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Journal of Chromatography A, 677 (1994) 345–354

JOURNAL OF
CHROMATOGRAPHY A

Analytical strategy by coupling headspace gas chromatography, atomic emission spectrometric detection and mass spectrometry Application to sulfur compounds from garlic

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First received 20 January 1994; revised manuscript received 13 April 1994

Abstract

Numerous sulfur-containing molecules are present in garlic cloves, alliine being the most important. The characteristic aroma of garlic is obtained after the reaction of the enzyme alliinase which converts alliine into allicine. This very unstable molecule is rapidly converted into a series of numerous other odorous sulfur-containing molecules. This complex mixture of volatile compounds was analysed using GC–atomic emission spectrometric detection and GC–MS after introduction with a headspace device. The analysed products were non-peeled and peeled whole garlic cloves, peeled and crushed garlic cloves and garlic juice obtained by crushing the cloves. A comparison was made between the heating times of the headspace device (5 and 45 min). The two-stage analytical strategy used for this kind of analysis is described: (i) location of the sulfur-containing compounds from the specific sulfur chromatograms obtained by monitoring the sulfur atomic emission line with an atomic emission spectrometer coupled to a gas chromatograph and (ii) structural analysis of the located and selected compounds by mass spectrometry after transposing the chromatographic method to GC–MS.

1. Introduction

Garlic, *Allium sativum* L. [1–3] has been known since antiquity for its medicinal properties and its characteristic flavour. The chemical composition of garlic cloves has been described in numerous studies and its pharmacological properties have been documented and established. These properties are associated with the presence of sulfur-containing molecules which are the main components of garlic. Their formation and degradation processes remained unknown for a long time because of the complexity of the chemical mechanisms giving rise to their formation during garlic use. Alliine [4–9], which is the major component, is converted into allicine by alliinase [10], an enzyme released when the garlic cloves are crushed. Allicine, which is very unstable chemically, allows the formation of a complex mixture of volatile sulfur-containing compounds.

The influence of the starting product was studied by measuring quantitatively and qualitatively the various mono-, di- and trisulfides formed from the garlic cloves. Their identification was carried out simultaneously by measure-

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SSDI 0021-9673(94)00382-J

ment of the C/S and H/C inter-element ratios using both atomic emission and mass spectrometry. The results shown here demonstrate the use of the dual analytical strategy of applying both atomic and mass spectrometric methods.

2. Experimental

2.1. Headspace device

The headspace device used was an HP 19395A from Hewlett-Packard. The temperature of the oil-bath was set at 70°C and that of the injector valve at 85°C. Two time intervals (5 and 45 min) were used for sample heating. The injector cycle was set as follows: pressurization, 10 s; ventilation, 10 s; injection, 130 s.

2.2. Gas chromatograph

The gas chromatographs used were HP 5890 Series II models. They were equipped with a capillary column (HP5, 25 m × 0.31 mm I.D., film thickness 0.53 μm, diphenylpolysiloxane 5%, dimethylpolysiloxane 95%). Helium (99.999%) was used as the mobile phase. The injector temperature was set at 220°C. Injection was performed according to the split mode (splitting ratio 1:40). The oven temperature was programmed as follows: 40°C for 2 min, then increased at 3°C/min up to 70°C, 7.5°C/min up to 205°C and 25°C/min up to 250°C. The temperature of the transfer line was 250°C.

2.3. Atomic emission spectrometric detector

The atomic emission spectrometric (AES) detector was an HP 5921A equipped with a Beenakker-type resonant cavity. The frequency of the microwave beam was 2.75 GHz and the plasma energy was 70 W. The plasma induced by microwaves was generated from helium (99.9999%). Oxygen and hydrogen were used as reactant gases under a pressure of 100 kPa (purity 99.998% for both gases). The discharge tube was cooled by water (60°C). The data were processed using an HP 59970 Chemstation. For atomic emission detection the following emission lines were monitored: carbon, 193.03 nm; sulphur, 180.7 nm; and hydrogen, 486.1 nm.

