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## Volatile Organosulfur and Nutrient Compounds Derived From Garlic (*Allium sativum*) According To Cultivating Areas and Processing Methods

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### ABSTRACT

The objectives of this study were the extraction of the volatiles organosulfur compounds of garlic (*Allium sativum*) according to different cultivating area and processing method by solvent extraction, and qualitative and quantitative analysis. The organosulfur compounds and calcium content of garlic bulbs were determined using Gas Chromatography-Mass Spectrometry (GC-MS) and Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Solvents were extracted from homogenates of fresh garlic cloves by cultivating area, which showed major volatile sulfur compounds such as diallyl disulfide (DADS), diallyl sulfide (DAS), diallyl trisulfide (DATS), methyl allyl disulfide (MADS) and methyl allyl trisulfide (MATS). As for the DADS content, Uisung garlic was significantly lower than the others. As for the DAS content, Danyang garlic was significantly higher than the others. As for the DMDS content, Uisung garlic was significantly higher than the others.

**Keywords:** Garlic (*Allium sativum*), Organosulfur compounds, Cultivating areas, Processing methods

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## INTRODUCTION

Garlic (*Allium sativum*) has been used in herbal medicine for thousands of years, and it appears to contain a number of active organosulfur compounds for medicinal use [1, 2]. It is known that garlic has its specific pungent flavor and odor as well as several medicinal properties including antithrombotic, antiatherosclerotic, anticarcinogenic, antihypertensive, hypolipidemic, and antimicrobial potential among other plants [1, 3, 4].

Several techniques have been used to isolate and finally identify the components of garlic that mainly consists of organosulfur compounds. Among them, allicin (allyl 2-propenethiosulphinate or diallyl thiosulphinate) is the major thiosulfinate compound found in garlic homogenate and it is known to be the principal bioactive compound [5]. In order to identify the odor of garlic, various isolation techniques have been used. Among them, the simple solvent extraction method of raw garlic homogenate was identified by various organic solvents [6, 7] and oil macerated garlic [8]. Also distillation setups such as simultaneous distillation extraction (SDE) and microwave-assisted hydrodistillation (MWHd) have been used. Several experimental data from these techniques revealed the existence of thermally unstable organosulfur compounds. On the other hand, the amount of organosulfur compounds in fresh garlic is highly variable. It has been known that the content of organosulfur compounds obtained from garlic samples from various regions is very variable [9]. Especially, the minimum allicin content to ensure pharmaceutical and economic viability of garlic powder products is 4.5 mg/g [9]. Therefore, the objectives of this study were the extraction of the volatiles organosulfur compounds of garlic according to cultivating area and processing method by solvent extraction, and qualitative and quantitative analysis by GC-MS.

## MATERIALS AND METHODS

### Materials and processing methods

Raw garlic bulbs were harvested from the Danyang, Uisung and Seosan area in Korea. They were kept in cooled bags for transport to the laboratory. The materials were packed in 5000 ml glass vessels and kept in cold storage until use. The garlic juice was prepared by homogenizing the required amount of dehusked garlic cloves in an appropriate volume of water to give a concentration of 12.5 mg/ml [10]. The homogenate was centrifuged at 3000 rpm for 10 min to remove garlic sediment and the supernatant fraction and garlic sediment were used for the experiment, respectively. As for preparation of thermal-dried garlic, garlic samples of about 1 cm thickness were dried in an oven at 50°C for 24 hrs. As for preparation of freeze-dried garlic powder, samples of about 1 cm thickness were frozen on trays and freeze-dried (Edwards Alto Vouto, Mini Fast 1700, Milan, Italy) at a residual pressure of 20 Pa, which allowed a sublimation temperature of -34°C. At the end of the drying process the chamber pressure was restored with dry nitrogen. Aliquots of about 10 g of freeze-dried samples were hermetically sealed in a nitrogen atmosphere (Audionvac, Gandus, Milan, Italy) in plastic bags, and stored at 4°C until experimental use. Garlic vines to be sun-dried were placed in the greenhouse for 3 days.

