

Flavor Quality of Cultivated Strawberries: The Role of the Sulfur Compounds

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For determination of the importance of the different aroma components for strawberry flavor at several times, different varieties of strawberries were organoleptically evaluated for flavor intensity and quantitative GC-MS analyses were performed. Interpretation of the results consisted of correlating the organoleptic data with the concentrations of the flavor components, representatively isolated by headspace concentration on Tenax. In some comparisons significant sensory flavor intensity data could be related to the sum of the concentration of the volatiles, released when macerating the fruits. In other cases, especially with "older" varieties, the difference in amount of sulfur-containing compounds, which compared to the esters were present at a much lower concentration, had to taken into account. The methylthiol esters, methylthiol acetate and methylthiol butyrate, are reported for the first time in strawberries.

The volatiles of cultivated and wild strawberries have been extensively studied by different authors. Recently, a summary of the literature has been given by Pyysalo et al. (1979), on the occasion of a study of a wild variety. From the different studies one may state that esters, alcohols, and carbonyls are important for the fruity and green notes of the strawberry flavor. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (Sundt, 1970) and 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (Pyysalo et al., 1979) are considered to be of the most important aroma constituents so far reported in strawberries. The volatile composition of wild strawberries, which has also been studied by Drawert et al. (1973), mainly differs from the cultivated varieties by a higher amount of 2-alkanones and 2-alkanols and the occurrence of fewer aliphatic carboxylic acids.

To the authors' knowledge, the only sulfur compounds which have been determined in strawberries are hydrogen sulfide, methanethiol, and dimethyl disulfide (Winter, 1963). Although sulfur compounds, particularly thiols, sulfides, disulfides, and isothiocyanates, play an important role in the vegetable flavors [e.g., Schreyen et al. (1976a,b)], very few volatile sulfur compounds have been identified in fruits. Some interesting examples are methyl and ethyl 3-methylthiopropionates in pineapple (Connell, 1964) and alkyl thioesters in grapes (Stern et al., 1967).

Flavor quality of fruits and vegetables is a combination of an impression on the tongue (taste), mainly determined by the acid/sweet ratio, an impression in the nose (aroma), due to the volatiles that we unconsciously send to the nose while eating, and the mouth feel impression, which is a physical texture characteristic. For strawberries the aroma part and the acidity are likely to be the most important parameters. An important aspect of objective flavor quality measurement by GC-MS and analyses is the isolation of a representative aroma mixture. For this purpose a promising technique is headspace concentration on Tenax of volatiles, liberated while macerating the fruits (Dirinck et al., 1977). Furthermore, for estimation of the importance of the different flavor components for the overall flavor, the safest procedure is to perform a relatively high amount of comparisons between sensory analyses (taste testing) and chemical analyses. Therefore, over the seasons 1977, 1978, and 1979, at several times different

varieties of strawberries were organoleptically evaluated for flavor intensity. When significant differences were obtained in the sensory analyses, titratable acidity and quantitative flavor analyses were performed. Interpretation of the results consisted of correlating the organoleptic data with the concentration of flavor components and the titratable acidity. This work is part of a long-term project, of which the aim is to study the organoleptic quality of fresh fruits and vegetables on an objective basis, the problem being the decreasing flavor quality as a result of excessive production, selection of varieties for optimal external quality and optimal production, and illness resistance without concern for flavor quality.

EXPERIMENTAL SECTION

Materials. Over the seasons 1977, 1978, and 1979, at several times different varieties of fresh ripe strawberries were organoleptically evaluated and analyzed by GC-MS analyses. The strawberries were field grown, greenhouse grown, or grown under plastic funnels. The strawberries were harvested at the ripe stage of maturation and stored in the refrigerator at 5 °C before taste testing and analyses. A survey of the different varieties used in the comparisons is given in Table I.

Sensory Analyses. For sensory testing scaling tests were performed (Kramer, 1974). The panel varied from 10 to 12 members, who after sniffing and tasting rated the fruits according to a 1-9 scale for flavor intensity: 1 = extremely weak strawberry flavor; 9 = extremely strong strawberry flavor. The panelists were blindfolded in order to mask the rather important appearance differences. As it is very difficult to compare taste characteristics of products, which have been tasted with important time intervals, significant differences could only be determined between varieties available at the same moment. Therefore, the individual scores for flavor intensity were transformed in rank numbers, and statistical analysis was performed by summing the rank numbers and comparing the rank sums to the totals required for statistical significance (Kramer, 1974). It should also be stressed that in order to exclude individual preferences the panelists ranked the samples on the basis of typical strawberry flavor intensity and not for flavor quality. However, as nowadays the problem of the commercially cultivated varieties is their "watery" and tasteless character, there would probably be a good relation between strawberry flavor intensity and flavor quality.

