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Characteristics and Antioxidative Activity of Volatile Compounds in Heated Garlic (*Allium sativum*)

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Abstract The aroma characteristics and antioxidative activity of volatile compounds in heat-treated garlic (*Allium sativum* L.) were evaluated. The garlic was heated to various temperatures (100, 110, 120, and 130°C) for different lengths of time (1, 2, and 3 hr). The volatile compounds of heated garlic were extracted by simultaneous steam distillation extraction (SDE). Aroma compound profiles were analyzed by gas chromatography/mass spectrometry (GC/MS) and antioxidative activity was measured by 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) cation decolorization assay. The major aroma compounds were sulfur compounds such as dimethyl disulfide, 2-propen-1-ol, methyl-2-propenyl disulfide, dimethyl trisulfide, diallyl disulfide, methyl-2-propenyl trisulfide, and di-2-propenyl trisulfide. DPPH radical scavenging activity (EDA, %) and the ascorbic acid equivalent antioxidant activity (AEAC) of volatile compounds in heated garlic increased significantly with the increase of temperature and time ($p<0.001$). The EDA (%) and AEAC of raw garlic were 26.8%/10 mg garlic and 39.05 mg ascorbic acid equivalent per g sample. After heat treatment, the highest values were 40.50%/10 mg garlic for EDA (%) and 46.43 mg ascorbic acid equivalent per g sample for ABTS.

Keywords: garlic (*Allium sativum*), heat treatment, simultaneous steam distillation extraction (SDE), aroma characteristics, antioxidative activity

Introduction

Garlic (*Allium sativum* L.) may be the most widely referenced herb in the literature for medicinal properties (1). The purported health benefits of garlic are numerous, including cancer chemopreventive, antibiotic, antihypertensive, and cholesterol-lowering properties (2-5). The characteristic flavor and pungency of garlic are due to an abundance of oil- and water-soluble sulfur-containing elements which likely produce the various medicinal effects. Intact, undisturbed bulbs of garlic, however, contain only a few medicinally active components. Although the pharmacological effects are poorly understood, it is clear that most are derived from the sulfur-containing components of garlic. Many of these active compounds have been identified in garlic and other *Allium* species and belong to one of three groups: dithiines, allyl sulfides, and ajoenes (2). Garlic oils used for medicinal purpose are mostly prepared by hydrodistillation of raw garlic homogenate. The garlic oil is insoluble in water and can therefore be separated or extracted after the distillation process. Garlic oil consists of diallyl, allyl methyl, and dimethyl mono to hexa sulfides (2, 6). Garlic oil from oil-macerated or ether-extracted garlic homogenate contains the 2-vinyl-[4H]-1,3-dithiin, 3-vinyl-[4H]-1,2-dithiin, allyl sulfides, and ajoenes (Z and E) (6).

Aroma is one of the most important sensory attributes of fruits and vegetables and flavors are particularly sensitive to compositional alterations. The volatile compounds that contribute to the flavors of fruits and vegetables are produced through metabolic pathways during ripening, harvest, postharvest, and storage and are influenced by many factors related to species, variety, and technological treatments (7, 8).

Garlic contains 0.1-0.4% volatile oil (7), carbohydrates (accounting for 75% of the dry matter), proteins (15-17% of the dry matter) such as peroxidase, myrosinase, and especially alliinase, lipids (less than 1.2% of the dry matter), and high concentrations of minerals (particularly potassium and phosphorus), vitamins, and glucosinolates (9). The volatile oil consists of sulfur-containing compounds: allicin (2-propene-1-sulfinothioic acid S-2-propenyl ester; $C_6H_{10}S_2$), allyl disulfide (4,5-dithia-1,7-octadiene; $C_6H_{10}S_2$), allyl trisulfide ($C_6H_{10}S_3$), among others. These volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic. In the last 15 years, the majority of studies on garlic have focused on investigating its properties and identifying its volatile compounds which are of interest in the fields of cardiovascular and cancer research (10, 11).