### Determination of nutrient and mineral contents

Table 1: Operating conditions for mineral content analysis by ICP-AES

Instrument	ICP-AES (Varian-Vista)
RF power	0.7-105kW (1.2-1.3kW for axial)
Plasma gas flow rate (Ar)	10.5-15 l/min (radial) 15 l/min (axial)
Auxiliary gas flow rate (Ar)	1.5 l/min
Viewing height	5-12
Copy and reading time	1-5s (max 60s)
Copy time	3s (max 100s)

The chemical properties of garlic bulbs were determined according to AOAC [11]. As to the calcium content, about 0.5 g of thermal and freeze-dried garlic and ground garlic was put into a burning cup and 15 ml of pure  $\text{HNO}_3$  was added. The samples were incinerated in a MARS 5 Microwave Oven at 200°C and the solution was diluted with the certain volume of water. Concentrations were determined using an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) [12]. Table 1 shows the operating conditions for mineral content analysis by ICP-AES.

#### Determination and identification of the volatile compounds from garlic by gas chromatography-mass spectrometry (GC-MS)

About 30 g of crushed garlic bulb and 10 g of freeze and thermal dried garlic powder were put into a small bottle, 90 ml of mixture solution (*n*-pentane : diethyl ether = 1 : 1, v/v) was added and extracted for 12h at room temperature. The mixture was cooled at 0°C for 1h and moisture was removed from the filtrate with anhydrous sodium sulfate. Then the samples were injected into a gas chromatograph (GC). The quantity of sample injected is 1 ml. The Agilent Technologies 6290N gas chromatograph equipped with a HP-5MS non-polar capillary column (30 m×0.25 mm i.d.×0.25 μm df) and a mass selective detector (MSD) was used to analyze the volatile compounds. The operating conditions were as follows: injector temperature, 250°C; detector temperature, 300°C; and column head pressure, 20 psi. The oven temperature was programmed from staying 40°C for 3min and then 40°C to 150°C at an increasing rate of 2°C /min, and 150°C to 200°C at an increasing rate of 4°C /min and staying at 200°C for 10min. Helium was used as carrier gas at a flow rate of 1.0 ml/min. A split ratio of 1:20 was used. The extract was analyzed by Gas chromatography-Mass spectrometry (GC-MS) equipped with a direct split interface and the same column used for the GC. The GC conditions were the same as described above. Mass spectra were obtained by electron ionization at 70 eV and an ion source temperature of 200°C. The mass range (m/z) of 30-350 was used. Diallyl disulfide (DADS), diallyl sulfide (DAS) and dimethyl disulfide (DMDS) were obtained from Aldrich Chemical Company. All chemicals used were of the highest purity grade available. Stock solutions (100μg/ml) of DADS, DAS and DMDS were prepared by diluting the standard DADS, DAS and DMDS with the mixture solution of diethyl ether and *n*-pentane (1:1, v/v). Working solutions were prepared just before use by serial dilution of the stock solutions. The identification and the structural assignment of the volatile organosulfur compounds in the extract were mainly based on the GC-MS information and were accomplished by comparing the mass spectra data with those of authentic compounds available from the WileyNBS computer library.

#### Statistical analysis

All data were presented as the means ± standard deviation (SD), and statistical analyses were performed using Statistical Analysis System version 8.0 (SAS Institute, Cary, NC, U.S.A). The differences between means were assessed by the Duncan's multiple range tests, and statistical significance was defined at  $P < 0.05$ .

## RESULTS AND DISCUSSION

#### Nutrient compositions of garlic by regional groups

The nutrient compositions of northern garlic by regional group are shown in Table 2. The calorie was lower in Danyang than Uisung and Seosan ( $P < 0.05$ ). It is considered that the fat content of garlic in Danyang was lower than that in Uisung and Seosan. The crude fat and crude carbohydrate contents in Danyang was significantly lower than those in Seosan ( $P < 0.05$ ,  $P < 0.05$ , respectively). The crude protein was higher in Uisung than in other areas ( $P < 0.05$ ). Shin *et al.* [13] reported that the crude protein, crude fiber and ash contents in Uisung were higher than those in Uisung and Namhae. On the other hand, it was reported that the moisture and crude ash contents in garlic cloves were not significantly different among the regions. In general, it is well known that the moisture content of garlic is needed to be maintained below 64% for long-term storage [14]. Also Calvey *et al.* [15] reported that the moisture contents of fresh ramp bulbs and garlic cloves were 77-81% and 62-68%, respectively. In this study, the crude fiber and calcium contents were significantly higher in Danyang than in Uisung and Seosan ( $P < 0.05$ ,  $P < 0.05$ , respectively). Especially, the calcium content in Danyang was higher than that in other areas. It was because of the regional characteristics of the Danyang area, which is rich in limestone. Haciseferoğulları *et al.* [16] reported that crude protein, crude oil, crude fiber and crude energy of garlic were 17.3%, 0.34%, 2.17% and 410.7 kcal/100g, respectively. The

