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Determination of organic sulphur compounds in garlic extracts by gas chromatography and mass spectrometry

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Gas chromatography and mass spectrometry were used to study organic sulphur compounds present in extracts of *Allium sativum* (garlic) plants. The limits of detection were between 1·76 and 9·40 ng for allyl methyl sulphide and dimethyl disulphide, respectively. Extraction of aqueous solutions with diethyl ether yielded percentage recovery rates ranging from 74·4% (allyl methyl sulphide) to 90·3% (dipropyl disulphide). The composition and yield of organic sulphur compounds present in extracts of garlic were studied using gas chromatography and mass spectrometry.

INTRODUCTION

One of the first studies of the composition of flavour in the genus *Allium* was performed in 1844 by Wertheim, who examined the essential oil of garlic and established the term 'ally' for the C_3H_5 configuration. In 1892 Semmler established the importance of diallyl disulphide and diallyl trisulphide in the flavour of garlic distillate (Block, 1985). These compounds are formed when the cellular tissue is disrupted, enabling the enzyme allinase to come in contact with non-odorous precursors (*S*-alkyl-L-cysteine sulphoxides). This enzyme (EC 4.4.1.4) has been characterised, and different inhibitors have been studied (Jansen *et al.*, 1989*a,b*). *S*-allyl-L-cysteine sulphoxide (alliin) has been identified as the main precursor (Stoll & Seebeck, 1947); this compound is converted to 2-propenyl-2-propenethiol-sulphinate (allicin), but there are also at least four more precursors (Virtanen, 1965). These precursors are located in cytoplasmic compartments (Lancaster *et al.*, 1989) and are produced by the splitting of α-glutamyl peptides by peptidases and transpeptidases (Whitaker, 1976); these peptides are formed in turn from soil sulphates prior to their reduction to cysteine (Lancaster & Shaw, 1989). Allicin is very unstable and its decomposition proceeds through several pathways (Block, 1985).

The best method for measuring flavour is probably gas chromatography, although this method measures secondary compounds of the enzymatic reaction such as sulphides, disulphides and trisulphides. Diallyl disulphide has been identified as the main component in garlic oil (Teleky-Vamossy & Petro-Turza, 1986); the influence of processes such as lyophilisation, desiccation and different extraction techniques in the composition of the flavour of this vegetable have been studied (Yu *et al.*, 1988).

The aim of the present study was to elucidate the analytical behaviour of pure sulphur compounds for their subsequent analysis in ether extracts of *Allium sativum* (garlic).

MATERIALS AND METHODS

Apparatus

Gas chromatography was done with a Perkin–Elmer model Sigma 3B apparatus equipped with a flame-ionisation detector and a fused-silica Carbowax 20 M column (50 m × 0·25 mm). The carrier gas was nitrogen (1·5 ml/min). An injection temperature of 200°C, detector temperature of 200°C and initial column temperature 50°C, raised by 1°C/min to 150°C, were used. The chromatograms were recorded on a Perkin–Elmer model LCI 100 data processor. For identification by mass spectrometry a Hewlett Packard model 5980 gas chromatograph equipped with a Hewlett Packard model 5988 A mass spectrometer was used. Operational parameters were as follows: carrier gas, helium; ionisation voltage, 70 eV; ion source temperature 200°C.

Reagents

Dimethyl sulphide (DMS), diethyl ether (Merck), allyl methyl sulphide (AMS), *p*-cymene (Janssen), diallyl sulphide (DAS), diallyl disulphide (DADS) (Fluka) and dipropyl disulphide (DPDS) (Aldrich), all of analytical grade, were used as standards.

Method

Linearity and sensitivity of the detector were calculated from a series of solutions from 2×10^{-2} M to 1×10^{-4} M in diethyl ether. The quantitative analyses of sulphur derivatives were performed using *p*-cymene as the internal standard.

To determine the extraction yield from aqueous solutions, 10^{-5} mol of the pure compounds AMS, DMS, DAS, DPDS and DADS were dissolved in 100 ml distilled water. The solution was extracted three times with 150 ml diethyl ether, and the extracts were pooled and concentrated to a volume of 4 ml in a Kuderna–Danish apparatus set in a water bath (40°C), then further concentrated under a nitrogen current to a final volume of 1 ml. To this volume was added 20 μl of a 5×10^{-3} M solution of *p*-cymene, and a 0·2 μl aliquot was injected into the chromatograph under the conditions described above.

