sugar concentration is a multiple regression equation using the individual nonreducing sugars sucrose and stachyose, and the total reducing sugars.

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ONION FLAVOR AND ODOR

The Volatile Flavor Components of Onions

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A number of the more important volatile flavor components of onions, *Allium cepa*, have been isolated by gas-liquid partition chromatography and identified by infrared methods and chemical derivatization. In particular, methyl disulfide, methyl trisulfide, methyl-*n*-propyl disulfide, methyl-*n*-propyl trisulfide, *n*-propyl disulfide, and *n*-propyl trisulfide were isolated and identified. Neither monosulfides nor allylic disulfides could be detected.

SEMMLER (*11*), in 1892, studied the composition of an onion oil and reported that the principal odoriferous volatile component was allyl *n*-propyl disulfide. This identification has apparently been accepted for many years without question and many textbooks and reference books (*7*) repeat this statement. Kohman (*8*), in 1947, found that propionaldehyde was an important volatile component obtained from onions, and Challenger and Greenwood (*3*), in 1949, demonstrated the presence of *n*-propyl mercaptan. More recently, Niegisch and Stahl (*9*) using the mass spectrometer for identification found hydrogen sulfide, sulfur dioxide, acetaldehyde, propionaldehyde, methyl alcohol, *n*-propyl alcohol, *n*-propyl mercaptan, and traces of *n*-propyl disulfide. No evidence for allylic disulfides could be obtained.

This paper describes the isolation and characterization of a number of the important volatile flavor components of the onion, *Allium cepa*. Both gas-liquid partition chromatography and conventional precipitation methods were used in the isolation of compounds. Two extraction methods were used, by carbon adsorption and with isopentane. As adsorption on carbon can lead to decomposition and rearrangement of the volatile components, a second milder procedure was used to confirm the results. Isopentane was chosen because of its low boiling point which should minimize thermal decomposition and its nonpolar nature which should minimize mercaptan-disulfide exchange reactions. Owing to the extreme lability of the materials responsible for the lachrymatory effect of freshly bruised onions, this effect is altered or lost during isolation and the final concentrate approaches more nearly that of a cooked onion than of a fresh onion.

Experimental

Extraction of Onions by Carbon Adsorption. Two separate batches of ca. 142 pounds each (peeled weight) of Sunspice onions, a strain of Improved Southport White Globe onions, were diced to pieces of ca. $^3/_8$ inch and each batch was treated as follows: The diced onions and 25 gallons of water were poured into a stainless steel vacuum pot of 100-gallon capacity which was exhausted through two parallel stainless steel tubes (1.5 inches I.D. and 33 inches long) to a very efficient vacuum system. Approximately 400 grams of activated carbon (Columbia AC brand, National Carbon Co.), 6 to 14 mesh, were placed in each tube. The onion slurry was then distilled for 40 hours, in vacuo at a pressure of 29 inches of mercury. Steam was bled in very slowly to agitate the thick slurry and to maintain a temperature of approximately 25° C. At the end of the distillation, the second batch of onions was treated similarly with fresh carbon in the adsorption tubes. The carbon was then dried in vacuo with an oil pump and a dry ice trap at temperatures of 25° C. and lower. The carbon was then extracted in Soxhlet extractors with peroxide-free ether for 40 to 44 hours. These extractions were performed in the dark to minimize decomposition. The combined ether extracts, 3 liters, were dried over anhydrous calcium sulfate and most of the ether was stripped off with a 15-plate Oldershaw column to give 115 ml. of concentrate. Further concentration was obtained by distilling off most of the remaining ether with a small, vacuum-jacketed column packed with glass helices. The final concentrate, 14 grams, was a pale yellow oil. It had a very slight dextrorotation $[\alpha]_D^{25} = +0.02°$(1 dm.). The oil was then distilled in vacuo at 1 mm. pressure with a bath temperature of 25° to 120° C.—most of the material distilling between 40° and 60° C. The yield of distillate was 8.7 grams, representing

