(28) Sadikov, V. S., Shoskin, A. F., Sterukina, K. M., Livshitz, M. I., *Compt. Rend. Acad. Sci., USSR* **3,** 39 (1934).
(29) Sands, A. E., Grafius, M. A., Wainwright, H. W., Wilson, M. W., "Determination of Low Concentrations of Hydrogen Sulfide in Gas by the Methylene Blue Method," U. S. Dept. Interior, Bureau of Mines, Rept. **4547** (1949).
(30) Schönberg, A., Moubacher, R., *Chem. Revs.* **50,** 261 (1952).
(31) Schönberg, A., Moubacher, R., Mostafa, A., *J. Chem. Soc.* **1948,** p. 176.
(32) Self, R., Rolley, H. L. J., Joyce, A. E., *J. Sci. Food Agr.* **14,** 8 (1963).
(33) Sjöström, L. B., Cairncross, S. E., Caul, J. F., in Monosodium Glutamate Symposium 2, p. 31, 1955.
(34) Speck, J. C., Jr., *Advan. Carbohydrate Chem.* **13,** 79 (1958).
(35) Stahl, C. R., Siggia, *S., Anal. Chem.* **29,** 154 (1957).
(36) Stotz, E., Raborg, J., *J. Biol. Chem.* **150,** 25 (1943).

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TOMATO FLAVOR

Formation of Volatile Sulfur Compounds in Processed Tomato Products

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The rate and amount of hydrogen sulfide and methyl sulfide produced in canned tomatoes and canned tomato juice were determined when these tomato products were stripped at various temperatures and pressures. The hydrogen sulfide was measured by a colorimetric technique involving the formation of methylene blue. The methyl sulfide was absorbed in concentrated sulfuric acid. The acid was then diluted, and the methyl sulfide in the headspace above it was determined by gas liquid chromatography. At 100° C. and atmospheric pressure, 150 p.p.b. hydrogen sulfide and 2 to 6 p.p.m. of methyl sulfide formed in an hour. Commercially canned tomatoes and juices contained 1.6 to 7.9 p.p.m. methyl sulfide. These amounts are far above their aroma thresholds in water, and probably both compounds modify the over-all aroma of processed tomato products.

Papers reporting on tomato volatiles (*3, 14, 20, 27, 28, 31, 32*) do not list any sulfur compounds, but they do show the complexity of the volatile fractions of raw and processed tomatoes. Chromatographic techniques indicate that there are at least three dozen compounds, and possibly several dozen more remain undetected. At least two dozen compounds including alcohols, aldehydes, ketones, esters, and several terpene-type compounds have been identified (*3, 14, 20, 27, 28, 31, 32*).

Sulfur compounds, although not yet shown in tomato products, have been reported in the volatiles of many other processed foods including vegetables (*7, 10, 13, 30*), citrus fruits (*17*), milk (*11*), eggs (*18*), chickens (*4, 25*), salmon (*1*), beef (*30*), coffee (*30*), tea (*30*), wine (*36*), and beer (*5*). Most of the vegetables (*30*) contained four or more volatile sulfur compounds. Potatoes may contain 10 such compounds, and onion volatiles showed eight sulfur compounds (*7, 13*).

The human nose can detect some of these sulfur compounds at extremely low concentrations. For example, the olfactory thresholds of methyl mercaptan and methyl sulfide in water are 0.02 and 0.33 p.p.b., respectively (*12*). Because of these very low thresholds, such volatile sulfur compounds appear to be important components of food aromas. For instance, one panel detected 5 p.p.b. of added hydrogen sulfide in the aroma of beer (*5*), and another panel detected 125 p.p.b. of the same compound added to chicken broth and believed that it intensified the broth aroma (*22*).

The strong odor of organic sulfides was detected in the cold traps (−78° and −196° C.) used to trap stripped volatiles from canned tomato juice. Gas chromatographic analysis showed that the trap odor might be caused by 3 to 4 p.p.m. methyl sulfide in the tomato juice. This concentration of methyl sulfide would be ten thousand times its odor threshold in water (*12*) and, therefore, probably quite important in the aroma of tomato products. This paper reports which volatile sulfur compounds and how much were present in some tomato products.