Recent studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activities due to various chemical changes during heat treatment (12-19). In addition, a few studies have examined the chemical and physical properties of garlic treated with both high temperature and pressure (HTPT). The latest studies have reported that heat treatment increased total polyphenolic and flavonoid contents and antioxidant activities in ginseng (12), garlic (13), pears (14), licorice (15), tomatoes (16), citrus peel (17), oranges (18), and *shiitake* mushroom (19). However, no studies have identified the aroma compounds released during heat

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treatment or determined the antioxidant activity of these aroma compounds. The goals of this study were to investigate the changes in aroma components of garlic during high-pressure steam treatment and to evaluate the antioxidant activities of the garlic aroma compounds.

Materials and Methods

Sample treatments Garlic was purchased at a farmer's market in Danyang, Chungbuk, Korea. The samples were packed in a commercial polyethylene bag and stored at -20°C. The garlic was putted into bottle and sealed tightly. Sample bottle was put into the instrument and heat treatment was performed using high-pressure steam generated by temperature and pressure controlling apparatus (Jisico, Seoul, Korea). The samples were heated to different temperatures (100, 110, 120, and 130°C) for varying lengths of time (1, 2, and 3 hr) (12-15).

Isolation of the volatile compounds Homogenized heated garlic (50 g) and 1,000 mL of distilled water were extracted into 50 mL of diethyl ether for 2 hr using a Likens-Nickerson (20) type simultaneous distillation and extraction (SDE) apparatus (Kontes, Vineland, NJ, USA). The extract was dried over anhydrous sodium sulfate, and the solvent was then concentrated to 1 mL with a gentle stream of nitrogen gas (99.99% purity). α-Pinene (1 μL, Sigma-Aldrich, St. Louis, MO, USA) was added to the final extract as an internal standard for GC/MS analysis.

Tentative identification of volatile compounds Tentative identification of the volatile compounds was performed using an Agilent 6890 gas chromatograph/5973N mass selective detector (Palo Alto, CA, USA). Volatiles were separated on a HP-FFAP capillary column (30 m length × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was set at 50°C for 5 min, raised to 230°C at 5°C/min, and held at this temperature for 20 min. The detector and injector temperatures were 250°C. The ionizing energy of the mass selective detector was 70 eV, with a scanning mass range of *m/z* 35-500. Most peaks were identified using the computer library (Wiley 275L program). Chromatographic peaks were checked for their homogeneity with the aid of mass chromatograms for the characteristic fragment ions. Semi-quantitative determinations of the volatile compounds were calculated by relating the peak intensities to the intensity of the internal standard and were expressed as micrograms per g of garlic. Mean values for the individual constituents were calculated from triplicate analyses of each sample.

Scavenging activity on DPPH radicals (electron donating activity) The antioxidant activity of heated garlic volatile compounds by 2,2-diphenyl-2-picrylhydrazyl (DPPH; Sigma-Aldrich) radical scavenging activity was estimated using the method described by Tepe *et al.* (21) and Lim *et al.* (22), with some modifications. Aliquots (0.8 mL) of 0.2 mM DPPH methanol were mixed with 0.2 mL of the extracts. The mixtures were vigorously shaken and left to stand for 30 min under subdued light. The absorbance at 520 nm was measured against diethyl ether as a blank and converted into percentage antioxidant activity using the following formula:

Electron donating activity (EDA, %)
= [1 - (absorbance of sample at 520 nm)/(absorbance of control at 520 nm)] × 100

The mean values were obtained from triplicate measurements.

ABTS radical scavenging activity The total antioxidant activity of heated garlic volatile compounds by 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS, Sigma-Aldrich) radical scavenging activity was estimated using the method of Re *et al.* (23), with some modifications. The ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate solution, and leaving the mixture to stand overnight in the dark at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.4 to 1.5 at 414 nm (molar extinction coefficient, e = 3.6×10^4/mol·cm) (24). Diluted ABTS radical cation solution (1 mL) was added to 50 μL of the extract or ascorbic acid standard solution. The absorbance was measured at 414 nm after 30 min. The ABTS radical cation scavenging activity was expressed as ascorbic acid equivalent antioxidant activity (AEAC) and defined as the mg of ascorbic acid equivalents per g of sample (19).

