

Volatile sulphides in subgenus *Allium*, sections *Allium* and *Cepa* and their use in numerical taxonomy

(Sulfida volatil dalam subgenus *Allium*, seksi *Allium* dan *Cepa* serta kegunaannya dalam taksonomi numerik)

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Key words: volatile sulphides, gas-liquid chromatography, numerical taxonomy

Abstrak

Dengan menggunakan kaedah kromatografi gas-cecair, penentuan dibuat terhadap komposisi dan kepekatan sulfida volatil di dalam tisu hirisan 24 aksesori *Allium* dalam seksi *Allium* dan *Cepa*. Spesies-spesies dalam seksi *Cepa* boleh dianggap mempunyai corak spektrum sulfida volatil yang sama. Sebaliknya, spesies-spesies dalam seksi *Allium* tidak menunjukkan spektrum sulfida volatil yang sama. Beberapa spesies dalam seksi *Allium* bercorak taburan sulfida volatil yang sama seperti spesies-spesies dalam seksi *Cepa* manakala bagi spesies lain corak taburan sulfida volatilnya seperti yang ditunjukkan oleh *A. sativum* yang corak taburan sulfida volatilnya dianggap mewakili seksi *Allium*. Pada keseluruhannya, bolehlah diandaikan bahawa taburan spektrum sulfida volatil dalam bebawang pelbagai spesies *Allium* tidak menunjukkan perbezaan yang ketara antara spesies atau seksi.

Abstract

Using gas-liquid chromatography, determinations were made on the proportions and concentrations of volatile sulphides from chopped tissues of 24 accessions of *Allium* belonging to sections *Allium* and *Cepa*. It could be said that species in sect. *Cepa* share essentially the same volatile spectrum pattern. On the other hand, species in sect. *Allium* do not show similar volatile pattern. Some species in sect. *Allium* possess volatile distribution patterns similar to those species in sect. *Cepa* while in other species, the volatile distribution patterns are similar to that of *A. sativum*, the volatile distribution pattern of which is usually considered to be typical for sect. *Allium*. On the whole, it could be concluded that the distribution of volatile sulphides in the bulbs of various *Allium* species did not show clear-cut delimitation between the species or sections.

Introduction

One of the characteristics of the genus *Allium* is that the plants produce a strong odour when the plant tissue is injured. This odour is mainly due to the presence or production of volatile organic sulphur compounds.

Jacobson et al. (1964) isolated and identified several aliphatic disulphides from various *Allium* species. Later, these investigators developed a rapid gas-liquid chromatographic (GLC) method for the separation and identification of sulphides

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from *Allium* vapours, and surveyed the common food species.

Saghir et al. (1964) noted that generally the odour of the various *Allium* can be related to the nature of their sulphide contents. Further investigations by Saghir et al. (1965) have indicated that neither the habitat, stages of growth nor plant part appreciably affect the proportions of alkyl sulphide radicals in the vapour of chopped *Allium* tissues.

The identification of volatile components produced by injured tissues of *Allium* has been reported for the common onion, *A. cepa* (La Croix et al. 1967) and for garlic, *A. sativum* (Cavallito and Bailey

1944). Saghir et al. (1966) related the composition of volatiles to the taxonomy of American *Allium*. El-Gadi (1976) compared the volatiles in several species in sect. *Rhizirideum* and El-Sheik Hussein (1978) compared the volatiles in several species in sect. *Codonoprasum*. It remains, however, that most of the investigations on *Allium* volatiles were carried out on cultivated species notably *A. cepa*, *A. sativum* and *A. porrum*.

This study explores the variation in proportions and composition of volatile constituents in the species listed in *Table 1* and their possible utilization in classification of these species.