Experimental Procedure

Canned Tomatoes and Tomato Juice. The canned juice or tomatoes, listed by variety name in the tables and figures, were prepared at this laboratory. Ripe tomatoes for juice were carefully washed, trimmed, and put through a pulper with 0.033-inch screen. This cold break juice was deaerated and sealed in 202 × 204 cans under 27 inches of Hg vacuum. The cans were processed from zero time to 80 minutes in boiling water and immediately cooled in ice water.

Four varieties of tomatoes were canned. Whole tomatoes were heated in boiling water for 2 minutes, cooled in tap water, peeled, cored, and quartered. After mixing, the quartered tomatoes were canned in juice from the same tomatoes, vacuum sealed in 303 × 406 cans, processed for periods from zero time to 90 minutes in boiling water, and cooled in ice water. The headspace from canned juice and tomato samples was analyzed by GLC and some samples, processed 60 minutes, were used to determine the rate and amount of hydrogen sulfide and methyl sulfide production when stripped at boiling temperatures ranging from 30° to 100° C. under pressures of 30 to 760 mm. of Hg (Figures 2, 3, and 4 and Table I).

The canned tomato products analyzed for Table II were from retail markets or from processors.

Stripping Apparatus. The apparatus shown in Figure 1, with modifications as required, was used to strip the volatiles from tomato juice or canned tomatoes. Stripping was accomplished by boiling at pressures ranging from 30 to 760 mm. of Hg, with nitrogen bled through the tomato product at 60 to

Table I. Stripping Conditions and Amounts of Hydrogen Sulfide and Methyl Sulfide from Can Headspace, and Stripping of Tomato Products

Expt. No.	Sample	Stripping Temp., ° C.	Stripping Time, Hours	H_2S[a], P.P.B.	Me_2S[b] before Stripping, P.P.M.	Me_2S[c] Recovered, P.P.M.	Me_2S Recovered, %
1	Commercial juice	40	4.5	0	...	2.4	...
		90	7	46	...	2.6	...
2	VF-36[d] canned tomatoes[e]	40	6	0	...	...	...
		85	6	78	...	2.4	...
3	Raw VF-36[d] tomatoes	30	3.5	0	...	0.0	...
		100	13	720	...	5.9	...
4	CPCT2[d] canned tomatoes[e]	35	4	...	5.8	0.3	103
		97	8	630		5.7	
5	A 1[d] canned tomatoes[e]	50	5.5	...	1.7	1.6	95
6	A 1[d] canned tomatoes[e]	38	6	...	1.7	1.6	95
7	VF-36[d] canned tomatoes[e]	45	6	...	1.6	1.4	88
8	C mmercial juice	100	6	...	15.7	15.3	97

[a] Measured by methylene blue method.
[b] Measured by GLC of the headspace.
[c] Trapped in concentrated sulfuric acid.
[d] The letter-+-number code name varieties.
[e] Processed 60 minutes in boiling water.

Table II. Methyl Sulfide in Commercial Tomato Products

Type of Product	No. of Samples	Range Me_2S, P.P.M.	Av. of Me_2S, P.P.M.	Me_2S at 5.7% Tomato Solids
Tomatoes	6	1.8–7.9	3.9	3.6
Juice	10	1.6–6.3	3.8	4.1
Puree	5	0.4–8.1	3.1	1.3
Paste	7	3.6–10.9	6.7	1.5

300 ml. per minute depending on pressure. The 30-inch inner-and-outer condenser was cooled with ice water during refluxing. Very little moisture passed over with the vapors to dilute the absorbing solids or solutions in the traps.

Gas Chromatographic Apparatus. A dual-column hydrogen flame ionization chromatograph as reported by McWilliam and Dewar (*21*) was used. The component parts were made and assembled at this laboratory. The columns were 10-foot × 0.105-inch i.d. stainless steel packed with 20% Apiezon M on 60- to 80-mesh Chromosorb P. Nitrogen at 30 ml. per minute was the carrier gas. Hydrogen at 18 ml. per minute and air at 600 ml. per minute were used in the detectors. The injection block was at 130° C., the columns were at 100° C., and the dual flame-detector chamber was at 125° C. A 1-mv. recorder was used to record the detector response. This gas chromatograph was used to determine methyl sulfide in various traps in the stripping experiments, in headspace above canned tomato products (*6*), and in and above different samples pertinent to the analysis for methyl sulfide. Three replicate samples of 50 to 250 μl. of headspace vapors were injected with gas-tight syringes.

