

## Additional Volatile Components of Cabbage, Broccoli, and Cauliflower

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The vacuum steam volatile oils of cooked cabbage, broccoli, and cauliflower were analyzed by the combination of capillary gas chromatography and mass spectrometry. Major components characterized that had not been previously reported in these vegetables included nonanal and octanal in cauliflower and broccoli and 3-methylthiopropyl cyanide in cauliflower and cabbage, 4-methylthiobutyl cyanide, 2-phenylethyl cyanide, and 2-phenylethyl isothiocyanate in all three vegetables. The most unusual compound characterized was  $\text{CH}_3\text{SCH}_2\text{SSCH}_3$ . Odor thresholds in water solution were determined on the major compounds characterized.

Studies (Lipton and Harris, 1976) to improve the storage time of cauliflower (*Brassica oleracea L.*, Botrytis group) have indicated the need for an improved knowledge of the compounds contributing to its flavor. As cauliflower is closely related to cabbage (*Brassica oleracea L.*, Capitata group) and broccoli (*Brassica oleracea L.*, Italica group) it was thought of some value to compare the volatiles of all three vegetables. Of the three, cabbage has been the most investigated.

Table I lists the compounds characterized previously in cabbage by spectral methods. Apparently no spectral evidence had been obtained previously for broccoli and cauliflower. Evidence based on GLC retention times was obtained by Self et al. (1963) for acetaldehyde, propanal, acetone, 2-methylpropanal, hydrogen sulfide, methanethiol, ethanethiol, propanethiol, and dimethyl sulfide in the volatiles of cauliflower. Additional evidence based on GLC retention times for cauliflower volatiles was obtained by Macleod and Macleod (1970) for formaldehyde, acetaldehyde, dimethyl sulfide, propanal, acetone, acrolein, butanal, 2-butanone, ethanol, pentan-3-one, butane-2,3-dione, but-2-enal, dipropyl sulfide, dimethyl disulfide, hexanal, allyl alcohol, pent-2-enal, prop-1-enyl cyanide, allyl cyanide, but-2-en-1-ol, hex-2-enal, methylpropyl sulfide, methyl isothiocyanate, pent-3-en-1-ol, acetoin or octanal, butylmethyl sulfide or propyl isothiocyanate, pent-2-en-1-ol, hexanol, allyl isothiocyanate, hex-3-enol, butyl isothiocyanate, and hept-2-enol. The same authors obtained GLC retention time evidence for essentially the same list of compounds in broccoli. Evidence based on GLC retention times, although useful, by itself cannot characterize a compound with any degree of certainty because there are so many compounds with the same or closely similar retention times.

The present work was aimed at obtaining fairly certain evidence for the identities of components with boiling points above that of hexane.

### EXPERIMENTAL SECTION

**Materials.** Fresh cabbage, broccoli, and cauliflower were obtained from local markets and from commercial growers. Authentic compounds were obtained from reliable commercial sources or synthesized by well-established methods. 3-Methylthiopropyl cyanide was synthesized by the short-wavelength uv light-catalyzed

Table I. Compounds (Characterized by Spectral Methods) Reported Previously as Components of the Volatile Oil of Cabbage

Sulfides and mercaptans <sup>a, b</sup>	Isothiocyanates <sup>a-c</sup>
Hydrogen sulfide	Methyl isothiocyanate
Methanethiol	Allyl isothiocyanate
Ethanethiol	(prop-2-enyl isothiocyanate)
Propanethiol	Butyl isothiocyanate
Dimethyl sulfide	But-3-enyl isothiocyanate
Diethyl sulfide	3-Methylthiopropyl isothiocyanate
Dibutyl sulfide	Cyanides <sup>a</sup>
Carbon disulfide	Allyl cyanide
Dimethyl disulfide	(prop-2-enyl cyanide)
Methylethyl disulfide	Aldehydes and ketones <sup>b</sup>
Diethyl disulfide	Acetaldehyde
Methylpropyl disulfide	Propanal
Ethylpropyl disulfide	Pent-2-enal
Dipropyl disulfide	Hexanal
Propylbutyl disulfide	Hex-2-enal
Propyl but-3-enyl disulfide	Acetone
Dibut-3-enyl disulfide	Butanone
Dimethyl trisulfide	Pentan-3-one
Diethyl trisulfide	Butane-2,3-dione
Alcohols <sup>b</sup>	
Methanol	
Ethanol	
Hex- <i>cis</i> ,3-enol	