2.4. Mass-selective detector

The analytical system used was an HP 5970 B mass-selective detector with a quadrupole mass filter, operating in the electron impact mode (ionization energy 70 eV). Mass spectra were recorded in the scan mode (one scan per second from m/z 35 to 300). The data were processed using an HP 59970 Chemstation.

3. Results

3.1. Elemental chromatograms: comparisons of carbon and sulfur profiles

AES detection when coupled with GC allows elemental chromatograms to be obtained from the detection and continuous monitoring of the specific atomic line of an atom or isotope [11,12]. Fig. 1 displays the chromatograms recorded by monitoring the 193-nm line for carbon and the 181-nm line for sulfur from the compounds evaporated from a peeled garlic clove placed inside the headspace device. The heating time of the sample was 45 min. The carbon chromatogram is a non-specific chromatogram and allows the detection of all the organic compounds that are present in the sample.

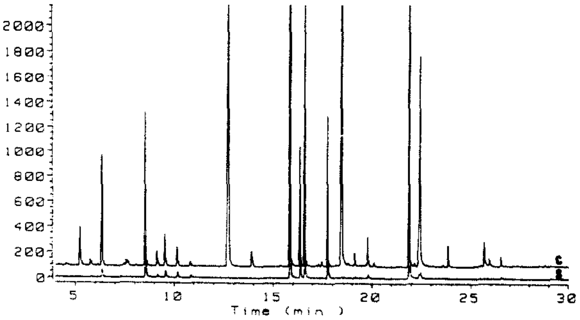

Fig. 1. Elemental chromatograms of carbon (193.03 nm) and sulfur (180.7 nm) for a whole peeled garlic clove.

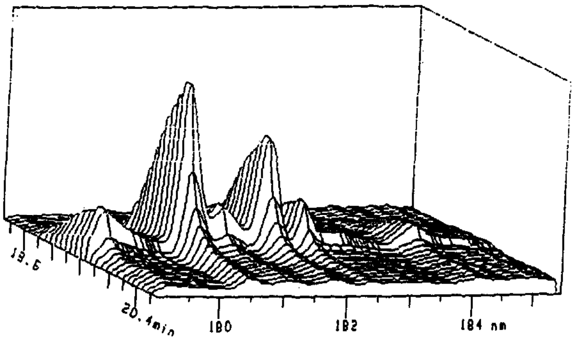

Fig. 2. Spectrum of the sulfur-containing compound eluted at 19.8 min.

When detection is carried out with the 180.7-nm line, which is characteristic of sulfur, only the sulfur-containing compounds are specifically detected. The presence of sulfur is verified by recording the three-dimensional emission spectrum of the chromatographic peak (Fig. 2) using the potential of an HP 5921A diode-array spectrophotometer [13,14]. On this spectrum the three characteristic emission lines of sulfur can be observed at 180.7, 182.0 and 182.7 nm.

Fig. 3 shows carbon and sulfur chromatograms obtained from the aroma of a crushed garlic clove. It can be observed that the compound with a retention time of 2.4 min contains sulfur atoms whereas that at 3.5 min does not contain sulfur because of the absence of a peak at 180.7 nm. This sulfur elemental chromatogram reveals nineteen sulfur-containing compounds. The compositions of the garlic effluvium from peeled cloves, non-peeled whole cloves, crushed cloves and garlic juice were compared. The results are given in Tables 1 and 2 and Figs. 4 and 5.

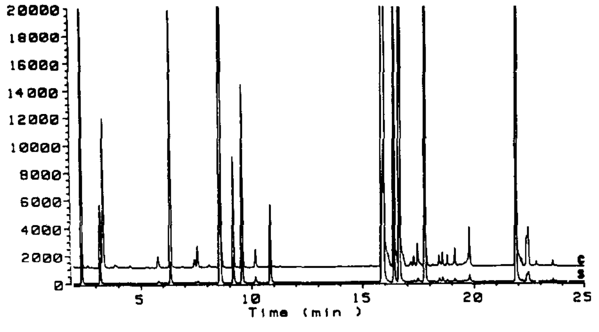

Fig. 3. Elemental chromatograms of carbon (193.03 nm) and sulfur (180.7 nm) for a crushed garlic clove.