Collection of Volatile Sulfur Compounds. Types of volatile sulfur compounds in tomato juice were determined by passing the effluent nitrogen and volatiles from the refluxing tomato juice or macerate through a train of traps containing: anhydrous calcium chloride, lead acetate (solid), 4% mercuric cyanide solution, 3 or 6.5% mercuric chloride solution, and sometimes concentrated sulfuric acid. These absorbents separate hydrogen sulfide, mercaptans, and organic mono- and disulfides from each other (*10*). When tomato juice or macerate was refluxed at atmospheric pressure, the lead acetate soon darkened owing to formation of lead sulfide from hydrogen sulfide. The mercuric cyanide solution remained clear with no precipitate forming even after several hours' stripping. This indicated little if any volatile mercaptans. On the other hand, a considerable amount of white precipitate formed in the mercuric chloride solution, showing the presence of organic sulfide in the volatiles. The white precipitate was filtered from the mercuric chloride solution, rinsed, and filtered three times with fresh mercuric chloride solution, and dried. The melting point was compared with that of authentic methyl sulfide–mercuric chloride complex and the mixed melting point of the two. All melted between 156.5° and 157.5° C. Literature values are 157° and 158° C. (*9*). Small samples of the unknown precipitate were dissolved in 3*N* H_2SO_4 and in 3*N* NaOH. GLC analysis of the headspace above these solutions showed only methyl sulfide.

For more sensitive determination of hydrogen sulfide in tomato products, a colorimetric procedure used by Brenner, Owades, and Golyzniak (*5*) was adopted. The hydrogen sulfide was absorbed in 2% zinc acetate. The zinc sulfide was treated with *p*-aminodimethyl aniline and ferric chloride. This reaction produces methylene blue. It is very specific because sulfide ion must be present to cause the reaction and to become part of the methylene blue. The absorbance of the solution was read at 745 mμ in a spectrophotometer. The amount of sulfide present in the solution was determined from a calibration curve giving the absorbance of methylene blue from known amounts of sulfide. This procedure is sensitive to less than 0.1 μg. of sulfide per ml. of absorbing solution.