Table 1. Sources of *Allium* species in sect. *Allium* and sect. *Cepa* studied

Acc. no.	Species	Source/origin
Section <i>Cepa</i>		
261	<i>A. cepa</i> cv. The Queen	Dobbies Seed Company, U.K.
203	<i>A. cepa</i> cv. White Spartan	Sutton Seed Company, U.K.
7	<i>A. cepa</i> var. <i>viviparum</i>	Botanischer Garten de Techichen Hochschule, Aachen, Germany. Acc. no. 338
314	<i>A. fistulosum</i>	Hortus Botanicus Univ., Budapest, Hungary (HBU). Acc. no. 2335
16	<i>A. schoenoprasum</i>	Hortus Botanicus Instituti Scientiarum, Lithuana, U.S.S.R. (HBIS), Acc. no. 153
258	<i>A. roylei</i>	Beltsville, U.S.A. Acc. no. C 502
363	<i>A. galanthum</i>	National Vegetable Research Station, Wellesbourne, U.K. (NVRs) Acc. no. Do 134
259	<i>A. vavilovii</i>	Beltsville, U.S.A. Acc. no. P1405035
14	<i>A. altaicum</i>	HBIS, Acc. no. 138.
360	<i>A. sibiricum</i>	HBU, Acc. no. 2402
319	<i>A. ledebourianum</i>	HBU, Acc. no. 2353
365	<i>A. pskemense</i>	NVRs, Acc. no. Do 316
5	<i>A. ascalonicum</i>	—
376	<i>A. chinense</i>	NVRs
Section <i>Allium</i>		
372	<i>A. ampeloprasum</i>	Galilee, Israel.
297	<i>A. atroviolaceum</i>	HBU
207	<i>A. porrum</i>	Sutton Seed Company, U.K.
371	<i>A. babingtonii</i>	NVRs
338	<i>A. sativum</i>	HBU, Acc. no. 2391
2	<i>A. longicuspis</i>	HBIS
269	<i>A. sphaerocephalon</i>	Marden Nurseries, Kent, U.K.
366	<i>A. scorodoprasum</i>	NVRs
370	<i>A. vineale</i>	NVRs
310	<i>A. jailae</i>	HBU, Acc. no. 2345

Materials and methods

Materials

Allium species listed in Table 1 were grown in the field at the Winterbourne Research Garden, University of Birmingham, United Kingdom. Dormant bulbs were collected at the end of the growing season (October–November). The outer tunics, roots and the upper part of the leaves were excised immediately before the volatile preparation. Samples of 15 g of the bulbs were taken and finely chopped using a sharp razor blade.

This method gave a high proportion of injured cells, allowing maximum production of volatiles. The chopped material was placed in a 35 mL flask and for replicate samples a mixture of alkanes consisting of equal proportions of the even numbered C⁶–C¹⁶ alkanes were added. The flask was then incubated in a water bath for 30 min at 40 °C. The flask was sealed with a rubber septum, allowing the vapour sample to be withdrawn for GLC analysis.

Identification and measurement of volatiles

A Pye Series 304 dual column GLC with flame ionization detectors was used. The relative retention volumes of sulphides used for identification of chromatographic peaks were established from commercial reagent grade products (Table 2). Methylene numbers of all peaks were calculated according to the formula of Dalgliesh et al. (1966), which states that:

$$\text{Methylene No.} = n + (2y) + x$$

where, n = no. of carbon atoms in previous alkane eluted

x = distance between peaks of adjacent alkanes

y = distance between the alkane peak C_{*n*} to the peak being measured

The relative amounts of volatiles present in each vapour sample were estimated from the chromatograms by measurement of peak areas. From these areas, the proportions of methyl, n-propyl and allyl radicals in the sulphides of each