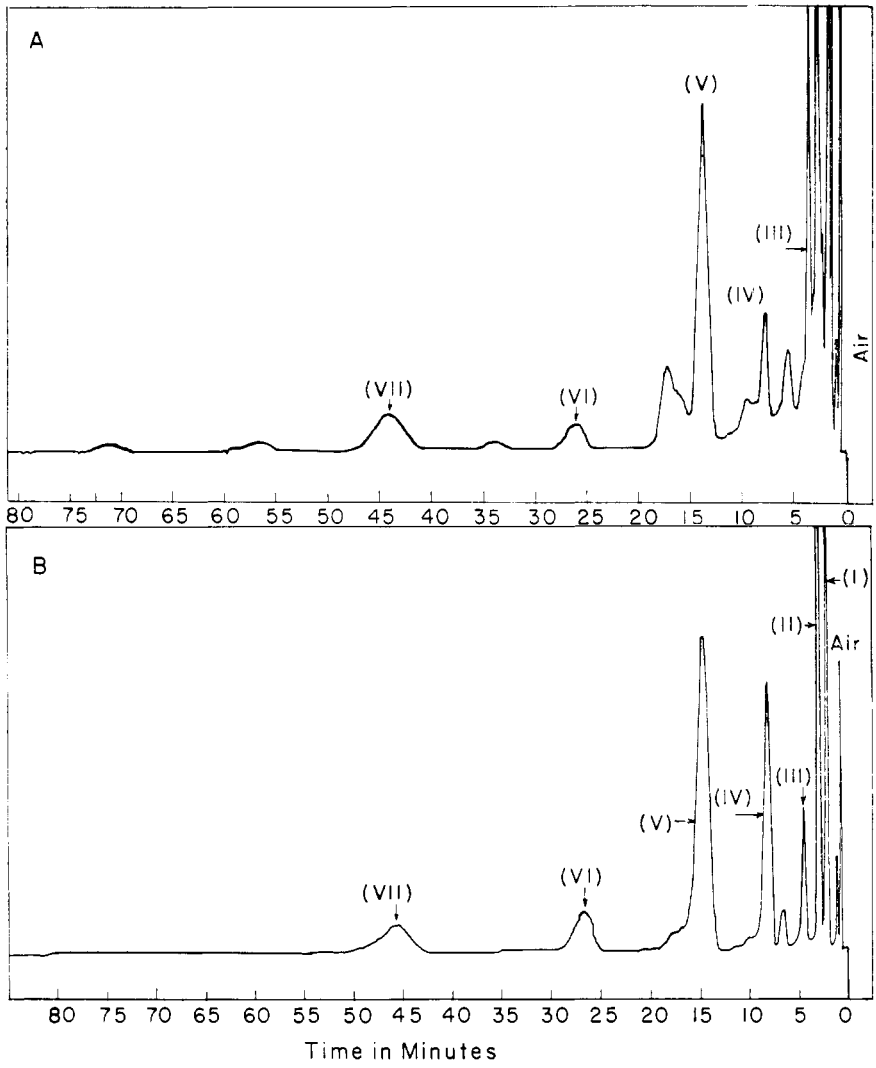

Figure 1. Gas-liquid partition chromatograms of onion volatiles

A. Isopentane extraction, Carbowax-1540 column
B. Carbon adsorption, Reoplex 400 column, 1/4 inch × 6 feet; temperature 150° C., flow rate 45 ml. of helium per minute; filament current 225 ma., 4-mv. full-scale deflection. Sample volume 5 μl.
Peak identification:
I. Ethyl and isopropyl alcohols. II. *n*-Propyl alcohol. III. Methyl disulfide. IV. Methyl-*n*-propyl disulfide. V. *n*-Propyl disulfide and methyl trisulfide. VI. Methyl-*n*-propyl trisulfide. VII. *n*-Propyl trisulfide

75 p.p.m. based on the fresh weight of onion.