Statistical analysis The results were reported as the means ± standard deviation (SD). The significance of differences among treatment means was determined by analysis of variance (one-way ANOVA) using SAS version 9.1 (SAS Institute, Cary, NC, USA) with a significance level of 0.05. Correlations between the parameters using regression analysis were also determined.

Results and Discussion

Aroma characteristics The typical total ion chromatograms of aroma extracts in raw (A) and heated garlic (B, treated at 120°C for 2 hr) are shown in Fig. 1. The volatile compounds identified with GC/MS are listed in Table 1, which presents the semi-quantitative composition (α-pinene as I.S.) of the garlic. The major volatile compounds of raw and heated garlic were sulfur compounds such as dimethyl disulfide, 2-propen-1-ol, methyl-2-propenyl disulfide, dimethyl trisulfide, diallyl disulfide, methyl-2-propenyl trisulfide, and di-2-propenyl trisulfide. With increased temperature and time, these sulfur compounds changed from di- or tri-sulfur compounds to mono-sulfur compounds. Generally, the amounts of benzaldehyde, furfural, 2-propen-1-ol, 3,3'-thiobis-1-propene, and pyrazine-type compounds increased with heating time. However, the amounts of sulfur compounds such as dimethyl disulfide, methyl-2-propenyl disulfide, dimethyl trisulfide, diallyl disulfide, methyl-2-propenyl trisulfide, and di-2-propenyl trisulfide decreased with heating time. The compounds most affected by heating temperature were diallyl disulfide and di-2-propenyl trisulfide, which both showed a steady decrease with increasing temperature. The amounts of 3-methyl-thiophene and 2-furanmethanol increased only at

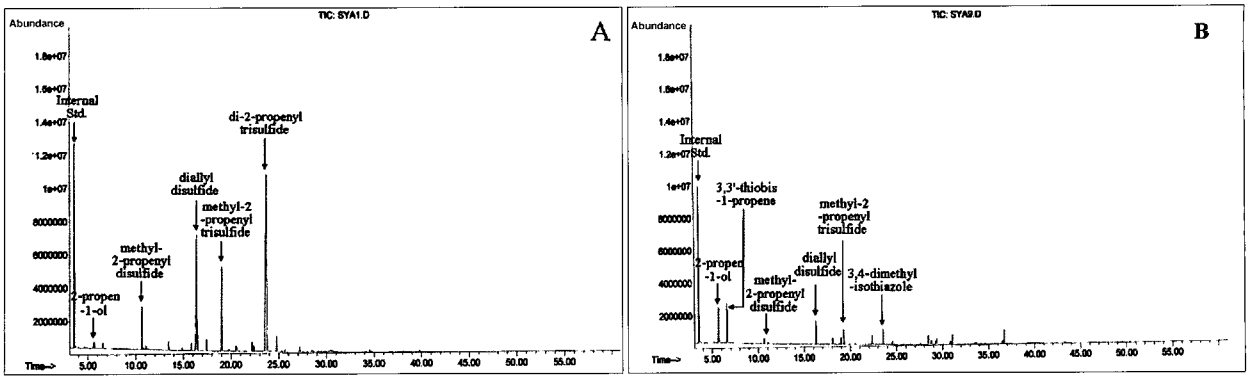

Fig. 1. Total ion chromatograms (TIC) of the volatile compounds found in raw garlic (A) and garlic heated at 120°C for 2 hr (B).

