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Analytical Methods Secction

Comparative study by gas chromatography–mass spectrometry of methods for the extraction of sulfur compounds in *Allium cepa L.*

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The influence of two extraction processes on the composition and yield of organic sulfur compounds present in the bulb of *Allium cepa L.* was evaluated using gas chromatography–mass spectrometry. The direct extraction method with diethyl ether, using chopped and macerated plant tissue, yielded methyl propyl disulfide as the major component, with a mean concentration of 34·37 μg per 100 g of fresh tissue. Prior steam distillation followed by extraction of the distillate identified the same components with the exception of methyl propyl disulfide, as in direct extraction method. The major component of the steam distillate was dipropyl disulfide, with a mean concentration of 110·2 μg per 100 g of fresh tissue.

INTRODUCTION

The flavor of onions (*Allium cepa L.*) is caused by the action of the enzyme alliinase (E.C.4.4.1.4.) (Nock & Mazelis, 1989) which reacts with nonodorous precursors (Schwimmer & Mazelis, 1963) when the vegetable tissue is destroyed. The precursors, *S*-alkyl-L-cysteine sulfoxides, are located in cytoplasmic compartments (Lancaster *et al.*, 1989) and are produced by the splitting of γ-glutamyl peptides (Whitaker, 1976), which are formed from sulfates derived from the soil prior to their reduction to cysteine (Lancaster, 1989). The γ-glutamyl peptides contain almost 90% of the vegetable sulfide, and are involved in plant growth.

1-Propenyl-L-cysteine sulfoxide is the main known precursor (Schwimmer, 1969) and produces the lacrymatory factor. This is an unstable compound which is probably either 1-propenyl sulfenic acid (Spare & Virtanen, 1963) or thiopropanal-*S*-oxide (Brodnitz & Pascale, 1971). When broken down, it produces 2-methyl-2-pentenal as well as the sulfur compounds responsible for the flavour (Boelens *et al.*, 1971).

One of the first studies of the composition of the flavor of onions was performed in 1892 by Semmler, who found several sulfur compounds in distillations of this vegetable (Block, 1985). The development of chromatographic techniques and especially of gas chromatography and mass spectrometry has allowed the identification of sulfides and disulfides (Saghir *et al.*, 1963; Bandyopadhyay, *et al.*, 1970; Freeman & Whemham, 1975; Tewari & Bandyopadhyay, 1977), dimethyl thiophenes (Galleto & Hoffman, 1976), and 3,5-diethyl-1,2,4-trithiolane (Kaemoka & Demizu, 1979) in vegetable extracts of onions. It has also been possible to determine the effect of dehydration, frying and boiling on the composition of onion extracts (Bernhard, 1968; Mazza & Le Maguer, 1979).

The main aim of this work was to compare two extraction processes on the composition and yield of organic sulfur compounds from *Allium cepa L.* In method **A** cold macerated plant tissue is extracted with diethyl ether and in method **B** an atmospheric pressure steam distillate from macerated plant tissue is extracted with ether.

The isolated components were analyzed using gas chromatography and mass spectrometry and quantified using an internal standard method.

EXPERIMENTAL

Apparatus

A Perkin-Elmer model Sigma 3B gas chromatograph equipped with a flame-ionisation detector and a fused-silica Carbowax 20 M column (50 m × 0·25 mm) was

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used. The operating conditions were: injector 200°C, detector 200°C, initial column temperature 50°C, raised by 1°C min^{-1} to 150°C, carrier gas (nitrogen) flow rate 1·50 ml min^{-1} split ratio 1:20, injection volume 0·2 μl. 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; ionization voltage, 70 eV; ion source temperature, 200°C.

METHOD

Direct extraction (Method A)

Clean onion tissue was chopped in a food processor to an approximate particle size of 4 mm. Two hundred and fifty gram aliquots of chopped onion were macerated in 100 ml of distilled water for periods of 0, 1, 2 and 3 h. The mixtures were kept at room temperature and away from light. When the appropriate time had elapsed each sample was filtered and the aqueous solution was extracted with diethyl ether (3 × 150 ml). The ether extracts were passed through anhydrous sodium sulfate and then concentrated in a Kuderna-Danish apparatus in a water bath at 50°C to a volume of 4 ml. Further concentration to a volume of 0·5 ml was achieved under a nitrogen current. Ten microlitres 5×10^{-3} M *p*-cymene as an internal standard was added, and 0·2 μl was injected into the chromatograph under the conditions described previously.

Extraction with prior steam distillation (Method B)

To determine the effect of the extraction technique on the composition of the flavour compounds, samples were steam distilled prior to extraction. Two hundred and fifty grams of clean, chopped onion were macerated in 100 ml of distilled water for 30 min and then steam distilled until the sample was completely reduced (no more than 2 h). The distillate was collected in a flask immersed in a bath of melting ice and then extracted as for the direct extraction method.

