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Quantitative Studies on Origins of Fresh Tomato Aroma Volatiles

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A method developed for the quantitative analysis of tomato volatiles has been extended to cover the C_5-C_{13} range of fresh tomato aroma volatiles. This method showed that the pulp and skin (+ some adhering pulp) had the highest concentrations of volatile aroma compounds of the tomato. The fluid showed smaller concentrations of (Z)-3-hexenal. No significant contribution was found from the seeds. A study of some unusual varieties containing high and low levels of carotenoids showed differences that were generally consistent with the view that 6-methyl-5-hepten-2-one, geranylacetone, β -ionone, and related compounds are derived from oxidative carotenoid breakdown.

Although there have been numerous qualitative studies of the aroma volatiles of foods, there have been very few quantitative studies. This is especially true with tomatoes. This is partly due to the complex mixtures of components involved, their usual very low concentration, and the variability with different isolation methods. A knowledge of the concentrations of aroma volatiles in a food is, however, very important to the understanding of each compound's role in the perceived aroma of that food. Some of the authors had previously developed a workable method for the quantitative analysis of C_5 - C_9 tomato volatiles (Buttery et al., 1987). It seemed desirable to extend this method to a wider range of volatiles and to apply the method to determine the principal location of volatile aroma compound production in the tomato. This information could be useful in the basic understanding of tomato aroma.

Considerable evidence regarding the lipids as the source of the C_6 aldehydes in tomatoes has been published by other authors (Kazeniac and Hall, 1970; Galliard and Matthew, 1977) and also for other foods such as tea leaves (Hatanaka and Harada, 1973). Buttery et al. (1969) and Stevens (1970) have thought that C_8 , C_{13} , and C_{18} terpenoid-like aroma volatiles of tomato might be derived by oxidative breakdown of carotenoids. The availability of the method for the quantitative analysis of tomato volatiles gave the authors the opportunity of supplying some additional information in this area.

EXPERIMENTAL SECTION

Materials. Tomatoes were grown on experimental fields at Davis, CA, during 1987. Tomato breeding lines used included the following: E6203, LA1563, ACC29 (Ace Yellow), ACC36 (High Beta), FM785, V80007-2-2-4, ACC81, XPH5498, Lassen, and GS-12 (Goldsmith-12). Freshly picked vine-ripe tomato samples were stored in the laboratory at room temperature under normal laboratory (fluorescent) lighting and used within 3 days. Authentic reference chemicals were obtained from reliable commercial sources or synthesized by well-established methods. They were purified by GLC separation before use. Freshly distilled diethyl ether and saturated CaCl₂ solutions were prepared as described previously (Buttery et al., 1987) except that it was found necessary to distill the ether through a fairly efficient (ca. 10-plate) glass helices packed column to remove trace amounts of volatile impurities.

Isolation of Volatiles from Tomato Samples. From Whole Tomatoes. Isolation was carried out largely as previously described (Buttery et al., 1987). The whole tomato sample (100 g at 25 °C) of pieces cut from three different tomatoes was blended for 30 s. The mixture was allowed to stand at room temperature for 180 s longer, then saturated CaCl₂ solution (100 mL) added, and the mixture blended for 10 s. A standard solution (5.0 mL) containing 20.0 ppm 2-octanone, 20.0 ppm 3-pentanone, and 5.0 ppm anethole in water was then added and the mixture blended again for 10 s. The resultant mixture was then poured into a round-bottomed 1-L flask containing an efficient magnetic stirrer. Purified air (3 L/min) was then led into the flask and passed over the vigorously stirred mixture and out of the flask through a Tenax trap (14-cm length ×

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2.2-cm i.d.). All connections and equipment were either Pyrex glass or Teflon. The isolation was carried out for 60 min and the trap then removed and eluted with 100 mL of diethyl ether. The ether extract was concentrated to ca. 50 μ L with a warm water bath and Vigreux distillation column. The Tenax trap was reactivated by passing a stream of purified nitrogen through it at 200 °C for 1 h.

Pulp, Locular Fluid, and Skin. Tomato line GS-12 was used for the pulp and locular fluid while line FM785 was used for the skin. Separation of each part of the tomato was generally done on different occasions to keep the manipulation time as short as possible (<5 min). In all cases a number of tomatoes were used (varying from at least three tomatoes in the case of pulp to ca. 5 kg in the case of skins). The fresh vine-ripe tomatoes were opened with knives. The pulp (100 g) was fairly readily obtained by simply physically removing the skin and fluid with the knives. The locular fluid (100 g) was obtained from ca. 10 tomatoes and filtered through stainless steel screen to remove the seeds. Enough skin (100 g) was obtained by peeling ca. 5 kg of tomatoes with sharp knives. The isolation of volatiles from the pulp, skin, and fluid was done exactly as described above for the whole tomato.