<sup>a</sup> Bailey et al. (1961). <sup>b</sup> Macleod and Macleod (1968).  
<sup>c</sup> Clapp et al. (1959).

condensation of allyl cyanide and methyl mercaptan as outlined by Kjaer and Conti (1954). 3-Methylthiopropyl isothiocyanate was synthesized by a similar condensation of allyl isothiocyanate and methyl mercaptan (cf. Kjaer et al., 1955a). 4-Methylthiobutyl cyanide was synthesized by the reaction of sodium methyl mercaptide with 1,4-dibromobutane to give 4-methylthiobutyl bromide and then reaction of this compound with KCN (cf. Kjaer et al., 1955b). 4-Methylthiobutyl isothiocyanate was synthesized by lithium aluminum hydride reduction of 3-methylthiopropyl cyanide to 4-methylthiobutylamine and then reaction of this amine with thiophosgene to give the corresponding isothiocyanate (cf. Kjaer and Gmelin, 1955). But-3-enyl isothiocyanate was synthesized in a similar way to the last compound from allyl cyanide. 2-Phenylethyl isothiocyanate was synthesized from 2-phenylethylamine using thiophosgene as described by Gilbert and Nursten (1972). 2,4,5-Trithiahexane ( $\text{CH}_3\text{SCH}_2\text{SSCH}_3$ ) was synthesized by the reaction of sodium methyl mercaptide with  $\text{ClCH}_2\text{SCl}$  (prepared from  $\text{CH}_3\text{SSCH}_3$  and  $\text{SO}_2\text{Cl}_2$ ) using procedures similar to those outlined by Brintzinger and Schmahl (1954) for the synthesis of 3,5,6-trithiaoctane. All compounds were purified by GLC separation.

**Isolation of the Volatile Oil.** Standard conditions were used for all three vegetables. The vegetable (1.8 kg) was cut up into pieces about  $3 \times (1.5)^2$  in. and placed in

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Table II. Compounds Characterized in the Steam Volatile Oils of Cabbage, Broccoli, and Cauliflower<sup>a</sup>

