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The problem of tracing normal food odors and off-odors to their source must ultimately be solved by actual identification and isolation of chemical components for individual study. To quote Turk (11): "Much of our present information on constituents of common odors is purely nominal and based on old and uncomplete research." Such is the case for the onion.

Thus Semmler (9), in 1892, claimed to have obtained a sulfide of unknown constitution from the onion, of the same molecular formula, but different boiling point than the allyl propyl disulfide which he reports in garlic oil. Kohman, (7) quoting Semmler as identifying allyl propyl disulfide as the onion flavor, measured this quantitatively by oxidizing the product of steam distillation with bromine to the sulfate state and determining sulphur gravimetrically. On the basis of the quantity of sulphur, allyl propyl disulfide was calculated. In an earlier paper Kohman (6) attributed the lachrymating properties of the onion to a thioaldehyde (thiopropionaldehyde or thioallylaldehyde) which he determined by the expedient of distilling the onion in vacuo at 50° C., oxidizing a weighed sample of the distillate residue with bromine and determining the sulphur present. The amount of sulphur was equivalent to that in the thioaldehyde which he hypothesized on the basis of the various observations made. Propionaldehyde was also identified by isolation and formation of its dinitrophenylhydrazone. Challenger and Greenwood (2) then detected n-propylthiol by absorption of the thiol in mercuric cyanide. They were not able to determine whether it occurs in the undamaged bulb or whether it is liberated by enzyme action after slicing.

Since little positive information has been published concerning the volatile constituents of onions, it was decided to apply the methods of instrumental analysis with the intention of outlining a technique which may be applicable to most other food odor problems. The following work is a report on a mass spectrometric and infrared spectroscopic analysis of vapors trapped at various low temperatures in the absence of air.

EXPERIMENTAL PROCEDURE

A model 21-103B Consolidated analytical mass spectrometer and a Baird infrared spectrometer were used in the present work. Volatile constituents of onions were collected by trapping the vapors at -30°, -80°, and -190° C. in a system as shown in Figure 1. The contents of the traps were individually analyzed mass spectrometrically, and then condensed into the distillation tube, or examined by gas partition chromatography (GPC). The distillation technique yielded between 3 to 5 cuts from the contents of each trap. A trapping system made possible the collection of gases separated by GPC.

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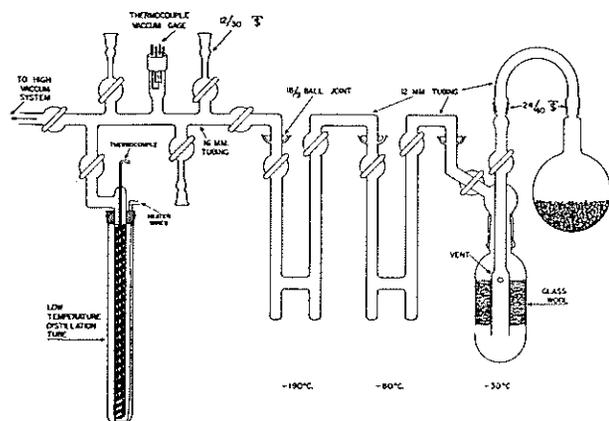


Figure 1. High vacuum trapping system and low temperature-low pressure distillation tube for prefractionation.

Preparation of system. Approximately 100 to 140 g. of onion are frozen in liquid nitrogen, minced, and placed in the 500 cc. distillation flask (Figure 1). Liquid nitrogen coolant is placed around the flask while the system is evacuated to less than 0.1 micron of mercury. The flask is isolated from the traps, and the onion is permitted to warm to room temperature. A heat lamp or water bath may be used to speed the warming process provided care is taken to prevent overheating the sample. At no time should the sample temperature exceed 30° C. It was found that slow cooling of the onion at this time to -30° C. ruptures the cells and yields a greater concentration of volatile matter.

Collection of vapors. Coolant is applied to the first trap and the onion is permitted to warm up and begin to distill into the first trap. The second and third traps are likewise cooled and stopcocks are opened to permit collection of the volatile emanations of the onion. The "H" type trap was used for the latter stages of trapping in order to maximize the cooling surface to volume ratio. The function of the glass wool used in the first trap was also to create a greater effective cooling surface for this stage, and to entrain micro-particles of frozen water.