crude calory, crude protein and crude oil contents were found to be considerably higher than Danyang garlic, but the crude fiber content was considerably lower. In a previous study, the total mineral content of garlic samples were in a range of 7112.6-9067.3 mg%, and the potassium content showed the highest concentration in a range of 4117.3-5175.3 mg% [13]. Based on the egg-box model, Ca<sup>2+</sup>-pectate is formed according to the cross-linkage between poly-galacturonic acid sequences of pectin chain and the Ca<sup>2+</sup> ion and this increases the hardness of cell wall and decreases the approaching of pectin esterase for pectin or other components of cell wall [17]. The half of the Danyang area consists of soil rich in limestone. With this characteristic of soil in Danyang, the tissue of the six pieces of garlic in Danyang was harder and tighter than that of other regions. Therefore, the calcium content can be the characteristic of garlic quality among garlic cultivars.

**Table 2: Nutrient compositions of garlic by regional groups**

	Danyang	Uisung	Seosan
Calorie (kcal/100g)	342.74±19.21 <sup>a</sup>	352.1±13.54 <sup>b</sup>	350.72±20.64 <sup>b</sup>
Moisture (%)	69.35±8.25 <sup>N.S.</sup>	68.09±7.46	65.58±8.12
Crude protein (%)	6.41±0.18 <sup>b</sup>	7.15±0.31 <sup>c</sup>	5.64±0.84 <sup>a</sup>
Crude fat (%)	0.06±0.00 <sup>a</sup>	0.46±0.04 <sup>b</sup>	0.36±0.01 <sup>b</sup>
Crude ash (%)	1.56±0.24 <sup>N.S.</sup>	1.39±0.16	1.46±0.13
Crude fiber (%)	11.27±2.86 <sup>b</sup>	9.77±3.04 <sup>a</sup>	9.85±3.54 <sup>a</sup>
Carbohydrate (%)	11.35±1.87 <sup>a</sup>	13.14±2.19 <sup>ab</sup>	17.11±1.96 <sup>b</sup>
Ca (ppm)	128.30±14.68 <sup>b</sup>	112.40±16.21 <sup>a</sup>	118.11±12.28 <sup>ab</sup>

Values are mean ± SD. Means in each column not sharing a common superscript letter are significantly different at  $P < 0.05$  by Duncan's multiple range test. <sup>NS</sup> Not significantly different among the groups.

**Nutrient compositions of Danyang garlic by part and by processing methods**

**Table 3: Nutrient compositions of Danyang garlic by processing methods**

	Garlic cloves	Garlic vines	Freeze drying
Calorie (kcal/100g)	342.74±19.21 <sup>b</sup>	127.83±9.87 <sup>a</sup>	315.38±17.64 <sup>b</sup>
Moisture (%)	69.35±8.25 <sup>c</sup>	7.43±0.52 <sup>b</sup>	2.43±0.02 <sup>a</sup>
Crude protein (%)	6.41±0.18 <sup>a</sup>	6.28±0.31 <sup>a</sup>	18.84±2.59 <sup>b</sup>
Crude fat (%)	0.06±0.00 <sup>a</sup>	3.03±0.18 <sup>b</sup>	0.82±0.02 <sup>a</sup>
Crude ash (%)	1.56±0.24 <sup>a</sup>	11.87±2.54 <sup>c</sup>	4.65±0.97 <sup>b</sup>
Crude fiber (%)	11.27±2.86 <sup>a</sup>	48.09±9.21 <sup>b</sup>	12.88±1.48 <sup>a</sup>
Carbohydrate (%)	11.35±1.87 <sup>a</sup>	23.3±4.97 <sup>b</sup>	60.38±9.54 <sup>c</sup>
Ca (ppm)	128.30±14.68 <sup>a</sup>	2839.71±101.32 <sup>c</sup>	459.48±21.79 <sup>b</sup>

Values are mean ± SD. Means in each column not sharing a common superscript letter are significantly different at  $P < 0.05$  by Duncan's multiple range test.

