Quantitative and Sensory Studies on Tomato Paste Volatiles

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The concentrations of 44 of the major aroma components of tomato paste were determined. Comparison with studies for fresh tomatoes showed marked decreases in concentration for some compounds, particularly aliphatic aldehydes ((Z)-3-hexenal and hexanal >400 times lower) in the paste. Other compounds, however, such as the potent odorant β-damascenone (also 6-methyl-5-hepten-2-one, other ketones, linalool, and α-terpeneol) show severalfold increases in concentration in the paste. The observation by other workers of the formation of relatively large (0.5–10 ppm) concentrations of dimethyl sulfide in processed tomato was confirmed. Considerations of the odor thresholds of components and their determined concentrations indicated that the compounds most important to tomato paste aroma include dimethyl sulfide, β-damascenone, β-ionone, 3-methylbutanal, 1-nitro-2-phenylethane, eugenol, methional, 3-methylbutyric acid, 6-methyl-5-hepten-2-one, phenylacetaldehyde, and linalool.

A major portion of the tomato crop in California is converted to tomato paste. This forms a relatively stable concentrated form of processed tomato that can be used in the preparation of a number of tomato products. The volatiles of fresh tomatoes have been extensively studied [see the review by Petro-Turza (1986–87)]. Some specific studies on processed tomatoes had previously been carried out by Miers (1966), Guadagni and Miers (1969), Wobben et al. (1974), Chung et al. (1983), Kazeniac and Hall (1970), Buttery et al. (1971), and Sieso and Crouzet (1977). Strong evidence of the importance of dimethyl sulfide to tomato juice aroma had been established by Guadagni and Miers (1969).

We have completed some recent studies on quantitative and qualitative analyses of fresh tomato volatiles (Buttery et al., 1988a,b, 1989) and have extended these in the present work to processed tomato paste.

EXPERIMENTAL SECTION

Materials. Tomato paste samples were obtained from three different processors located in California. Samples were also obtained from local retail markets. These pastes showed 28–30% total solids content. The exact lines of tomato used for the commercial manufacture of the paste are not known but were probably largely FM785, GS-12, or related Californian lines. Authentic reference chemicals were obtained from reliable commercial sources or synthesized by well-established methods. They were purified by gas–liquid chromatographic (GLC) separation and their identities verified by spectral (mass or infrared) means.

Isolation of Volatiles from Tomato Paste Samples. *1. General Method.* The method used for tomato paste is similar to that previously used by us for fresh tomato (Buttery et al., 1988b). Tomato paste (100 g) was mixed thoroughly with water (200 mL) in a 1-L beaker and the mixture poured into a 1-L round-bottom flask containing an efficient magnetic stirrer. A volume (5 mL) of a stock solution of internal standards (containing 20.0 ppm 3-pentanone, 20.0 ppm 2-octanone, and 5.0 ppm anethole) in water was then added. A suitable Pyrex glass head was attached to the flask, which allowed purified air (3 L/min flow rate) to enter the flask and pass over the vigorously stirred mixture and out through a Tenax trap (Tenax-GC, 60–80 mesh, 10 g, 14-cm length × 2.2-cm i.d.). The isolation was carried out for 60 min at 25 °C and the trap then removed and eluted with 100 mL of freshly distilled diethyl ether (containing ca. 0.001% Ethyl antioxidant 330). The ether extract was then concentrated to ca. 20 μL with a warm water bath and Vigreux distillation column. The Tenax trap was regenerated by passing a stream of nitrogen through it at 200 °C for 1 h.

2. Modified Method for Free Acids. The procedure was the same as that above except that 25 mL of 2.5 N HCl was added just before the isolation to bring the mixture to ca. pH 2. The isolation was continued for 24 h instead of 1 h.

Determination of Relative Recovery–GLC Response Factors. Overall combined recovery–GLC response factors relative to the internal standards were determined in the following way. Stock solutions of purified compounds were made by dissolving the compound in water (usually at the 10.0 ppm level). Measured portions of the stock solutions (stored at 2 °C) were added to 300 mL of water together with a volume (usually 5 mL) of the internal standard solution. The isolation of volatiles was then carried out on the Tenax trap system as for the tomato paste.