Extraction of Onions with Isopentane. Trimmed peeled onions, 114 pounds, were chopped in a commercial dicer set for slices 1/4 × 5/32 × 5/32 inch and the mixture was allowed to stand for 1 hour. Onion juice was pressed from the mixture by means of a hydraulic press to yield 23 liters of juice. The juice was then steam-distilled in 3-liter quantities at temperatures not over 42° C. under a vacuum of 29 ± 0.5 inches of mercury. The receiver was packed in dry ice. The volume of distillate obtained was approximately half of the charge. The distillate was then extracted continuously with isopentane for 30 hours, the extract was dried with calcium sulfate and then stripped of isopentane in the same manner as previously described for the ether extract of the carbon adsorption procedure. The yield was 5 ml. of concentrate. Distillation of this material in vacuo yielded 3 ml. of liquid, distilling at 25° C. or less at a pressure of 1 mm. of mercury and 0.9 ml. of liquid distilling at bath temperatures from 25° to 100° C. That extraction of volatiles was not complete at this stage was shown by the fact that ether extraction of the residual aqueous solution yielded about as much distillate as the original isopentane extraction.

Apparatus. The gas-liquid partition chromatography apparatus was described in a previous publication (*2*). Columns used were of stainless steel, 1/4- or 1/2-inch O.D., and 5 to 6 feet long. These lengths were used rather than the customary 10-foot columns to decrease the time spent in the column and to minimize decomposition of disulfides and trisulfides. The stationary phases consisted of firebrick (C-22, Johns-Manville Co.) ground to 40 to 60 mesh, acid washed, heated to 400° C., and impregnated with the appropriate organic phase (dissolved in acetone or hexane) in the ratio of firebrick to liquid phase of 4 to 1 (by weight) for the 1/4-inch column. Three liquid phases were employed, a polyethylene glycol (Carbowax 1540, Union Carbide Chemicals Co.), a polyoxyalkylene adipate (Reoplex 400, Geigy Pharmaceutical Division, Geigy Chemical Corp.), and an extreme nonpolar phase, Apiezon M. In the early studies, Carbowax was used extensively, but in later work most of the separations were on Reoplex columns which were somewhat more stable.

For isolation purposes, a 1/2-inch column with Carbowax or Reoplex was very effective for preliminary separations. Quantities of 150 to 200 μl. of onion oil were injected with a helium flow rate of 180 ml. per minute and a column temperature of 140° or 150° C. Fractions were collected and then purified by chromatography on a 1/4-inch O.D. column of the same packing. At this stage, 15 to 20 μl. samples were injected. Obviously, the injection of samples of this magnitude is overloading the column for most effective separation, but it was necessary for the purpose of isolation in quantities sufficient for infrared as well as chemical derivatization.

Figure 1,*B*, shows a gas-liquid partition chromatogram (Reoplex column) of the volatile components of a commercial dehydrating onion (Improved Southport White Globe). This concentrate has been isolated by adsorption on carbon and subsequent extraction with ether. For this particular chromatogram, the column was operated at an optimum temperature, 150° C., for the separation of the higher boiling components. There are many low-boiling components which are not resolved.

Figure 1,*A*, shows the resolution of the volatile materials obtained by isopentane extraction of the same onion on a Carbowax column.

Preparation of Compounds. For purposes of infrared comparison and relative retention times, methyl disulfide and *n*-propyl disulfide were Eastman chemicals purified by gas-liquid chromatography. Methyl trisulfide, methyl-*n*-propyl disulfide and trisulfide, *n*-propyl trisulfide, and allyl-*n*-propyl disulfide were synthesized as described previously (*2*).