Seeds. It required ca. 0.5 h to separate enough seeds (100 g wet weight) from ca. 7 kg of tomatoes (line GS-12). The seeds were washed well with water to remove adhering fluid and then placed in the blender for the isolation with 50 mL of water (the seeds did not contain enough fluid of their own for blending). The rest of the procedure for the isolation of volatiles was exactly as described for the whole tomato.

Capillary GLC Analysis. Two commercially obtained capillary GLC columns were used; both were 60-m length \times 0.32-mm i.d. fused silica. One was coated with DB-1 and the other with DB-wax. The GC oven programming conditions for the DB-1 coated column were to hold the temperature at 30 °C for 25 min and then increase the temperature at 4 °C/min to 190 °C and hold this temperature for another 30 min. The GC oven conditions for the DBwax coated column were to hold the temperature at 30 °C for the first 5 min and then increase the temperature at 4 °C/min to 170 °C and hold this temperature for 30 min. Carrier gas (He) velocity was 22 cm/s for the DB-1 coated column and 32 cm/s for the DB-wax coated column. Sample size was $1 \,\mu L$ split 1/20. The injector temperature was 170 °C. The gas chromatographs were Hewlett-Packard series 5880 and 5890 instruments with electronic peak area measurements.

Capillary GLC-MS Analysis. This was carried out on a 60 m \times 0.32 mm (i.d.) fused silica capillary column coated with DB-1. The mass spectrometer used was a Finnigan MAT 4500 (quadrupole) GC-MS. GLC (Kovats) indices were used along with the mass spectral data to confirm the identities of components.

RESULTS AND DISCUSSION

The method developed previously by Buttery et al. (1987) for the quantitative analysis of tomato volatiles was extended in the present work to higher boiling compounds such as geranylacetone, methyl salicylate, and β -ionone. It was also extended to evaluate additional minor compounds such as geranial and β -cyclocitral. Twenty-five volatile compounds were quantitatively analyzed in each tomato sample. Identities of components were verified by capillary GLC-MS. All compounds in the tables had been identified previously in tomato [for a recent review see Petro-Turza (1986–1987)] except for β -cyclocitral, which was identified in tomatoes for the first time in the present work. The unknown had mass spectra (major ions at m/e

Table I. Comparison of Concentrations (ppb) of Volatiles in Vine-Ripe Tomato Parts Pulp, Fluid, and Seed with That in the Whole Tomato Using the Standard Isolation Procedure (GS-12 Tomato Line)

compound	whole	pulp	fluid	seed
2- + 3-methylbutanals	36	10	25	0
1-penten-3-one	550	350	280	1
hexanal	3500	8100	3500	36
isobutyl cyanide	17	44	30	1
(E)-2-pentenal	86	40	37	0
(Z)-3-hexenal	10000	11000	6000	10
1-penten-3-ol	74	140	71	1
2- + 3-methylbutanols	380	350	460	17
(E)-2-hexenal	330	660	190	70
pentanol	150	220	190	0
(E)-2-heptenal	54	61	38	1
1-nitro-3-methylbutane	53	101	54	1
6-methyl-5-hepten-2-one	150	200	230	2
hexanol	6	230	22	1
(Z)-3-hexenol	140	850	200	21
2-isobutylthiazole	99	97	73	0
6-methyl-5-hepten-2-ol	18	35	46	0
β -cyclocitral	3	1	2	0
linalool	4	3	4	0
neral	2	1	4	0
geranial	25	25	20	0
methyl salicylate	20	8	12	0
geranylacetone	78	83	110	0
2-phenylethanol	1500	1900	1300	0
β-ionone	4	3	3	0

41, 137, 109, 81, 152 (M⁺), 67, 123, 55) and GLC Kovats retention index (1190 on DB-1) consistent with that of an authentic sample of β -cyclocitral. Three internal standards, 3-pentanone, 2-octanone, and anethole, were used to cover the volatiles from ca. C₅ to C₁₃. All analysis figures listed in the tables are averages from at least three separate complete isolations from different lots of tomatoes. Further discussion of the isolation method is included later.