Capillary GLC Analysis. The main study was carried out with a commercially obtained 60 m × 0.25 mm fused silica capillary GLC column wall coated with a bonded methyl silicone, DB-1. The gas chromatography oven programming conditions were 25 min at 30 °C, increasing at 4 °C/min until 200 °C and finally 5 min at this upper limit. The injector temperature was 170 °C, and the carrier gas (He) flow velocity was 22 cm/s. Sample size was 1 μL split 1/20.

For analysis of free acids (which did not resolve well on the DB1 column) a DBwax wall coated fused silica capillary was used, which was otherwise similar in dimensions to the DB1 column.

Quantitative Analysis of Dimethyl Sulfide. Tomato paste (50 g) was mixed with water (140 mL) and the mixture stirred thoroughly until uniform. This mixture was then placed into a 250-mL wide-neck flask containing a ground joint side arm where a thermometer was inserted. An internal standard (10 mL) of a 10.0 ppm solution of benzene in water was then added. The flask neck was covered with aluminum foil and the mixture warmed to 40 °C by heating on a steam bath with continuous swirling of the flask. When the mixture reached 40 °C (0.5 °C), a glass syringe was pushed through the aluminum foil and a 5-mL vapor sample obtained and injected directly into the GLC column (splitless injector). The column was a laboratory-constructed 150-m length × 0.66-mm i.d. Pyrex glass capillary wall coated with Silicone OV-3. The column was held at 30 °C with an inlet pressure of 10 psi of He. The system was calibrated by using known concentrations of dimethyl sulfide in water and carrying through the isolation process as described above.

Capillary GLC–MS Analysis. This was carried out as previously described with use of a modified Consolidated 21-620 cycloidal type mass spectrometer.

Odor Threshold Determinations. These were carried out (with samples purified by gas chromatographic separation) with

