

Table II. Retention Times of Carbonyl Compounds Regenerated from 2,4-DNPS by Exchange with Levulinic Acid

Carbonyl Compound	Retention Time on Column, Min.			
	Diisodecyl Phthalate Column at 100° C.		Carbowax 20M Column at 75° C.	
	Unknown	Known	Unknown	Known
Formaldehyde	1.5	1.5	2.0	2.1
Acetaldehyde	1.8	2.0	3.4	3.4
Propanal	2.6	2.7	5.2	5.1
Acetone	2.8	2.8	5.9	5.9
2-Methylpropanal	3.4	3.4	6.8	6.6
Butanal	4.2	4.3	8.4	8.3
2-Butanone	4.6	4.5	9.7	9.7
3-Methylbutanal	6.3	6.2	11.0	10.8
5-Carbon carbonyls	8.1	8.2	16.0	15.7

beef cooked in water and fat had a sweet, faint caramel odor. Formaldehyde, acetaldehyde, propanal, acetone, 2-methylpropanal, butanal, 2-butanone, and 3-methyl butanal were identified from both fat-cooked and water-cooked beef. Another peak corresponding to the 5-carbon aldehydes and ketones, which could contain pentanal, 2-pentanone, 3-pentanone, or any combination of the three compounds, was observed. As 2,3-butanedione and other known di- and polycarbonyl compounds gave either broad or reverse peaks, they could not be used for identification. Five small peaks were also obtained but, because of the small sample size, tentative identification was not possible. The water-cooked beef yielded more butanal than 2-butanone, whereas the fat-cooked sample usually had more 2-butanone than butanal and was less consistent in the amounts of carbonyls volatilized. Hornstein and Crowe (4) noted similar variation in volatile carbonyl compounds released from heated lamb fat. A typical chromatograph of the carbonyl

compounds from beef cooked in water and fat is shown in Figure 2.

Although beef cooked in fat has a greater yield of volatile carbonyls than water-cooked beef, both cooking procedures produced the same number of aldehydes and ketones in varying proportions. Butanal, 2-butanone, and the 5-carbon carbonyls were found to be the major carbonyl compounds, with smaller amounts of 3-methylbutanal, 2-methylpropanal, acetaldehyde, formaldehyde, acetone, and propanal. The low concentrations of formaldehyde and acetaldehyde, especially the former, were undoubtedly due to losses by volatilization. Sweeping the surface of the cooking meat slurry with air or oxygen instead of nitrogen might have produced a greater yield of carbonyl compounds. However, neither air nor oxygen entrainment, nor even vacuum distillation of flavor volatiles, paralleled so-called "normal" cooking any more closely than nitrogen entrainment. Perhaps the differences in the flavor volatiles produced by beef cooked in water and fat may

arise from the quantity and composition of the aldehydes and ketones released on heating. However, no attempts were made in the present study to relate the differences in the flavor volatiles associated with the two methods of cookery to the different flavors developed.

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FLAVOR COMPOUND

Isolation of S-Methyl Methionine Sulfonium Salt from Fresh Tomatoes

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A sulfonium compound has been isolated from fresh tomato fruit. Infrared spectra, melting points, and paper chromatograms establish that it is a salt of S-methyl methionine sulfonium. The compound decomposes to yield homoserine and dimethyl sulfide. The behavior of the isolated sulfonium salt and its concentration in the tomato suggest it is an important precursor in tomato aroma.

INCREASING RESEARCH in flavors is uncovering organic sulfur compounds that are important volatile flavor components of foods (2, 6, 10). Although these compounds are by nature malodorous in large quantities, trace quantities are often pleasant. The principal flavor component of garlic (*Allium sativum*), for example, has been reported to be allylthio allylsulfinate (4);

those of onions (*Allium cepa*) are methyl and n-propyl thiosulfonates and corresponding disulfides (2). Vegetables of the *Brassica* species (cabbage, etc.) give off varying amounts of methyl sulfide as well as isothiocyanates, which are found also in radishes (8, 23). Small amounts of methyl sulfide are given off by raw tomato fruit and appreciable amounts are produced during cooking or

prolonged heating (12). Indeed, there is strong evidence that methyl sulfide is the principal volatile flavor component of processed tomato products (12).

Sulfur-bearing amino acids and their derivatives have drawn renewed interest, especially as precursors of volatile sulfur components of flavor (19). Allyl cysteine sulfoxide has been shown to be the flavor precursor in garlic (16).

S-Methyl, propyl, and propenyl cysteine sulfoxides (3, 20, 27) are important flavor precursors in onions. These amino acids decompose enzymatically to yield thiosulfonates and ultimately disulfides (75). Others have reported the isolation of *S*-methyl cysteine sulfoxide from turnip and cabbage (73, 77).

This paper reports isolation of an *S*-methyl methionine sulfonium salt from fresh tomatoes. The literature reports the finding of sulfonium compounds in seaweed (6), brackens, and horsetail rushes (7). Recently, Kiribuchi and Yamanishi reported recovery of a methyl methionine sulfonium salt from extracts of green tea and identified it as the precursor of methyl sulfide in green tea flavor (9). Challenger and Haywood isolated a sulfonium salt from asparagus (5), and McRorie *et al.* (11) found it in varying amounts in certain other vegetables. However, an extensive search of the literature shows that such a compound has not been reported before in the tomato.