DISCUSSION AND RESULTS

Direct extraction (Method A)

At time zero the products released from the vegetable tissue were minimal. The components started to appear after 1 h of maceration (Fig. 1) and reached the highest levels at 2 h. After 3 h the chromatograms were no longer reproducible, therefore chopped onion was macerated for 2 h before extraction.

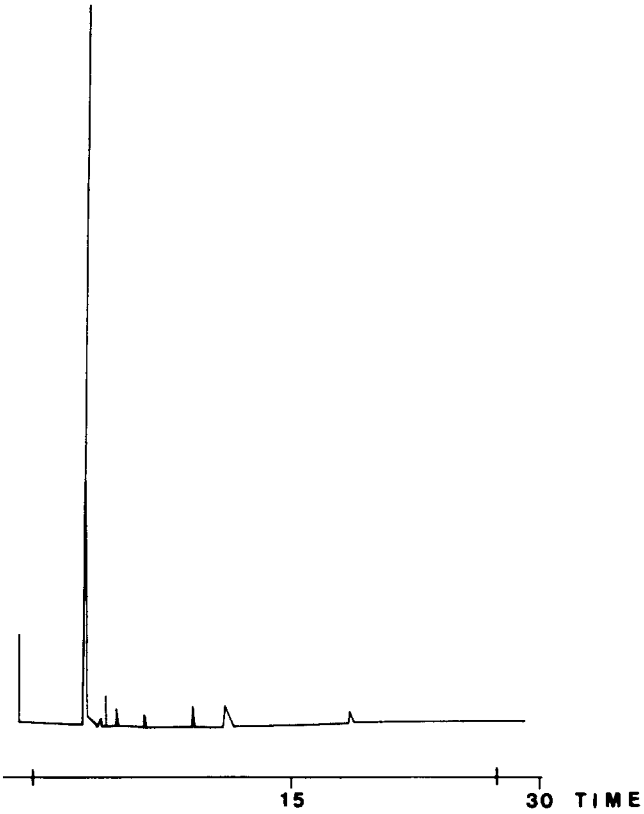

Fig. 1. Chromatogram of onion extract after 1 h of maceration.

The identification of the components was based on the retention times of standard stock solutions and mass spectrometry (Table 1). Quantification was based on *p*-cymene as the internal standard. Table 2 presents the mean concentrations (μg per 100 g) of five extractions, together with the statistical parameters. The principal sulfur compound was methyl propyl disulfide, with a mean concentration of 34·4 μg per 100 g compared to 1·2 μg per 100 g for dimethyl trisulfide. It should be noted that in the case of *cis-trans* isomeric compounds, such as 2-propenyl propyl disulfide and 3,5-diethyl-1,2,4-trithiolane, the concentration given is the sum of both isomers.

Table 1. Composition of volatiles isolated from onion

Peak	Compound
1	Methyl 1-propenyl sulfide
2	2-Methyl 2-pentenal
3	Methyl propyl disulfide
4	2,4-dimethylthiophene
5	Methyl 1-propenyl disulfide
6	Dipropyl disulfide
7	Dimethyl trisulfide
8	2-Propenyl propyl disulfide (*cis*)
8'	2-Propenyl propyl disulfide (*trans*)
9	Dipropyl trisulfide
10	3,5-Diethyl-1,2,4-trithiolane (*cis*)
10'	3,5-Diethyl-1,2,4-trithiolane (*trans*)