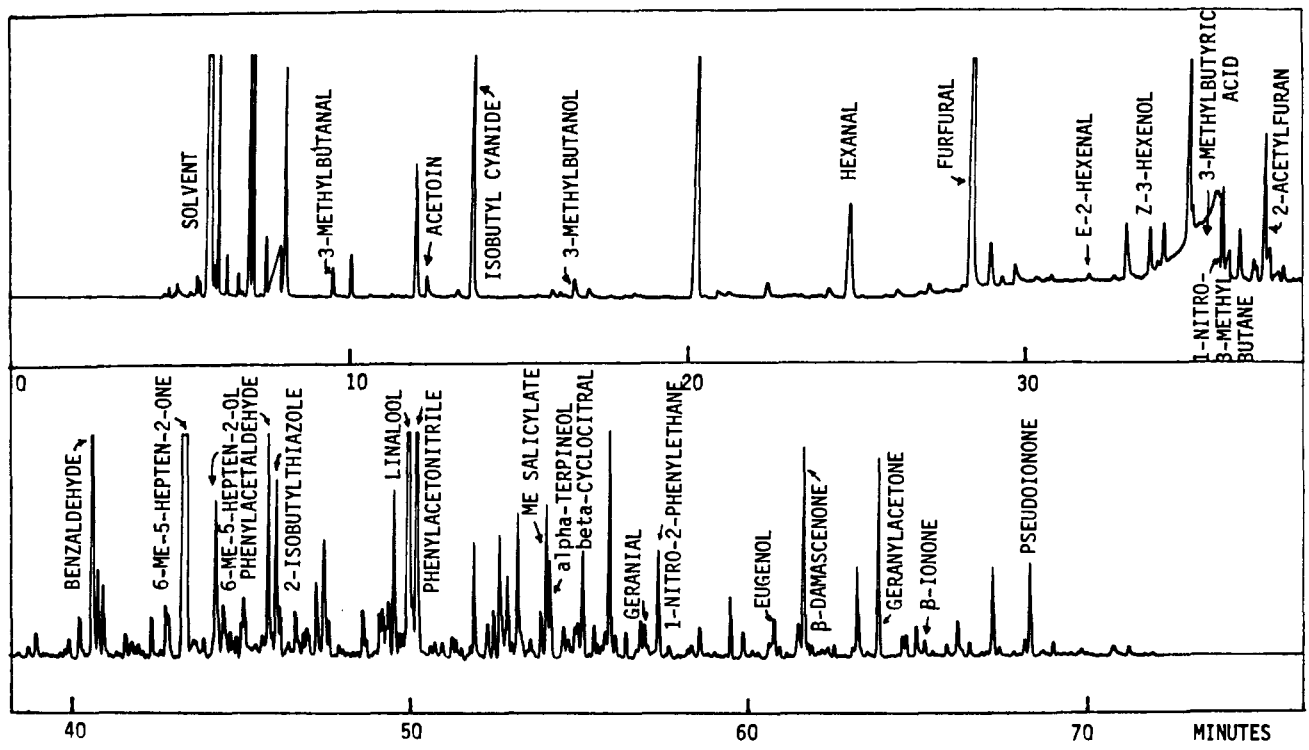

Figure 1. Capillary GLC analysis of the Tenax-trapped volatile concentrate from tomato paste. GLC conditions are listed in the Experimental Section.

methods previously described (Buttery et al., 1971) with a panel of 16–20 judges.

RESULTS AND DISCUSSION

The volatiles were isolated from tomato paste (diluted with 2× its volume of water) following closely the procedure previously described by the authors for fresh tomato volatiles using Tenax trapping (large 10-g Tenax trap) and high sweep gas flow rates (3 L/min air). The method used for tomato paste differed in that no saturated $CaCl_2$ solution (used with fresh tomatoes to deactivate enzymes) was used with the paste because the enzyme systems were already inactivated by the temperatures of the paste process.

Qualitative studies were carried out by the usual method of capillary GLC–mass spectrometry. These studies confirmed the presence of previously identified compounds (Petra-Turza, 1986–87) including β-damascenone and 1-nitro-2-phenylethane also recently identified in fresh tomatoes (Buttery et al., 1988a, 1989). Figure 1 shows a capillary GLC analysis of tomato paste volatiles isolated by the Tenax trap procedure described in the Experimental Section.

Quantitative analyses using internal standards were carried out and results are listed in Table I. For comparison, an analysis of the volatiles from a processing line (FM785) of fresh tomatoes previously obtained (Buttery et al., 1988b) is also listed. The figures are averages from at least three separate isolations from different cans of the same brand of paste. They are also reasonably representative of a large number of analyses carried out on many different samples of fresh tomato and paste. Some idea of the range of concentrations found with different paste samples is also given in parentheses. It can be seen from Table I that many of the compounds present in the fresh tomatoes are greatly decreased in concentration by the manufacture of the paste.

Tomato paste is usually made in California processing plants by running the macerated tomato down the walls of three consecutive towers (so called first, second, and third effect towers) with heating and reduced pressure. The first effect tower is close to atmospheric pressure whereas the third effect tower is at a much lower pressure (ca. 100 mmHg). Conversion of fresh tomatoes (ca. 6% solids) to paste (ca. 29% solids) gives a ca. 5 times concentration of solids. Major loss of volatile compounds by steam volatilization would be expected, however, and is apparent in Table I for most fresh tomato volatiles.

One of the most marked differences between the fresh tomato and the paste is the almost complete loss of (*Z*)-3-hexenal (the major contributor to fresh tomato aroma) in the paste. The conditions used in the production of the paste would be expected to be very unfavorable to (*Z*)-3-hexenal, which is known to be unstable to heat and metal surfaces. Other aliphatic aldehydes, such as hexanal, (*E*)-2-hexenal, and (*E*)-2-heptenal, which are more stable under these conditions, are also markedly reduced in concentration. Aromatic aldehydes such as benzaldehyde and phenylacetaldehyde and ketones such as 6-methyl-5-hepten-2-one seem more able to survive the processing conditions.

The general loss by volatilization, as might be expected, is most marked with the very volatile compounds such as 1-penten-3-one (ca. 500 times lower in the paste) and 3-methylbutanol (ca. 20 times lower in the paste). There is also considerable loss of compounds with more average volatility such as 2-isobutylthiazole (7 times lower in the paste).