Volatiles from Different Tomato Parts. The tomato fruit can be divided into four main components: the pulp, i.e. all solid tissue left after removal of fluid, seeds, and skin; the fluid, i.e. the locular fluid or gel; the seeds; the skin. None of these parts are completely separable from one another in their undamaged fresh condition. Washing of the seeds with cold water removed most of the tomato fluid. The skin, however, could not be completely removed from the attached pulp. Some of the fluid was probably retained in the rather porous pulp. The separation of the different parts was carried out as rapidly as possible, and the isolation of volatiles started within 5 min (except for seeds, which took ca. 30 min). The rapid separation was necessary to minimize the volatiles produced by the separation process that would cause some tissue damage. The concentrations of volatiles (in parts per billion of the tomato, ppb) found in these separated parts (GS-12 line) are listed in Table I and compared to that found in the whole tomato and discussed below.

Pulp. The pulp forms about 70% of the tomato. In Table I it can be seen that the quantitative analysis of the pulp showed very similar values to that found in the whole tomato except that the pulp has a considerably higher concentration of C_6 alcohols and (E)-2-hexenal, which may be the result of cell damage unavoidably occurring during the separation of the pulp.

Fluid. The fluid (ca. 20% of the tomato) was obtained by opening the tomato with a knife and filtering the fluid through a stainless steel screen (to remove seeds) before isolating the volatiles by the standard procedure. The (Z)-3-hexenal, (E)-2-hexenal, and 1-penten-3-one concentrations found in the fluid were about half that found in the whole tomato. Concentrations of most other compo-

Table II. Comparison of Concentrations (ppb) of Volatiles from Tomato Skin with Those of the Whole Vine-Ripe Tomato (FM785 Line)

compound	whole	skin	
2- + 3-methylbutanals	27	27	
1-penten-3-one	520	400	
hexanal	3100	9700	
isobutyl cyanide	13	22	
(E)-2-pentenal	140	140	
(Z)-3-hexenal	12000	20000	
1-penten-3-ol	110	210	
2- + 3-methylbutanols	380	410	
(E)-2-hexenal	270	950	
pentanol	120	280	
(E)-2-heptenal	60	80	
1-nitro-3-methylbutane	59	160	
6-methyl-5-hepten-2-one	130	320	
hexanol	7	710	
(Z)-3-hexanol	150	2900	
2-isobutylthiazole	36	86	
6-methyl-5-hepten-2-ol	16	12	
β -cyclocitral	3	2	
linalool	2	4	
neral	2	4	
geranial	12	28	
methyl salicylate	48	17	
geranylacetone	57	250	
2-phenylethanol	1900	1900	
β-ionone	4	5	

nents were, however, fairly comparable.

Seeds. The seeds (ca. 0.3% of the tomato) were obtained by opening the tomatoes with a knife, filtering the seeds on a stainless steel screen, and washing them well with water. Most of the water was pressed off, but the wet weight of the seed was used for the determination of the concentrations shown in Table I. Water (50 mL) was added in the blending part of the standard isolation procedure to allow the seeds to be broken up properly. It can be seen that the concentration for the seeds is of the order of 100 times lower than that of the whole tomato for most compounds. Only (E)-2-hexenal occurs in the same order of concentration as that of the whole tomato. But the seeds are such a small percentage of the whole tomato (ca. $1/_{30}$ th the weight) that none of the seed volatiles could contribute significantly to the volatiles of the whole tomato.

Skin. A similar but different tomato line (FM785) was used for the study of skin volatiles, and the comparison is made against the whole tomato of that variety in Table II. The tomatoes were peeled with sharp knives as close as possible to the surface, but appreciable amounts of pulp, ca. 1-2 mm thick, still remained attached to the skin. The skin obtained in this way formed ca. 10% of the tomato. Other more effective methods of removing the skin, such as the commonly used processing methods of steam or alkaline peeling, however, would produce abnormal volatiles. Results in Table II show that the skin (and adhering surface pulp) had a considerably higher concentration of C_6 aldehydes and a several-fold higher concentration of C_6 alcohols than the whole tomato or the interior pulp. The possibility that this may partly be the result of tissue damage occurring during peeling cannot be excluded.

Quantitative Studies with Different Tomato Lines. Samples of tomatoes from more than 10 different lines of tomatoes have been studied by the authors. Although a thorough statistical analysis was not made, no marked differences were observed for the concentrations of C_5-C_{13} volatiles found for the vine-ripe samples of the commercial lines studied. The analyses found (except for the three unusual lines discussed below) were all similar to that for the whole tomatoes (GS-12 and FM785 lines) shown in

Table III.	Comparison of	Concentratio	ns of Vola	tiles (ppb)
for Differe	ent Tomato Lin	es Containing	Different	Levels of
Carotenoid	s	Ū.		