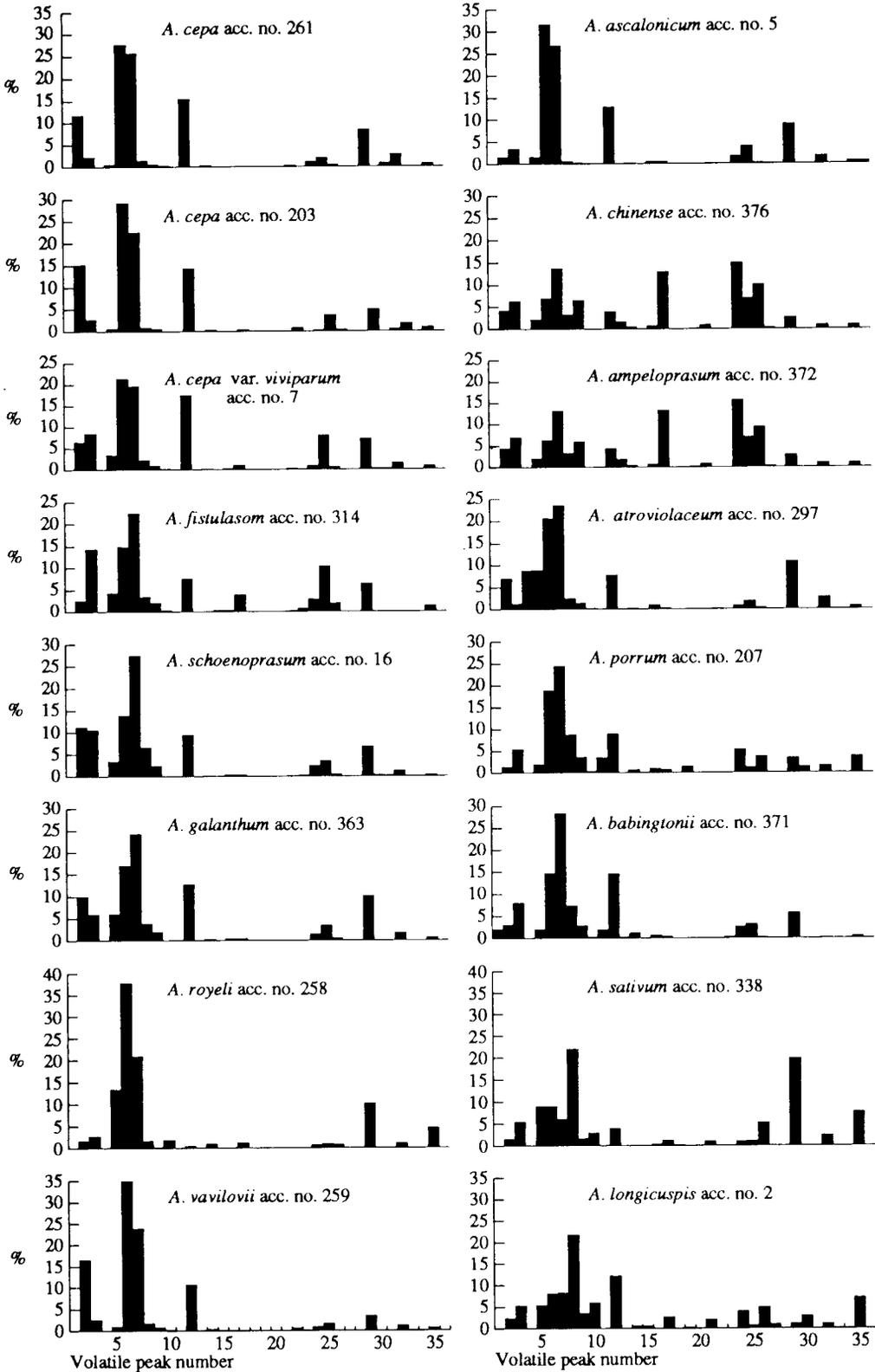
gas sample were calculated. For example, the total n-propyl was determined by adding the appropriate peak areas of each sulphide: n-propyl disulphide + one-half methyl-n-propyl disulphide + one-half n-propyl allyl disulphide. The proportions are expressed as a percentage of the total area of all the sulphides identified on each chromatogram.

The data of bulb volatiles obtained were then used in numerical analysis. The data were coded as presence or absence of a

Table 2. Methylene numbers of allium sulphide peaks and volatile compounds identified

Peak no.	Methylene no.	Compounds
1.	5.57	–
2.	6.00	–
3.	6.29	–
4.	6.91	–
5.	7.37	Methyl sulphide
6.	8.00	Methyl–propyl sulphide
7.	8.29	Dipropyl sulphide
8.	8.62	Diallyl sulphide
9.	9.07	–
10.	9.43	–
11.	9.68	–
12.	10.00	Dimethyl disulphide
13.	10.19	–
14.	10.35	–
15.	10.45	–
16.	10.53	Propanol
17.	10.64	Methyl disulphide
18.	10.94	–
19.	11.01	–
20.	11.38	–
21.	11.49	–
22.	11.70	–
23.	11.90	–
24.	12.00	–
25.	12.39	Propyl–allyl sulphide
26.	12.87	Diallyl disulphide
27.	13.35	–
28.	13.62	–
29.	13.78	Propyl disulphide
30.	14.00	propyl allyl disulphide
31.	14.16	–
32.	14.65	–
33.	15.32	–
34.	15.45	–
35.	15.69	Allyl disulphide
36.	16.00	–