Increases in concentrations of linalool and α-terpineol in heated tomato has been reported previously (Buttery et al., 1971). It can be seen that linalool shows a ca. 10 times increase in tomato paste (Table I). α-Terpineol was not detected in the fresh tomato. There is also an increase in the concentrations of 6-methyl-5-hepten-2-one and some other ketones in the paste. The most notable increase is with the potent odorant, β-damascenone,

Table I. Comparison of Quantitative Analyses of Major Volatiles of Fresh Tomato and Tomato Paste (Figures in Parentheses Show the Range Found in a Variety of Pastes)

compound	concn,[a] ppb: fresh tomato	concn,[a] ppb: tomato paste	Kovats index (DB1)
dimethyl sulfide	0	2000 (800–10000)	508
3-methylbutanal	27	24 (10–74)	627
1-penten-3-one	520	1 (0–8)	658
1-penten-3-ol	110	2 (0–5)	662
acetoin[b]	0	200 (100–300)	674
isobutyl cyanide	13	6 (2–20)	689
2-methyl-2-butenal	75	<1	708
3-methylbutanol	380	16 (5–50)	714
(*E*)-2-pentenal[c]	140[c]	0	723
2-methylbutanol[c]	100[c]	8 (2–20)[c]	724
pentanol	120	10 (0–50)	744
(*Z*)-3-hexenal	12000	0.7 (0–3)	765
hexanal	3100	8 (4–14)	772
furfural	0	140 (70–210)	800
(*E*)-2-hexenal	270	1.2 (0–5)	822
(Z)-3-hexenol	150	5 (0–15)	834
3-methylbutyric acid[b]	200[b]	2000 (800–8000)[b]	830
hexanol	7	0	848
1-nitro-3-methylbutane	59	4 (2–12)	856
methional[b]	0	3 (1–4)[b]	856
2-acetylfuran	0	45 (18–80)	870
benzaldehyde	31	14 (8–22)	926
(*E*)-2-heptenal	60	1 (0–4)	927
6-methyl-5-hepten-2-one	130	310 (50–1030)	961
6-methyl-5-hepten-2-ol	16	5 (0–15)	974
hexanoic acid[b]	–[d]	60 (40–80)[b]	975
2-pentylfuran	0	7 (3–13)	977
phenylacetaldehyde	15	18 (5–40)	1006
2-isobutylthiazole	36	5 (3–9)	1013
6-methyl-3,5-heptadien-2-one	0	6 (2–14)	1074
linalool	2	20 (16–40)	1083
2-phenylethanol[b]	1900[b]	1000 (500–2000)[b]	1081
phenylacetonitrile	3	43 (14–130)	1088
methyl salicylate	48	3 (0–8)	1166
α-terpineol	0	36 (9–54)	1170
β-cyclocitral	3	3 (2–6)	1194
neral	2	<0.5	1213
geranial	12	4 (3–4)	1242
1-nitro-2-phenylethane	17	66 (44–99)	1255
eugenol[b]	–[d]	100 (20–400)[b]	1326
β-damascenone	1	14 (2–25)	1360
geranylacetone		18 (6–26)	1428
β-ionone	4	2 (0–4)	1462
pseudoionone	2	4 (0–6)	1550

[a] Concentration in parts (mL) of compound per 10^9 parts of tomato or tomato paste. [b] Accuracy limited because of poor recovery of compounds. [c] Accuracy limited because not separated from other components. [d] Not determined.

Table II. Odor Thresholds of Tomato Paste Volatile Components and log Odor Unit Values (Concentration/Threshold) for Components in Tomato Paste

compound	odor threshold, ppb in water[a]	log U_o for paste[b]
dimethyl sulfide	0.3	3.8
β-damascenone	0.002	3.8
β-ionone	0.007	2.5
3-methylbutanal	0.2	2.1
1-nitro-2-phenylethane	2	1.5
eugenol	6	1.2
methional	0.2	1.2[c]
3-methylbutyric acid	250	0.9
6-methyl-5-hepten-2-one	50	0.87
phenylacetaldehyde	4	0.65
linalool	6	0.5
(*Z*)-3-hexenal	0.25	0.4
hexanal	4.5	0.3
2-isobutylthiazole	3.5	0.16
1-penten-3-one	1	0
2-phenylethanol	1100	–0.04
2-pentylfuran	6	0.07
methyl salicylate	40	–1
β-cyclocitral	5	–0.2
geranylacetone	60	–0.5
acetoin	800	–0.6
geranial	32	–0.9
(*E*)-2-heptenal	13	–1
α-terpineol	330	–1
(*E*)-2-hexenal	17	–1.2
(*Z*)-3-hexenol	70	–1.2
3-methylbutanol	250	–1.2
furfural	3000	–1.3
phenylacetonitrile	1000	–1.4
1-nitro-3-methylbutane	150	–1.6
hexanoic acid	3000	–1.7
6-methyl-3,5-heptadien-2-one	380	–1.8
isobutyl cyanide	1000	–2.2
pseudoionone	800	–2.3
1-penten-3-ol	400	–2.3
2-acetylfuran	10000	–2.4
benzaldehyde	3500	–2.4
pentanol	4000	–2.6
6-methyl-5-hepten-2-ol	2000	–2.6
2-methyl-2-butenal	500	–2.7