Table 1 (and Fig. 4) allows one to compare the relative compositions (%) of sulfur-containing compounds for the four types of sample according to the two heating conditions (5 and 45 min). Table 2 (and Fig. 5) gives the normalized results, the more abundant compounds being taken as the 100% value. These results show that both the type of sample and the heating time have an influence on the qualitative and quantitative composition of the garlic aroma.

Whole non-peeled garlic clove

When the heating time in increased from 5 to 45 min, new sulfur-containing compound are formed and eluted from the whole non-peeled clove. A significant variation in the relative proportions of each component can be observed with an important decrease in the principal compound with a retention time of 15.9 min (Table 1). Increasing the heating time inside the headspace device allows more sulfur-containing compounds to be generated with different relative proportions. The dry envelope partially prevents the sulfur-containing molecules from escaping from the clove.

Whole peeled clove

In the whole peeled garlic glove, most of the sulfur-containing compounds are detected with a heating time of 5 min at 70°C in the headspace device. The variations induced by a longer heating time are less important than those obtained from a non-peeled garlic clove. Increasing the heating time decreases this screening effect.

Crushed garlic clove

Important quantitative variations can be observed according to the heating time when crushed cloves are analysed. Qualitative differences can also be observed: five new sulfur-containing compounds are located on the elemental chromatograms and the overall area of all the chromatographic peaks corresponding to

Table 1
Relative percentages of the sulfur-containing compounds eluted, with heating for 5 and 45 min

t_r (min)	Non-peeled clove		Peeled clove		Crushed clove		Juice	
	5 min	45 min	5 min	45 min	5 min	45 min	5 min	45 min
2.4	12.91	2.98	15.61	11.73	0.74	3.64	5.2	4.35
3.2	0	0	0	0.32	1.76	2.25	1.82	1.68
5.8	0	0	0	0	0	0.28	0	0
6.4	5.84	2.69	1.67	4.94	0.52	2.1	6.35	5.87
8.6	8.83	14.64	7.85	8.86	18.05	12.96	1.93	2.58
9.2	0	0	0	0.22	0.32	2.56	0.62	0.68
9.6	0	0	0	0.24	3.31	4.11	6.08	5.77
10.2	0	0	0	0.23	0.5	0.63	0	0
10.9	0	0	0	0.21	0.25	3.97	2.79	2.85
15.9	66.93	57.81	69.06	53.54	35.17	21.87	13.89	14.74
16.4	0	1.87	1.03	1.82	1.68	5.42	2.25	2.68
16.7	0	4.19	2.05	3.29	20.32	11.38	15.59	15.76
17.5	0	0	0	0	0.9	0.32	0	0
17.8	0	3.86	0	2.43	3.01	12.33	15.97	15.77
19.14	0	0	0	0	1.77	0.35	0	0
19.8	0	0	0	0.21	3.12	0.76	0	0
21.9	5.49	11.95	2.72	11.7	7.71	13.19	26.26	25.87
22.4	0	0	0	0.28	0.48	0.62	0	0
22.5	0	0	0	0	1	1.03	0.68	0.73
23.6	0	0	0	0	0	0.24	0.48	0.57
Sample code	S1	S2	S3	S4	S5	S6	S7	S8

the sulfur containing molecules is multiplied fourfold. Hence crushing the clove allows the release of a larger amount of volatile sulfur compounds.

Garlic juice

The number of volatile compounds is qualitatively lower than when the crushed clove is analysed. The compounds with retention times at 5.8, 10.2, 17.5, 19.2, 19.8 and 22.4 min are either evaporated or decomposed before analysis. The two compounds with retention times of 10.2 and 19.8 min (Tables 1 and 2) are absent from the juice. The variation of the relative proportions of the various compounds with the heating time of the sample is smaller than with the other samples studied. After a 45-min heating time, the quantitatively major compounds are those with the longer retention times.