Compound	Cabbage <sup>b</sup>	Broccoli <sup>b</sup>	Cauliflower <sup>b</sup>
<b>Sulfides</b>			
Dimethyl disulfide (2)	MS, RT (<0.5%)	MS, ir, RT (<0.5%)	MS, ir, RT (<0.5%)
Dimethyl trisulfide (13)	MS, RT (0.7%)	MS, RT (2%)	MS, ir, RT (1%)
2,4,5-Trithiahexane (28)	MS, RT (0.5%)	MS, RT (0.5%)	MS, RT (<0.5%)
<b>Isothiocyanates</b>			
Allyl isothiocyanate (11) (prop-2-enyl isothiocyanate)	MS, RT (10%)		MS, ir, RT (3%)
But-3-enyl isothiocyanate (19a)	MS, RT (30%)		MS, RT (<0.5%)
3-Methylthiopropyl isothiocyanate (44)	MS, RT (7%)		MS, ir, RT (16%)
4-Methylthiobutyl isothiocyanate (48)	MS, RT (3%)	MS, ir, RT (14%)	MS, RT (2%)
2-Phenylethyl isothiocyanate (50)	MS, RT (19%)	MS, ir, RT (3%)	MS, RT (0.7%)
<b>Cyanides</b>			
3-Methylthiopropyl cyanide (35)	MS, RT (1%)		MS, ir, RT (7%)
Phenylacetoneitrile (41)	MS, RT (0.6%)		MS, RT (<0.5%)
4-Methylthiobutyl cyanide (43)		MS, RT (20%)	MS, RT (2%)
2-Phenylethyl cyanide (45)	MS, RT (10%)	MS, ir, RT (13%)	MS, RT (2%)
<b>Aliphatic aldehydes</b>			
Hex-2-enal (6)	MS, RT (<0.5%)	MS, RT (<0.5%)	MS, RT (<0.5%)
Octanal (8)			MS, RT (<0.5%)
Hepta-2,4-dienal, <i>trans,cis</i> - (19a)	MS, RT (<0.5%)	MS, RT (<0.5%)	MS, RT (<0.5%)
Hepta-2,4-dienal, <i>trans,trans</i> - (22)	MS, RT (3%)	MS, ir, RT (3%)	MS, RT (1.4%)
Nonanal (19)		MS, RT (8%)	MS, RT (11%)
Oct-2-enal	MS, RT (<0.5%)		
Deca-2,4-dienal, <i>trans,trans</i> - (40)	MS, RT (1%)	MS, RT (<0.5%)	MS, RT (<0.5%)
<b>Aliphatic alcohols</b>			
Hexanol (14)			MS, RT (1%)
Hex- <i>cis</i> ,3-enol (15)	MS, RT (0.5%)	MS, ir, RT (2%)	MS, RT (4%)
Octanol (26)		MS, RT (1%)	MS, RT (2%)
Nonanol (32)			MS, RT (2%)
<b>Aromatic Compounds</b>			
Benzaldehyde (22)	MS, RT (0.5%)	MS, RT (0.2%)	MS, RT (<0.5%)
Acetophenone (28)			MS, RT (<0.5%)
Phenylacetaldehyde (28a)	MS, RT (1%)	MS, RT (2.5%)	MS, RT (<0.5%)
1,2-Dimethoxybenzene (33)		MS, RT (0.7%)	MS, RT (1%)
<i>p</i> -Methylacetophenone (34)		MS, RT (<0.5%)	MS, RT (1%)
Benzothiazole		MS, RT (<0.5%)	
<i>p</i> -Vinylguaiaicol (49)			MS, RT (1%)
Indole (58)			MS, RT (1%)
<b>Miscellaneous</b>			
2-Pentylfuran (7)	MS, RT (<0.5%)	MS, RT (<0.5%)	MS, RT (<0.5%)
Limonene (6a)		MS, RT (<0.5%)	MS, RT (<0.5%)

<sup>a</sup> Peak numbers corresponding to the peaks in Figure 1 are shown in parentheses immediately after the compound name.

<sup>b</sup> MS, ir, RT = mass spectral, infrared absorption spectral, and GLC retention evidence, respectively. Evidence cited is consistent with that of an authentic sample.

a 12-l. flask with 6 l. of odor free water. The mixture was brought to a boil as rapidly as possible and boiled gently for 5 min. The flask was then cooled rapidly to about 50 °C by immersion in an ice bath. A Likens-Nickerson steam distillation continuous extraction head was then attached to the flask and the volatiles extracted under reduced pressure (80–100 mm, product at 45–50°C) for 3 h using hexane as the extracting solvent. The hexane extract was then dried over sodium sulfate and concentrated in the usual way using low hold up distillation columns.

**Capillary GLC–Mass Spectral Analysis.** This was carried out in a similar manner to that reported previously by the authors (Buttery et al., 1975). The column was a 150 m × 0.75 mm i.d. Pyrex glass capillary column coated with Tween 20 containing 5% Igepal CO-880 and 5% Silanox. Several different runs were made on each vegetable using different temperature programming conditions but the main one used was 70–170 °C at 0.5 °C per min. A silicone membrane molecular separator was used to couple the end of the capillary GLC column to the mass spectrometer which was a modified Consolidated 21-620 cycloidal type.

**Packed Column GLC–Infrared Spectral Analysis.** Samples were separated for ir spectra using a 1 m × 0.64 cm aluminum column packed with 80–100 mesh Chromosorb P coated with 10% Tween 20 (temperature programmed at 50–170 °C). Spectra were measured with

ultramicrocavity cells and a reflecting beam condenser using a Perkin-Elmer Model 237 instrument.

