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Headspace profile of fresh garlic bulbs, stems, leaves and commercial garlic granules

Running title: Headspace Volatile Profile of Garlic Tissues and Granules

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ABSTRACT

The composition of headspace volatile (HSV) components was determined by direct HS-GC/MS analysis of fresh garlic leaves, stems, and bulbs, as well as granules obtained by extraction of dried bulbs. In all tested samples of fresh garlic parts, the most abundant HSV constituent was diallyl disulfide (65.6 – 46 %), while in granules, diallyl disulfide (27.1 %) and diallyl trisulfide (29.5 %) were the most abundant in approximately equal amounts. It has been shown that automated direct HS-GC/MS analysis can be used to determine volatile constituents of fresh garlic parts and commercial garlic granules.

Keywords: garlic, headspace, GC-MS, sulfides, disulfides, trisulfides

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Introduction

Garlic (*Allium sativum* L.) is used all over the world not only as food and spice but also for the prevention and treatment of numerous disorders of the human body. El-Saber Batiha et al. (2020) have very comprehensively analyzed published research on the composition and activity of garlic bulbs' components and extracts. Research has shown that the most important chemical constituents are: sulfur-containing compounds such as alliin, allicin, ajoenes, vinyl dithiins, and sulfides. Extracts and individual components reported to possess immunomodulatory, anti-inflammatory, anticarcinogenic, antioxidant, antidiabetic, renoprotective, anti-atherosclerotic, antibacterial, antifungal, antiprotozoal, and antihypertensive activities. The question arises: can anything new be discovered about the chemical composition of garlic? Several papers have been published describing different headspace methods for the analysis of volatile components of garlic bulbs: derivatization (Warren et al., 2013), solid phase extraction (Najman et al., 2022; Keleş et al. 2014; Clemente et al., 2011) and loop system method (Molina-Calle et al., 2016). ResearchGate and ScienceDirect index databases listed only one paper related to the analysis of garlic granules by direct HS analysis (Jovanović et al., 2020). With the aim of supplementing the data on the composition of volatile constituents of garlic of individual garlic organs and the possibility of using the direct HS method for their analysis, this paper presents the results of direct HS analysis of volatile components of fresh garlic leaves, stems, bulbs and granules in an automated process without any prior treatment such as derivatization, solid-phase adsorption, or sampling HS components via a so-called loop.

Experimental

Five whole fresh garlic plants and a garlic granule were purchased at a local market. Fresh garlic is divided into bulbs, stems, and leaves, then chopped into similar-sized pieces with a knife. Five hundred mg of each plant part and granules were weighed, and each one was separately placed in a 20 mL HS vial. Only one batch was measured per sample, and each batch was injected only once. The samples were immediately subjected to the following automated HS-GC-MS program:

In the thermostat of the instrument Agilent 7890 gas chromatograph with 7000B GC-MS-MS triple quadrupole system, operating in MS1 scan mode, and equipped with a fused-silica capillary column Agilent HP-5 MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness), they were heated at 70 °C for 20 minutes with shaking for 5 seconds and pausing for 2 seconds. After that, the 500 μL of volatiles with a split ratio 2:1, was injected into the GC. The GC was operated under the following conditions: injector temperature 250 °C; GC-MS interface temperature 300 °C; oven temperature programmed 50 °C for 2 minutes, then to 150°C at 5 °C/min, to 200 °C at 10°C/min (carrier gas He, 1.0 mL/min, constant flow mode). MS conditions were as follows: ionization voltage of 70 eV; acquisition mass range 40-440; scan time 0.32 seconds. HSV were identified from TIC by comparison of their linear retention indices relative to C_8-C_{40} n-alkanes recorded on the same column/temperature program with literature values - NIST Chemistry WebBook and Adams 2007, and their mass spectra with those of standards from Wiley 6, NIST02, Adams by the application of the AMDIS software (the Automated Mass Spectral Deconvolution and Identification System, Ver. 2.7, distributed within software package for 7890-7000 BGC-MS/MS triple quadrupole system). The percentage composition was computed from the TIC peak areas.

Results and Discussion

Fourteen organosulfur compounds were identified using the direct HS-GC/MS method (Table 1). The identified compounds are formed through a series of reactions from S-alk(en)yl-L-cysteine via S-alk(en)yl-L-cysteine-S-oxides, among which alliin is the most abundant. The allinase enzyme is activated during chopping and transforms allin into intermediates that condense in the intracellular aqueous environment into thiosulfinates, among which allicin predominates. These unstable compounds produce a large number of volatile sulfur compounds, among which the most common are diallyl mono-, di- and trisulfides (Figure 1).

