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STUDIES ON FLAVOUR COMPONENTS OF ONION (*ALLIUM CEPA*)

I. THIN-LAYER CHROMATOGRAPHIC INVESTIGATION OF ONION OIL

C. BANDYOPADHYAY, A. N. SRIRANGARAJAN AND A. SREENIVASAN
Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay (India)
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SUMMARY

Studies on the flavouring constituents of onion (*Allium cepa*) by gas chromatography, thin-layer chromatography, elemental analysis as well as infrared spectroscopy have been undertaken. Gas chromatographic analysis of volatile components of onion oil trapped in the head space of a simple glass apparatus under specified conditions revealed that more volatile components were present in a fresh rather than a stored onion. A thin-layer chromatographic technique has been developed as a new approach to the evaluation of flavouring constituents of onion oil that was separated after extraction into seven major components which were determined directly on the silica gel plate by sensory tests. Elemental analysis as well as infrared spectra of these components showed that organic compounds other than sulphur compounds are also responsible for the flavour of onion.

INTRODUCTION

As early as 1892 SEMMLER[1] obtained an oily product having the characteristic onion odour by steam distillation of fresh bulbs of *Allium cepa*; this was identified as allyl propyl di- and trisulphides. KOHMAN[2], on the other hand, showed the presence of unstable thioaldehydes as major odoriferous components. NIEGISCH AND STAHL[3] reported 3-hydroxythiopropionaldehyde as the major flavour component of onion.

The origin of these sulphur compounds was not clear until STOLL AND SEEBECK[4,5] demonstrated that the characteristic odoriferous components of onion are absent in intact tissue and are produced by enzymatic cleavage of alliins, only when the tissue is injured. Alliins undergo spontaneous reactions by the enzyme alinase giving rise to allicin, which, in turn, readily forms the sulphides[6]. S-alkyl and S-allyl cysteine sulphoxides have been isolated from raw onion[7–9] which after splitting by alinase led to the formation of saturated as well as unsaturated di- and trisulphides.

Gas chromatographic analyses of flavour components of onion have been carried out by many workers[10–12]. CARSON AND WONG[11,12] identified methyl propyl di- and trisulphides from dehydrated onion. The composition of sulphides in the vapour of freshly chopped onion was studied by SAGHIR *et al.*[13] who found that allyl propyl

disulphide amongst allyl and allyl methyl disulphides imparted the typical onion flavour. MACKAY *et al.*[14] while analysing by gas chromatography (GC) vapour of freshly comminuted onion trapped in the head space reported that not only disulphides but also other components are responsible for onion odour and that rapid changes in the composition of vapour were found with time. Recently with the help of preparative GC followed by infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopy (MS) analyses BRONDNITZ *et al.*[15] conclusively reported the presence of seventeen components in commercial onion oil, amongst which propenyl propyl disulphide existing both in the *cis* and *trans* forms is the main source of flavour.

Investigations by thin-layer chromatography (TLC) of onion oil have not been hitherto reported in the literature, although several organic sulphur compounds have been studied by this technique. CURTIS AND PHILLIPS[16] separated various nonpolar and polar thiophene derivatives on Alumina G as well as silica gel plates using petroleum ether and benzene–chloroform (9:1), respectively, as solvent. The chromatogram was visualised under UV light followed by spraying with a ninhydrin or isatin solution. Separation by TLC of thiols, sulphides and disulphides has been achieved by PRINZLER *et al.*[17]. They used an Aluminium Oxide D layer with petroleum ether as solvent as well as a 5 % cetan-impregnated Kieselguhr layer with varying proportions of chloroform–methanol as solvent on which spots were detected by spraying with an alkaline solution of Bromothymol Blue and a potassium permanganate solution, respectively. Recently FUJIMAKI *et al.*[18] utilised this technique to study the pyrolyses of sulphur-containing amino acids.

The present paper deals with the GC analyses of volatile components of onion trapped in the head space of a simple apparatus and also with the TLC separation of components of onion oil followed by their characterisation with the help of IR and other analyses.