The nutrient compositions of Danyang garlic by processing method are shown in Table 3. The calorie of garlic vines was significantly lower than that of garlic cloves and freeze dried garlic powder ( $P < 0.05$ ,  $P < 0.05$ , respectively). The moisture content of garlic cloves was significantly higher than the others ( $P < 0.05$ ). The crude protein and crude carbohydrate contents of freeze dried garlic powder were significantly higher than those of garlic cloves and garlic vines ( $P < 0.05$ ,  $P < 0.05$ , respectively). The crude fat and the crude fiber contents of garlic cloves were significantly lower than those of garlic vines ( $P < 0.05$ ,  $P < 0.05$ , respectively). However, the crude ash and calcium contents of garlic vines were the highest among the groups ( $P < 0.05$ ). This is considered that garlic vines contain a large amount of fiber. Kim and Chung [18] reported that the proximate compositions of garlic shoot from both cold and warm regions were crude protein 2%, crude lipid 0.4%, crude ash 1.1% and 1.5%, crude fiber 1.3% and 1.5%, respectively. Kimbaris *et al.* [19] reported that in case of ultrasound-assisted extraction, the major essential oil components were found to be 2-vinyl-[4H]-1,3-dithiin (38.1%) and 3-vinyl-[4H]-1,2-dithiin (32.7%). And polysulfides present in minor amounts were diallyl disulfide (8.2%), diallyl trisulfide (0.2%), methyl allyl trisulfide (0.1%), *cis* and *trans* methyl allyl disulfide (0.6%),

diallyl sulfide (0.3%), dimethyl disulfide, dimethyl trisulfide, diallyl tetrasulfide, and traces of 1-dimethyl tetrasulfide. Also ethyl vinyl sulfide was found in a measurable percentage (3.2%). It is well known that these molecules are highly reactive. Moreover, they change spontaneously into other organic sulfur compounds, which take part in further transformations [20].

**Quantitative analysis of Diallyl disulfide (DADS), Diallyl sulfide (DAS) and Dimethyl disulfide (DMDS) in whole garlic cloves by cultivating areas**

The quantitative analysis of diallyl disulfide (DADS), diallyl sulfide (DAS) and dimethyl disulfide (DMDS) from garlic cloves using GC-MS by cultivating area is shown in Fig. 1. The DADS content was 43.85ppm for Danyang, 35.85ppm for Uisung, and 45.14ppm for Seosan, so the content for Uisung was significantly lower than the others ( $P < 0.05$ ). The DAS content was 0.86ppm for Danyang, 0.75ppm for Uisung and 0.79ppm for Seosan, so the content for Danyang was significantly higher than the others ( $P < 0.05$ ). On the other hand, the DMDS content was 0.10ppm for Danyang, 0.79ppm for Uisung and 0.10ppm for Seosan, so the content for Uisung was significantly higher than the others ( $P < 0.05$ ). In a previous study, it was reported that the alliin content was higher in Danyang and Uisung. And their alliin content was about twice as high as that of the same cultivars in the same area Suwon [21]. These results indicated that the characteristics of garlic cultivars are affected by their native cultivating area. Although alliin was not detected in our study, Wi [22] reported that in various garlics the alliin content was 0.34~0.73% of fresh weight. Also Baghalian *et al.* [9] reported that the range of the alliin content had a considerable variation between 2.71% and 6.44%. Furthermore the potential of alliin production within all evaluated ecotypes was higher than the international standard (4.5mg/g). Also it seems that environmental and genetic parameters are both effective in the production of the alliin content. Kimbaris *et al.* [19] reported that essential oils obtained by simultaneous distillation extraction (SDE) is richer in diallyl disulfide (DADS, 28.4%), diallyl trisulfide (DATS, 20.4%), methyl allyl trisulfide (MATS, 16.3%), *cis* and *trans* methyl allyl disulfide (MADS, 9.3%), diallyl sulfide (DAS, 2.3%), dimethyl disulfide (DMDS, 2.2%), dimethyl trisulfide (DMTS, 2.0%), diallyl tetrasulfide (DATTS, 0.7%), 1,4-dimethyl tetrasulfide (DMTTS, 0.4%), and traces ethyl vinyl sulfide (EVS).