**Odor Thresholds.** These were measured in water solution (on the GLC purified compounds) as previously described (Guadagni et al., 1963) using Teflon bottles and tubing for the odor solutions.

## RESULTS AND DISCUSSION

As the amount and composition of the volatile oil produced from this type of vegetable depended considerably on the time of cooking, etc., standard conditions were used for all three vegetables. These conditions were also chosen to be similar to those normally used for cooking these vegetables in the U.S. The volatiles were then isolated by vacuum steam distillation continuous extraction of the cooled vegetables. Under these conditions all three vegetables gave the same order of concentration of volatile oil at about 2 ppm of the vegetable. Blending of the vegetable beforehand could give as much as ten times this amount of material apparently from enzyme action.

Figure 1 shows the analysis of the volatile oil of cauliflower. Table II lists the compounds characterized for all three vegetables together with the approximate relative percentages found in each oil. There is some variation with different samples and the quantitative figures are only meant to give a general idea of what might be considered

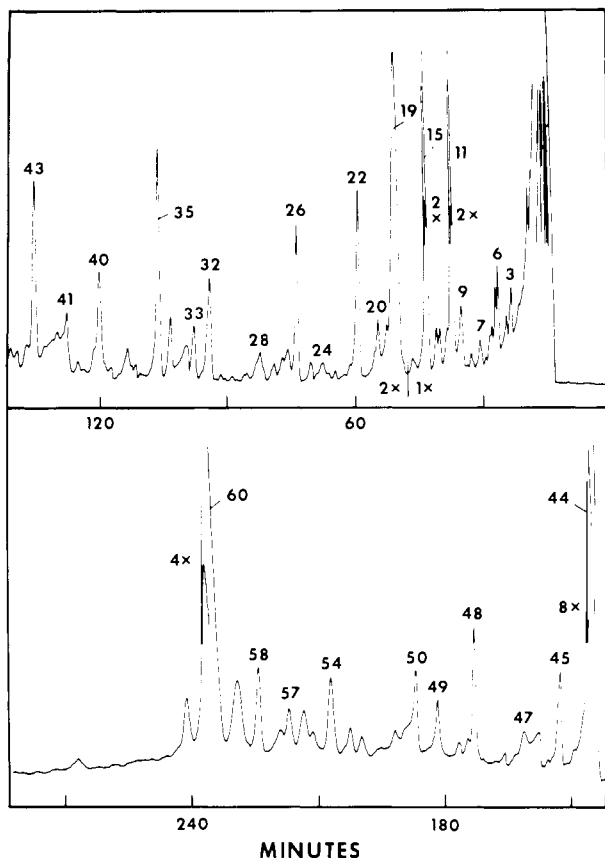


Figure 1. Capillary GLC analysis of the steam volatile oil of cauliflower using a Tween-20 coated, 150 m  $\times$  0.75 mm i.d. Pyrex glass capillary column, temperature programmed from 70 to 170  $^{\circ}$ C at 0.5  $^{\circ}$ C per min.

to be a fairly typical sample.

Notable of the compounds not previously reported in cauliflower and broccoli is nonanal which is a major component of cauliflower volatiles and is also prominent in broccoli volatiles. Octanol was also a major component of cauliflower not previously noted. Other major components not previously reported in any of the three vegetables include 3-methylthiopropyl cyanide, 4-methylthiobutyl cyanide, 2-phenylethyl cyanide, and 2-phenylethyl isothiocyanate. The only cyanide previously reported in any of the three vegetables was allyl cyanide. It was not prominent in any of the volatile oils in the present work and indeed was not detected (we did not, however, make a special effort to detect it). Cyanides can apparently be enzymatically produced from the same plant thioglucosides which lead to isothiocyanates depending on the conditions (pH, etc.) in the vegetable (Schwimmer and Friedman, 1972).

Notable of compounds reported previously by other workers but not found in the present work was butyl isothiocyanate. This compound has the same molecular weight and elemental composition as 3-methylthiopropyl cyanide whose mass spectrum may have been misinterpreted as that of butyl isothiocyanate in the earlier work.