Table 1. The volatile compounds found in garlic under various heating conditions

Retention time (min)	Compound	Content (μg/g)											
		Raw	100°C			110°C			120°C			130°C	
			1 hr	2 hr	3 hr	1 hr	2 hr	3 hr	1 hr	2 hr	3 hr	1 hr	2 hr	3 hr
4.012	2-Butanal	ND[1)]	0.16	0.34	0.13	0.01	0.05	ND	ND	ND	ND	ND	ND	ND
4.263	3-Hexanal	ND	ND	ND	0.13	ND	0.25	ND	0.06	ND	ND	0.15	0.12	ND
4.639	Dimethyl disulfide	0.22	ND	0.00	ND	0.03	ND	ND	ND	ND	ND	ND	ND	ND
5.189	2-Methyl-2-butenal	ND	ND	0.04	0.01	ND	ND	0.19	0.11	0.26	0.07	0.17	0.16	ND
5.635	2-Propen-1-ol	1.31	6.35	10.27	15.24	10.41	14.80	13.05	11.85	15.46	14.59	13.93	13.34	12.42
5.860	3-Methylthiophene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.41	0.35
6.570	3,3'-Thiobis-1-propene	0.93	2.11	6.43	8.00	6.91	11.61	13.04	12.11	11.79	7.72	5.90	2.64	0.75
7.211	4-Pentenylbutanoic acid	ND	0.01	0.41	0.31	0.34	0.34	0.15	0.38	ND	ND	ND	ND	ND
10.249	2-Methylpyrazine	ND	ND	ND	ND	ND	ND	ND	0.11	ND	0.19	0.33	0.60	0.94
10.661	Methyl-2-propenyl disulfide	8.62	0.35	1.00	2.88	3.20	2.52	2.07	2.33	1.38	0.51	0.37	0.11	ND
11.063	3-Methylpyridine	0.88	ND	0.10	0.10	0.02	0.20	0.24	0.05	0.33	0.24	ND	ND	ND
12.059	2,6-Dimethylpyrazine	ND	ND	ND	ND	ND	ND	0.06	ND	0.13	0.23	0.53	0.97	1.14
13.448	Dimethyl trisulfide	1.50	0.07	0.25	0.27	0.20	0.20	0.17	0.11	0.18	0.07	ND	ND	ND
13.544	3-Ethylpyridine	ND	ND	0.23	0.03	0.12	0.13	0.26	0.14	0.29	ND	0.48	ND	ND
16.094	Furfural	ND	ND	0.01	0.01	0.06	0.05	ND	ND	ND	ND	0.13	0.83	2.63
16.310	Diallyl disulfide	53.70	9.06	15.91	21.83	22.57	16.20	8.70	13.08	5.87	2.05	1.53	0.47	0.16
19.050	Methyl-2-propenyl trisulfide	23.24	3.95	8.65	6.45	4.50	3.82	1.76	2.27	1.78	0.55	0.36	0.12	1.05
19.267	2,2-Dimethyl-1,3-dithiane	ND	ND	ND	ND	ND	ND	3.02	0.63	4.30	5.59	5.35	5.94	5.11
19.916	2-Methyl-benzaldehyde	ND	0.03	0.24	0.17	0.19	0.19	ND	0.17	0.18	0.08	0.14	ND	ND
20.526	2-Acetylthiazole	1.95	ND	0.23	0.76	0.47	0.49	0.41	0.33	0.55	0.34	0.49	0.52	ND
20.890	2-Furanmethanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.34	0.93	1.48
22.375	Propanoic acid	1.09	0.26	0.43	1.27	0.94	2.21	2.83	2.21	2.92	2.77	3.24	2.50	2.28
23.574	3,4-Dimethyl-isothiazole	ND	ND	ND	ND	ND	ND	ND	9.69	4.48	1.19	0.93	0.49	0.47
23.764	Di-2-propenyl trisulfide	105.44	37.86	66.26	36.23	23.52	18.15	5.29	ND	ND	0.08	ND	0.11	ND
24.773	3-Vinyl-1,2-dithiocyclohex-5-ene	2.87	0.79	1.15	0.49	0.41	0.27	0.14	0.20	0.12	1.12	0.98	1.25	1.32

[1)]Not detected.

130°C, and those of 2,6-dimethyl pyrazine, 2,2-dimethyl-1,3-dithiane, and 2-methyl-benzaldehyde increased at temperatures above 120°C. 3,4-Dimethyl-isothiazole was created at 120°C and decreased in concentration at higher temperatures.