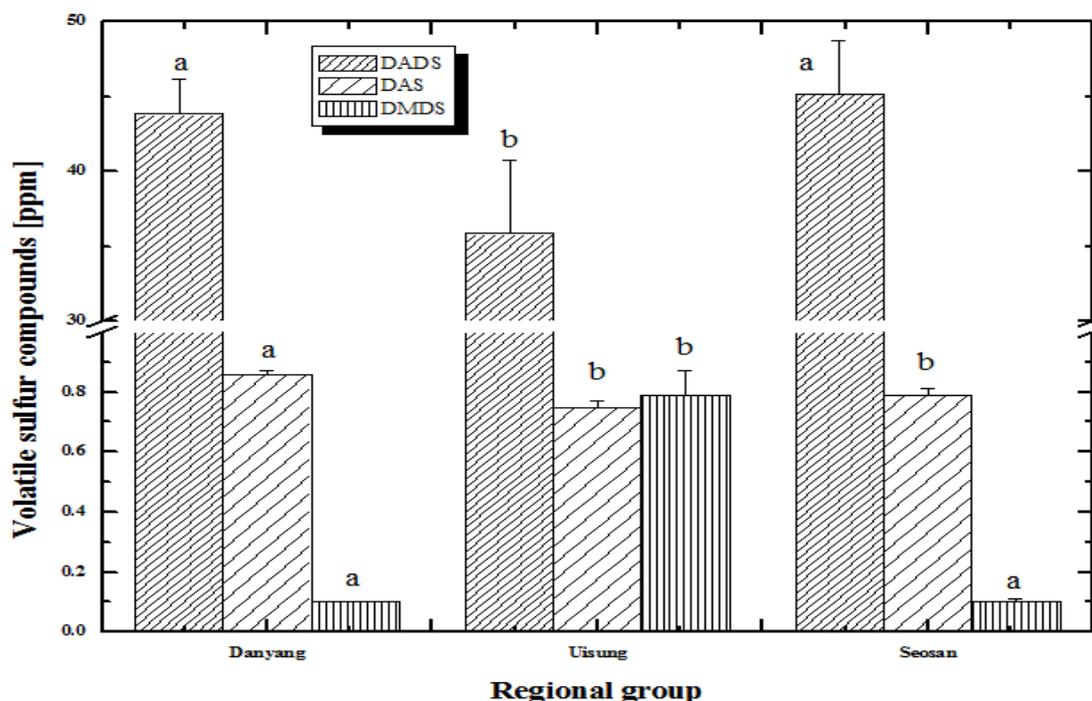


Figure 1: The contents of Diallyl disulfide (DADS), Diallyl sulfide (DAS) and Dimethyl disulfide (DMDS) in whole garlic by region. Data are presented as means  $\pm$  SEM bar. <sup>a, b</sup> Means with different superscript among the groups are different ( $P < 0.05$ ).

**Quantitative analysis of Diallyl disulfide (DADS), Diallyl sulfide (DAS) and Dimethyl disulfide (DMDS) in Danyang garlic by processing methods**

The quantitative analysis of diallyl disulfide (DADS), diallyl sulfide (DAS) and dimethyl disulfide (DMDS) in Danyang garlic by processing method is shown in Fig. 2. The DADS content was 39.47ppm in garlic juice, 24.97ppm in garlic sediment, 31.45ppm in freeze dried garlic powder, 0.30ppm in thermal dried garlic powder and 41.64ppm in garlic vines. The DAS content was 0.67ppm in garlic juice, 0.27ppm in garlic sediment, 0.51ppm in freeze dried garlic powder, 0.30ppm in thermal dried garlic powder, and 0.82ppm in garlic vines. The DMDS content was 0.32ppm in garlic juice, 0.15ppm in garlic sediment, 0.31ppm in freeze dried garlic powder, 0.31ppm in thermal dried garlic powder, and 0.43ppm in garlic vines. Kim and Ahn [23] reported that most of the thiosulfinate and pyruvate existed in storage leaf (90.4%) but little in protective leaf. On the other hand, Kim and Yang [24] reported that the heat process is very effective to reduce odorous volatile organic compounds including DADS and DMDS in garlic. Especially, it was reported that volatile compounds decreased in heat-treated garlic as the heating temperature was raised. Garlic heated at 70, 80 and 90°C had 20%, 31%, and 49% less volatile compounds, respectively [25]. In other previous studies, chopped garlic boiled for 30 min and 60 min had more volatile sulfur compounds than fresh garlic, while 2-vinyl-4H-1,3-dithiin decreased by boiling. However, whole garlic boiled for 30 min and 60 min had less volatile sulfur compounds than fresh garlic while allyl methyl trisulfide and diallyl trisulfide increased by boiling [6]. In recent previous studies, it was reported that the total amount of volatile organic compounds in garlic cloves decreased during freeze-drying from 853.42mg/kg to 802.21mg/kg, and the composition of major components were nearly same in fresh and freeze-dried garlic. Especially the amount of sulfur containing compounds decreased during freeze-drying but methyl propyl trisulfide, 3-allylthiopropionic acid, cyclopentyl ethyl sulfide, ethyl acetate, ethanol, 2-propenol, 2-propenal and hexanal increased in freeze-dried garlic [7]. Sohn *et al.* [26] also reported that pressure-treated garlic at 500 MPa had little pungency and sulfuryl odor compared to raw garlic. These results showed a rapid decrease in the DADS content by the thermal dry process compared to that by the freeze dry process. Also it is considered that garlic vine contains a good deal of organosulfur compounds such as DADS, DAS and DMDS, so it can be recycled as a source of feed additives.