	ACC29:	V80007	ACC36
	low	high	high
compound	caroten	lycopene	β -carotene
2 mothylbutonol	20	15	07
1 nonton 2 ono	840	650	21 500
1-penten-5-one	1400	600	020 1000
nexanal	1400	2500	1300
isobutyi cyanide	63	5	20
(E)-2-pentenal	77	120	61
(Z)-3-hexenal	11000	13000	4800
1-penten-3-ol	210	91	62
2- + 3-methylbutanols	550	160	1710
(E)-2-hexenal	480	230	120
pentanol	99	100	230
(E)-2-heptenal	62	80	48
1-nitro-3-methylbutane	240	31	230
6-methyl-5-hepten-2-one	2	73	7
hexanol	3	7	29
(Z)-3-hexenol	120	190	140
2-isobutylthiazole	70	33	32
6-methyl-5-hepten-2-ol	2	18	2
β -cyclocitral	0	2	3
linalool	1	2	19
neral	0	4	1
geranial	0	21	2
methyl salicylate	22	10	230
geranylacetone	11	48	45
2-phenylethanol	330	230	990
β-ionone	1	3	17

Tables I and II. The main quantitative differences that the authors have encountered seemed to be caused by variations in degrees of ripeness or by storage conditions (Buttery et al., 1987).

Three unusual experimental lines also studied were ACC36, a high-\beta-carotene line; V80007-2-2-4, a high-lycopene line; and ACC29, a low-carotenoid line. The carotenoid content of these tomato lines had been determined by other workers (Stevens, 1970, 1986). Analyses for these lines are listed in Table III. These can be compared with those of the common commercial lines in Tables I and II. The high β -carotene line (ACC36) showed the highest concentrations of β -ionone (17 ppb) and β -cyclocitral (30 ppb), both known to result from biological or chemical degradation of β -carotene. The high-lycopene line (V80007), however, did not show any significantly higher concentration of the suspected lycopene degradation products 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, or geranylacetone. It did show a significantly higher value for geranial (21 ppb) compared to that of the common FM785 line (12 ppb). Geranial could be considered a lycopene degradation product. The concentration of all of the above carotenoid fragments was appreciably lower in the low-carotenoid line ACC29.

It would be interesting to study the volatile concentrations in tomato lines varying in unsaturated fatty acid content to note the effect on unsaturated fatty acid degradation products such as (Z)-3-hexenal, hexanal, 1-penten-3-one, etc. However, the authors are not aware of where such tomato lines could be obtained.

Quantitative Isolation Method. The isolation procedure, previously used by Buttery et al. (1987) for C_5-C_9 tomato volatiles, was extended in the present study for C_5-C_{13} volatiles. Three internal standards were used to cover this range. The most volatile was 3-pentanone, which was used as an internal standard for 2- and 3-methylbutanals, 1-penten-3-one, and (*E*)-2-pentenal. The second internal standard, 2-octanone, was used for the middle range of compounds from hexanal to geranial listed in Tables I-III (except for (*E*)-2-pentenal). The third internal

Table IV. Percent Recovery Found for Standard Solutions Relative to Respective Internal Standards, 3-Pentanone, 2-Octanone, and Anethole, and Absolute Recovery Using Standard Tenax Trap Isolation Procedure

compound	rel % rec ^a	abs % rec
3-methylbutanal ^b	50	19
1-penten-3-one ^b	83	31
isobutyl cyanide ^c	118	96
(E)-2-pentenal ^b	116	43
hexanol	82	66
1-penten-3-ol°	64	52
linalool	106	86
geranial ^c	92	75
geraniol	70	57
β -cyclocitral ^c	84	68
methyl salicylate ^d	60	35
geranylacetoned	63	36
2-phenylethanol ^d	3	2
A-ionone ^d	76	44

^a The absolute recovery of 2-octanone was 81%, 3-pentanone was recovered 46% relative to 2-octanone, and anethole was recovered 71% relative to 2-octanone. ^bRelative to 3-pentanone. ^cRelative to 2-octanone. ^dRelative to anethole.