EXPERIMENTAL

After removing the skin, 100 g of onion (red variety) were shreaded into small pieces using a Master mixer and were put into an apparatus as shown in Fig. 1. The apparatus consisted of a 250-ml conical flask fitted with an adaptor which was provided with a side tube having a stopcock and a small aperture at the top. The aperture was closed with the help of self-sealing rhodorsil silicon elastomar septum (Société des Usines Chimiques, Paris). The flask was then kept at $-35°$ for 30 min and thereafter rapidly put under vacuum (20 in. Hg) to avoid the escape of volatile components. Thereafter, when the flask reached room temperature, it was kept in an oven at $50 \pm 1°$ for 2 h. Vapour (10 ml) was then removed by inserting an hypodermic syringe previously kept at $50 \pm 1°$ through the septum into the gas trapped in the head space. It was injected directly into the column. A gas chromatograph equipped with a flame ionisation detector (Model BARC) was fabricated in our research centre. A glass column (6 ft. × 1/4 in. O.D.) was packed with 10 % Carbowax 20M supported on acid-washed Chromosorb W (60–80 mesh). The carrier gas was nitrogen with a flow rate of 25 ml/min. The oven was equilibrated at 68°. Chromatograms were recorded on a 10-in. recorder with a sensitivity of $A \times 10^{-10}$. A few internal standards, *e.g.* formaldehyde, acetaldehyde, ethyl mercaptan, allyl sulphide and ethanol were run simultaneously.

An ethyl ether extract from fresh onion was used for preparative TLC. Crushed

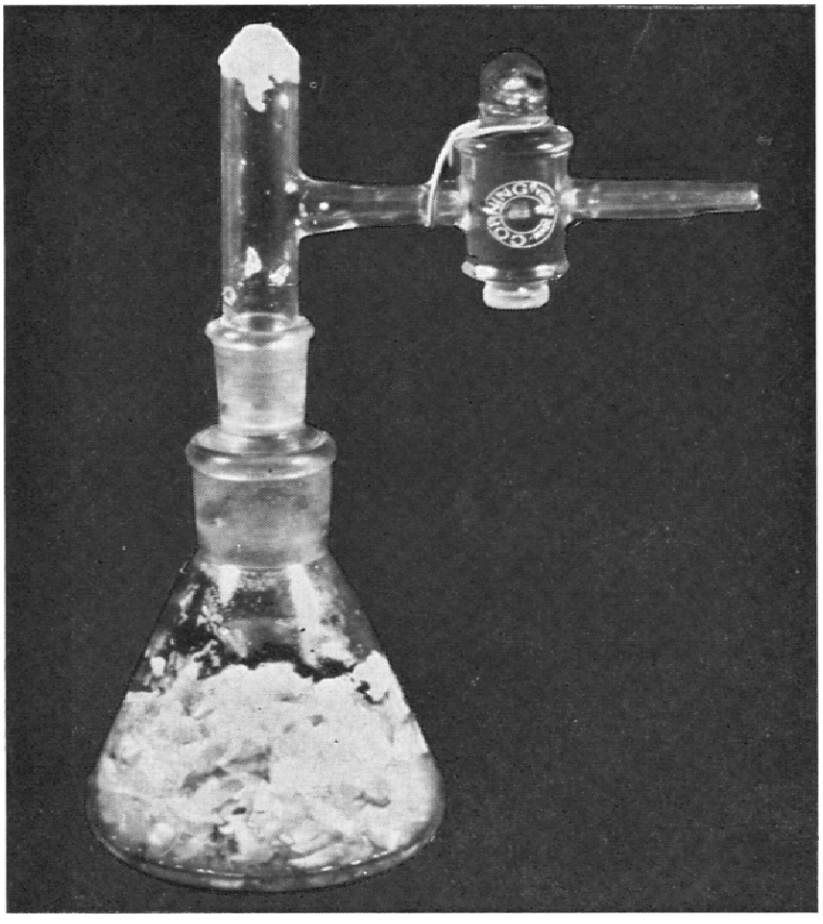

Fig. 1. All-glass apparatus for the determination of volatile components in the gas of freshly chopped onion found in the head space of the apparatus.

onion (500 g) was macerated with 250 ml of peroxide-free ether in a Waring blender at room temperature. The pulpy mass was squeezed with the help of mull cloth to obtain a filtrate consisting of ether and the aqueous layer. The operation was repeated two times with the residue until the final residue was odourless. The filtrate was allowed to separate into layers in a separatory funnel. The aqueous layer was drawn off, reextracted with an equal volume of ether, and the ether layer was collected after centrifuging the whole mass, washed with distilled water, dried over anhydrous sodium sulphate and filtered. After evaporation of ether from the filtrate under a stream of nitrogen at room temperature, a light-yellow pasty product having an intense onion flavour was obtained.