It should be noted that the system may be constantly evacuated or isolated from the vacuum source as desired while collecting sample. Both techniques were employed, but it was found that the latter provides cleaner separations especially with respect to water carryover, whereas the former technique yields a greater concentration of collected vapors.

At least eight hours are required to provide adequate samples for analysis, and overnight runs usually dehydrate the onion sufficiently to provide more than enough condensate.

A portion of the vapor phase contents of each trap is analyzed directly on the mass spectrometer and the residual is condensed into the distillation tube or separated by vapor phase chromatography. The amount used for mass spectral analysis is quite small (approximately 5×10^{-3} moles) and does not represent serious reduction of sample size.

The distillation tube. The tube itself is an adaptation of a design originally used by the Bureau of Standards as an isothermal distillation tube (10). A copper constantan thermocouple measures the internal temperature in the bottom of the distillation tube; the column is packed with stainless steel balls, and a nichrome ribbon is wrapped

around the tube to supply the heat necessary to conduct a distillation. A variac is used in series with a dropping resistor in a 115 V AC power source to supply approximately 15 to 20 watts to the heater, depending upon the speed of distillation desired. It is advisable to allow at least 6 hours for a single distillation from -190° C. to +25° C. The tube has a capacity for approximately 1 ml. of liquid, but less material than this is desired since only 1 to 5 mm. mercury of vapor is collected in a 250 ml. gas sampling bottle for further analysis. Normally the sample size is too small for a liquid-vapor phase equilibrium to exist in the column, so the purpose of the column as used herein is to allow the vapors to fractionally diffuse through the packing. The gas bottle is equipped with an oblique bore high vacuum stopcock and a 12/30 standard taper joint.

Distillation. After analyzing the contents of one of the traps mass spectrometrically, the trap is attached to the high vacuum manifold and is cooled with liquid nitrogen. The system is evacuated and a bulb to bulb distillation condenses the contents of the trap into the lower portion of the tube. The entire contents of the -80° and -190° traps are condensed into the tube, but since approximately 85% of the mass of the onion is collected in the -30° trap, it is necessary to obtain a small aliquot from this trap for distillation. A fraction is collected when the pressure in the system exceeds 200 microns of mercury, and the pressure does not appreciably rise with increasing temperature. Needless to say, the contents of each trap is distilled separately. The fractions are then analyzed mass spectrometrically. Table 1 illustrates the results obtained by applying this technique to an onion fraction.

TABLE 1
Contents^a of a -190° C. trap analyzed before and after separation by use of the distillation tube

| Temp. degrees C. | Mole per cent | | | | | |
|-----------------------|---------------------|--------------------|------|------|------|------|
| | Before distillation | After distillation | | | | |
| | +25 | -117 | -93 | -90 | -42 | +25 |
| Propyl mercaptan..... | 0.1 | 0 | 0 | 0 | 1.0 | 0.6 |
| Propionaldehyde..... | 21.2 | 3.3 | 1.3 | 0.1 | 53.1 | 21.4 |
| Acetaldehyde..... | 0.8 | 0 | 0 | 0 | 42.2 | 0.1 |
| Carbon dioxide..... | 65.8 | 94.1 | 98.1 | 99.8 | 1.5 | 0.1 |
| Methyl alcohol..... | 12.1 | 0.9 | 0.5 | 0.1 | 2.1 | 77.8 |
| Hydrogen sulfide..... | 0 | 1.7 | 0.1 | 0 | 0 | 0 |

^a Calculated air and water free. Zero values indicate less than 0.05% detectable.

Gas partition chromatography. A column (10 mm × 2 meter), packed with Celite 545 having diethyl hexyl sebacate as the non-mobile phase, was used with a Gow-Mac thermal conductivity cell as the detector. A flow rate of 50 ml. per minute of helium was maintained, and the column was thermostated at 25° C. The gas phase of each trap was condensed into a special inlet system designed to minimize the volume of the mixture. The sample was then vaporized and introduced into the column by displacement with helium. Fractions were collected of each peak appearing on the chromatogram and analyzed mass spectrometrically.^b Figure 3 depicts a gas-partition chromatogram obtained, with subsequent mass spectral analysis of each fraction presented in Table 2.