Garlic extract

To obtain garlic extract a technique described in a previous paper was used (Artacho *et al.*, 1992).

RESULTS AND DISCUSSION

Table 1 summarises the analytical characterisation of standard solutions, the linear equations obtained, the minimum detection limits as a function of the volume of injection (0·2 μl) and the percentage recoveries from aqueous solutions. The linear response for the internal standard was characterised by the equation $y = 65{\cdot}11 \times 10^6 x + 16{\cdot}90 \times 10^3$, with a correlation coefficient of 0·9960.

A gas chromatogram of garlic extract is shown in Fig. 1. Volatile components were identified by comparing

Table 1. Analytical characterisation of standard solutions

Compound[a]	$y = ax + b$		Correlation coefficient	Minimum detection limit (ng/0·2 μl)	% recovered ± SD
	a	*b*			
AMS	$25{\cdot}75 \times 10^6$	$1{\cdot}67 \times 10^3$	0·9975	1·76	74·39 ± 3·03
DMDS	$11{\cdot}94 \times 10^6$	$4{\cdot}18 \times 10^3$	0·9994	9·40	80·69 ± 2·91
DAS	$39{\cdot}72 \times 10^6$	$7{\cdot}37 \times 10^3$	0·9952	2·28	83·37 ± 3·51
DPDS	$38{\cdot}94 \times 10^6$	$5{\cdot}77 \times 10^3$	0·9973	3·00	90·31 ± 1·52
DADS	$25{\cdot}36 \times 10^6$	$10{\cdot}90 \times 10^3$	0·9995	2·92	86·54 ± 1·78

[a]AMS, allylmethyl sulphide; DMDS, dimethyl disulphide; DAS, diallyl sulphide; DPDS, dipropyldisulphide; DADS, diallyldisulphide.

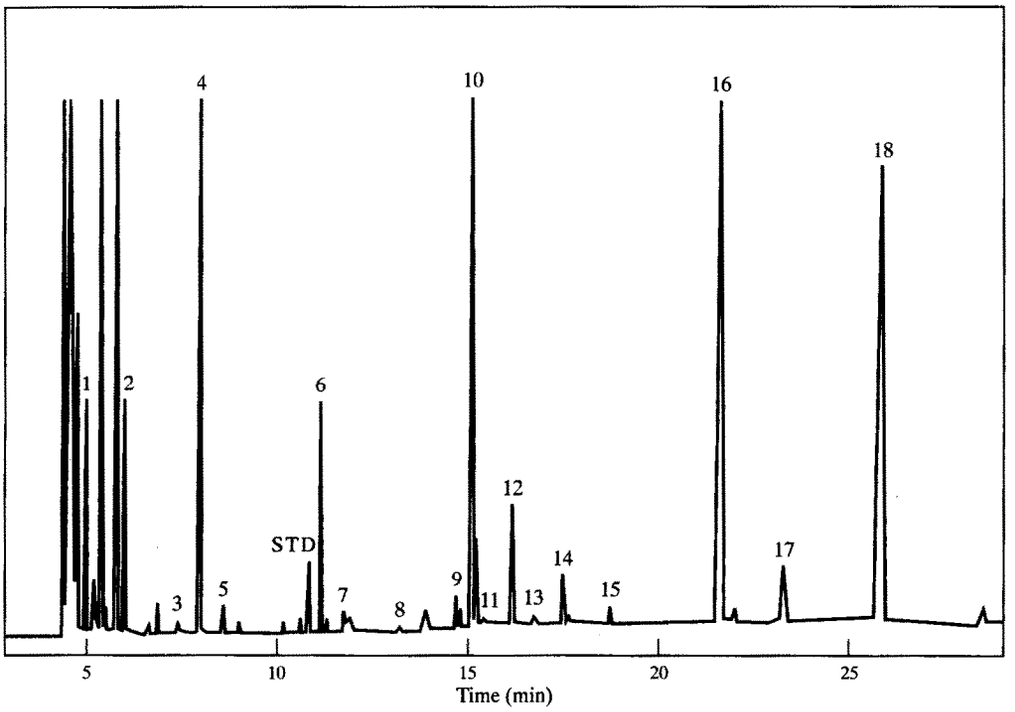

Fig. 1. Capillary gas chromatogram of volatile components of garlic extract.