Allicin (diallyl thiosulfinate) is the major thiosulfinate

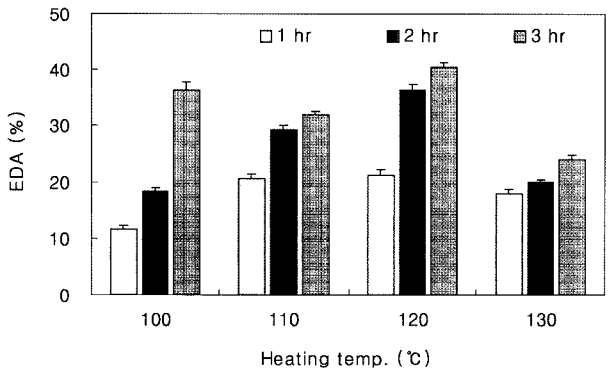

Fig. 2. Change in EDA (%) by DPPH assay on the volatile compounds of garlic under various heating conditions. The EDA (%) on the volatile compounds of raw garlic was 26.82± 1.09/10 mg garlic extract.

compound found in garlic homogenate and is thought to be the principal bioactive compound in aqueous garlic extracts (2). Allicin was affected by heating temperature; the allicin content in raw garlic was 105.44 μg/g, and this content decreased with temperature and time to 110°C for 3 hr. The typical aroma of garlic comes not from just one or a few impact aroma compounds, but from numerous volatiles present at certain concentrations and certain proportions. Thus, garlic aroma is the result of the combined perception of several aromatic constituents. Although over 20 compounds have been identified in the aroma of garlic (25), only a few volatiles (sulfur compounds) appear to be important contributors to garlic aroma. Our study revealed that heating temperature has a profound effect on the production of these aroma compounds. Garlic appears to produce higher levels of mono-sulfur compounds when subjected to higher temperatures for longer periods of time.

Antioxidant activities The use of DPPH free radicals is a common method to evaluate antioxidant activities in a relatively short time compared to other methods. The reduction capability of DPPH radicals was determined by the antioxidant-induced decrease in their absorbance at 520 nm. The DPPH free-radical scavenging potentials of the garlic aroma extract (GAE) on the 10 mg of garlic are shown in Fig. 2. The DPPH radical scavenging activity (EDA, %) of the GAE on the raw sample of 10 mg garlic was 26.82%. After heating at 100 and 120°C for 3 hr, the EDA (%) increased to 36.47 and 40.50%, respectively. The EDA (%) of the GAE treated at 120°C for 3 hr was about 1.5-fold higher than that of raw garlic, and the EDA (%) at 100°C for 3 hr was about 1.3-fold higher than that of raw garlic. With increasing temperature (100-120°C) and time (1-3 hr), the EDA (%) of the GAE increased significantly ($p<0.001$; Table 2). The EDA (%) of the GAE gradually increased with an increase in temperature from 100 to 120°C over 3 hr, and than decreased. The EDA (%) of the GAE of 10 mg garlic at 100°C for 1 hr was 11.67%, about 2.2-fold lower than that of raw garlic. This result suggests that the EDA (%) had a low value at low temperatures but a high value at high temperatures.

The EDA (%) of GAE across both temperature and time was found to be statistically significant (Table 2). In addition, antioxidant activity appeared to be more affected by heating temperature than heating time. The EDA (%) of GAE increased significantly with heat treated temperature (X_1, 100-130°C) and time (X_2, 1-3 hr) ($p<0.001$). Regression coefficients of the second-order polynomial equation were as follows:

$$Y_{EDA(\%)} = -30.83 + 17.12X_1 + 66.31X_2 - 8.06X_1X_2 - 12.20X_2^2 \quad (R^2 = 0.8342)$$