Sampling Procedure and Sample Injection of Strawberry Volatiles. For correlation with the flavor

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Table I. Compared Strawberry Varieties at Different Dates

compared varieties	sampling dates (cultivation technique)						
	May 3, 1977 ^a	June 31, 1977 ^b	July 25, 1977 ^c	June 27, 1978 ^c	June 21, 1979 ^c	July 3, 1979 ^c	July 12, 1979 ^c
Confitura	X	X	X				
Gorella	X	X	X	X	X	X	X
Induka	X	X	X				
Jesco	X	X	X		X		X
Primella	X		X				
Senga Gigana	X						
Sivetta	X	X	X	X	X		X
Red Gauntlet	X	X	X			X	
Souvenir de Charles Machiroux				X		X	X
Bel Rubi				X	X		
Geko				X			
Domanil						X	
Hapil						X	

^a Greenhouse grown. ^b Grown under plastic funnels. ^c Field grown.

intensity scores, the occurrence and concentration of the strawberry volatiles, representatively isolated from the food matrix, were determined. For isolation of a fresh representative flavor mixture, the volatiles, liberated while macerating the strawberries, were adsorbed on Tenax GC, 60–80 mesh, as described before (Dirinck et al., 1977). When helium was passed through the adsorption column (i.d. 16 mm; length 10 cm, with an adsorbent content of 5 g) at a flow rate of 500 mL/min during 10 min, the volatiles were quantitatively adsorbed. (Loaded columns should be closed for avoiding contamination with laboratory air pollutants and can be stored in the refrigerator for several weeks without loss of volatiles.)

The flavor components were desorbed at 220 °C in a helium stream (flow 20 mL/min) and transferred to a cooling trap. The sample injection system was modified as to the previous publication. For sample injections the adsorption tube was placed in a separate oven (gradually warmed up to 220 °C), which was connected to a two-position six-port, high-temperature injection valve (Valco Instruments Co., Houston, Tx), also by 1/16 in. thermally isolated zero-volume fittings. The injection valve was constantly kept at 250 °C in a separate oven. In the transfer position the volatiles were desorbed from the Tenax adsorber and collected in a trap cooled with liquid nitrogen. For injection the liquid nitrogen was replaced by an injection oven while the six-port injection valve was switched from the collecting position to the injection position.

Gas Chromatography. The volatiles from the different strawberry samples were separated on a Varian 3700 gas chromatograph, equipped with a flame ionization detector (FID) and a 200 m × 0.6 mm i.d. glass column coated with SE-30. Further operating conditions were as follows: linear temperature programming from 20 to 220 °C at 2 °C/min; carrier gas N₂, 3 mL/min; injection valve temperature, 250 °C; detector temperature 250 °C.

Quantitative determination of the different strawberry volatiles was performed by electronic integration (Infotronics 204) and by relating the peak intensities of *n*-dodecane and *n*-tridecane as internal references. Therefore, before desorption 1 μL of a 1% solution of the *n*-alkanes in carbon disulfide (1 μg of each hydrocarbon/μL of carbon disulfide) was injected on the adsorption column for quantitation.

Besides analyses on a universal FID, for some varieties specific analyses for sulfur compounds on a Tracor 560 gas chromatograph equipped with a flame photometric detector were also performed. The Tracor 560 gas chromatograph was equipped with a 125 m × 0.6 mm i.d. capillary

Table II. Significant Differences in Flavor Intensity Obtained by the Ranking Procedure between the Evaluated Strawberries

sampling date	varieties with significantly different flavor intensity ^b	
	more intense flavor	less intense flavor
May 3, 1977	Confitura (5%) ^a	Gorella (1%)
June 31, 1977	Jesco (1%)	
June 27, 1978	Souvenir DCM (1%)	Gorella (5%)
June 21, 1979	Jesco (1%)	Gorella (1%)
June 3, 1979	Souvenir DCM (5%)	Hapil (5%)

^a The significance level is given in parentheses. ^b The compared varieties at the different sampling dates are given in Table I.

coated with SE-30. The flame photometric detector (FPD) is 100–1000 times more sensitive for sulfur compounds than the FID.