Mass spectrometric analysis. The gas bottles containing the various fractions obtained by distillation or by chromatographic separations are introduced into the mass spectrometer and a record is obtained which represents the fragmentation pattern of the different molecules present in the sample. By applying classic techniques it is possible to identify the components present from their fragmentation pattern. It is not the intention of the authors to discuss in greater detail the theory, calculations of data,

^b This technique is described in detail in a paper submitted to *J. Agr. Food Chem.* by Stahl, Niegisch, and Levy.

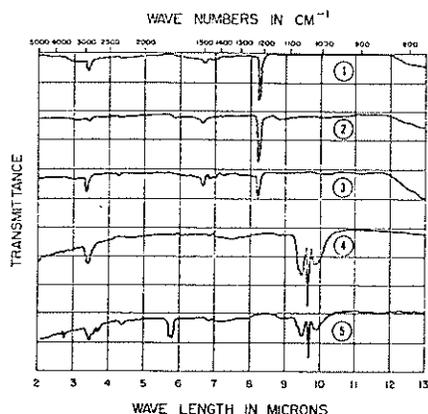


Figure 2. Infra-red spectra indicating the presence of n-propyl mercaptan and methanol in onions. Top to bottom: 1) 0.05% propyl mercaptan in CC14; 2) CC14 extract of onion; 3) CC14 extract of water solution collected in -20°C . trap from domestic yellow onion; 4) Methanol vapor phase; 5) -190°C trap contents of Spanish onion, vapor phase.

or operation of the mass spectrometer. The literature has excellent articles which discuss the subject in great detail (3, 8). By operating the instrument at high sensitivity it is possible to detect less than 5×10^{-7} moles of a particular molecular species.

Infrared analysis. Considerable difficulty was encountered in detecting small quantities of material present in addition to water in the -30°C . trap because of the preponderance of water. A carbon tetrachloride extract of the contents of this trap was analyzed in a 0.1 sodium chloride cell against a carbon tetrachloride reference cell. The resulting spectrum is shown in Figure 2 and may be compared with a carbon tetrachloride extract of a whole Waring blended onion, and a spectrum of 0.05% n-propyl mercaptan. It is seen that the characteristic frequencies related to n-propyl thiol are found in the onion samples examined. The vapor phase contents of a -190°C . trap is also shown in Figure 2 and comparison with a spectrum of methanol indicates the presence of methanol in the trap. A 10 cm. sodium chloride cell was used for this analysis.

TABLE 2
Contents¹ of a -190°C . trap analyzed before and after separation by vapor phase chromatography

| Cut number | Mole per cent | | | | |
|-----------------------|-------------------|------------------|------|------|------|
| | Before separation | After separation | | | |
| | | 1 | 2 | 3 | 4 |
| Propyl mercaptan..... | 0 | 0 | 0 | 0 | 0.1 |
| Propionaldehyde..... | 3.2 | 0 | 0 | 0 | 89.9 |
| Acetaldehyde..... | 2.3 | 0 | 98.4 | 6.3 | 1.6 |
| Carbon dioxide..... | 92.7 | 100.0 | 1.6 | 1.7 | 0 |
| Methyl alcohol..... | 0.8 | 0 | 0 | 92.0 | 8.4 |

¹ Calculated air and water free. Zero values indicate less than 0.05% detectable.

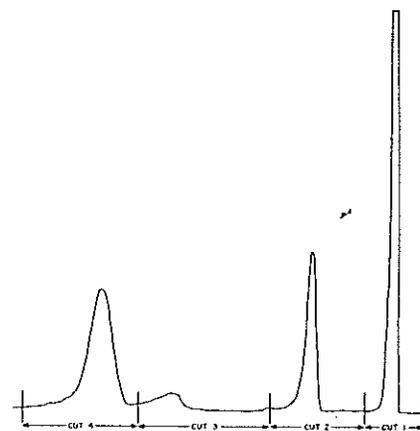


Figure 3. Chromatogram of the contents of a -190°C . trap fraction. (See analysis of cuts obtained in Table 2.)

Adsorption. An attempt was made to employ various adsorbants such as Drierite, Ascarite, Linde Molecular Sieves, alumina, etc., to facilitate the removal of the large amounts of carbon dioxide and water which tend to mask the detection of the odoriferous onion components. It was found by passing the contents of a -80°C . trap over a column of potassium hydroxide pellets, that the exit gases contained a small amount of di-(n-propyl) disulfide.