Method

S-Methyl methionine sulfonium (3-amino-3-carboxypropyl dimethyl sulfonium) ion was isolated from the tomato by a modification of the procedure described by McRorie *et al.* (11). Ten kilograms of fresh ripe tomatoes were disintegrated in a blender with 500 ml. of distilled water per kg. of fruit. The slurry was mixed with diatomaceous earth and filtered with aspiration to give a clear, pale straw-colored extract. This extract was percolated through Amberlite IRC-50 (NH₂) resin columns (56 × 400 mm.) at the rate of 1 liter per hour. The columns were next thoroughly washed with distilled water, and the adsorbed material was eluted with 1*N* acetic acid. The eluate was concentrated in vacuo, without heating, to a white solid. Removal of ammonium acetate at this point did not appear to be critical. Indeed, prolonged holding of the extracted material under the conditions described for sublimation of the ammonium acetate resulted in prohibitive losses of the sought-after sulfonium compound. Minimal volumes of methanol were used to dissolve the extracted solids; the insoluble material was discarded. Isopropanol was added to the methanolic solution 6 to 1. Any precipitate formed after 4 hours at +34° F. was filtered and discarded. The alcoholic solution was either passed through alumina (Alcoa, grade F-20, 80–200 mesh), from which the adsorbed material was eluted with water, or taken directly to dryness in vacuo without heating. In many instances, the use of alumina (17) was not necessary. After either operation, the solids obtained were redissolved in water, and the pH was adjusted to 4.0 with hydrochloric acid. Phosphotungstic acid was added in excess,

and, after being stirred for several hours, the mixture was allowed to precipitate overnight in the refrigerator. The solids were removed, washed, recovered by centrifugation, and then suspended in 50% aqueous acetone. Tetraethyl ammonium bromide at four times the weight of the phosphotungstate precipitate was added to the rapidly stirred suspension. After one hour, the mixture was centrifuged. The supernatant was filtered and concentrated in vacuo to a viscous sirup or to a porous white solid. The methyl methionine sulfonium salt was obtained from ethanol-water solutions of this crude material as the bromide in the form of small, thin platelets or clusters of tiny needles. The volume of sirup was usually about 80 to 85 ml. while the material, if in the solid form, was dissolved by the addition of 50 ml. of hot water. Hot absolute ethanol was slowly added to the first sign of opalescence, and the solution was allowed to cool slowly. The yield was 250 mg. from 10 kg. of fresh tomatoes. Excess tetraethyl ammonium bromide in the crude solids interfered with crystallization of the product and had to be removed by repeatedly concentrating the mother liquors and allowing the unwanted bromide to crystallize out.

The methyl methionine sulfonium bromide was washed with cold absolute ethanol and recrystallized by dissolving it in a minimal volume of hot water followed by addition of absolute ethanol. The product crystallized out of solution after being held at refrigerator temperature overnight or longer. Recovery from 250 mg. of original crystals was 195 mg. or 78%.

Results and Discussion

On a Koeffler-type apparatus, the

product gave m.p. 139–140° d as compared with an authentic sample m.p. also 139–140° d—139° d reported (74). Mixed melting point showed no change. Picrates prepared from the experimental and authentic samples gave identical m.p.'s 158–9° d—158° d reported (5, 6).

The infrared spectrum of the product in a Beckmann IR-5 infrared spectrophotometer (KBr pellet) was identical with that of the authentic material (Figure 1).

This agreement of spectra and the melting point data firmly established that the isolated product is *S*-methyl methionine sulfonium bromide. This identity is further supported by descending paper chromatograms (17, 18) developed with butanol–acetic acid–water (63:10:27), and *sec*-butyl alcohol–*tert*-butyl alcohol–water (2:2:1). Whatman No. 1 and S & S No. 589 Blue Ribbon papers were used and developing times were 18, 24, and 48 hours at 23 ± 0.5° C. Since, in many instances, the solvent had run off the papers at the end of the development period, methionine was the reference standard. All the *R_f*'s were calculated with respect to the migration of this amino acid. Table I shows the relative *R_f*'s.

Aqueous solutions of the isolated methyl methionine sulfonium salt give off dimethyl sulfide with varying ease when heated in a water bath (5, 10). At pH 8 and higher, dimethyl sulfide comes off readily, but longer heating is required when the pH is 6–7. At pH 4, close to the pH of tomatoes, the dimethyl sulfide comes off slowly under prolonged heating. This pattern is very similar to that observed in heated tomato macerates and juice (12).