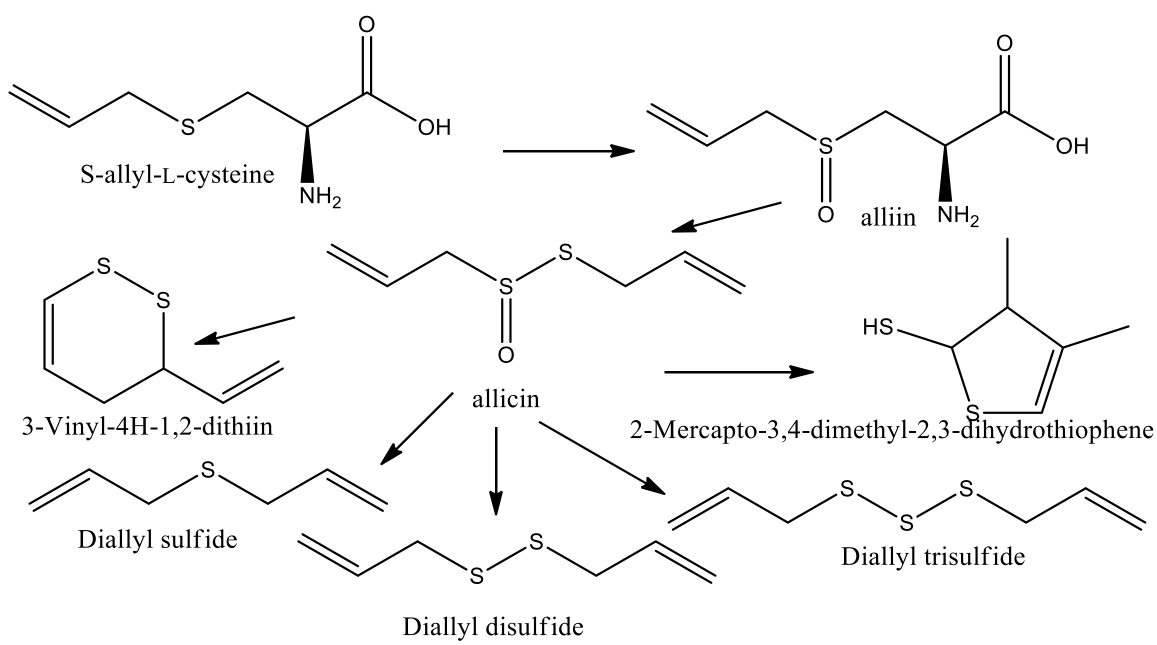

Figure 1. The reaction sequence of the formation of garlic volatile organic compounds

The distribution of organosulfur compounds in leaves differs from their content in the stem and bulbs, which are similar to each other (Table 1). Namely, sulfides and disulfides are more abundant in leaves, while trisulfides are found only in traces. Diallyl disulfide is the most abundant constituent in all three organs examined. Its content accounts for about half of the total content in the stem and bulb and about two-thirds of the content of HSV (headspace volatiles) in the leaves. The abundance of constituents in garlic granules differs significantly from the abundance of the same components in the bulb. The comparison was made only with garlic bulbs, given that the manufacturer's declaration states that the granules were obtained by grinding dried garlic bulbs. The sulfide content is about 8 times higher, the trisulfide content is about 5 times higher, while the disulfide content is about 2.5 times lower. The results obtained are in agreement with those from direct HS analysis on the same apparatus, although the experiment differs in that the previous analysis was performed by adding 1 mL of water to a vial containing 0.2 g of granules (Jovanović et al., 2020). Observed differences in abundance between fresh and dried bulbs indicate that at oven-drying temperatures, thiosulfinates are transformed more rapidly into stable sulfides and trisulfides than into disulfides. The different compositions of fresh and dried bulbs also determine different tastes. Namely, fresh bulbs have a pungent, spicy, and earthy flavor, while granulated garlic is milder and more balanced, with a savory and aromatic taste.

Jimenez-Amezcua et al. (2025) found that the garlic diallyl sulfide content decreases with aging time under controlled temperature (<65 °C) and humidity (60–90 %) conditions from 5.5 µg g^{-1} to 1 µg g^{-1} for 40 days, which is not in accordance with our results (2.2 % fresh bulbs, 7.9 % granules). The diallyl disulfide content also decreases from 38 µg g^{-1} to 10 µg g^{-1}, which is in agreement with the results of this work (46 % fresh bulbs, 27.1 % granules). For diallyl trisulfide an increase from 23 µg g^{-1} to 30 µg $g^{-1}$was observed after 14 days of aging, and then its content dropped to 7.2 µg g^{-1}after 40 days of aging. The results of our samples show an increase from 5.3 % for fresh bulbs to 29.5 % for granules. Radulovic et al. (2015) examined *Allium ursinum* L. essential oils of fresh, air-dried, and oven-dried aerial parts. In this work, it was published that fresh aerial part oil contains 0.6 % sulfides, 1 % disulfides and 0.7 % trisulfides. The sulfides, disulfides, and trisulfides contents of air-dried samples were 60.3 %, 21.0 %, and 27.1 %, respectively. The oven-dried samples contained 28.2% sulfides, 34.6 % disulfides and 57 % trisulfides.