3.2. Recording of mass spectra corresponding to the retention times of sulfur-containing compounds

Chromatograms obtained under the same conditions by the spectrometric sulfur emission at 180.7 nm and by mass-selective detection were compared. Fig. 6 shows the chromatogram obtained from a crushed garlic clove after a heating time of 45 min. Fig. 7 shows the total ion current recorded by GC from the same sample. Comparing the retention times from both profiles allows one to locate the sulfur-containing compounds on the AES profile and record the mass spectra at the various retention times of the previously located peaks.

The flow-rate inside the chromatograph column has to be optimized according to the type of detector used in order to obtain the same retention times because the pressures at the end of

Table 2
Absolute intensities of the sulfur-containing compounds eluted, with heating for 5 and 45 min

t_r (min)	Non-peeled clove		Peeled clove		Crushed clove		Juice	
	5 min	45 min	5 min	45 min	5 min	45 min	5 min	45 min
2.4	19.29	5.15	22.60	21.91	2.10	16.64	19.80	16.81
3.2	0.00	0.00	0.00	0.60	5.00	10.29	6.93	6.49
5.8	0.00	0.00	0.00	0.00	0.00	1.28	0.00	0.00
6.4	8.73	4.65	2.42	9.23	1.48	9.60	24.18	22.69
8.6	13.19	25.32	11.37	16.55	51.32	59.26	7.35	9.97
9.2	0.00	0.00	0.00	0.41	0.91	11.71	2.36	2.63
9.6	0.00	0.00	0.00	0.45	9.41	18.79	23.15	22.30
10.2	0.00	0.00	0.00	0.43	1.42	2.88	0.00	0.00
10.9	0.00	0.00	0.00	0.39	0.71	18.15	10.62	11.02
15.9	100.00	100.00	100.00	100.00	100.00	100.00	52.89	56.98
16.4	0.00	3.23	1.49	3.40	4.78	24.78	8.57	10.36
16.7	0.00	7.25	2.97	6.14	57.78	52.03	59.37	60.92
17.5	0.00	0.00	0.00	0.00	2.56	1.46	0.00	0.00
17.8	000	6.68	0.00	4.54	8.56	56.38	60.81	60.96
19.14	0.00	0.00	0.00	0.00	5.03	1.60	0.00	0.00
19.8	0.00	0.00	0.00	0.39	8.87	3.48	0.00	0.00
21.9	8.20	20.67	3.94	21.85	21.92	60.31	100.00	100.00
22.4	0.00	0.00	0.00	0.52	1.36	2.83	0.00	0.00
22.5	0.00	0.00	0.00	0.00	2.84	4.71	2.59	2.82
23.6	0.00	0.00	0.00	0.00	0.00	1.10	1.83	2.20
Sample code	S1	S2	S3	S4	S5	S6	S7	S8