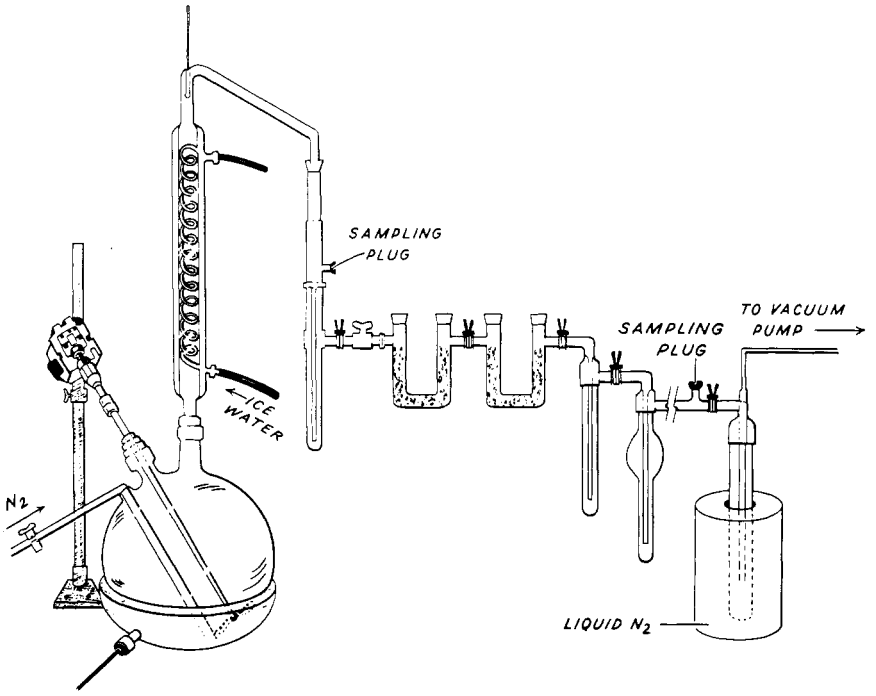

Figure 1. Stripping assembly

The methyl sulfide–mercuric chloride coordinate complex easily identified methyl sulfide as the only organic sulfide present. However, this complex could not be used to measure the methyl sulfide quantitatively because mercuric chloride solutions do not absorb methyl sulfide completely. Methyl sulfide can always be smelled above the dried or aqueous dispersions of the complex. Therefore, the absorbing action of concentrated sulfuric acid was tested (*15, 26*).

Concentrated sulfuric acid frequently contains volatile impurities. These volatile impurities in the concentrated sulfuric acid were removed by bleeding nitrogen gas through the acid for several hours. Redistilled commercial methyl sulfide (1 ml.) was added at 0° C. to 99 ml. of concentrated sulfuric acid at 0° C., and the two were thoroughly mixed. The resulting solution was colorless and odorless, and the headspace above it contained no methyl sulfide detectable by the gas chromatograph. When this concentrated acid solution was diluted to 0.02% acid (2 p.p.m. of methyl sulfide), it possessed a strong methyl sulfide odor, and the peak from the vapor of this diluted acid solution was equivalent to that of the same amount of methyl sulfide in boiled distilled water.

After the above tests, volatiles stripped from tomato juice or macerate were absorbed in degassed concentrated sulfuric acid. Five-milliliter aliquots of this acid were pipetted into 95 ml. of ice-cold, boiled distilled water in a 125-ml. Erlenmeyer flask which was then stoppered with a serum cap. The flask was warmed to 25° C. Three replicate samples of 50 to 250 μl. of headspace gas were analyzed with the gas chromatograph. The response to the methyl sulfide in this diluted sulfuric acid was compared with the response to headspace vapors above known amounts of methyl sulfide in 5% sulfuric acid. Results are expressed as parts per million calculated on a volume basis.

The methyl sulfide in canned tomato products was also determined by headspace analysis at 25° C. A 1/4-inch hole was punched near the edge of the lid, and the hole plugged immediately with a small serum cap. Fifty to 250 μl. of the headspace gas were withdrawn through the cap and analyzed with the gas chromatograph. The response was compared with the response to headspace above 100 ml. of standard solutions of methyl sulfide in water. Corrections were made because of the increase in the vapor pressure and GLC response to methyl sulfide when dissolved in a tomato product instead of water. Thus, the GLC response to methyl sulfide in a tomato product relative to the response for the same amount of methyl sulfide in water is 1.08 for a juice of 6% tomato solids, 1.21 for a puree of 12% solids, and 1.85 for a paste of 25% solids.

Results and Discussion

Hydrogen Sulfide. Stripping raw or processed juice or tomatoes for several hours at 100° C. produced 600 to 700 p.p.b. hydrogen sulfide (Table I). On the other hand, when the same samples were first stripped for several hours at 30° to 40° C. no hydrogen sulfide evolved. If the stripping temperature was 85° or 90° C., a slight amount, less than 100 p.p.b., of hydrogen sulfide came off during 6 or 7 hours of stripping.

The rates and amounts of hydrogen sulfide recovery at 30° and 100° C. are shown in Figure 2. These curves show that the rate is zero at 30° C.; the rate is quite high during the first 4 hours at 100° C., but then falls off sharply, and probably only a small part of the hydrogen sulfide precursor changes to hydrogen sulfide during ordinary processing of tomato products. Further heating converted some of the remaining precursor to hydrogen sulfide in experiments 1, 2, and 4 of Table I.

No hydrogen sulfide was found in canned juice or tomatoes unless the stripping temperature was 85° C. or more. The disappearance of hydrogen sulfide produced in the processing of canned tomato products would be expected since it reacts with the tin and iron (*2, 35*).