Gas Chromatography–Mass Spectrometry. The GC–MS apparatus consisted of a Varian 2700 gas chromatograph linked to a MAT 112 mass spectrometer. The mass spectrometer was directly coupled to the gas chromatography by means of a glass tubing capillary (length 50 cm; 0.133-mm i.d.) heated at 250 °C and allowing a constant flow of 2 mL of effluent into the MS source. In order to avoid disturbance of the monitogram due to the water content of the sample, we installed an effluent splitter to send ~50% of the total effluent to a FID. Operating conditions for GC–MS were as follows: 250 m × 0.6 mm i.d. glass column, coated with OV-1; linear temperature programming from 20 to 220 °C at 2 °C/min; carrier gas He, 2.8 mL/min and make up to 4 mL/min before FID–MS splitter and to 30 mL/min before FID; injector temperature, 250 °C; interconnecting line temperature, 250 °C; ion source temperature, 250 °C; ion source pressure, 10⁻⁵ mmHg; trap current, 300 μA; filament voltage, 70 V; scan speed, 100 masses/s.

RESULTS AND DISCUSSION

Panel Evaluations. In Table II only the varieties with a significantly different flavor intensity score of the different comparisons (see Table I) are given. These significant differences were obtained by the ranking procedure of the individual flavor intensities.

Quantitative GC–MS Analyses. A typical gas chromatogram for the strawberry variety Sivetta obtained by adsorption on Tenax in standard conditions (see Experimental Section) of the volatiles liberated while macerating the fruits is given in Figure 1. The peak numbering is based on retention indexes and corresponds with the in-

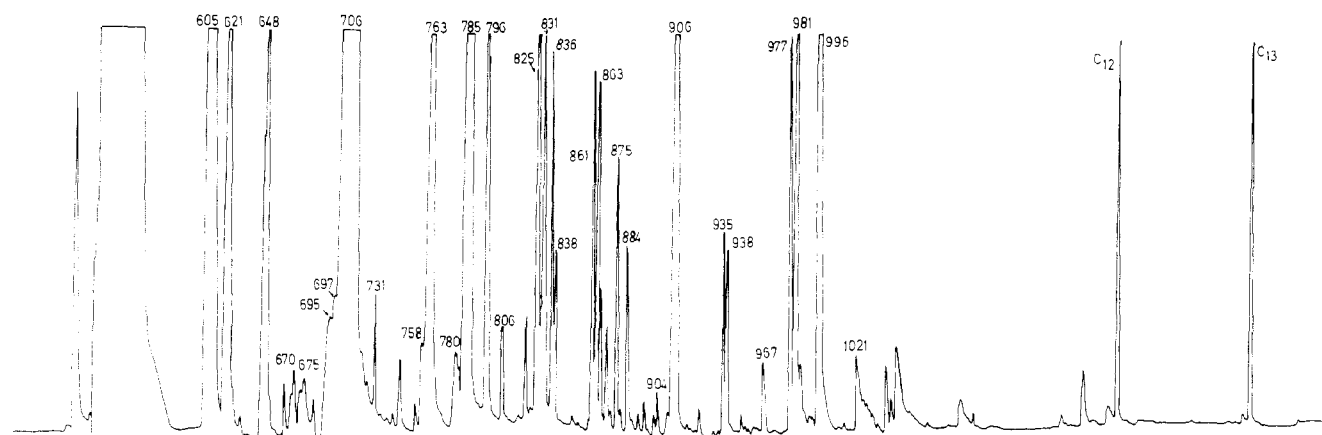


Figure 1. Typical gas chromatogram of the variety Sivetta. Peak numbering is by means of retention indexes. Dodecane (C_{12}) and tridecane (C_{13}) are internal standards.

Table III. Relative Amounts of Flavor Components, Isolated in Standard Conditions for the Sampling of May 3, 1977

