

Short communication

Solid-phase microextraction–gas chromatographic–mass spectrometric analysis of garlic oil obtained by hydrodistillation

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Abstract

Garlic (*Allium sativum* L.) is highly consumed worldwide. This crop is mainly known for its flavor and odor, although the many medicinal properties that are attributed to it, including anticarcinogenic, antiatherosclerotic, and antithrombotic potential, among several others, have called the attention of scientists since very early times. It is known that sulfur-containing volatiles are the principal compounds responsible for such properties. The aims of this work were to develop a solventless extraction method for sulfur-containing volatiles from garlic, as well as their chemical characterization. Since garlic volatiles are rather thermolabile, low-pressure hydrodistillation was chosen as the extracting method. The analysis of all compounds was performed on an HP-FFAP chromatographic column mounted in a GC–MS system. For volatile transfer and injection method, solid-phase microextraction was selected, with the use of eight different fibers. The most abundant volatile compound was diallyl disulfide, followed by diallyl trisulfide. Among the 47 totally identified compounds, 18 were linear sulfur-containing volatile compounds, 6 were of non-sulfur nature, and the other 23 were cyclic compounds. However, linear sulfur volatiles accounted for 94% of the total amount. © 2004 Elsevier B.V. All rights reserved.

Keywords: *Allium sativum*; Solid-phase microextraction; Garlic oil; Oils; Hydrodistillation; Organosulfur compounds; Volatile organic compounds

1. Introduction

Garlic is among the best known medicinal plants. Its flavor and odor, as well as its several medicinal properties, including anticarcinogenic, antithrombotic, antiatherosclerotic, hypolipidemic, antihypertensive, and antimicrobial potential among others; have gained to this plant a wide popularity throughout the world [1–4]. Since early times, it is known that most of such properties are given by volatile sulfur-containing compounds that are not present in the intact plant, but are formed after tissue disruption via enzymatic reactions [3,4]. Solid-phase microextraction (SPME) is a rather recent developed technique that has a number of advantages like rapidity, easiness, inexpensiveness, the lack of need for the use of solvents or other pre-concentration steps, and the capability to develop assays in the field. This technique is based on the affinity of a fiber (covered by a polymer) for the different analytes in a sample. It has been

reported that large differences have been obtained after sampling a material with different fibers [5].

The objectives of this study were extraction of garlic volatiles by hydrodistillation and analysis by SPME–GC–MS.

2. Experimental

2.1. Extraction by hydrodistillation

Five kilograms of peeled garlic cloves were grinded in 5 l of water during 10 min. The mixture was heated to 60 °C for 2 h while pressure was slowly decreased to 200 mmHg (1 mmHg = 133.322 Pa). A brilliant yellow-greenish crystal-clear liquid was obtained.

2.2. SPME

One microliter of essential oil was placed inside a 4 ml vial, sealed tightly with a screw-top septum-containing cap, and allowed to stand at 30 °C for 1 h. The SPME needle was

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then inserted through the septum, the holder was secured, and the fiber was exposed to the headspace. After 30 min of sampling at 30 °C, the fiber was retracted and immediately inserted into the inlet of a GC for thermal desorption. Injection was accomplished by desorption of the fiber for 15 min at 220 °C, while the injector operated in the splitless mode for 1 min. Additional 14 min of exposure time in the injection

port allowed the fiber to be cleaned of any compound that may not have been desorbed during the initial minute.

The following fibers were used: polydimethylsiloxane 100 μm (PDMS 100); polydimethylsiloxane 30 μm (PDMS 30); polydimethylsiloxane 7 μm (PDMS 7); polydimethylsiloxane–divinylbenzene 65 μm (PDMS–DVB); polyacrylate 85 μm (PA); Carboxen/polydimethyl-