RESULTS AND DISCUSSION

From the data presented in Figure 2 and by mass spectral analysis, the presence of n-propyl mercaptan and methyl alcohol is unequivocally established. Table 2 also conclusively proves the presence of propionaldehyde, acetaldehyde, and carbon dioxide by virtue of the relatively large concentrations found in the chromatographic cuts (see Figure 3) when analyzed mass spectrometrically, and by calibrating compounds by gas-partition chromatography (not shown).

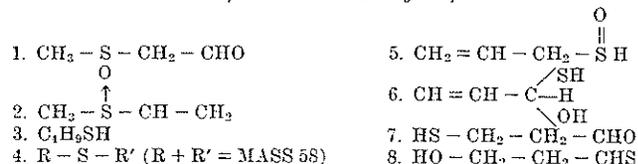
Because of the ability of the mass spectrometer to detect very small amounts of material in the presence of large diluents under certain conditions evident to a mass spectrometrists, the detection of other materials is reported below, although no verifying data applying other techniques is offered. Extreme caution was exercised by the authors to eliminate all evident sources of possible contamination, and thus to permit the claim that the following are also true components of onion: propyl alcohol, sulphur dioxide, hydrogen sulfide and di(n-propyl)disulfide. The latter has a definite "sweet" odor typical of disulfides, and when combined with n-propyl mercaptan, a creditable onion odor is produced.

Theoretical predictions. By applying the technique of structural analysis to the mass spectral scans, it is postulated that a hexenal may also be present in onions. Structural deductions drawn from a composite spectrum

calculated from more than 20 scans of various onion fractions indicated that the compound may be 4-hexen-1-al. It is certain that the unknown is not the "leaf aldehyde" (2-hexen-1-al) discussed by Guenther (5) because of the dissimilar mass spectra of these two compounds. It was also ascertained that the unknown is not α -methyl, β -methyl acrolein, which is the condensation product of propionaldehyde, and was first suspected, but also eliminated because of differences in the ionization fragmentation pattern.

Kohman (6) suggested that the lachrymatory principle of the onion was due to a thiolaldehyde. There was no evidence from any data obtained in this investigation that either thiopropionaldehyde or thioallylaldehyde was collected in any of the traps. However, a compound of mass 90 has been observed several times in the -80° traps which is known from isotopic measurements to contain one sulphur atom in the molecule. There are eight obvious mass type isomers containing carbon, hydrogen, sulphur and/or oxygen atoms which would have a mass of 90. Molecules containing nitrogen atom were eliminated because those which have one nitrogen atom have uneven molecular weights, and compounds containing more than one atom of nitrogen and one sulfur atom do not have molecular weights of 90.

Isomers of Mass 90 Containing Sulfur



The spectrum containing the mass 90 unknown is also characterized by the presence of mass 73 and 45 which are not common breakdown masses. They could conceivably arise from cleavage of a hydroxyl group to give a mass 73, and either a carboxyl, $-\text{CSH}_2$, $-\text{CH}_2\text{CH}_2\text{OH}$, $-\text{CH}_2 - \text{O} - \text{CH}_3$,

$\text{OCH}_2 - \text{CH}_2$ or $-\overset{\text{H}}{\text{C}} = \text{S}$ to produce a mass 45 fragment. It is readily seen that the first four compounds above do not meet both these requirements. Structural analysis also eliminates the presence of an ether linkage. Compound five, allyl sulphenic acid, has never been isolated and is usually considered an intermediary indicating oxidation of a disulfide to a sulfenic acid. It is highly unlikely that this compound is present from the existing information regarding sulfenic acids.

Attempts to synthesize compound six have failed. However, the hemithial of propionaldehyde, $\text{CH}_2 = \text{CH}_2 - \overset{\text{SH}}{\underset{\text{OH}}{\text{C}}}$, was readily prepared by

the addition of hydrogen sulfide to propionaldehyde (80% yield). The structure was deduced from information obtained by infrared observation

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of the decrease of the carbonyl band and formation of water accompanied by increasing cloudiness of the solution on standing. This observation would imply the addition of hydrogen sulfide to form the hemithial, with subsequent release of water and trimerization of the resulting propanthial. Analysis of the mixture mass spectrometrically showed no ionization to mass 90 although the data obtained by infrared indicated 80% of the hemithial present. Instead, only water, hydrogen sulfide and propionaldehyde were found. This indicates the instability of the hemithial in the high vacuum (10^{-6} mm mercury) of the mass spectrometer ionization chamber. The hemimercaptal prepared in this Laboratory by the reaction of acetaldehyde and methyl mercaptan analyzes similarly by infrared and mass spectrometric techniques. It is assumed that the hemithial of acrolein, structure six, will behave in a similar manner in the mass spectrometer, and will never ionize to the molecular weight mass. It is interesting to note that the hemithial of propionaldehyde has a strong persisting onion-like odor.