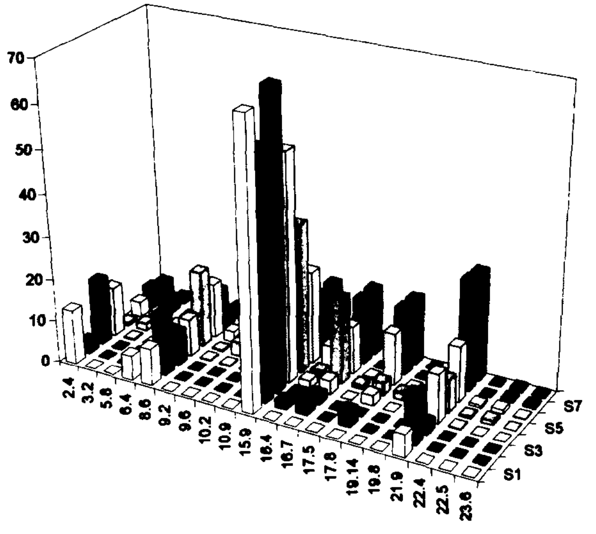

Fig. 4. Histogram of relative percentages of sulfur-containing compounds eluted. S1 = non-peeled garlic clove (5 min); S2 = non-peeled garlic clove (45 min); S3 = peeled garlic clove (5 min); S4 = peeled garlic clove (45 min); S5 = crushed garlic clove (5 min); S6 = crushed garlic clove (45 min); S7 = garlic juice (5 min); S8 = garlic juice (45 min). y-Axis represents %, x-axis: time in min.

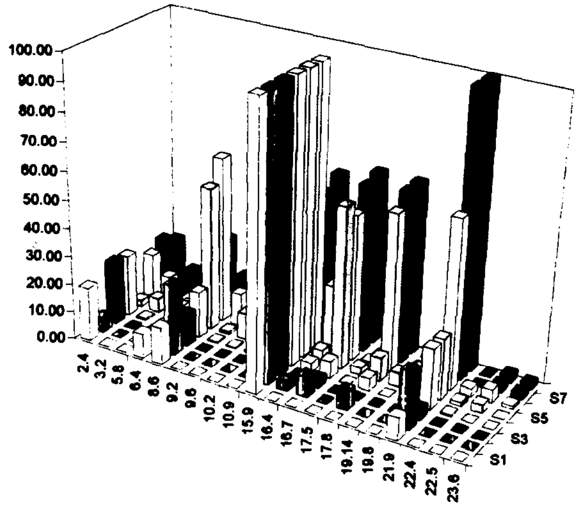

Fig. 5. Histogram of the absolute intensity of the sulfur-containing compounds eluted. S1–S8 as in Fig. 4. y-Axis represents %, x-axis: time in min.

the column are different when AES and MS detectors are connected. The characteristic ions of the compounds eluted from the whole garlic

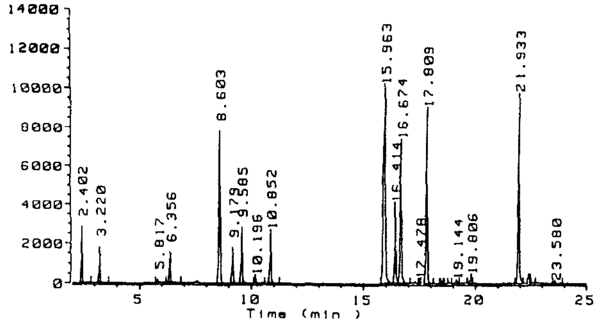

Fig. 6. Elemental chromatograms of sulfur (180.7 nm) from a crushed garlic clove. Heating time in the headspace device: 45 min.

clove and crushed cloves are given in Tables 3 and 4, respectively. Two compounds were easily identified from these spectra, at a retention time of 5.97 min allyl sulfide ($m/z = 114$; $C_6H_{10}S$) and at 16.28 min diallyl disulfide ($m/z = 146$; $C_6H_{10}S_2$). The identification of all other sulfur-containing molecules was achieved using data from both AES (inter-element ratios) and MS.

3.3. Inter-element ratio

The analytical response of the AES detector is proportional to the concentration and to the number of emitting atoms present in the analysed sample. When two elements are simultaneously monitored, their relative response depends only on the number of atoms. The ratio between the response areas of these atoms affords the inter-element ratio [15–17].