Volatile sulphides and their use in numerical taxonomy



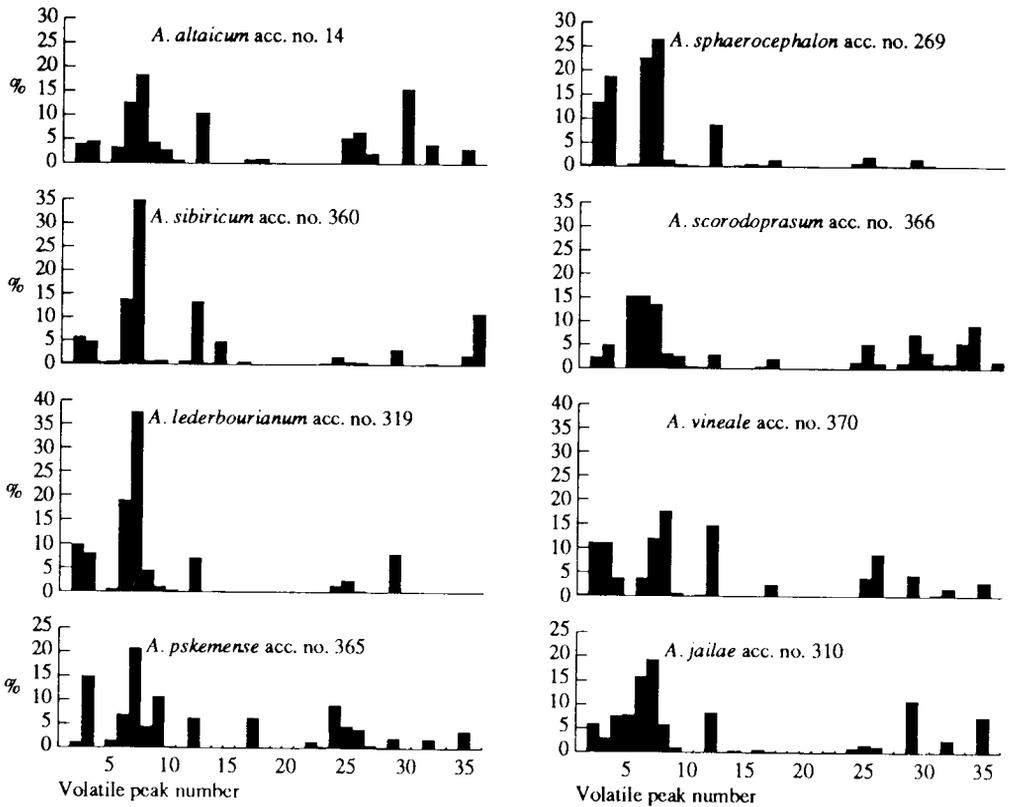


Figure 1. Proportions, based on measurements of peak areas, of 36 components present in the vapour of chopped *Alliums*

particular peak, and for several compounds a coding of 1 to 5 was given to take into account their relative amounts present.

Numerical analysis was carried out using CLUSTAN 2 programme. CLUSTAN is an integrated package of FORTRAN IV for the collective study and use in various cluster analysis and other multivariate methods. The programme incorporates several different types of analysis, two of which will be utilized here. Firstly, hierarchical cluster analysis, based on the matrix of similarity values (Euclidean distance), was carried out and the results presented as dendrograms. The dendrograms were formed through Ward's (1963) clustering method. Secondly, the data matrix, standardized to zero mean and unit variance as with the clusterings, was used for principal component analysis. Here, the multivariate relationships of all taxa can be

expressed with a reasonable degree of accuracy in just a few dimensions (components), providing that a substantial proportion of the total variance is accounted for by those few components and those components should have eigenvalues greater than one. Plots of the first against the second eigenvectors and the third against the fourth eigenvectors were compared.

Results and discussion

Results of the analyses of the various species are presented here in the form of histograms of the proportions of the various sulphides eluted (Figure 1). The proportions of methyl, n-propyl and allyl radicals in the vapours from chopped alliums are presented in Table 3. In sect. *Cepa*, there are four prominent sulphides, i.e. dipropyl sulphide, methyl-propyl sulphide, dimethyl disulphide and propyl disulphide. Dipropyl sulphide