Since tomatoes contain about 100 p.p.m. total sulfur (*16, 19*), it appears from experiments 3 and 4 of Table I that less than 1% of the total sulfur can be obtained as hydrogen sulfide. This limitation indicates that the precursor for hydrogen sulfide is probably present in a small amount compared with the total sulfur. However, other competitive reactions may also be using the same precursor.

Methyl Sulfide. Tomato products contain much more methyl sulfide (0.4 to 10.9 p.p.m.) than hydrogen sulfide (Tables I and II). These amounts range from 1200 to 33,000 times the odor threshold of methyl sulfide in water (*12*). One can hardly avoid the conclusion that this compound must be important in the aroma of tomato products. Research showing this importance will be reported elsewhere.

When concentrated sulfuric acid proved a quantitative absorber of methyl sulfide, some strippings were made to see if the amount of methyl sulfide in the tomato product as determined by headspace analysis agreed with the amount recovered by stripping the product and absorbing the methyl sulfide in concentrated sulfuric acid. In experiments 5, 6, and 7 of Table I, methyl sulfide in the can headspace of the tomato products was determined before stripping; then the methyl sulfide already present was stripped off at a low temperature, absorbed in concentrated sulfuric acid, and determined. In experiments 4 and 8, cans from the same samples that were stripped were heated in boiling water for 8 hours. The methyl sulfide was then determined from the headspace at 25° C. in these cans and is listed in the third column from the right in Table I. The unheated but normally processed cans were stripped at 100° C.; the methyl sulfide was absorbed in the concentrated sulfuric acid and determined. This amount is listed in the second column from the right in Table I. The methyl