standard, anethole (4-propenylanisole), was used for the last four compounds in Tables I-III. The three internal standards were dissolved in water to make a standard solution containing 20.0 ppm 3-pentanone, 20.0 ppm 2octanone, and 5.0 ppm anethole. The internal standard solution was stored in the dark at 2 °C. Usually 5.0 mL of this standard solution was added to 100 g of blended tomato or test solution (after first adding 100 mL of saturated CaCl₂ solution to deactivate enzyme systems). It had been previously found that an absolute recovery of 81% of 2-octanone was obtained by the isolation method developed and that an average of 46% 3-pentanone was recovered relative to 2-octanone (Buttery et al., 1987). In the present study it was found that an average recovery of 71% of anethole was recovered relative to 2-octanone. The recoveries reported previously were reconfirmed except for (Z)-3-hexenal, which showed a 64% recovery (relative to 2-octanone) in the present study (apparently because of shorter storage times of the standard water solution). Recoveries found for additional tomato components relative to their respective internal standard are listed in Table IV. Most compounds, including the higher boiling compounds, are recovered reasonably well. A notable exception is 2-phenylethanol, which gave an average of only 3% recovery relative to anethole. This is apparently due to the low air to water partition coefficient (K)of 2-phenylethanol (the combination of its low vapor pressure with its relatively high water solubility). To obtain a 64% recovery would require ca. 50 times the volume of sweep gas used in the present study according to the theoretical relation previously derived by Buttery et al. (1987). For a 64% recovery this relation simplifies to V_a = V_w/K , where V_a is the volume of sweep gas, V_w is the volume of water solution, and K is the air/water partition coefficient. A 50-h trapping period is impractical for the large number of samples needed, and there is the possibility that there could be some changes in the blended tomato-saturated CaCl₂ mixture over such a long period. For 2-phenylethanol and also for all other compounds, a correction factor based on the relative percent recovery obtained was used for the figures in Tables I-III. The errors involved would be expected to be greater for compounds such as 2-phenylethanol where the percent recovery is so low.

GLC Conditions. In the previous publication (Buttery et al., 1987), a 30 m \times 0.25 mm (i.d.) DB-wax (Carbowax type) coated fused silica capillary column was used. It was

found in the present study that a 60-m DB-1 (methyl silicone type) coated fused silica capillary was more suitable for most compounds and by holding the column at 30 °C for the first 25 min gave sufficient separation between (Z)-3-hexenal and hexanal. The DB-1 column is more stable and the retention data are more reproduceable over long periods. However, a 60-m DB-wax column was also used for the analysis of certain specific compounds and for confirmation that the retention time identity was correct in the routine analysis of large numbers of samples. The DB-1 column also has the advantage that the aldehyde peaks do not show the tailing that they do on DB-wax, allowing for more accurate integration. The 60-m columns also give higher resolution of peaks, which decreases errors caused by overlapping of peaks.

The accuracy of the quantitative analysis found for standard solutions of most compounds in Tables I–III on the average was within ca. $\pm 8\%$ of the known concentration as reported previously (Buttery et al., 1987). The error involved with 2-phenylethanol could be considerably higher because of its poor recovery, and the figures in Tables I-III for 2-phenylethanol can only be considered to be of the right order. With the very small amounts of volatile tomato components involved, there can be problems in evervday routine analyses with trace contamination of the equipment. Occasional unexplained specific adsorption of certain compounds especially in regard to the higher boiling polar compounds can also cause problems. Some of these sources of error are minimized by the use of two GLC instruments with different polarity columns such as DB-1 and DB-wax.

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Registry No. 2-Methylbutanal, 96-17-3; 3-methylbutanal, 590-86-3; 1-penten-3-one, 1629-58-9; hexanal, 662-5-1; isobutyl cyanide, 625-28-5; (E)-2-pentenal, 1576-87-0; (Z)-3-hexenal, 6789-80-6; 1-penten-3-ol, 616-25-1; 2-methylbutanol, 137-32-6; 3-methylbutanol, 123-51-3; (E)-2-hexenal, 6728-26-3; pentanol, 71-41-0; (E)-2-heptenal, 18829-55-5; 1-nitro-3-methylbutane, 627-67-8; 6-methyl-5-hepten-2-one, 110-93-0; hexanol, 111-27-3; (Z)-3-hexenol, 928-96-1; 2-isobutylthiazole, 18640-74-9; 6-methyl-5-hepten-2-ol, 1569-60-4; β -cyclocitral, 432-25-7; linalool, 78-70-6; neral, 106-26-3; geranial, 141-27-5; methyl salicylate, 119-36-8; geranylacetone, 3796-70-1; 2-phenylethanol, 60-12-8; β -ionone, 79-77-6.

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