Allyl isothiocyanate is of course a well known constituent of mustard. Each of the isothiocyanates in Table II had been found in one or other of the following materials, horseradish (Gilbert and Nursten, 1972), rutabaga (Lichtenstein et al., 1962), water cress (Gadamer, 1899), and related plants (Kjaer, 1958).

It might be noted that vacuum isolation of the volatile oil from cabbage and cauliflower without prior cooking gave much larger relative amounts of allyl and but-3-enyl

isothiocyanates and somewhat larger amounts of dimethyl trisulfide. In this case the enzyme systems are not deactivated and are acting over the whole time (3 h) of isolation. Isolation of the volatile oil at atmospheric pressure for 3 h tended to give much greater amounts of dimethyl di- and trisulfide as well as related sulfides and also (with cabbage and cauliflower) somewhat greater amounts of allyl and but-3-enyl isothiocyanates. Normal cooking in the U.S. is to avoid overcooking of these vegetables. In the present work (and in the normal cooking of these vegetables) some volatile material is lost during the initial 5-min cooking period.

A very unusual sulfide found in all three vegetables was the compound 2,4,5-trithiahexane ( $\text{CH}_3\text{SCH}_2\text{SSCH}_3$ ). As far as the authors can determine this compound had not previously been reported in natural products. It was synthesized by the reaction between  $\text{CH}_3\text{SNa}$  and  $\text{ClCH}_2\text{SCH}_2\text{Cl}$ . It had been synthesized previously by Block and O'Connor (1973). This compound and dimethyl disulfide and trisulfide were the only simple alkyl sulfides found in the vacuum steam volatile oil. Too volatile to occur in the vacuum steam volatile oil, dimethyl sulfide was also characterized (MS and GLC-RT) by capillary GLC-mass spectrometry of the vapor above cooked cauliflower, broccoli, and cabbage (estimated concentration 5 ppm of cauliflower).

Although all three vegetables have qualitatively generally the same volatile components, there are some distinct and interesting differences. Cabbage is the only one with appreciable amounts of but-3-enyl isothiocyanate. It is also the only one with relatively large amounts of allyl isothiocyanate under the conditions of isolation used in the present paper. We could not detect any allyl or but-3-enyl isothiocyanate in broccoli.

Each of the vegetables seems to have its own characteristic high boiling isothiocyanate. Cabbage steam volatile oil has relatively high concentrations of 2-phenylethyl isothiocyanate and its corresponding cyanide. Broccoli has high concentrations of 4-methylthiobutyl isothiocyanate and corresponding cyanide. Cauliflower has high concentrations of 3-methylthiopropyl isothiocyanate and corresponding cyanide. We found negligible amounts of 3-methylthiopropyl isothiocyanate in broccoli and only a relatively small amount of 4-methylthiobutyl isothiocyanate in cauliflower. Cabbage on the other hand has moderate amounts of the aliphatic isothiocyanates as well as the aromatic. These facts probably follow from the particular thioglucoside composition of each of the three vegetables.

Glass capillary GLC columns and glass insert injectors were used throughout the work reported here. Preliminary work with stainless steel capillary GLC columns indicated that there was considerable loss of the higher boiling isothiocyanates and other sulfur compounds probably due to adsorption on the stainless steel.

The mass spectra of most of the compounds listed in Table II are well enough known except for the following compounds whose mass spectra are listed below (two most intense ions every 14 mass units above  $m/e$  34, intensities in parentheses, molecular ion in boldface type): 3-methylthiopropyl cyanide, 41 (50), 47 (32); 48 (24), 61 (100); 62 (33), 74 (12); 115 (47); 4-methylthiobutyl cyanide, 41 (24), 45 (24); 55 (30), 61 (100); 63 (6), 75 (5); 82 (30), 87 (7); 102 (1), 114 (3); 129 (34); phenylethyl cyanide, 39 (12), 41 (2); 50 (5), 51 (11); 63 (5), 65 (14); 77 (5), 89 (1); 91 (100), 103 (1); 131 (23); 3-methylthiopropyl isothiocyanate, 41 (92), 45 (48); 59 (10), 61 (58); 72 (53), 73 (22); 86 (4), 88 (2); 99 (16), 101 (100); 147 (11), 149 (1); 4-methylthiobutyl