Preparative Silica Gel G plates (20 × 20 cm) were prepared according to a method described elsewhere[19]. 150–200 mg of the above extract in 10% chloroform were applied as a band on three plates which were allowed to develop at room temperature in a chamber containing petroleum ether–diethyl ether (80:20) as solvent.

When the solvent front attained a height of 14 cm, the plates were taken out, and the solvent on the plate was allowed to evaporate under a stream of nitrogen. The chromatograms were exposed to iodine vapour for a short time. Each band visualised was scraped off of the plate using a sharp-edged stainless-steel spatula and collected in separate tubes. Then they were extracted five times each with 3–5 ml

of chloroform and filtered through anhydrous sodium sulphate. Each fraction thus obtained was tested for its homogeneity by TLC using a silica gel plate and a different solvent system of benzene–chloroform (9:1) (ref. 16). Finally, chloroform was completely evaporated from each tube under a stream of nitrogen at room temperature, and the products were stored under nitrogen at 0°. For documentation, 50 and 200 μg, respectively, of a 2 % chloroform solution of onion oil were applied both as spots and bands on the Silica Gel G plate (250 μ thick); and, after development with the above solvent system and evaporation of solvent from the plate under a stream of nitrogen, the plate was finally charred with 50 % sulphuric acid at 140°.

IR analyses of ether-extracted onion oil and its fractions obtained by preparative TLC were carried out using a thin film of these solutions on a sodium chloride prism. A Perkin-Elmer Infracord spectrophotometer, Model 137B, was employed.

A portion of each fraction and of onion oil itself after removal of chloroform was tested for the presence of the carbonyl group according to the method of LAPPIN AND CLARK[20]. Further, using this technique, the vapour of chopped onion obtained from the head space as described above was also analysed for its carbonyl content by removing 50 ml of gas with a syringe and injecting it into a few milliliters of carbonyl-free methanol.

Qualitative elemental analysis of sulphur and nitrogen in onion oil and its chromatographically separated components was performed by conventional methods[21].

Sensory assessment of fractions of onion oil separated in band form was carried out directly on the silica gel plate by a panel of six members. The fractions were judged according to their flavour intensity.

RESULTS AND DISCUSSION

Fig. 2 shows a chromatogram of vapour, removed from the head space, of fresh onion and onion stored for five months at ambient temperature, obtained by GC under