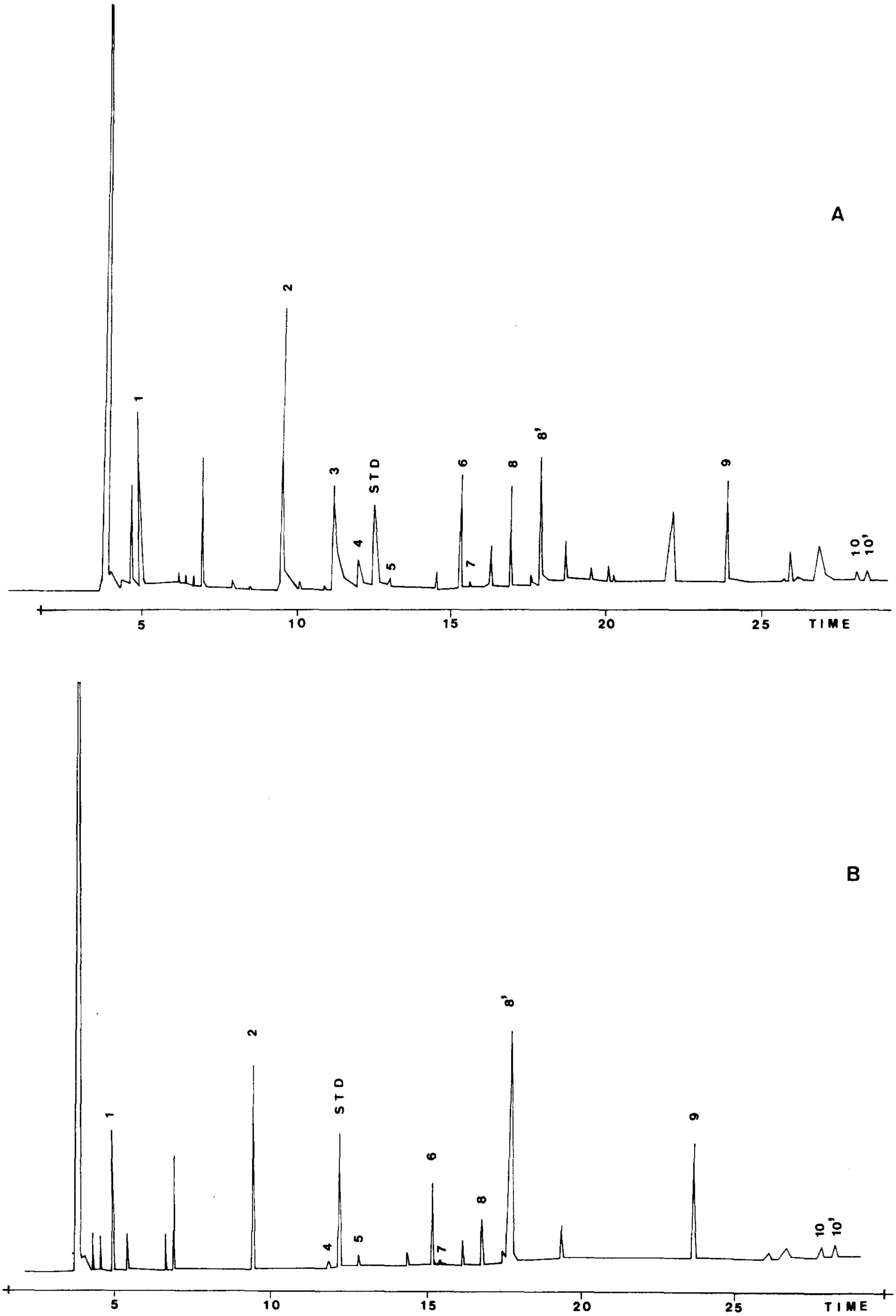

Fig. 2. Chromatogram of onion volatiles isolated by direct extraction and by prior steam distillation (methods A and B).

Table 2. Comparison of the two extraction procedures (μg per 100 g fresh tissue ± S.D.

Peak	Method A	Method B
1	23·70 ± 3·18	23·09 ± 5·06
2	25·04 ± 1·46	76·99 ± 13·92
3	34·37 ± 2·96	—
4	7·26 ± 0·49	4·57 ± 1·53
5	4·16 ± 0·26	7·78 ± 3·60
6	27·54 ± 3·51	110·22 ± 43·76
7	1·23 ± 0·16	6·10 ± 2·75
8	13·50 ± 1·70	51·56 ± 19·73
9	9·37 ± 0·74	82·14 ± 19·56
10	1·35 ± 0·32	12·73 ± 3·51

Extraction with prior steam distillation (Method B)

The mean concentrations of five extractions from the distillate are given in Table 2. In this case the principal sulfur compound was dipropyl disulfide with a mean concentration of 110·2 μg per 100 g, while the component present in the lowest quantities was 2,4-dimethylthiophene.

The similarity in the first part of the chromatogram obtained with each of the two methods indicates that the distillation temperature was not high enough to break methyl 1-propenyl disulfide down into 2,4-dimethylthiophenes. This process was studied by Boelens *et al.* (1971) who subjected an onion distillate to high temperatures. However, the absence of methyl propyl disulfide in our distillates may have been due to destruction of the compound. Our findings agree with those of Block (1985), who also obtained dipropyl disulfide as the major component in steam distillates of onion.

With prior steam distillation (Method **B**), the concentration of all compounds with GLC retention times longer than that obtained for dipropyl disulfide were considerably increased. This can be explained by the increased solubility of the volatile compounds with increasing temperature (Mazza & Le Maguer, 1979). Propyl propenyl disulfides possess the flavor of cooked onion; the aroma of onion distillate is much closer of that of cooked than fresh onion.

Our findings for 3,5-diethyl-1,2,4-trithiolanes concur with those of Kaemoka & Demizu (1979), who also identified these compounds in onion distillates extracted with diethyl ether.

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