Identification of Disulfides and Trisulfides. The presence of disulfides or trisulfides in a given peak was suggested by odor and established by color development with Grote's reagent and cyanide (*6*). Peak V (in Figure 1,*A* or *B*) containing methyl trisulfide and *n*-propyl disulfide could not be resolved

Table I. Relative Retention Times of Chromatograph Peaks and of Pure Disulfides and Trisulfides

Peak	Compound	Retention Times Relative to That of Cyclohexane: Reoplex	Apiezon M
IV[a]		0.676	1.11
	$CH_3S_2nC_3H_7$	0.658	1.03
V_a[b]		1.19	1.46
	$CH_3S_3CH_3$	1.22	1.44
V_b[b]		1.17	2.63
	$nC_3H_7S_2nC_3H_7$	1.13	2.59
VI[a]		2.11	3.68
	$CH_3S_3nC_3H_7$	2.17	3.69
VII[a]		3.88	9.79
	$nC_3H_7S_2nC_3H_7$	3.81	9.83

[a] These refer to labeled peaks of Figure 1, *A* and *B*.
[b] Peak V of Figure 1, *A* or *B*, was split into two peaks by rechromatographing on Apiezon M. The faster moving component is labeled V_a and the slower V_b.

Table II. Volatile Components Found in Onions

Hydrogen sulfide	Methyl trisulfide
n-Propyl mercaptan	Methyl *n*-propyl trisulfide
Ethyl alcohol	*n*-Propyl trisulfide
n-Propyl alcohol	Acetaldehyde
Isopropyl alcohol	Propionaldehyde
Methyl disulfide	*n*-Butyraldehyde
Methyl *n*-propyl disulfide	Acetone
n-Propyl disulfide	Methyl ethyl ketone

with the polar phases, Carbowax or Reoplex, but was easily separated on Apiezon M when the two peaks were at least 10 minutes apart. The faster moving component was labeled V_a and the slower one V_b. The purified fractions were first chromatographed on a Reoplex column and the ratios of the retention times to that of cyclohexanone were calculated and compared with the ratios for the pure compounds. The procedure was then repeated with a column of widely different character such as Apiezon M (*2*).

In Table I, the relative retention times for peaks IV, V_a, V_b, VI, and VII and for the relevant disulfides and trisulfides are listed. Ratios were not determined for peak III, but its identity was demonstrated by infrared spectra and by chemical characterization. The structure of each fraction was also established by comparing infrared spectra with spectra of the authentic compounds determined in liquid cells with sodium chloride optics on a Beckman IR-3 recording spectrophotometer.

The structures of the isolated fractions were also determined by chemical derivatization as previously described (*1*). This consists in reduction of the disulfide or trisulfide with lithium aluminum hydride to the mercaptides and reaction of these with 2,4-dinitrochlorobenzene to yield the crystalline 2,4-dinitrophenylsulfides.

In a typical example, 50 μl. from peak VI were dissolved in 15 ml. of dry ether in a 50-ml. round-bottomed flask equipped with a reflux condenser and drying tube. Approximately 0.3 gram of lithium aluminum hydride was added over a 30-minute period accompanied with a vigorous evolution of hydrogen. The mixture was refluxed gently for 2 hours and, after cooling, the excess hydride was destroyed by the addition of 1 ml. of ethyl acetate, followed by a solution of 5 ml. of ethyl alcohol and 2 ml. of water. The suspension was then poured into 50 ml. of ethyl alcohol containing 0.22 gram of 2,4-dinitrochlorobenzene, and the mixture was heated in a water bath at 65° C. for 15 minutes. The suspension was filtered from salts and hydroxides, and the filtrate was taken to a dry solid in vacuo. Interfering substances including dinitrophenol were removed by extracting a benzene solution (200 ml.) with two 75-ml. portions of 5% NaOH. The dried benzene solution was concentrated in vacuo to 40 ml. and applied to a 48 × 300 mm. column of silicic acid (Mallinckrodt Analytical Grade) containing 1% of a fluorescent zinc sulfide (*1*). Development with 1300 ml. of hexane–ethyl acetate (15 to 1) yielded a slow band which had moved 7.0 cm. and a fast band which had moved 22.0 cm. The bands were carved from the column and extracted with acetone. Removal of solvent and crystallization from ethyl alcohol yielded from the slow band 30 mg. of methyl-2,4-dinitrophenylsulfide and from the fast band 46 mg. of *n*-propyl-2,4-dinitrophenylsulfide. The compounds were identified by microscopic observation of fusion behavior. Similarly, the methyl *n*-propyl disulfide fraction (peak IV) yielded the same two derivatives. The methyl disulfide fraction (peak III) and methyl trisulfide fraction (V_a) yielded only the methyl derivative and the *n*-propyl disulfide (V_b) and trisulfide (VII) yielded only the propyl derivative.