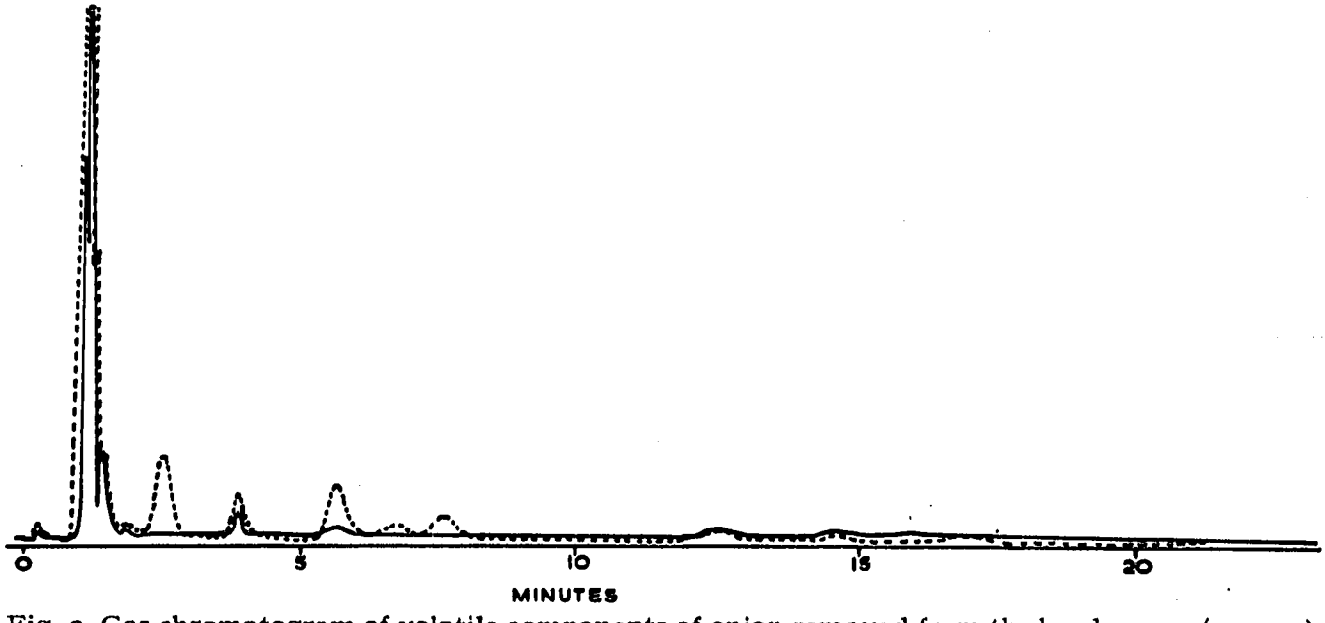

Fig. 2. Gas chromatogram of volatile components of onion removed from the head space. (– – – –), fresh onion; (————), stored onion. Glass column (6 ft. × 1/4 in. O.D.) packed with 10% Carbowax 20M on Chromosorb W; temperature maintained at 68°.

specified conditions. It can be seen that the gas of stored onion consisted of fewer volatile components in comparison with that of fresh onion. By taking into account all the components responsible for flavour, there should be an obvious discrimination between those of fresh and stored onion and hence in preferential acceptability by the consumer, based on flavour intensity. This, in fact, corroborates with the sensory evaluation of the gas of both fresh and stored onion, in which a more intense flavour has been noted in the volatile components of fresh onion. GC analyses further show that intensity of flavour is not only restricted to a variation in the amount of the components present but also to the production of other flavouring components, as indicated by an increase in the number of peaks in the case of fresh onion. A chemical test of gas from the head space indicates the presence of carbonyl compounds. By comparing the peak with those obtained with some internal standards using the gas chromatograph, the major peak in both gases have been characterised as acetaldehyde, while ethyl mercaptan and allyl sulphide are absent.

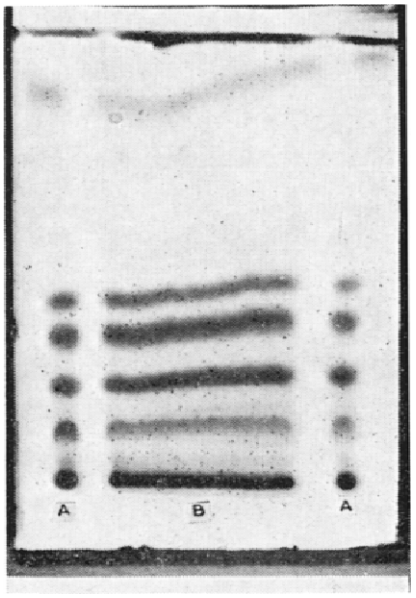

Fig. 3. TLC separation of ether-extracted onion oil on a silica gel plate using petroleum ether–diethyl ether (80:20) as developing solvent. 50 and 200 μg of a 2% chloroform solution of onion oil were applied as a spot or band as represented by A and B, respectively. The chromatogram was detected by spraying the plate with 50% sulphuric acid followed by charring.

Separation by TLC of ether-extracted onion oil both as a spot and band is shown in Fig. 3. Seven distinct spots or bands, including those at the origin and at the solvent front, numbered consecutively from the base, are obtained. Since separation is based on the polarity of the compounds present, the highly polar compounds obviously do not migrate with the solvent system used here, and hence there is the possibility that the spot at the base consists of more than one component. While charring the chromatogram for 10 min at 140° with 50% sulphuric acid, different colours of individual fractions have been observed which, however, turned black after prolonged heating. Chemical analyses of the fractions, their R_F values and sensory evaluation, as well as corresponding colours developed during charring with sulphuric acid, are given in Table I.

TABLE I

R_F VALUE, CHEMICAL ANALYSIS, SENSORY EVALUATION AND COLOUR OF CHROMATOGRAM OF FRACTIONS OF ONION OIL SEPARATED ON A SILICA GEL G PLATE, DEVELOPED AFTER 10 MIN OF CHARRING AT 140° WITH 50% SULPHURIC ACID

Spot No.[a]	*R_F value*	*Colour during charring with 50% sulphuric acid*	*Test for carbonyl group*[b]	*Test for sulphur*	*Test for flavour evaluation*
Onion oil			++	++	++
1	0.0	Yellowish black	++	++	++
2	0.11	Yellow	++	++	++
3	0.21	Blueish violet	+	—	+
4	0.33	Violet	—	—	+
5	0.42	Violet	+	—	+
6	0.86	Yellow	++	++	++
7	0.96	Black	—	—	+

[a] Numbered consecutively from origin upwards.
[b] ++ represents strong response; + represents weak response; — represents absent.