Compound seven, β -mercapto propionaldehyde, has been reported to have a definite onion-like odor (4) and was suspected as an onion constituent. The predicted fragmentation pattern for this compound coincides generally with the postulated fragmentation pattern of the unknown, except for the lack of a hydroxyl group which would account for a mass 73 breakdown. Compound eight, β -hydroxy propanthial, satisfies all of the requirements of a predicted fragmentation for the unknown. In addition, this compound would be expected to polymerize readily, accounting for the low concentration of this material present in the volatile onion vapors collected. It is conceivable that the allyl thial predicted by Kohman could easily add water to produce the β -hydroxy propanthial postulated in this investigation.

As it is well known that mercaptans oxidize readily to disulfides, it was deemed necessary to show that dipropyl disulfide found could not be formed under the conditions of isolation existing in this investigation. Therefore, *n*-propyl mercaptan was deliberately diluted to contain 20% air, and the mixture was run through wet potassium hydroxide, which were the conditions existing during the initial detection of the disulfide. No trace of the disulfide was found upon analysis of the scrubbed gases.

All onions used in this work were the common domestic yellow with the exception of one determination made on a Spanish onion from which the infrared data establishing the presence of methanol was shown. It was found that the domestic yellow generally produced the greatest amount of condensate other than water and carbon dioxide.

All data presented have been obtained in the absence of air at room temperature, and samples were analyzed entirely at very low pressures to reduce the possibility of oxidation, reduction or other chemical changes which may take place. Vapors were condensed and stored at low temperatures, which would retard the physical changes which may take place under conditions existing in other investigations. All data were obtained within 48 hours or less after collection of sample. This would suggest that all of the compounds found, exist *per se* in the onion in contrast to the data of Buck and Joslyn (1) wherein they suspect the source of acetaldehyde in

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broccoli to be brought about by enzymatic decarboxylation after long term storage in the frozen state.

It is difficult to assay the data obtained quantitatively, yet something should be said as to the relative concentration of each material reported. The following is a relative estimation of each component present, in decreasing order of concentration. The gross amounts of carbon dioxide and water are not included in this tabulation.

| Very abundant | Abundant | Small | Trace |
|---------------------------------------|---|----------------------|---|
| CH ₃ OH | CH ₂ -CH ₂ -CH ₂ -SH | H ₂ S | SO ₂ |
| CH ₂ -CH ₂ -CHO | | CH ₂ -CHO | C ₆ H ₆ -S-S-C ₆ H ₆ |
| | | | C ₆ H ₅ OH |
| | | | CH ₂ -CH=CH-CH ₂ -CH ₂ -CHO ¹ |
| | | | HOCH ₂ -CH ₂ -CH ₂ -CHO ¹ |

¹ Suggested structures.

CONCLUSION

It is evident from the data presented in this paper that the analysis of food odors is greatly simplified by applying modern instrumentation to a quantity of starting material many orders of magnitude less than that required by classic chemical methods. In addition, it is particularly well suited to the analysis of gases and vapors which are commonly overlooked by other methods. It may be suggested to the laboratory only set up for classical chemical methods that all of the instrumental techniques presented need not be applied simultaneously. Indeed, infrared data on GPC or distillation cuts can be obtained quite successfully without mass spectrometer analysis provided larger samples are obtained.

There can also be no ambiguity as existed in the reports of earlier investigators. The authors make no claim that this work reports all of the volatile odors emanating from onions. However, no evidence of the existence of allyl propyl disulfide reported by other investigators was found despite the great care taken in processing samples and searching for this compound. It is very likely with the advent of greater sensitivity of gas partition chromatography that better separations will be possible in the near future which will facilitate the detection of materials present in extremely small concentrations.

Acknowledgments

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