flavor components	rel amount of flavor components, $\mu\text{g}/5\text{ L}$ of headspace							
	Ip-SE ₃₀	Confitura	Gorella	Induka	Jesco	Primella	Senge Gigana	Sivetta
ethyl acetate	605	182.585 ^a	1.566	3.637 ^a	2.067	36.663 ^a	0.631	15.329
methyl propionate	621	2.872 ^a	1.759	0.415 ^a	0.930	3.738 ^a	0.150	2.867
isopropyl acetate	648	9.227	3.653	0.522 ^a	3.611	4.120 ^a	0.139	1.406
methyl isobutyrate	670	—	0.629	—	—	1.319	—	0.170
methylthiol acetate	675	—	—	—	—	—	—	0.170
ethyl propionate	695	—	—	—	—	—	—	—
propyl acetate +	697	6.390	0.674	0.211 ^b	0.180	1.411	—	2.032
methyl butyrate	706	17.444	17.345	1.532 ^b	96.240	22.019	22.074	94.433
ethyl isobutyrate	743	1.983	—	—	—	—	—	—
isobutyl acetate	758	3.244	0.457	0.190 ^b	0.436 ^b	0.884	0.081	0.341
methyl isopentanoate	761	—	0.404	—	—	—	0.143	—
methyl 2-methylbutyrate	763	2.439	2.250	0.152 ^b	1.146 ^b	1.740	0.102	8.550
ethyl butyrate	785	35.494	0.796	0.817	4.224 ^b	11.153	3.875	20.117
butyl acetate	796	5.219	2.198	2.670	9.492 ^b	2.660	0.287	2.782
methyl pentanoate	806	0.212	—	—	0.324 ^b	0.280	0.245	0.402
isopropyl butyrate	825	1.780	0.350	0.105	8.870 ^b	1.394	0.527	1.727
ethyl 2-methylbutyrate	836	8.696	—	0.111	0.191	0.666	—	0.707
ethyl isopentanoate	838	0.662	—	0.160	—	—	—	0.494
isoamyl acetate	861	2.817	0.947	0.300	0.773	0.586	—	0.872
2-methylbutyl acetate	863	1.334	0.542	0.304	0.363	0.505	—	0.764
methyl isohexanoate	875	0.240	—	—	0.407	0.280	—	0.534
propyl butyrate	884	—	—	—	0.306	—	—	0.393
pentyl acetate	896	0.186	0.069	—	0.082	0.093	—	—
isopent-2-enyl acetate	904	0.519	0.278	—	0.152	0.199	—	0.108
methyl hexanoate	906	13.918	6.581	0.333	9.221	15.232	11.530	7.308
isobutyl isobutyrate	938	—	—	—	—	—	—	0.479
isohexyl acetate	967	—	0.151	—	0.187	—	—	0.287
butyl butyrate	977	0.119	0.030	0.200	3.459	0.986	0.162	1.101
ethyl hexanoate	981	25.299	0.187	0.238	0.685	7.656	2.777	1.762
cis-3-hexenyl acetate	987	0.378	0.236	0.188	0.101	0.216	0.108	0.164
hexyl acetate	996	7.185	8.242	4.412	3.674	5.254	4.625	3.991
isopropyl hexanoate	1021	—	—	—	0.482	0.463	—	0.528
2-methylbutyl isobutyrate	—	—	—	—	—	—	—	0.236
trans-2-hexenyl acetate	—	—	—	—	—	—	—	—
sum		330.247	49.344	17.172	147.603	119.517	47.636	170.054

^a Contaminated peak. ^b Disturbed base line.

dexes in Tables III and IV. By relating the electronic integration of the different aroma components to the intensity of 1 μg of hydrocarbon (mean of *n*-dodecane and *n*-tridecane), we calculated relative amounts and listed them in the tables. As an illustration, the relative amounts expressed as $\mu\text{g}/5\text{ L}$ of headspace of the liberated flavor components of the sampling of May 3, 1977, and of June 27, 1978, are respectively given in Tables III and Table IV.

The strawberry flavor is composed of a whole range of volatile organic esters, some aldehydes, *trans*-2-hexenal, *n*-hexanal, and 2,4-hexadienal, and sulfur-containing compounds (dimethyl disulfide, methylthiol butyrate, and

methylthiol acetate), which in the FID chromatogram are present in lower concentrations and can only be distinguished in some varieties. As can be seen from the tables, important qualitative and quantitative differences occur between the varieties. Some varieties have characteristic patterns, e.g., Jesco is the only variety with a more important concentration of butyl butyrate compared to ethyl hexanoate. The 2-alkanones, 2-pentanone, and 2-heptanone are also typical components, which only occur in the varieties Souvenir DCM and Domanil.