[a] Parts (mL) of compound per 10^9 parts (mL) of water. [b] Log of concentration in tomato paste divided by odor threshold in water solution (odor unit value, U_o). [c] Exact concentration and log U_o value uncertain.

which shows a ca. 10-fold increase in concentration in the paste.

The formation of linalool and other volatiles during the heating of grape juice has been thoroughly studied by Williams et al. (1982), who have shown this to result from the hydrolysis (via heat and pH ~5 conditions) of glycosidic precursors. Hydrolysis of glycosides may also explain the formation of many volatile compounds in heated tomato products. Braell et al. (1986) have evidence that β-damascenone is probably formed in grape juice by hydrolysis of glycosides. Schwab and Schreier (1988) have recently reported studies on the glycoside origins of a number of apple volatiles including 3-hydroxy-β-damascone (which can, in theory, dehydrate to β-damascenone). It seems likely that the β-damascenone present in heated tomato products also originates from the hydrolysis of a glycoside. The understanding of the mechanism of this release of volatile aroma compounds could be of considerable importance to the control of flavor in tomato paste. The possibility exists that this release of important aroma compounds could be manipulated in the paste manufacturing process.

Odor Contribution of Volatile Components to Paste. The odor thresholds in water solution listed in Table II of most of the tomato paste volatiles had been determined in previous studies of fresh tomato and other food volatiles (Buttery et al., 1971, 1989). Dividing the concentration data from Table I by the threshold data gives the value called odor units (U_o). U_o is equal to the number of threshold concentrations of each compound present in the paste. Listed in Table II as their logarithmic forms, they are arranged in decreasing order of the log U_o values; i.e., the compounds with most odor units are placed first and those with the least last.

The ranking by log U_o clearly shows the prominence of dimethyl sulfide and β-damascenone. It can be seen that the first 16 compounds in Table II occur at or above their threshold concentration in the paste (log $U_o > 0$). All other components occur in the paste at less than their odor threshold concentrations (log $U_o < 0$) and so are unlikely to contribute significantly to the paste odor.

A "synthetic paste essence" consisting of a water solution mixture of seven of the compounds with the most

odor units (from Table II) was found (by panel evaluation) to closely duplicate the odor of tomato paste. This solution contained dimethyl sulfide, β-damascenone, 3-methylbutanal, 1-nitro-2-phenylethane, eugenol, methional, and 3-methylbutyric acid in the proportions 140:1.0:1.7:5.0:7.1:2.1:140, adjusting the concentration (according to Table I) so that the dimethyl sulfide was at 2000 ppb (2 ppm). It is interesting that although 3-methylbutyric acid is usually considered an off-flavor, in the right proportions and concentration it was found to be an essential component of this mixture and undoubtably is an important contributor to the desirable aroma of most processed tomato products.

Importance of Dimethyl Sulfide. Wong and Carson (1966) have reported that dimethyl sulfide found in heated tomato is formed by thermal decomposition of the natural fresh tomato component (3-amino-3-carboxypropyl)dimethylsulfoniumion, $(CH_3)_2S(+)CH_2CH_2CH(NH_2)COOH$.