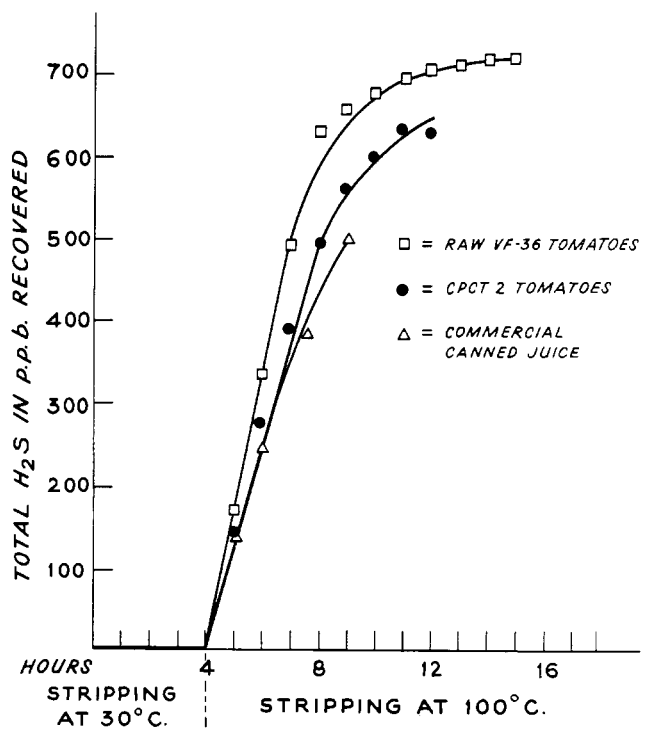

Figure 2. Hydrogen sulfide recovery during stripping

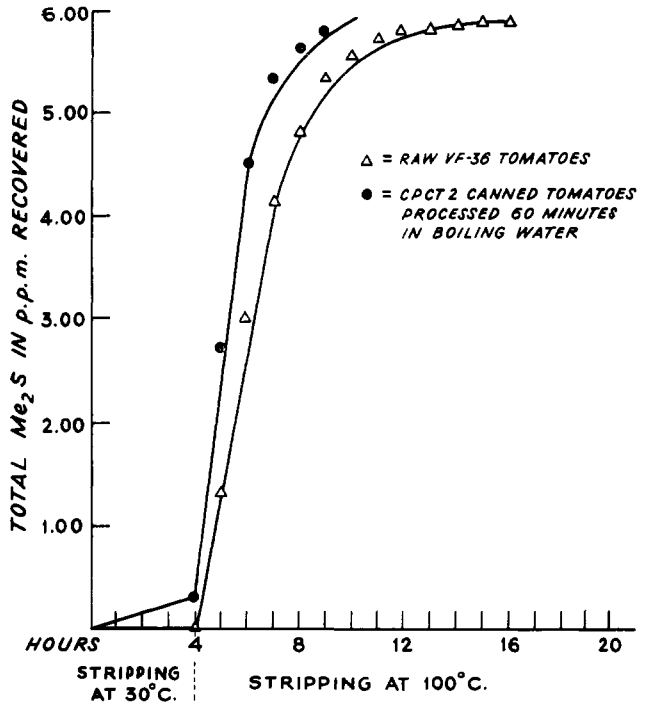

Figure 3. Methyl sulfide recovery during stripping

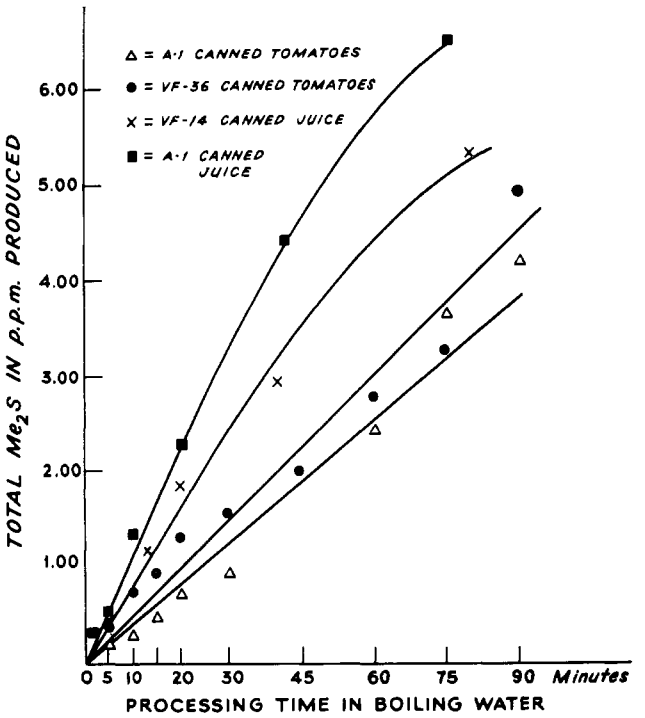

Figure 4. Methyl sulfide formation during processing of canned juice and canned tomatoes

sulfide recovered from the sulfuric acid was 88 to 103% of the amount indicated by the original headspace analysis. This recovery is quite good since the stripped products still contained 3 to 5% of the original methyl sulfide according to GLC analysis of the headspace above the tomato product before and after stripping.

Figure 3 shows the rate at which methyl sulfide was recovered in stripping the samples first at 30° and then at 100° C. It is recovered quite fast for 4 hours at 100° C., but hardly at all in the next 4 hours. Also, the curves show that the total methyl sulfide precursor is much greater than what is expended in the normal processing of tomato products. No methyl sulfide was in the raw tomatoes before heating. The headspace above several other samples of raw tomatoes never showed a peak for methyl sulfide although an extremely small peak for methyl sulfide could have been hidden under the preceding peak in the chromatograms. On the other hand, the methyl sulfide peak in the chromatograms of the headspace above canned tomato products indicates that several times more methyl sulfide is present than any other single component in the headspace.

Figure 4 shows the methyl sulfide content determined by headspace analysis after laboratory canned tomatoes and juices were processed in boiling water for varying times. The spread of the points for these samples indicates considerable variability, about threefold, in the methyl sulfide content at any one processing time. An even greater variability in methyl sulfide content was found in four different types of commercially canned tomato products listed in Table II. The average methyl sulfide content for each type is shown in the third column. The figures in the last column show the average methyl sulfide content recalculated for a standard product containing 5.7% tomato solids for each type of product. These canned tomatoes and juices contained about three times as much methyl sulfide per unit of tomato solids as the purees and pastes contained. Such results were expected since the methyl sulfide would evaporate in processing the purees and pastes, but would not evaporate in the processing of canned tomatoes and juice.