The ABTS radical scavenging activity of the GAE of raw sample, expressed as AEAC and defined as the mg of ascorbic acid equivalents per g of garlic, was 39.05 mg ascorbic acid (AA) equivalents (eq) per g of sample. After heating at 100°C for 3 hr, and at 110 and 120°C for 2 hr, the AEAC values were 45.09, 43.55, and 46.43 mg AA eq/g sample, respectively. With increased temperature (100-120°C) and time (1-3 hr), the AEAC of the GAE increased significantly ($p<0.001$); it gradually increased from 100 to 120°C for 2 hr and than decreased afterward. The AEAC of the GAE, as determined by ABTS radical scavenging activity, are presented in Fig. 3. The AEAC of GAE across both heating temperature and time parameters was found to be statistically significant (Table 2). Moreover, the AEAC appeared to be affected more by heating temperature than heating time. The AEAC of GAE increased significantly with heating temperature (100-130°C) and time (1-3 hr) ($p<0.001$). Regression coefficients of the second-order polynomial equation were as follows:

$$Y_{AEAC} = -24.90 + 29.29X_1 + 27.13X_2 - 4.20X_1^2 - 3.88X_1X_2 \quad (R^2 = 0.6111)$$

Table 2. Analysis of variance for EDA (%) by DPPH assay and AEAC by $ABTS^{\cdot+}$ decolorization assay under various heating conditions

	Variables[1)]	df	Sum of squares	Mean square	F-value	R-square
EDA (%)	X_1	3	1,596.59	532.20	35.48***	0.8342
	X_2	2	839.24	279.75	18.65***	
AEAC	X_1	3	1,098.77	366.26	12.18***	0.6111
	X_2	2	769.23	256.41	8.53***	

[1)]X_1, heating temperature (°C); X_2, heating time (hr); ***$p<0.001$.

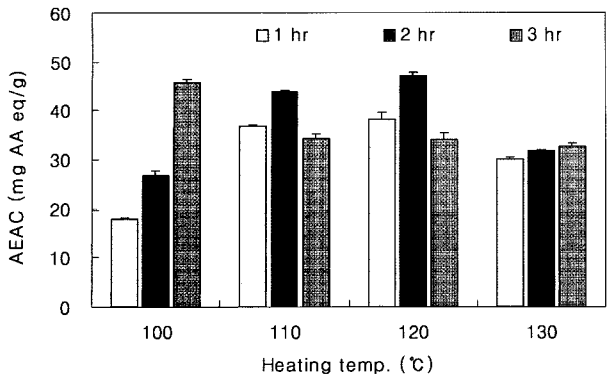

Fig. 3. Change in AEAC by ABTS cation decolorization assay on the volatile compounds found in garlic exposed to various heating conditions. AEAC on the volatile compounds of raw garlic was 39.05±0.69 mg AA eq /g garlic extract.

Gorinstein *et al.* (26) reported that antioxidant activity with heated garlic at 100°C for 20 min in a common oven decreased. In this result, antioxidant activity with heating at 100°C for 1 hr showed the similar tendency. However, we have shown that increasing heating temperature and time significantly enhanced the overall antioxidant activities of GAE. This could be explained by the increased amount of antioxidant compounds (for example, 2-propen-1-ol and 3,3'-thiobis-1-propene etc.) produced by heating. These volatile compounds were increased with heating temperature and time, and the highest values were showed at 110°C for 2 and 3 hr. Many antioxidant compounds in plant materials are present mainly in a covalently bound form with insoluble polymers (18). Therefore, heat treatment may disrupt cell walls and liberate antioxidant compounds from insoluble portions of garlic, increasing the pool of bioaccessible antioxidant compounds. Another reason for improved antioxidant activity could be the formation of novel compounds possessing antioxidant activity during heat treatment or thermal processing. In this study, nonenzymatic browning reaction products may have been formed during prolonged heat treatment, with the improvement in antioxidant activity. Recently, another study carried out on tomato and coffee found that prolonged heat treatment enhanced the antioxidant activity of these food items (25); browning and antioxidant activities of the tomato and coffee samples increased with heating and roasting time. In the last decade, many studies have examined the antioxidant activities after heat treatment and showed that heating products exhibit chain-breaking and oxygen-scavenging activities (13-19, 27, 28). The activities of these components increase after heat treatments due to the low-molecularization effects of the heating process, and thus these active low-molecular weight components are readily extracted. Therefore, it is possible that the release of antioxidant activities from aroma components of garlic after heat treatmen.

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