Guadagni and Meirs (1969) have found by panel methods that the most desirable concentration of dimethyl sulfide for tomato juice flavor was the range 0.5–2 ppm. The desirable range may be different for tomato paste or the various forms of tomato sauce, etc., obtained by diluting tomato paste. The authors have some preliminary evidence suggesting that storage of canned tomato paste at room temperature for long periods may lead to the formation of higher concentrations of dimethyl sulfide. Higher concentrations of dimethyl sulfide may be desirable for tomato paste. Wong and Carson (1966) had determined the concentration of the (3-amino-3-carboxypropyl)dimethylsulfonium ion to be 16–35 ppm in the fresh tomatoes, an effective upper limit for the concentration of dimethyl sulfide obtainable in the paste. Considerable decomposition of this compound to dimethyl sulfide would likely occur during the "hot break" and first effect stages of the paste manufacture where the temperatures are close to 100 °C. Consequently, much of the dimethyl sulfide would be lost at these points by steam volatilization.

Volatile Acids. 3-Methylbutyric acid occurs at a moderate concentration (ca. 50–200 ppb) in fresh tomatoes but generally below its odor threshold value (250 ppb). It is difficult to get an accurate figure on its concentration because of its low recovery with the Tenax isolation procedure (see discussion later). In tomato paste, the concentration of 3-methylbutyric acid was found to be between 800 and 8000 ppb, considerably above its threshold concentration. Schwab and Schreier (1988) had found that 2-methylbutyric acid occurs in apples in the form of a glycoside and can be released by hydrolysis under conditions of heating (at pH ~5) or by specific enzymes. 3-Methylbutyric acid in tomatoes may also occur as a glycoside. Although 2-methylbutyric acid has not been identified in tomatoes, it probably also occurs but at a lower concentration.

Hexanoic acid was also detected in tomato paste but at levels below its odor threshold value (3000 ppb), and it seems unlikely that it contributes to the total aroma. No detectable amounts of (*E*)-2-hexenoic acid, a likely oxidation product of (*Z*)-3-hexenal and (*E*)-2-hexenal, were found.

Quantitative Method. The isolation method using Tenax trapping was very similar to that previously used by us for fresh tomato volatiles (Buttery et al., 1988b). The fresh tomato work found that most volatiles show a 10–100% recovery by the isolation procedure. However, some very polar compounds, such as 2-phenylethanol and eugenol, gave very poor recoveries. Processed tomatoes have some additional compounds that give poor recoveries. These include acetoin (3-hydroxy-2-butanone) and methional (3-(methylthio)propanal), which were found to give only a 1% recovery, and 3-methylbutyric acid, which gave only 5% recovery (at pH 5) in the 1-h trapping period. Acidifying with dilute hydrochloric acid to pH 2 and carrying out the Tenax trapping for 24 h increased relative recoveries to 38% for 3-methylbutyric acid and 22% for hexanoic acid.

Combined relative recovery–GLC response factors (relative to internal standards) for each of the components were obtained experimentally and used in the calculations of the concentrations in Table I. The values found for the tomato paste isolation procedure were different from those previously obtained by us (Buttery et al., 1988b) where saturated $CaCl_2$ solution was used. The previous use of $CaCl_2$ had given a better recovery of many components, particularly alcohols. The combined average relative recovery–GLC response factors found for the paste isolation method were as follows. (1) Relative to 3-pentanone: 3-methylbutanal, 50%; 1-penten-3-one, 80%; (*E*)-2-pentenal, 105%. (2) Relative to 2-octanone: 1-penten-3-ol, 28%; acetoin, 0.7%; isobutyl cyanide, 120%; 2-methyl-2-butenal, 50%; 3-methylbutanol, 19%; pentanol, 19%; (*Z*)-3-hexenal, 70%; hexanal, 77%; furfural, 14%; (*E*)-2-hexenal, 60%; (*Z*)-3-hexenol, 20%; 3-methylbutyric acid, 5%; hexanol, 20%; 1-nitro-3-methylbutane, 81%; methional, 1%; 2-acetylfuran, 11%; benzaldehyde, 84%; (*E*)-2-heptenal, 70%; 6-methyl-5-hepten-2-one, 80%; 6-methyl-5-hepten-2-ol, 13%; 2-pentylfuran, 80%; phenylacetaldehyde, 40%; 2-isobutylthiazole, 88%; (*E*,*E*)-6-methyl-3,5-heptadien-2-one, 50%; linalool, 51%; α-terpineol, 11%; β-cyclocitral, 61%; geranial, 80%; 1-nitro-2-phenylethane, 9%. (3) Relative to anethole: 2-phenylethanol, 0.3%; phenylacetonitrile, 14%; methyl salicylate, 63%; eugenol, 0.5%; β-damascenone, 53%; geranylacetone, 49%; β-ionone, 43%; pseudoionone, 55%.