Table 3. Porportions of alkyl constituents from allium volatiles

Acc no.	Species/Cultivar	Methyl	Propyl	Allyl	Unidentified	Total volatile content*
261	<i>A. cepa</i> cv. The Queen	29.27	48.08	3.43	19.23	3 720
203	<i>A. cepa</i> cv. White Spartan	29.57	43.57	3.90	22.96	3 807
7	<i>A. cepa</i> var. <i>viviparum</i>	32.32	41.35	7.58	18.75	5 889
314	<i>A. fistulosum</i>	23.06	43.32	11.87	21.75	4 981
16	<i>A. schoenoprasum</i>	19.99	43.11	8.99	27.91	5 303
258	<i>A. royeli</i>	32.52	50.28	7.36	8.84	4 478
363	<i>A. galanthum</i>	27.55	44.68	6.61	21.16	5 010
259	<i>A. vavilovii</i>	28.95	48.09	7.44	18.50	3 049
14	<i>A. altaicum</i>	20.87	44.59	13.42	21.12	5 454
360	<i>A. sibiricum</i>	20.68	45.63	3.26	30.41	3 008
319	<i>A. ledebourianum</i>	17.26	56.07	5.84	20.83	3 632
365	<i>A. pskemense</i>	17.34	28.48	14.27	39.91	7 542
5	<i>A. ascalonicum</i>	30.31	53.55	3.12	12.02	4 145
376	<i>A. chinense</i>	22.43	23.70	17.59	36.28	13 843
372	<i>A. ampeloprasum</i>	26.99	41.08	10.52	21.41	5 902
297	<i>A. atroviolaceum</i>	27.05	46.26	4.31	22.38	4 809
207	<i>A. porrum</i>	20.68	39.21	15.38	24.73	5 836
371	<i>A. babingtonii</i>	21.49	7.71	61.47	9.33	7 069
338	<i>A. sativum</i>	18.21	30.88	34.66	16.25	9 275
2	<i>A. longicuspis</i>	23.82	14.94	35.41	25.83	8 669
269	<i>A. shaperocephalon</i>	21.98	40.91	2.76	34.35	3 015
366	<i>A. scorodoprasum</i>	27.65	33.08	8.46	30.81	9 715
370	<i>A. vineale</i>	19.09	20.51	31.36	29.04	8 128
310	<i>A. jaitae</i>	23.96	39.48	15.25	21.31	5 945

* Total volatile content is expressed in arbitrary units and is related to the mm² of peak areas.

Table 4. Eigenvalues 1–10 and percentage and cumulative variance for the principal component analysis of 36 bulb volatile chromatographic constituents characters

Eigenvector	Eigenvalues	Percentage variance	Cumulative variance
1	6.81	18.91	18.91
2	4.13	11.49	30.40
3	3.24	9.00	39.40
4	2.85	7.91	47.31
5	2.41	6.68	53.99
6	2.26	6.28	60.27
7	1.72	4.77	65.04
8	1.58	4.39	69.43
9	1.37	3.81	73.23
10	1.32	3.66	76.89

forms the biggest proportion of volatile sulphides in most species of this section. In some species, i.e. *A. cepa*, *A. royeli* and *A. vavilovii*, however, methyl-propyl sulphide forms the biggest proportion while the proportion of dipropyl sulphide is slightly less than this major compound. The species of this section are also characterized by the

lack of diallyl sulphide and other compounds with allyl radicals in their volatiles (Table 4). The volatiles of each accession are classified into three radical groups. The table also indicates that compounds of the propyl group predominate in the volatiles from accessions of the *Allium* sect. *Cepa*.

There are, however, some variations from the above volatile spectrum distribution in a few species. The most notable of these is in *A. chinense*. In this taxon, besides possession of a relatively high level of compounds with allyl radicals, there were comparatively high proportions of methyl disulphide and an unidentified compound eluted at peak no. 24 in the chromatogram, as well as comparatively low levels of methyl-propyl sulphide and dipropyl sulphide. A similar variation occurs in *A. pskemense*, but in general it could be said that the pattern of volatile distribution is close to those of other members of the section.

Bernhard (1970) also found that members of sect. *Cepa* showed close correspondence in the general features of the volatile pattern, with an accession of *A. pskemense* and *A. royeli* showing slight deviations from the general features. He, however, found n-propyl disulphide to be the major constituent of volatiles of species in this section instead of dipropyl sulphide as was found in this investigation. The deviation might be due to error in identification of the sulphide peak since the dipropyl sulphide used for identification was of very old stock.