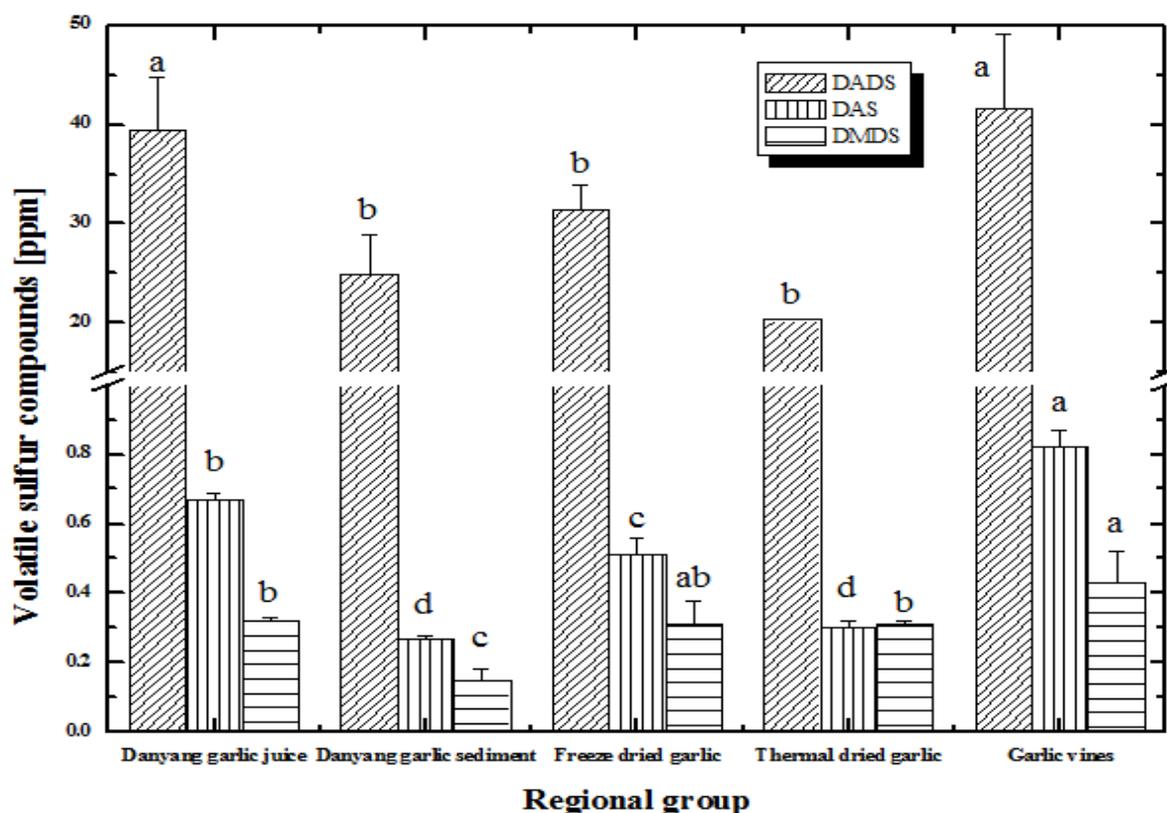


Figure 2: The contents of the Diallyl disulfide (DADS), Diallyl sulfide (DAS) and Dimethyl disulfide (DMDS) in Danyang garlic by processing methods. <sup>a, b, c, d</sup> Means with different superscript among the groups are different ( $P < 0.05$ ).

**CONCLUSIONS**

In conclusion, our results indicated that garlic vines and processed garlic as well as garlic cloves have considerable organosulfur compounds, which have several bioactivities. Therefore, these results may be useful for recycling the garlic vines and controlling the quality and biological activities of garlic in various food industries.

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