For each of the eluted compounds the C/S and the H/C elemental ratios were calculated. In order to calculate these ratios accurately, two compounds whose composition was given by mass spectrometry were taken as standards in order to obtain the coefficient of proportionality, K, between the elements. The $K_{C/S}$ value calculated from allyl disulfide was 2.12 and the value of $K_{H/C}$ calculated from allyl sulfide was 2.34 (means of three measurements). These values were calculated according to the following equation:

$$K_{C/S} = (\text{No. of S atoms}) \cdot (\text{area of carbon peak}) / (\text{No. of C atoms}) \cdot (\text{area of sulphur peak})$$

The inter-element ratios of all other compounds were calculated using these coefficients of proportionality in the following relationship:

$$\text{No. of S atoms/No. of C atoms} = K_{C/S} \cdot (\text{area of sulfur peak})/(\text{area of carbon peak})$$

The calculated and theoretical values are given in Tables 5 and 6. The identification of the other

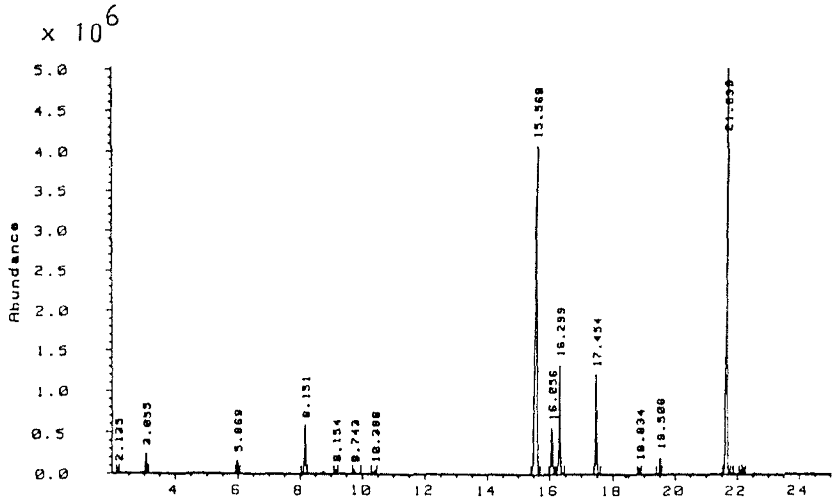

Fig. 7. Total ion current from a crushed garlic clove. Heating time in the headspace device: 45 min. x-Axis represents time in min.

Table 3
Retention times and characteristic ions (m/z and abundance, %) for sulfur-containing compounds from a whole peeled garlic clove

t_r (min) (MS)	No.	M_r	More abundant ions
2.14	1	88	88 (61), 73 (61), 61(20), 47 (32), 45 (100), 41 (62), 39 (86)
3.05	2	94	94 (75), 84 (29), 79 (52), 64 (20), 61 (16), 55 (32), 47 (37), 46 (47), 45 (100)
5.97	3	114	114 (13), 99 (14), 81 (8), 73 (33), 72 (25), 47 (19), 45 (100)
8.16	4	120	120 (31), 79 (10), 64 (11), 47 (13), 46 (13), 45 (48), 41 (100)
	5		
9.16	6	120	120 (52), 80 (21), 72 (33), 71 (25), 58 (13), 48 (17), 47 (31), 45 (100), 41 (36)
9.74	7		
10.39	8	126	126 (60), 79 (49), 64 (37), 47 (44), 46 (31), 45 (100), 44 (17)
15.58	9	146	146 (3), 113 (4), 105 (5), 81 (10), 79 (5), 76 (3), 72 (3), 71 (5), 47 (7), 45 (31), 41 (100)
16.04	10	146	146 (14), 105 (12), 81 (22), 79 (5), 73 (34), 72 (13), 71 (19), 61 (15), 47 (20), 45 (99), 41 (100), 39 (80)
16.28	11	146	146 (13), 105 (12), 81 (21), 73 (34), 71 (16), 61 (18), 58 (7), 47 (14), 45 (95), 41 (100), 39 (77)
17.45	12	152	152 (2), 120 (4), 111 (9), 105 (2), 87 (66), 79 (16), 64 (21), 47 (41), 45 (100), 41 (83), 39 (67)
18.83	13	144	144 (55), 111 (80), 97 (66), 85 (20), 79 (50), 77 (58), 71 (60), 58 (19), 45 (100), 39 (73)
19.5	14	144	144 (30), 111 (34), 72 (100), 71 (95), 45 (82), 39 (42)
21.83	15	178	178 (2), 113 (34), 105 (3)k, 73 (62), 71 (9), 64 (9), 47 (14), 45 (68), 41 (100), 39 (86)
22.17	16	NI[a]	

[a] Not identified.