The above pattern also appears to be consistent with the findings of Saghir et al. (1964; 1966), and Freeman and Whenham (1975). Freeman and Whenham (1975) found that in *A. porrum* and *A. scorodoprasum* the proportions of methyl, propyl and allyl radicals were not very much different from those of the species in sect. *Cepa* except that the proportions of allyl radical constituents were slightly higher. Similar results were obtained in this investigation for *A. porrum*, *A. scorodoprasum*, *A. jailae*, *A. ampeloprasum* and *A. atroviolaceum*. Similar proportions were also found in *A. ampeloprasum* (leek) by Saghir et al. (1964). These investigators, however, showed that in another accession of *A. ampeloprasum* (great-headed garlic), the proportion of

constituents with allyl radicals was very high, and that the distribution of volatiles was very much like that in *A. sativum*. The proportion of allyl constituents was not high in great-headed garlic (acc. no. 209), the distribution of volatiles in this accession being very similar to those of *A. porrum* and other accessions of *A. ampeloprasum*. Another species which did not show volatile proportions similar to *A. sativum* was *A. sphaerocephalon*. *A. sphaerocephalon* investigated was characterized by very low proportions of compounds of the allyl group, the proportion of constituents of allyl compounds being the lowest among the accessions investigated. There were no reports on investigations involving GLC for this species, therefore no comparison could be made here.

The dendrogram based on the hierarchical cluster analysis of 36 bulb volatile chromatographic constituents (Figure 2) shows that at a dissimilarity level of 19.288% (with reference to *A. cepa*, acc. no. 261) there is a split consisting of a group of taxa. Two of these taxa, *A. chinense* and *A. pskemense* belong to sect. *Cepa* while the rest belong to sect. *Allium*. All these taxa are characterized by the possession of comparatively large amounts of constituents eluted during the latter part of the chromatographic process and also comparatively large amounts of constituents of the allyl radicals. Within this cluster *A. chinense* and *A. pskemense* are closely related, (dissimilarity level of 4.25%) while the other species in this cluster, *A. babingtonii*, *A. sativum* and *A. longicuspis* were more closely related to each other than to the above two species.

The second split occurred at 10% dissimilarity level and consists of *A. scorodoprasum* alone. The chromatographic constituent pattern of this species is almost similar to that of those species in sect. *Allium* as above except for the absence of diallyl disulphide (peak no. 35) while possessing compounds eluted at peak numbers 33, 34, and 36 which were not