Table 2. Identity of sulphur compounds in garlic extract

Peak no.	Compound	*m/z* (Relative intensity)							Ref.[a]
2	Allyl methyl sulphide	88(100)	73(80)	45(50)	61(18)	47(17)			R
3	Dimethyl disulphide	94(100)	45(62)	79(57)	64(14)	61(13)	96(10)		R
5	Diallyl sulphide	45(100)	73(60)	114(47)	99(40)	72(38)	71(24)		R
6	Allyl methyl disulphide	120(100)	41(97)	45(47)	79(18)	47(14)	69(13)		P
8	Dimethyl trisulphide	126(100)	45(75)	79(68)	64(40)	47(38)	111(25)	80(17)	P
10	Diallyl disulphide	41(100)	81(53)	39(52)	113(39)	105(27)	45(35)	146(35)	R
14	Allyl methyl trisulphide	87(100)	45(95)	41(72)	73(72)	47(51)	111(21)	79(20)	P
16	3-Vinyl-4(*H*)-1,2-dithiin	45(100)	144(78)	97(70)	103(54)	77(48)	85(17)	111(10)	P
17	Diallyl trisulphide	73(100)	113(93)	41(36)	39(20)	138(18)	74(16)		P
18	2-vinyl-4(*H*)-1,3-dithiin	72(100)	71(78)	144(45)	45(41)	111(35)	97(16)		P

[a]P, proposed structure according to MS data; R, standard available.

their gas chromatographic retention times and mass spectra with those of authentic compounds and published data (Table 2); peak nos 1 and 4 are ethyl acetate and 2-propen-1-ol, respectively. The other peaks that do not appear in Table 2 are unknown, and were not quantified.

Mean values of five extractions, corrected in accordance with the extraction yield, are given in Table 3. The major sulphur compound was 2-vinyl-4(*H*)-1,3-dithiin, with a mean concentration of 839 mg/100 g; the least abundant compound was dimethyl trisulphide (0·075 mg/100 g).

Although 2-vinyl-(4*H*)-1,3-dithiin and 3-vinyl-(4*H*)-1,2-dithiin (peak nos 16 and 18) are considered artefacts from allicin during gas chromatography (Yu *et al.*, 1989), Block *et al.*, (1989) has explained a mechanism for their formation from allicin when there is no heat involved in the extraction procedure. We have observed that, upon heating, e.g. in the preparation of essential oil of garlic by steam distillation, allicin decomposes giving primarily diallyl disulphide (349 mg/100 g) (Artacho, 1990).

In conclusion, the analytical method described here makes it possible to identify and quantify the major components formed from chopped garlic bulbs. The method can be used as a tool to determine the components of this and possibly other species, and yields products suitable for further biological study. The products obtained with this method are currently being used to investigate iodine absorption by the thyroid gland, given that garlic is frequently consumed in areas of endemic goitre.

Table 3. Volatile sulphur compounds in garlic (mg per 100 g fresh tissue ± SD)

Peak no.	Sulphur compound	mg/100 g fresh tissue ± SD
2	Allyl methyl sulphide	0·167 ± 0·015
3	Dimethyl disulphide	0·204 ± 0·026
5	Diallyl sulphide	0·122 ± 0·014
6	Allyl methyl disulphide	1·193 ± 0·068
8	Dimethyl trisulphide	0·075 ± 0·011
10	Diallyl disulphide	5·297 ± 0·846
14	Allyl methyl trisulphide	0·562 ± 0·078
16	3-Vinyl-4(*H*)-1,2-dithiin	4·088 ± 2·266
17	Diallyl trisulphide	1·288 ± 0·183
18	2-Vinyl-4(*H*)-1,3-dithiin	830·069 ± 1·757

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