Table 4
Retention times and characteristic ions (m/z and abundance, %) of sulfur-containing compounds from a crushed garlic clove

t_r (min) (MS)	No.	M_r	More abundant ions
2.14	1	88	88 (61), 73 (61), 61(20), 47 (32), 45 (100), 41 (62), 39 (86)
2.91	2	94	94 (75), 84 (29), 79 (52), 64 (20), 61 (16), 55 (32), 47 (37), 46 (47), 45 (100)
5.97	3	114	114 (13), 99 (14), 81 (8), 73 (33), 72 (25), 47 (19), 45 (100)
8.16	4	120	120 (31), 79 (10), 64 (11), 47 (13), 46 (13), 45 (48), 41 (100)
ND[a]	5		
9.16	6	120	120 (52), 80 (21), 72 (33), 71 (25), 58 (13), 48 (17), 47 (31), 45 (100), 41 (36)
ND	7		
10.39	8	126	126 (60), 79 (49), 64 (37), 47 (44), 46 (31), 45 (100), 44 (11)
15.58	9	146	146 (3), 113 (4), 105 (5), 81 (10), 79 (5), 76 (3), 72 (3), 71 (5), 47 (7), 45 (31), 41 (100)
16.04	10	146	146 (14), 105 (12), 81 (22), 79 (5), 73 (34), 72 (13), 71 (19), 61 (15), 47 (20), 45 (99), 41 (100), 39 (80)
16.28	11	146	146 (13), 105 (12), 81 (21), 73 (34), 71 (16), 61 (18), 58 (7), 47 (14), 45 (95), 41 (100), 39 (77)
17.45	12	152	152 (2), 120 (4), 111 (9), 105 (2), 87 (66), 79 (16), 64 (21), 47 (41), 45 (100), 41 (83), 39 (67)
ND	13		
ND	14		
21.61	15	178	178 (2), 113 (34), 105 (3), 73 (62), 71 (9), 64 (9), 47 (14), 45 (68), 41 (100), 39 (86)
22.17	16	NI[b]	

[a] Not detected.
[b] Not identified.

Table 5
Retention times and calculated and theoretical C/S and H/C ratios from a whole peeled garlic clove

Parameter	t_r (min) (GC–AES)										
	2.4	3.22	6.35	8.56	9.18	10.19	15.86	16.38	16.61	17.76	21.89
Calculated C/S	4.14	1.53	6.14	2.05	1.85	ND[a]	2.98	3	2.84	1.32	2.08
Theoretical C/S	4	1	6	2	2	0.66	3	3	3	1.33	2
Calculated H/C	2.34	2.6	1.67	2.22	1.79	2.65	1.99	1.61	1.66	2.19	1.85
Theoretical H/C	2	3	1.67	2	2	3	1.66	1.67	1.67	2	1.67
Formula	C_4H_8S	$C_2H_6S_2$	$C_6H_{10}S$	$C_4H_8S_2$	$C_4H_8S_2$	$C_2H_6S_3$	$C_6H_{10}S_2$	$C_6H_{10}S_2$	$C_6H_{10}S_2$	$C_4H_8S_3$	$C_6H_{10}S_3$

[a] Not detected.