Samples of filtered tomato juice and solutions of authentic methyl methionine

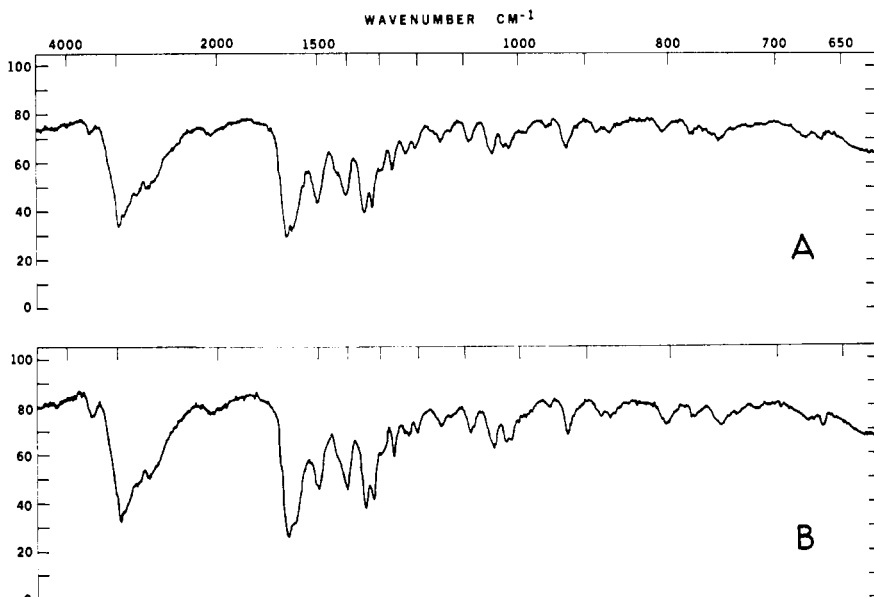


Figure 1. Infrared spectra of isolated and authentic *S*-methyl methionine sulfonium bromide

KBr pellet. A = authentic. B = isolated product

Table I. R_f 's of Isolated Sulfonium Salt and Authentic S-Methyl Methionine Bromide^a

Compound	Butanol-Acetic Acid-Water		Sec-Butyl alcohol- tert-Butyl alcohol-Water	
	Whatman No. 1	S & S 589 Blue Ribbon	Whatman No. 1	S & S 589 Blue Ribbon
Isolated sulfonium salt	0.09	0.10	0.12	0.11
S-Methyl methionine sulfonium bromide	0.09	0.10	0.12	0.11
Homoserine	0.28	0.28	0.47	0.40
Degradation product of isolated sulfonium	0.29	0.28	0.46	0.40

^a R_f 's calculated with respect to methionine.

sulfonium bromide and chloride were compared in an automatic amino acid analyzer (24). Authentic methyl methionine sulfonium chloride was obtained from the California Corporation for Biochemical Research and converted to the bromide using the procedure described for the isolation of the natural occurring salt. In the tomato juice, a prominent peak was found that coincided with the peak of the authentic material. When an aliquot of the tomato sample was heated at length in a water bath, the sulfonium peak disappeared, and an almost equimolar increase in homoserine, a known degradation product of methyl methionine sulfonium, took place (9, 10, 11).

Calculations based on the amino acid analyses indicate the concentration of the methyl methionine sulfonium salts in the tomato to be on the order of 16 to 35 p.p.m. Miers (12) found that exhaustive collection of the volatile materials from tomato preparations

indicate that dimethyl sulfide occurs within this range. These findings show that methyl methionine sulfonium salt plays an important role in the formation of flavor in heated tomato products.

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MUSCAT VOLATILES

Volatiles from Grapes. Muscat of Alexandria

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The volatile constituents found in Muscat of Alexandria have been determined with a capillary gas chromatograph attached to a mass spectrometer. Some 60 components were identified; the most abundant were 1-hexanol, geraniol, and linalool.

INVESTIGATION of fruit volatiles by the combination of capillary GLC and rapid scan mass spectrometry (4) has yielded a tremendous amount of information which, only a few years ago, would have been very difficult if not impossible to obtain. In the present investigation, analysis of the volatile constituents of Muscat of Alexandria (*Vitis vinifera*) has yielded the identity of some 60 components and has revealed the presence of many unknown components.

Muscat of Alexandria has been previously investigated by Cordonnier

(7) and Webb (6). By using liquid/solid adsorption chromatography, Cordonnier identified geraniol, terpineol, limonene, and linalool. Webb, employing distillation, derivative formation, and liquid/solid chromatography, identified 13 compounds along with several C₄ to C₁₂ carboxylic acid esters.

Experimental

Mature muscat grapes (*Vitis vinifera* var. Muscat of Alexandria) were harvested near Fresno, Calif., on the afternoon of October 6, 1964. From these grapes, 1000 gallons of free-run juice

(23° Brix) was obtained on the morning of October 7, 1964. The operation was carried out by a commercial winery with precautions to prevent contamination. All crushers, conveyors, pipes, tanks, etc.; were flushed with water before processing. After the pulp, seeds, skins, and stems had been filtered off, the juice was cooled to 2° C., then pumped into twenty 55-gallon drums lined with polyethylene liners. One pound of USP sodium benzoate was added to each barrel as a preservative. The juice was shipped to Albany, Calif., in a refrigerated van at 0° C., and was stored at the same temperature until stripped.