Differences in odour of components of onion oil when separated on a preparative thin-layer plate are remarkable. By sensory evaluation of the separated fractions on the plate, only band 6 in Fig. 3 gives the typical onion flavour, while other bands impart a flavour of a different nature. Elemental analysis reveals that mainly sulphur-containing fractions give strong odour and that all fractions of onion oil are devoid of nitrogen. Simple sensory evaluation of various flavour compounds could easily be achieved by utilising this technique on a large plate. It can be further deduced from Table I that sulphur compounds are not the only index of onion flavour but also other compounds without a sulphur linkage contribute to the overall onion flavour.

Fig. 4 represents IR spectra of ether-extracted onion oil and of its fractions, indicating effective separation of onion oil into its constituents by preparative TLC. It can be seen from the spectra that aromatic and mercaptan type compounds are absent, while, on the other hand, the carbonyl group is present in fractions 1, 2 and 6 as well as the hydroxyl group in fractions 1, 2 and 3. Further the presence of a long-chain hydrocarbon-containing ester group in fraction 7 can be inferred. In view of the chromatographic behaviour of onion oil components, based on their polarity in the solvent system employed here, the occurrence of the hydroxyl as well as the ester group in the fractions mentioned above can be further justified. However, the nature of the compound(s) present in fractions 4 and 5 cannot be interpreted in the present study.

The nature and composition of volatile components of onion oil are still controversial, although several flavour precursors believed to be alliins have been isolated and characterised. The spontaneous enzymatic reaction with alliins, giving rise to intermediate products which in turn undergo further reactions as well as eventual polymerisation, complicate exact characterisation of flavouring components of onion. Climatic, geographic and varietal differences might also play an important role in the composition of volatile components of onions in addition to differences in the isolation methods. The present isolation method, however, gives a yield of 1% oil in contrast to the reported value[22] of 0.046% obtained by steam distillation. Using

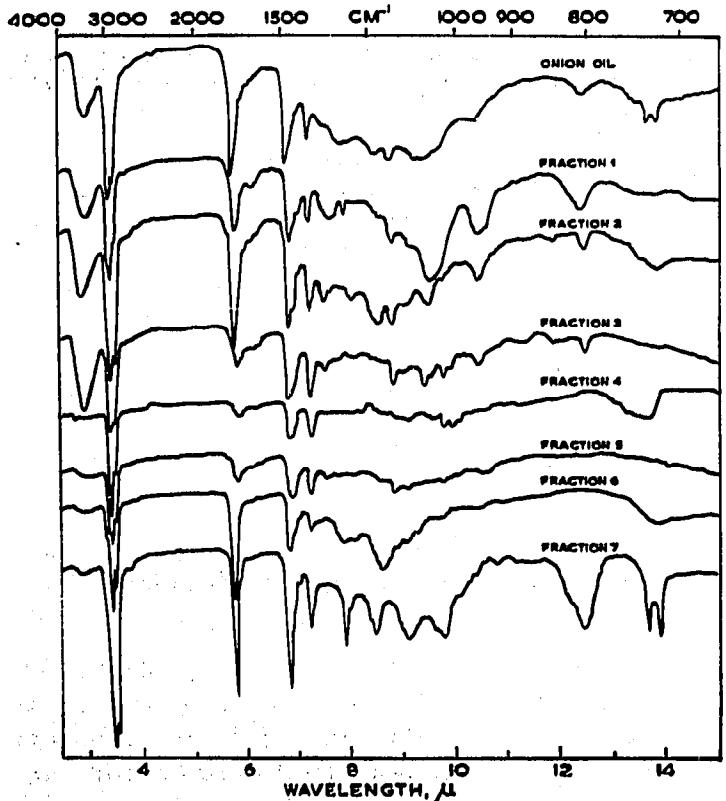

Fig. 4. IR spectra of onion oil and its fractions obtained from a preparative silica gel plate.

chloroform for extraction, a slight increase in yield of oil has been observed without a change in the pattern of separation of the oil on the thin-layer plate. While analysing onion oil extracted from different lots of onion (always the red variety), using diethyl ether and TLC described here, a few minor spots were occasionally located but the pattern of separation of major components, as shown in Fig. 3, is reproducible in all cases. Further studies regarding characterisation of fractions of onion oil obtained by the present method are in progress.

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