As pointed out previously, hydrogen sulfide produced in processing of tomato products disappears in the cans (*2*, *35*), but no evidence that methyl sulfide disappears in cans could be found. On the contrary, Table III indicates a significant increase of methyl sulfide during storage at 21° and 32° C. for 2 years. Such increases at these relatively low temperatures show that the precursor is not very stable, and this increase may explain in part the changes in flavor that tomato products undergo when stored at such temperatures (*24*).

Possible Precursors. Although it is now known that many food products produce one or more volatile sulfur compounds during processing, the specific precursors of these volatiles are unknown in many cases. However, enough work has been done with several food products to conclude that direct or indirect precursors in many cases are probably sulfur-amino acids and their derivatives

Table III. Increase in Methyl Sulfide during Storage of Commercially Canned Tomato Juice for Two Years

Storage Temp., ° C.	Me_2S[a] in Tomato Juice, P.P.M.	Increase per Month, %	Σ Increase, %
1 [Control]	5.3	...	...
21	7.5	1.8	42
32	10.6	4.2	100

[a] Determined by headspace analysis.

(*29*). Thus alliin, an unsaturated sulfoxide of L-cysteine, is the precursor of diallyldisulfide in garlic (*33*), (+) *S*-methyl-L-cysteine sulfoxide is the precursor of volatile sulfur compounds in cabbage (*34*), and the same sulfoxide and (+) *S*-*n*-propyl-L-cysteine sulfoxide are precursors of volatile sulfur compounds from onions (*8*).

Free amino acid determinations on tomato paste serum indicated the presence of several sulfur-amino acids such as cysteine, methionine, *S*-methyl cysteine sulfoxide or sulfone, methionine sulfone, and lanthionine (*23*). Methionine and *S*-methyl cysteine might give rise to dimethyl sulfonium type salts from which methyl sulfide could be produced by heat. Additional work on the identity of the precursors of the volatile sulfur compounds in tomato products will be reported.

Literature Cited

(1) Almy, L. H., *J. Am. Chem. Soc.* **47**, 1381 (1925).
(2) American Can Co., "The Canned Food Reference Manual," 3rd ed., p.