The average error for standard solutions of low–medium-polarity compounds for this type of analysis was within 8%. The error in calculating the figures for the high-polarity, low-recovery compounds would be expected to be greater.

It was not possible to analyze very volatile compounds such as dimethyl sulfide by the particular type of Tenax trapping method used. Instead, a procedure similar to that used by Guadagni and Meirs (1969) was utilized. A sample of the atmosphere above the diluted paste was directly injected into the gas chromatograph. In the present study, a measured quantity of a standard solution of benzene in water (10.0 ppm) was first added to the paste as an internal standard before taking the vapor sample. The method follows well-established laws of vapor-solution equilibria and had been used many years previously by some of us (Buttery and Teranishi, 1963) for other compounds. The method was calibrated on standard solutions of dimethyl sulfide in water. A critical part of the method is that the sampling be carried out at a fixed temperature and that the mixtures be well swirled or stirred to ensure that equilibrium is attained.

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Registry No. Dimethyl sulfide, 75-18-3; 3-methylbutanal, 590-86-3; 1-penten-3-one, 1629-58-9; 1-penten-3-ol, 616-25-1; ace-

toin, 513-86-0; isobutyl cyanide, 625-28-5; 2-methyl-2-butenal, 1115-11-3; 3-methylbutanol, 123-51-3; (*E*)-2-pentenal, 1576-87-0; 2-methylbutanol, 137-32-6; pentanol, 71-41-0; (*Z*)-3-hexenal, 6789-80-6; hexanal, 66-25-1; furfural, 98-01-1; (*E*)-2-hexenal, 6728-26-3; (*Z*)-3-hexenol, 928-96-1; 3-methylbutyric acid, 503-74-2; 6-methyl-5-hepten-2-one, 110-93-0; 6-methyl-5-hepten-2-ol, 1569-60-4; hexanoic acid, 142-62-1; 2-pentylfuran, 3777-69-3; phenylacetaldehyde, 122-78-1; 2-isobutylthiazole, 18640-74-9; 6-methyl-3,5-heptadien-2-one, 1604-28-0; linalool, 78-70-6; 2-phenylethanol, 60-12-8; phenylacetonitrile, 140-29-4; methyl salicylate, 119-36-8; α-terpineol, 98-55-5; β-cyclocitral, 432-25-7; neral, 106-26-3; geranial, 141-27-5; eugenol, 97-53-0; β-damascenone, 23726-93-4; geranylacetone, 3796-70-1; β-ionone, 79-77-6; pseudoionone, 141-10-6; hexanol, 111-27-3; 1-nitro-3-methylbutane, 627-67-8; methional, 3268-49-3; 2-acetylfuran, 1192-62-7; benzaldehyde, 100-52-7; (*E*)-2-heptenal, 18829-55-5.

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Water-Soluble Fluorescent Compounds in Rat Tissue Fed Cottonseed Flour Supplemented with Vitamin E

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The presence and accumulation of water-soluble fluorescent compounds in the tissue of vitamin E deficient rats and in rats fed gossypol were determined. Sixteen rats were divided into four groups and were fed a control diet, a vitamin E deficient diet, a cottonseed diet, or a cottonseed plus an excess of vitamin E diet for 8 weeks. Results show that more pigment accumulated in muscle, liver, and testes of rats fed the cottonseed and vitamin E deficient diet than in those of rats fed the control diet. There was no significant difference in heart pigment levels among rats fed the four diets.

The accumulation of lipofuscinic pigment found in animal tissues has been related to aging and vitamin E deficiencies. The presence of these pigments is considered to be a consequence of the autoxidation of intracellular compounds. Apparently, the dietary deficiency of antioxidants, such as vitamin E and selenium, results in the intracellular accumulation of similar autofluorescent pigments in some tissues. Most of these pigments were believed to be due to the Schiff base type compounds, with the greatest fluorescence at 435 nm called "solvent-

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