Mercaptan Determination. In this procedure (*3*, *4*), purified air was drawn by aspiration through cylinders filled with a total of 3 kg. of chopped onions. The entrained volatiles were conducted successively through traps containing anhydrous calcium chloride, dry 8- and 12-mesh lead acetate, 4% aqueous mercuric cyanide, and 3% aqueous mercuric chloride. The onions were aspirated for 48 hours. The lead acetate granules blackened quickly, demonstrating the presence of hydrogen sulfide. In the mercuric cyanide trap, mercuric mercaptide precipitated in a yield of 26 mg. per kg. of onion. Only a trace of material precipitated in the mercuric chloride trap. On treatment with acid, the precipitate from the mercuric cyanide trap yielded *n*-propyl mercaptan as the only thiol. No evidence of volatile sulfides could be obtained from the slight precipitate in the mercuric chloride trap. Gas chromatography of onion oils also failed to show sulfides.

Isolation of Carbonyl Components. The aspiration method of Challenger and Greenwood (*3*) was used. Each of several batches of chopped onions (totaling 5 kg. per batch) was aspirated for 48 hours and the volatiles were allowed to react with a 2% solution of 2,4-dinitrophenylhydrazine in 2*N* HCl. The dinitrophenylhydrazones were extracted into benzene and chromatographed on a 54 × 250 mm. column containing a 1 to 10 mixture of Celite and silicic acid (Mallinckrodt Analytical Grade) which was washed successively with ether and Skellysolve F prior to charging. The developing solvent was a mixture of ether and Skellysolve F (1 to 8). Six bands or zones, in addition to the stationary reagent zone, were obtained. Five of these bands yielded the pure crystalline 2,4-dinitrophenylhydrazones of acetone, acetaldehyde, propionaldehyde, methyl ethyl ketone, and *n*-butyraldehyde. The sixth and fastest moving band yielded a crystalline dinitrophenylhydrazone which has not been identified. The compounds were identified by crystallographic and infrared examination.

Results and Discussion

The compounds that have been identified are recorded in Table II. Of these, the most important for flavor are the compounds which contain sulfur. The presence of hydrogen sulfide and *n*-propyl mercaptan was demonstrated by the procedure of Challenger (*3*) as modified by Dateo *et al.* (*4*) confirming previous results. Surprisingly, no mercuric methyl mercaptide was obtained by this procedure, although both carbon adsorption of the volatiles and isopentane extraction of the steam distillate yielded disulfides and trisulfides containing methyl as well as propyl.

Ethyl and isopropyl alcohols were found to be in peak I of Figure 1 and *n*-propyl alcohol was found in peak II. These substances were purified by rechromatographing at 90° C. and their identity was established by infrared measurements.

The disulfides and trisulfides were identified by three methods: comparison of relative retention times (retention times relative to that of cyclohexanone as an internal standard) with the relative retention times of the compounds determined on two different column substrates (Table I); infrared determinations on the isolated samples; and chemical characterization as crystalline derivatives. In previous studies (*2*), methods were developed for the gas-liquid partition chromatographic separa-

tion of *n*-propyl disulfide, allyl-*n*-propyl disulfide, and allyl disulfide. Allyl-*n*-propyl disulfide, if present, would have appeared as a peak following peak V of Figure 1. Addition of this compound to the oil produced a separate peak at the expected position.