Table III. Odor Thresholds of Major Components in Water Solution

Compound	Odor	
	thresh- old, ppb	95% confi- dence interval
Dimethyl disulfide	12	10-14
Dimethyl trisulfide	0.01	0.008-0.012
Allyl isothiocyanate	375	292-482
(prop-2-enyl isothiocyanate)		
But-2-enyl isothiocyanate	380	320-452
3-Methylthiopropyl isothiocyanate	5	4.1-6.1
4-Methylthiobutyl isothiocyanate	3.4	2.7-4.3
2-Phenylethyl isothiocyanate	6	5-8
3-Methylthiopropyl cyanide	82	68-98
2-Phenylethyl cyanide	15	11-20
Octanal <sup>a</sup>	0.7	
Nonanal <sup>a</sup>	1	
Oct-2-enal <sup>a</sup>	3	
Deca-2,4-dienal <sup>a</sup>	0.07	
Hex-cis,3-enol <sup>a</sup>	70	
Benzaldehyde <sup>a</sup>	350	
Phenylacetaldehyde <sup>a</sup>	4	
2-Pentylfuran <sup>a</sup>	6	
p-Vinylguaiacol <sup>a</sup>	3	
p-Methylacetophenone	19	15-24

<sup>a</sup> Determined previously by the authors in studies with other vegetables.

isothiocyanate, 41 (27), 45 (38); 55 (40), 61 (100); 72 (49), 74 (20); 85 (37), 87 (27); 100 (5), 102 (4); 113 (17), 115 (71); 146 (5); 161 (13); 2-phenylethyl isothiocyanate, 39 (13), 45 (3); 50 (5), 51 (13); 65 (14), 72 (9); 77 (12), 79 (5); 91 (100), 103 (5); 105 (16); 163 (34); 2,4,5-trithiahexane, 45 (36), 46 (14); 59 (1), 61 (100); 63 (3), 64 (2); 76 (2), 79 (5); 93 (2), 94 (4); 107 (0.1), 108 (0.2); 125 (0.2); 140 (9).

#### Aroma Significance of Characterized Components.

Isothiocyanates in mustard and horseradish are known to cause the pungency or "hotness" of these products. In the dilute concentration in which they occur in cabbage, broccoli, and cauliflower (cooked as described in this paper) it is doubtful whether this property is functioning. To get some idea of how these and the other components contribute to the aroma (and hence flavor) of the vegetables, the odor thresholds of many of the compounds in Table II were determined in water solution. These are listed in Table III.

Considering the amounts present and the odor thresholds ( $t$ ) it seems that dimethyl trisulfide ( $t = 0.01$  ppb) is probably one of the most important components to the odor of all three vegetables. Nonanal ( $t = 1$  ppb) occurring in relatively high concentration in cauliflower probably contributes a considerable portion to its aroma. Nonanal's contribution to the aroma of broccoli is probably

also considerable. It seems, however, to be completely absent from cabbage. The high odor thresholds of allyl and but-3-enyl isothiocyanates would indicate that these compounds do not contribute appreciably to the aroma of any of the three vegetables. The other isothiocyanates in Table III, however, have relatively low thresholds and this coupled with their generally reasonable concentration indicates that they probably do contribute to the aroma. The relatively large percentage of 3-methylthiopropyl isothiocyanate ( $t = 5$  ppb) in cauliflower and its insignificant concentration in broccoli together with the reverse situation with 4-methylthiobutyl isothiocyanate ( $t = 3.4$  ppb) may influence the individual aroma character of these two vegetables somewhat. The predominance of 2-phenylethyl isothiocyanate in cabbage is probably an important factor to this vegetable's characteristic aroma.

Of the very volatile components, characterized by previous workers, which do not occur in the oil obtained under vacuum, the large concentration of dimethyl sulfide (ca. 5 ppm of cauliflower,  $t = 0.33$  ppb) must also have a major effect on the aroma of these vegetables.

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