Table 1. The chemical composition (%) of the garlic HSV

RI	RN	Compound	Leaves	Steam	Bulbs	Bulbs*	Granules	Granules**
864.6	859	Diallyl sulfide	5.8	2.5	2.2	6.7	17.9	2.3-26.6
915.3	919	Allyl methyl disulfide	13	12.3	16.4	0.04	6.8	3.8-9.0
929.4	932	Methyl propyl disulfide	t	t	0.5	-	t	-
937.5	940	Methyl (*E*)-1-propenyl disulfide	0.9	1.3	1.8	0.33[b]	t	0.0-0.2
967.6	972	Dimethyl trisulfide	t	t	t	0.18	0.9	0.6-6.5
1079.5	1077	Diallyl disulfide	65.6	48.7	46	24.8	27.1	10.8-28.6
1095.2	1097	Allyl propyl disulfide	5.4	4.9	4.7	-	1.0	-
1105.1	-	Allyl (*E*)-1-propenyl disulfide	9.1	19.1	19.5	-	-	-

1116.0	1110.3	(*Z*)-1-propenyl propyl disulfide	t	0.5	t	-	-	-
1127.2	1117.6	(*E*)-1-propenyl propyl disulfide	t	0.7	0.6	-	-	-
1138.6	1144	Allyl methyl trisulfide	t	1.3	1.8	4.6	7.9	11.3-27
1174.4	-	2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene	t	0.5	0.5	-	-	-
1212.1	-	3-Vinyl-4H-1,2-dithiin (Syn 3-Vinyl-1,2-dithiacyclohex-5-ene)	t	T	t	0.55	0.7	0-0.8
1301.8	1304	Diallyl trisulfide	t	7.5	5.3	18.5	29.5	23.2-59.9
		Sulfides	5.8	2.5	2.2	6.7	17.9	10.6±10.0
		Disulfides	94.0	87.5	89.5	26.87	34.9	25.0±6.2
		Cyclic disulfides	t	t	t	0.55	0.7	0.4±0.4
		Trisulfides	t	8.8	7.1	23.8	38.3	61.6±10.9

RI- Experimental linear retention indices relative to C_8–C_{40} alkanes on the HP-5MS
NI-retention indices from NIST Chemistry WebBook relative to HP-5MS
t -trace (≤0.1 %).
[b] -In the cited paper, it is not stated which stereoisomer it is

[*] Molina-Calle, M., Priego-Capote, F., & de Castro, M. D. (2016). HS-GC/MS volatile profile of different varieties of garlic and their behavior under heating. Analytical and bioanalytical chemistry, 408(14), 3843–3852. https://doi.org/10.1007/s00216-016-9477-0
[**] Jovanović, S. Č., Jovanović, O. P., Mitić, Z. S., Petrović, G. M., & Stojanović, G. S. (2020). Chemical composition and distribution of the headspace volatiles in commercial culinary herbs and spices: Chemometric approach. Journal of the Serbian Chemical Society, 85(8), 1001–1010. https://doi.org/10.2298/JSC191121007J

Molina-Çalle et al. (2016) examined the HSV constituents of fresh white, purple, and Chinese garlic varieties at an equilibrium temperature of 103 °C using headspace sampling via a loop. Within the organosulfur compounds, they found that diallyl disulfide and diallyl trisulfide were major garlic volatiles which represented a relative concentration of 26.4 % and 25.3 %, respectively, for Chinese garlic, 24.8 % and 18.5 %, respectively, for White garlic, and 26.4 % and 25.3 %, respectively, for Purple garlic. It can be assumed that the different experimental conditions are the cause of the not-so-good agreement of diallyl disulfide and diallyl trisulfide contents in their results and the results presented in this paper (diallyl disulfide 46 % and diallyl trisulfide 5.3 %).

Conclusion

By direct automated HS-GC/MS analysis, without pre-treatment, except for the chopping of individual organs of garlic, 14 organosulfur compounds were identified. It has been shown that their distribution in leaves is different from that in the stem and bulb. Furthermore, the content of trisulfides and sulfides is much higher in garlic granules compared to their content in the bulb from which the granules are obtained. It has also been shown that the composition of HSV constituents depends on the sampling method used, i.e. whether HSV constituents are injected directly from a thermostated vial or via a loop.

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Conflict-of-Interest Statement

The author declare no conflicts of interest.

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