284, American Can Co., New York, N. Y.
(3) Bidmead, D. S., Welti, D., *Research* **13,** 295 (1960).
(4) Bouthilet, R. J., *Food Res.* **16,** 137 (1951).
(5) Brenner, M. W., Owades, J. L., Golyzniak, R., *Am. Soc. Brewing Chemists Proc.* **1953,** p. 83.
(6) Buttery, R. G., Teranishi, R., *Anal. Chem.* **33,** 1439 (1961).
(7) Carson, J. F., Wong, F. F., J. AGR. FOOD CHEM. **9,** 140 (1961).
(8) Carson, J. F., *J. Org. Chem.* **26,** 4997 (1961).
(9) Challenger, F., Charlton, P. T., *J. Chem. Soc.* **1947,** p. 424.
(10) Dateo, G. P., Clapp, R. C., MacKay, D. A. M., Hewitt, E. J., Hasselstrom, T., *Food Res.* **22,** 440 (1957).
(11) Gould, I. A., Sommer, H. H., Mich. Agr. Expt. Sta. Tech. Bull. **164** (1939).
(12) Guadagni, D. G., Buttery, R. G., Okano, S., *J. Sci. Food Agr.* **14,** 761 (1963).
(13) Gumbmann, M. R., Burr, H. K., J. AGR. FOOD CHEM. **12,** 404 (1964).
(14) Hein, R. E., Fuller, G. W., "Conference on Advances in Flavor Research," Southern Utilization Research and Development Division, United States Department of Agriculture, New Orleans, La., 1963.
(15) Henze, M., *Z. Physiol. Chem.* **41,** 109 (1904).
(16) Hummel, F. C., Shepherd, M. L., Galbraith, H., Williams, H. H., Macy, I. G., *J. Nutrition* **24,** 41 (1942).
(17) Kirchener, J. G., Rice, R. G., Miller, J. M., Keller, G. J., *Arch. Biochem.* **25,** 231 (1950).
(18) Lowe, B., "Experimental Cookery," 4th ed., p. 343, Wiley, New York, 1955.
(19) Masters, M., McCance, R. A., *Biochem. J.* **33,** 1304 (1939).
(20) Matthews, R. F., "Gas and Paper Chromatography of Volatile Flavor Constituents of Several Vegetables," Ph.D. thesis, Cornell University, 1960; *Dissert. Abstr.* **21,** 1693 (1961).
(21) McWilliam, I. G., Dewar, R. A., "Gas Chromatography 1958," D. H. Desty, Ed., p. 142, Academic Press, New York, N. Y., 1958.
(22) Lineweaver, H., Pippen, E. L., "Chicken Flavor," Proceedings of a Symposium on Flavor Chemistry, p. 21–36, Campbell Soup Co., Camden, N. J., 1961.
(23) Nutting, M.-D., U.S. Department of Agriculture, Albany, Calif., private communication, 1965.
(24) Nutting, M.-D., Harris, J. G., Feustel, I. C., Olcott, H. S., *Food Technol.* **9,** 466 (1955).
(25) Pippen, E. L., Eyring, E. J., *Ibid.*, **11,** 53 (1957).
(26) Pohl, J., *Arch. Exptl. Pathol. Pharmkol.* **51,** 341 (1904).
(27) Pyne, A. W., Wick, E. L., *J. Food Sci.* **30,** 192 (1965).
(28) Rakitin, Y. V., *Biokhimiya (Moscow)* **10,** 373 (1945).
(29) Robinson, T., "The Organic Constituents of Higher Plants," p. 278, Burgess Publishing Co., Minneapolis, Minn., 1963.
(30) Self, R., Casey, J. C., Swain, T., *Chem. Ind.* **1963,** p. 863.
(31) Schormuller, J., Grosch, W., *Z. Lebensmitt. Untersuch.* **118** (5), 385 (1962); 126 (1), 38 (1964); 126 (3), 188 (1965).
(32) Spencer, M. S., Stanley, W. L., J. AGR. FOOD CHEM. **2,** 1113 (1954).
(33) Stoll, A., Seebeck, E., *Helv. Chim. Acta* **31,** 189 (1948).
(34) Synge, R. L. M., Wood, J. C., *Biochem. J.* **64,** 252 (1956).
(35) Thompson, M. H., *Food Technol.* **17,** 665 (1963).
(36) Thoukis, G., Stern, L. A., *Am. J. Enol. Viticult.* **13,** 133 (1962).

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FORAGE CARBOHYDRATES

Some Nonstructural Carbohydrates in Forage Legume Herbage

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The influence of stage of maturity on the content of some carbohydrate constituents in the herbage of alfalfa, red clover, Ladino clover, and birdsfoot trefoil was studied at Madison, Wis. Samples were taken at six stages of maturity and freeze-dried for subsequent analysis. Percentages of takadiastase enzyme extractable and 2% H_2SO_4 hydrolyzable carbohydrates generally were highest at early stages of maturity and tended to be higher in red clover and Ladino clover than in alfalfa and trefoil. The interrelationships of glucose, fructose, and sucrose were somewhat different in each of the four legumes.

IDENTIFICATION and quantitative determination of the major carbohydrate constituents of most forage legumes have been accomplished. General reviews include those of Hansen *et al.* (*3*) and Sullivan and Garber (*12*). Detailed studies have been made of alfalfa herbage by Hirst, Mackenzie, and Wylam (*5*) and Nalewaja and Smith (*8*), of red clover by Bailey (*1*), and of Ladino clover by Wilkins *et al.* (*14*). Little information is available regarding the carbohydrate composition of birdsfoot trefoil.

Results obtained by different investigators for the same species rarely are directly comparable because of seasonal and varietal differences, differences in geographic location, and variations in analytical methods used. To be most meaningful, comparisons of carbohydrate content in relation to species and stage of maturity should be made within a single study. Few studies have been found, however, where the carbohydrate contents at successive maturity stages of two or more forage legume species have been compared under the same experimental conditions. This study was conducted to provide such comparative information.

Materials and Methods

Alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), Ladino clover (*Trifolium repens* L.), and birdsfoot tre-

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