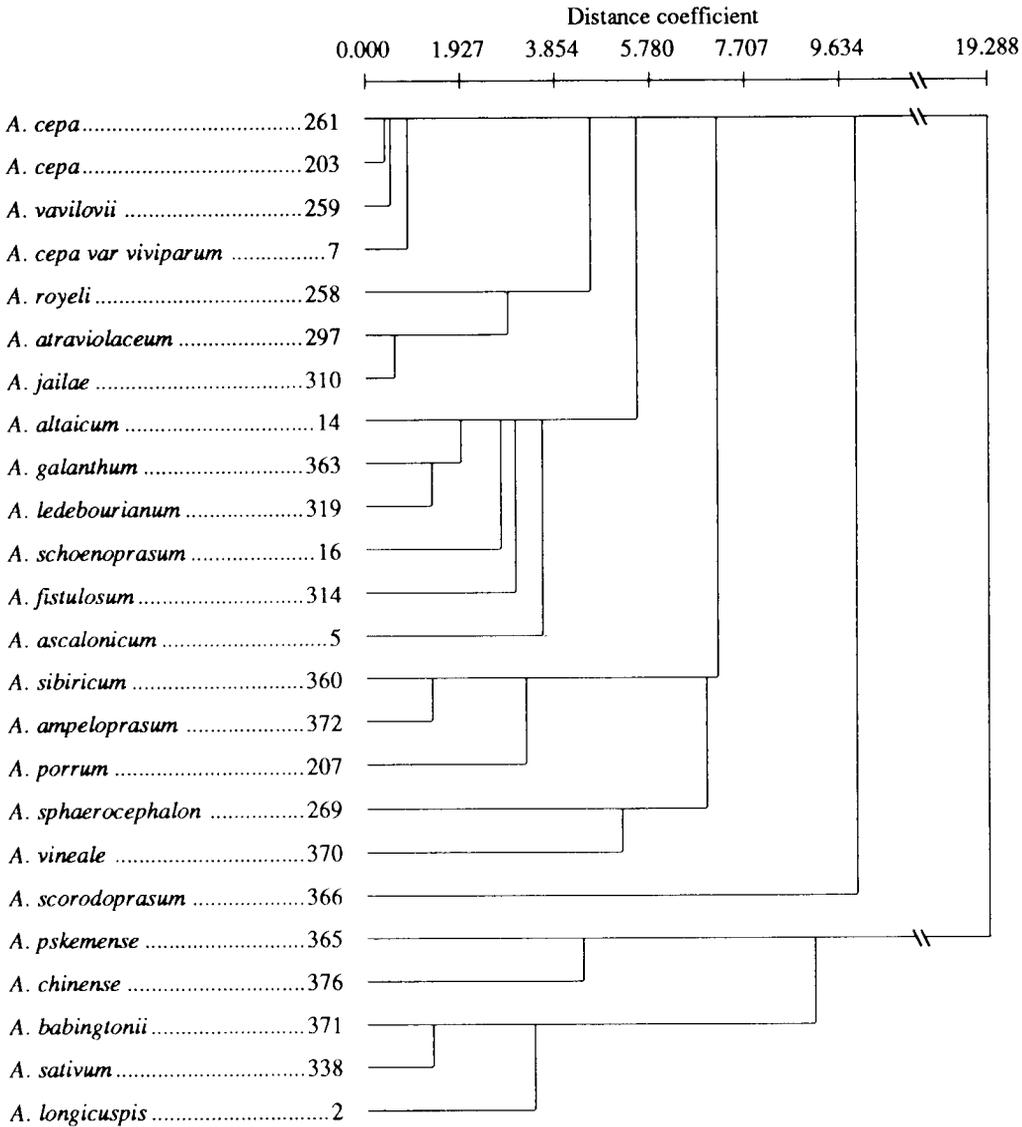


Figure 2. Dendrogram showing group average clustering of accessions of *Allium* using Euclidean distances based on the cluster analysis of chromatographic constituents of bulb volatiles

present in other species in sect. *Allium*.

The third split occurred at 7.4% dissimilarity level and constitutes all other species in sect. *Allium* with the exception of *A. sibiricum* (acc. no. 360). The inclusion of *A. sibiricum* in this cluster is unexpected since its chromatographic constituent pattern was not much different from other species in sect. *Cepa* except that it also possessed small amounts of compounds at peak

numbers 1 and 4 and of larger amounts at peak no. 36. Within this cluster, *A. porrum* and *A. ampeloprasum* accessions were more closely related to each other than to other species in the cluster.

Species clustered below 5.5% dissimilarity level can be considered as a unit, since this constitutes a small variation, although there are a few other taxa clustering below this level. Species below

the above dissimilarity level all belong to the sect. *Cepa* except for *A. atroviolaceum* and *A. jailae*.

Results of the principal component analysis of the bulb volatile chromatographic constituents (Table 4) show that less than half (47.31%) of the total variance is encompassed within the first four eigenvectors, the greatest proportion of which is encompassed by the first (18.91%). The above levels of variance are clearly not adequate for the first four components alone to be analysed.

Consequently, the plots of the first against the second eigenvector and the third against the fourth eigenvectors did not show much significance in the scattering of the species. The plot of factor 1 against factor 2, however, does show that those species producing large amounts of volatiles (Table 3, Figure 1 and Figure 3) as well as possessing relatively large amounts of allyl radical constituents are separated from the main body of the scatter of the taxa. On eigenvector 1, it was found that chromatographic peak numbers 21, 26 and 7 provided most of the variance while on factor 2, peak numbers 3, 24 and 13 provide most of the variance.

On the whole, it could be concluded that analysis of bulb volatile sulphides could not provide clear delimitation between the species or sections of *Alliums*.

In this investigation, there were no significant differences between species and cultivars in sect. *Cepa* in the proportions of sulphides, but there were large differences in the concentration of the volatile sulphides (Table 3). Differences within species in the concentration of volatiles could be due to differential loss of the volatiles during the primary slicing operation. Bernhard (1970) estimated that about 30–50% of the volatile components were lost during this process. Therefore, variation in the length of time taken to complete this process leads to variation in the concentration of the volatile components analysed. However, this variation does not appreciably affect the

analysis done in this investigation since the primary consideration is on the proportions and not on the concentrations of the volatiles.

Conclusion

Determination of the proportions and concentrations of bulb volatile sulphides in *Allium* confirms the fact that the proportions of the various volatiles in any particular species did not vary though the concentration of the volatiles varies with the method of volatile preparation. On the whole, the volatile spectrum pattern of species within sections showed close affinities with each other especially in sect. *Cepa*. However, this general trend is not exclusive. Numerical analysis using principal component analysis and hierarchical cluster analysis showed that some other species within the two *Allium* sections studied showed obvious deviations from the general trend with respect to volatile composition and concentration. Thus, in taxonomic studies, GLC of bulb volatiles have to be augmented with other studies, for example morphology, karyotype analysis, seed protein, isozyme electrophoresis and cross-compatibility studies in order to give clear delimitation of the species and sections in *Allium*.

