	〃
Normalvaleraldehyde	0.025	〃
Ethyl acetate	<0.3	〃
Isobutanol	<0.09	〃
Methyl isobuthyl ketone	<0.1	〃
Toluene	<1.0	〃
Xylene	<0.1	〃
Styrene	<0.04	〃
Propionic acid	0.009	〃
Normal butyric acid	0.0012	〃
Isovaleric acid	0.004	〃
Normalvaleric acid	0.004	〃

< Below detection limit

Table 2. Summary of bacteria growth inhibition by plants-derived volatile

Bacteria	*Pseudomonas*	*B. natto*	O157	MRSA	*C. albicans*
Garlic	-	-	-	-	
Onion	+	+	+	-	
Dokudami	+	-	+	-	
Japanese Horseradish	-	-	-	-	
Rubbed Sage	+	+	+	+	+
Whole Clove	+	-	-	-	-
Cinnamon	+	-	-	-	-
Control	+	+	+	+	+

(+): Bacteria (Growth), (—): Bacteria (No Growth)



Control (Growth) Garlic Gas (No growth)

Photo 2. Anti-bacteria activity of volatile released from garlic

Present microscopic view suggested that bacteria killing mechanisms by gas (volatile) are depending on types of gas included components from plants (Photo. 3).

Hiba tree (Japanese cypress) gas exposed bacteria produced rough cell surface and transformed shape, but formalin gas treated bacteria was flat in form like sheet implying of contents leakage. Probably bacteria killing actions of gas are variety depending on types of chemicals included in plant-released volatile.

Hiba tree (extracted oil) produces aromatic flavor, the physician now applies it to the waiting room in clinic for patients relaxation and disinfection in room based on our report in Japan.

Control (Intact O157) Hiba tree odor exposed Formalin gas exposed

Photo 3. Scanning electron micrograph of *E. coli* O157; H7 exposed to Hiba tree emitted odor and formalin gas

Lastly, we should refer to acrylamide amount contained in the black garlic for safety and security by showing our analyzing data. Its value was 0.28 ppm/kg, and this amount is enough safe level as an ingestion food compared to these of fried potato (0.38), instant coffee (0.68), and bread (0.02).

4. CONCLUSION

Chemical constituents in volatile released from black garlic processing was analyzed. Major three components were detected as ammonia, sulfide-group and aldehyde-group/ These three compounds occupied at 99% in black garlic processing gas. Vegetable (plants) originated gas tested had bacteria killing activity against *P. aeruginosa*, *B. natto*, MRSA, O157. Gas-exposed bacteria showed different appearance depending on types of gas. Acrylamide included in the black garlic was at safe level like other foods such as fried potato, instant coffee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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