Table 6
Retention times and calculated and theoretical C/S and H/C ratios from a crushed garlic clove

Parameter	t_r (min) (GC–AES)											
	2.4	3.22	6.35	8.56	9.18	10.85	15.96	16.41	16.67	17.81	19.81	21.93
Calculated C/S	4.11	1.53	6.13	2.05	1.85	0.55	2.98	3	2.84	1.33	3.25	2.15
Theoretical C/S	4	1	6	2	2	0.66	3	3	3	1.33	3	2
Calculated H/C	2.34	2.6	1.67	2.22	1.79	2.65	1.99	1.61	1.66	2.19	ND[a]	1.85
Theoretical H/C	2	3	1.67	2	2	3	1.66	1.67	1.67	2	1.33	1.67
Formula	C_4H_8S	$C_2H_6S_2$	$C_6H_{10}S$	$C_4H_8S_2$	$C_4H_8S_2$	$C_2H_6S_3$	$C_6H_{10}S_2$	$C_6H_{10}S_2$	$C_6H_{10}S_2$	$C_4H_8S_3$	$C_6H_8S_2$	$C_6H_{10}S_3$

[a] Not detected.

Table 7
Characteristic ions mostly encountered

Ion m/z	Formula
39	$CH_2{=}CHCH^+$
41	$^+CH_2CH{=}CH_2$ or $CH_2{=}CHCH_2^+$
45	$^+CH{=}S$
47	CH_3S^+
73	$^+SCH_2CH{=}CH_2$
79	CH_3SS^+ or $CH_3S^+{=}S$
105	$^+SSCH_2CH{=}CH_2$ or $S{=}S^+CH_2CH{=}CH_2$
111	$^+SSSCH_3$ or $S{=}S^+SCH_3$

sulfur-containing was carried out using their mass spectra and elemental data given by AES detection.

Most of the analysed molecules are unsaturated mono-, di- or trisulfides. Several noticeable fragment ions are characteristic of the various chemical groups: $m/z = 39$ and 41 (allyl), $m/z = 73$ (allyl sulfide), $m/z = 105$ (allyl disulfide), $m/z = 47$, 79, 111 for mono-, di- and trisulfides, respectively (Table 7). All the identified compounds are given in Table 8 [18].

4. Conclusions

The detection by AES of sulfur from aromas obtained by the headspace method from various garlic samples (whole non-peeled cloves, peeled cloves, crushed cloves and juices) allowed their sulfur-containing components to be located and compared. These results show the influence of the sample conditions and of the experimental conditions (temperature) on the analytical response when aromas containing very labile and unstable molecules react chemically or enzymatically during sample handling and analytical processes. These observations have to be taken in account for the correct interpretation of the data observed from samples such as garlic and for the use of this kind of product where sulfur-containing molecules are mostly responsible of the flavour and also of the pharmacological properties. The use of elemental profiles obtained from the same sample allows the calculation of inter-element ratios and the determination of the number of C, S and H atoms present in each of the molecules eluted from the GC column when AES data are coupled with MS data. These elemental data are very useful for the determination of the structures and compositions of molecules from complex mixtures such as aromas and natural extracts.

Table 8
Identification of sulfur-containing compounds eluted by the headspace device

No.	M_r	Formula	Name
1	88	C_4H_8S	Allyl methyl sulfide
2	94	$C_2H_6S_2$	Methyl disulfide
3	114	$C_6H_{10}S$	Allyl sulfide
4	120	$C_4H_8S_2$	Allyl methyl disulfide
5			
6	120	$C_4H_8S_2$	Methyl *trans*-propenyl disulfide
7			
8	126	$C_2H_6S_3$	Dimethyl trisulfide
9	146	$C_6H_{10}S_2$	Dipropenyl disulfide
10	146	$C_6H_{10}S_2$	Allyl propenyl disulfide
11	146	$C_6H_{10}S_2$	Allyl disulfide
12	152	$C_4H_8S_3$	Allyl methyl trisulfide
13	144	$C_6H_8S_2$	3-Vinyl-1,2-dithiin
14	144	$C_6H_8S_2$	2-Vinyl-1,3-dithiin
15	178	$C_6H_{10}S_3$	Allyl trisuflide

This example shows that a dual analytical strategy can be developed. The first step is the location of the compounds of interest by the specific detection of the atomic emission of one or several of their characteristic elements. In the second step, mass spectra of the selected compounds are recorded, then both mass spectrometric and elemental data are used simultaneously for the accurate determination of the unknown compounds.

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