Table 1
Identified compounds in garlic essential oil^a

RI ^b	Compound	PDMS–DVB	DVB–CAR–PDMS	PDMS 100	PDMS 30	PDMS 7	CW–PDMS	PA	CAR–PDMS
904	Allyl mercaptan	1426	1631	2171	827	179	4009	6153	2230
960	Methyl allyl sulfide	3712	2891	5882	1877	402	2729	6190	9228
1051	2-Butenal	1247	3744	1205	348	143	908	976	5171
1063	2-Methyl-4-pentanal	519	–	639	216	–	–	473	–
1078	Dimethyl disulfide	2052	1124	2709	799	185	1106	2127	1737
1097	2-Methyl-2-butenal	2317	1753	2888	875	181	1076	2043	4356
1110	2-Methylene-4-pentanal	4963	3670	6971	2149	482	2784	5416	8955
1132	2-Pentenal	176	178	230	59	–	82	–	346
1145	Diallyl sulfide	14453	7981	23228	7461	1623	6651	19372	31916
1210	Thiazole	10	–	–	–	–	–	–	26
1216	2-Methylfuran	–	93	–	–	–	–	–	203
1218	Methyl propyl disulfide	201	128	280	90	–	119	306	390
1244	2,4-Dimethylthiophene	336	486	409	153	27	413	917	1312
1261	Methyl allyl disulfide	66435	40422	90799	32953	6463	44999	94684	50286
1269	1-Propenyl methyl disulfide	19101	11835	22968	9031	1824	12756	23279	16433
1289	3-Methylpyridine	207	278	277	–	31	160	351	568
1329	2,5-Dimethylpyridine	315	178	369	–	–	93	201	301
1369	Dimethyl trisulfide	17560	12615	22620	7391	1353	15420	30687	13697
1397	2,4-Hexadienal	704	534	862	216	22	466	1079	–
1496	Diallyl disulfide	283087	187845	376053	176668	38538	211064	416572	216343
1518	Methyl allyl thioacetate	354	54	–	–	–	–	–	–
1527	2-Ethyl-1,3-dithiane	–	–	–	–	–	–	–	105
1533	Metoxymethyl isothiocyanate	12686	7339	16982	7382	1201	13099	22450	9457
1551	Cyclopentathiazole	362	184	589	159	19	252	541	223
1605	Methyl allyl trisulfide	111619	73281	142992	61198	10811	85040	168022	70051
1659	5-(Methylthio)-4-penten-2-ol	–	–	418	51	–	351	–	–
1666	Butylpropenyl sulfide	383	157	991	188	–	–	1385	–
1678	Methylthiocyclopentane	827	453	1542	372	–	586	1666	510
1679	2-Vinyl-1,3-dithiane	–	–	–	–	–	163	–	–
1703	3-(Methylthio)penta-2,4-dione	–	–	–	–	–	–	–	562
1704	Methylthiocyclohexane	431	179	930	197	–	290	1023	140
1714	2-Thiophenecarboxaldehyde	177	72	–	–	–	140	–	108
1740	3,5-Diethyl-1,2,4-trithiolane	1062	436	1845	–	–	702	1874	465
1750	3-Vinyl-1,2-dithiocyclohex-4-ene	10157	4528	13687	4930	587	6614	16540	5975
1763	Dimethyl tetrasulfide	1327	410	880	–	–	–	–	516
1778	2-Ethylidene-1,3-dithiane	804	585	2415	815	–	731	2991	721
1819	Diallyl trisulfide	156381	69166	178245	95345	17098	131124	171192	63807
1869	1,3,5-Trithiane	–	–	–	–	–	–	–	650
1874	3-Vinyl-1,2-dithiocyclohex-5-ene	13886	5306	18169	6486	768	8339	19376	5785
1910	2-(1-Propenylthio)thiophene	–	–	–	–	–	–	1799	351
1998	Ethyl-2-thiopheneacetate	–	–	–	–	–	–	–	564
2074	Cyclopentyl hexyl sulfide	–	–	–	–	–	–	4228	–
2191	4,5-Dimethylisothiazole	–	–	–	–	–	676	1547	–
2225	3-Methyl-1,1-bis(methylthio)-1,3-butadiene	–	–	–	–	–	–	2014	–
2254	N-Morpholinomethyl isopropyl sulfide	–	–	–	–	–	338	867	–
2467	1,3-Benzedithiol	–	–	–	–	–	–	–	49
2468	Benzothiophene	243	128	313	132	25	210	642	1069

^a Arbitrary units.

^b Linear retention indices.

siloxane 75 μm (CAR–PDMS); Carbowax–divinylbenzene 65 μm (CW–DVB); divinylbenzene–Carboxen–polydimethylsiloxane 50/30 μm (DVB–CAR–PDMS).

2.3. GC–MS

Analyses were carried on an HP 5890 Series II gas chromatograph directly coupled to an HP 5972 mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The capillary column used was an HP-FFAP (30 m \times 0.25 mm i.d., film thickness 0.25 μm ; Hewlett-Packard). Conditions were as follows: column temperature was held at 40 $^{\circ}\text{C}$ for 3 min and then increased at 5 $^{\circ}\text{C}/\text{min}$ to 220 $^{\circ}\text{C}$; helium was used as carrier gas at a linear flow of 2 ml/min. Mass spectra were obtained at 70 eV and the ion source was at 230 $^{\circ}\text{C}$. The linear retention indices of the volatile compounds were calculated with *n*-paraffin series as standard. All the essays were performed three times.

The identification of the analyzed compounds was accomplished by comparing their mass spectra with those of authentic compounds available from computerized spectral database (NIST/EPA/NIH 75K) or published literature [6–10].

3. Results and discussion

A total of 47 compounds were detected, which can be classified in sulfur, non-sulfur, and cyclic compounds (Table 1). It is worth to note that the general abundance of sulfur compounds was generally much higher than those of the compounds in other groups. Within this first group were detected 18 compounds, most of them reported as therapeutically active, mainly as anticancerigen and cholesterol-lowering agents [1–3]. Diallyl disulfide was the most abundant, and was best extracted by the PA fiber, followed by the PDMS 100 fiber. Next in abundance are diallyl trisulfide and methyl allyl trisulfide, whose levels were maximum after sampling

with the PDMS 100 fiber and the PA fiber, respectively. The second group had the lesser amount of compounds (six in total), which were also the less abundant. Many cyclic compounds were detected (23 in total), although their levels were generally low. Our results are in concordance with previous works regarding the chemical composition of garlic oil [3,4,6,9]. In general, several differences were found among SPME fibers. The polyacrylate fiber showed the highest sensitivity according to the total amount extracted, followed by the polydimethylsiloxane 100 μm fiber. However, the CAR–PDMS extracted the maximum number of compounds if compared to the rest of the fibers. It was also evidenced that polymer thickness is decisive in the amount of volatiles recovered, since in most cases there was a correlation between abundances of the different compounds and polymer thickness when the three PDMS fibers were compared.

In general, it can be said that extraction by hydrodistillation and analysis by SPME–GC–MS allowed the characterization of a wide number of pharmacologically important compounds from garlic.

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