The esters are obviously the qualitatively and quantitatively most important class of volatiles in the strawberry

Table IV. Relative Amounts of Strawberry Flavor Components of the Sampling of June 27, 1978

flavor components	rel amount of flavor components, $\mu\text{g}/5\text{ L}$ of headspace					
	Ip-SE ₃₀	Souvenir	Bel Rubi	Geko	Sivetta	Gorella
ethyl acetate ^a	605	0.573	6.989	2.996	6.583	2.017
methyl propionate ^a	621	0.186	0.459	0.651	1.620	1.693
isopropyl acetate	648	0.400	0.272	0.888	0.545	0.894
methyl isobutyrate	670	—	—	0.070	0.133	0.125
2-pentanone	670	0.349	—	—	—	—
methylthiol acetate	675	0.156	0.134	—	—	—
ethyl propionate	695	—	—	—	—	—
propyl acetate	697	—	—	—	—	—
methyl butyrate	706	11.086	27.428	40.055	24.782	7.794
dimethyl disulfide	731	0.223	0.134	0.120	—	0.030
ethyl isobutyrate	743	—	—	0.099	—	—
isobutyl acetate	758	—	—	0.073	0.181	0.118
methyl isopentanoate	761	0.109	2.192	—	1.156	—
methyl 2-methylbutyrate	763	—	—	1.173	1.156	0.812
<i>n</i> -hexanal	780	0.137	0.132	0.058	2.745	0.094
ethyl butyrate	785	0.850	6.315	2.326	2.023	0.164
butyl acetate ^a	796	—	3.526	2.740	1.723	0.423
methyl pentanoate	806	0.124	0.114	0.189	0.016	0.021
isopropyl butyrate	825	0.759	0.665	2.090	0.458	0.121
<i>trans</i> -2-hexenal	831	0.238	0.185	0.083	6.434	0.077
ethyl 2-methylbutyrate	836	—	0.217	0.127	—	0.054
ethyl isopentanoate	838	—	0.720	0.222	—	0.037
isoamyl acetate	861	0.422	0.595	0.424	0.312	0.390
2-methylbutyl acetate	863	0.228	0.616	0.411	0.533	0.280
methylthiol butyrate	871	0.333	—	—	—	—
2-heptanone	873	0.459	—	—	—	—
methyl isohexanoate	875	—	0.086	0.148	0.303	0.162
propyl butyrate	884	—	0.273	0.133	—	—
2,4-hexadienal	890	—	—	—	—	—
butyl propionate	892	—	0.115	—	—	—
amyl acetate	896	—	0.080	—	—	—
isopent-2-enyl acetate	904	—	—	—	—	—
methyl hexanoate	906	0.580	3.498	6.292	1.879	1.891
benzaldehyde	935	—	—	—	—	—
isobutyl isobutyrate	938	—	0.155	—	—	—
isohexyl acetate	967	—	0.078	—	—	—
butyl butyrate	977	0.100	0.264	0.305	0.078	—
ethyl hexanoate	981	0.174	0.710	0.305	0.065	—
<i>cis</i> -3-hexenyl acetate	987	—	0.161	—	—	—
hexyl acetate ^a	996	0.226	1.893	0.641	0.316	—
isopropyl hexanoate	1021	—	0.053	—	—	—
limoneen	1021	0.158	—	—	—	—
2-methylbutyl isobutyrate	—	—	—	—	—	—
isobutyl valerate	—	—	0.061	—	—	—
<i>trans</i> -2-hexenyl acetate	—	—	—	—	—	—
sum		17.870	58.380	62.629	51.938	17.197

^a Contaminated peak.

flavor. The main components are these formed from volatile organic acids with an even carbon number such as acetic, butyric, and hexanoic acid. For the alcohol part in the ester formation methanol, ethanol, 2-propanol, butanol, and hexanol are important. Esters, which occur in lower concentration, are formed from propionic, isobutyric, isopentanoic, 2-methylbutyric, isohexanoic, or 2-methylpentanoic acid. The amount of the different esters that occur is dependent on the variety; e.g., Gorella has a larger concentration of acetates, and Jesco a more important concentration of butyrates. The organoleptic importance of the different ester types for the overall flavor is not clear. Determination of threshold values for the different ester types could perhaps give some indication.

n-Hexanal and *trans*-2-hexenal show very important concentration fluctuations. These aldehydes are formed enzymatically during maceration from the unsaturated fatty acids linoleic and linolenic acid. The mechanism has been thoroughly studied for tomatoes (Bonnet and Crouzet, 1977) and grapes (Drawert, 1974). In the season 1978 we also followed, by the same technique as described here, the evolution of the volatiles in the function of ma-

turation on the plant and after harvesting at immature stages. These experiments showed totally different patterns and important fluctuations for both aldehydes in the function of the maturity stages. Also, the concentrations of *n*-hexanal and *trans*-2-hexenal cannot be considered as variety characteristics but are connected with the maturity stage of the strawberries. They may be important for the "green" character of these fruits.