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Factor 1

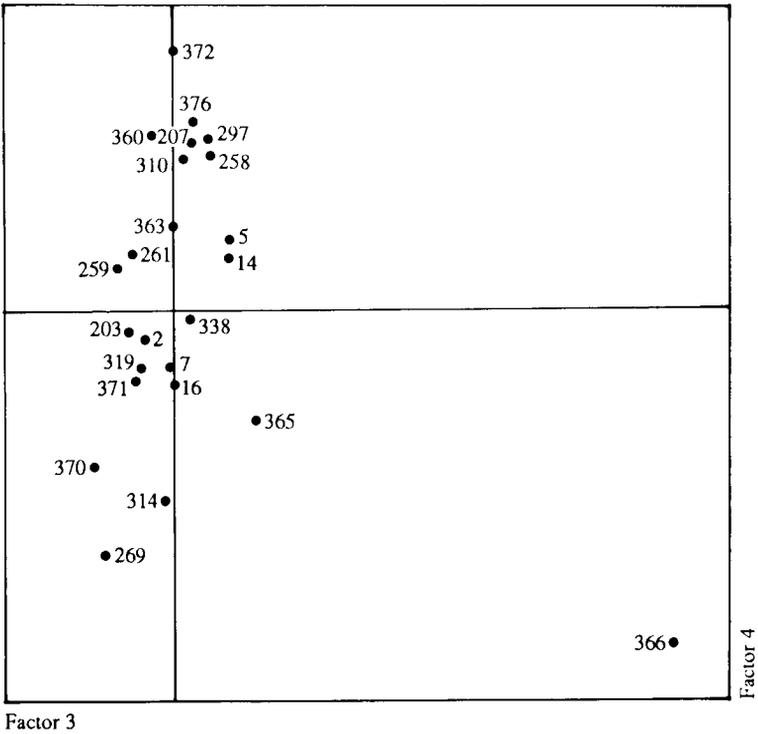
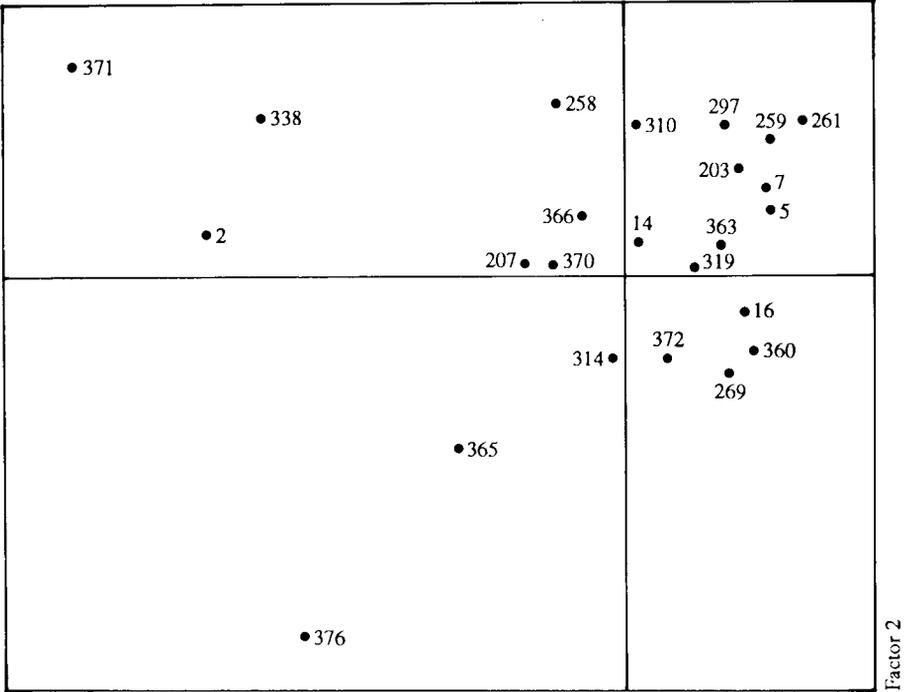


Figure 3. Principal component analysis of chromatographic constituents of bulb volatiles

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