The two different methods of isolation, carbon adsorption of the volatiles and isopentane extraction of steam distillates, yielded oils giving different patterns upon gas chromatography, but the same disulfides and trisulfides were found in each case. This establishes the fact that the trisulfides were not merely artifacts resulting from adsorption on carbon or subsequent desorption with ether. The poorly resolved peak following V in Figure 1,*A*, although appearing in the expected position for allyl-*n*-propyl disulfide, was shown not to contain any of this compound.

As explained in the experimental section, the five volatile carbonyl components were identified as dinitrophenylhydrazones.

The most important new findings are that allyl-*n*-propyl disulfide is not present in significant amounts; methyl as well as *n*-propyl derivatives occur; and substantial quantities of trisulfides corresponding to the disulfides are found.

The isolation of methyl as well as *n*-propyl sulfur derivatives is consistent with the findings of Fujiwara, Yoshimura, and Tsuno (*5*) who obtained evidence for the presence of methyl methanethiolsulfinate, *n*-propyl propanethiolsulfinate, and a mixed methyl *n*-propanethiosulfinate in freshly cut *Allium cepa*. These compounds are very labile and on decomposition will yield disulfides and thiolsulfonates. Significantly, these investigators could find no allyl thiolsulfinates in their onion samples. Virtanen and Matikkala (*13*) have recently demonstrated the presence of *S*-methyl-cysteine sulfoxide and *S*-propyl-cysteine sulfoxide from Finnish onions, and in this laboratory (*12*) a crude enzyme preparation from commercial onions was recently isolated, which decomposes both of these amino acids to thiosulfinates, pyruvate, and ammonia in a manner similar to that of alliinase from garlic.

Since enzymic breakdown of the sulfoxide amino acids produces thiolsulfinates which in turn decompose to thiolsulfonates and disulfides, both of which can further interact with thiols, the actual pattern of disulfides and trisulfides will probably vary with conditions of the enzyme reaction and with the method of extraction.

Although Semmler (*10*) presented evidence for the presence of allyl trisulfide in garlic oil based on elemental analysis of higher boiling fractions, the present work is believed to represent the first unequivocal isolation of pure, well-defined aliphatic trisulfides from plant sources. The authors have no explanation for the formation of these compounds. They are not artifacts of gas-liquid chromatography nor are they formed by a simple mercaptan-disulfide exchange. However, hydrogen sulfide or free sulfur may react with disulfides to yield polysulfides (*14*) and this may be the origin of these compounds in an onion macerate.

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OFF-FLAVORS IN FOODS

Effect of Pyrrolidonecarboxylic Acid on Flavor of Processed Fruit and Vegetable Products

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PYRROLIDONECARBOXYLIC ACID (PCA) in processed fruit and vegetable products is formed by the conversion of free glutamine during heat treatment and subsequent storage (*4*). Rice and Pederson (*8*) observed that a relationship might exist between the formation of PCA and the appearance of off-flavors and other undesirable changes in canned foods. Subsequent investigations by Shallenberger and coworkers (*10, 11*) revealed that off-flavors, described as bitter, medicinal, or phenolic, were significant in processed beet purees but not in raw beets, and that the intensity of off-flavor in beet purees varied directly with PCA concentration. An analysis of 22 processed fruits and vegetables, reported herein, revealed the presence of PCA in all products examined. The purpose of this study was to determine the effect of PCA on the flavor of several representative processed products.

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Methods

Determination of PCA in Commercially Processed Fruits and Vegetables. Commercially canned fruits and vegetables were purchased from local grocery stores. Drained weight and volume of brine were determined, after which the can contents were pureed in a Waring Blendor and filtered through Reeve Angel filter paper No. 202. PCA and glutamine were determined by column chromatography of the filtrate (*4*). Glutamine was determined only in high-acid foods, since it had been reported