Relation of Flavor Quality and Volatile Composition. In Table V the sum of the concentrations of volatiles, released when macerating the fruits, for the different varieties and sampling dates is given. Table V shows a good relationship between the sensory analyses data (significant more or less flavor intensive varieties per comparison) and the sum of the volatiles, isolated by headspace adsorption on Tenax for the comparisons in the season 1977. So up to then, the sum of the volatiles isolated by the described procedure could be used as an objective measurement method for flavor quality evaluation. However, when some "older" varieties were included in the comparisons, as was the case with Souvenir in the season 1978, the individual flavor composition had to be taken into account. There-

Table V. Sum of Liberated Strawberry Volatiles for the Different Varieties and Sampling Dates

variety	sum of volatiles, $\mu\text{g}/5\text{ L}$ of headspace, at sampling date						
	May 3, 1977	June 31, 1977	June 25, 1977	June 27, 1978	June 21, 1979	July 3, 1979	July 12, 1979
Confitura	330 ^a	273	—	—	—	—	—
Gorella	49 ^b	21	110	20	59 ^b	41	140
Induka	17	—	—	—	—	—	—
Jesco	148	930 ^a	143	—	335 ^a	—	355
Primella	120	—	18	—	—	—	—
Senga Gigana	48	—	—	—	—	—	—
Sivetta	170	77	—	17	238	—	386
Red Gauntlet	—	43	—	—	—	39	—
Souvenir de Charles Machiroux	—	—	—	18 ^a	—	161 ^a	201
Bel Rubi	—	—	—	58	241	—	—
Geko	—	—	—	63	—	—	—
Domanil	—	—	—	—	—	360	—
Hapil	—	—	—	—	—	27 ^b	—

^a Sum of volatiles of varieties with significantly higher flavor intensity scores. ^b Sum of volatiles of varieties with significantly lower flavor intensity scores.

fore, we compared the volatile compositions of Souvenir and Gorella, which in the comparison of the June 27, 1978, compositions were respectively significantly more and significantly less flavor intensive than the other varieties and which had about an equal amount of total volatiles. On comparison, the striking difference between these two varieties was the occurrence of 2-pentanone and 2-heptanone and of higher concentrations of the sulfur-containing compounds methylthiol acetate, dimethyl disulfide, and methylthiol butyrate in the FID chromatogram of Souvenir. Sulfur compounds have generally very low threshold value and are important contributors to the flavor of different foodstuffs (Shankaranarayana et al., 1974; Schutte, 1974; Maga, 1975a,b, 1976). This hypothesis was supported by an organoleptic perception. When disintegrating the Souvenir strawberries in a Waring blender and sniffing the headspace, we observed a rotten odor, which after only a few minutes was dominated again by the fruity character. As the sulfur compounds occurred in relatively small concentrations compared to the esters, specific analyses for sulfur-containing compounds on a flame photometric detector were also performed.

Specific Analyses of the Sulfur-Containing Compounds. In the season 1979, besides the analyses on a FID, analyses on a FPD were also performed for some varieties. As an illustration, the FPD chromatograms taken in analogue isolation and GC conditions for the variety Gorella and Souvenir are given in Figure 2. Up to now no quantitative data for the sulfur compounds have been obtained but several analyses in analogue conditions for some varieties indicated a much more important amount of sulfur compounds for Souvenir compared to the other varieties (Gorella, Hapil, and Red Gauntlet). As can be seen from the chromatograms, besides methylthiol acetate, dimethyl disulfide, and methylthiol butyrate methanethiol and dimethyl sulfide could also be observed in the FPD chromatograms. Methylthiol acetate and methylthiol butyrate have been synthesized and exhibited a typical sulfury smell, which, however, is less intense than that of methanethiol. Probably the "agreeable rotten" note of some varieties is mainly caused by methanethiol which is formed enzymatically (by thiol esterase activity) from the thiol esters during desintegration of the fruits in the mouth and in the headspace concentration procedure.

CONCLUSION

As a conclusion we may state that besides the high concentration of volatile esters sulfur-containing compounds, which occur in much lower concentrations, also play an important role for the strawberry flavor. Besides

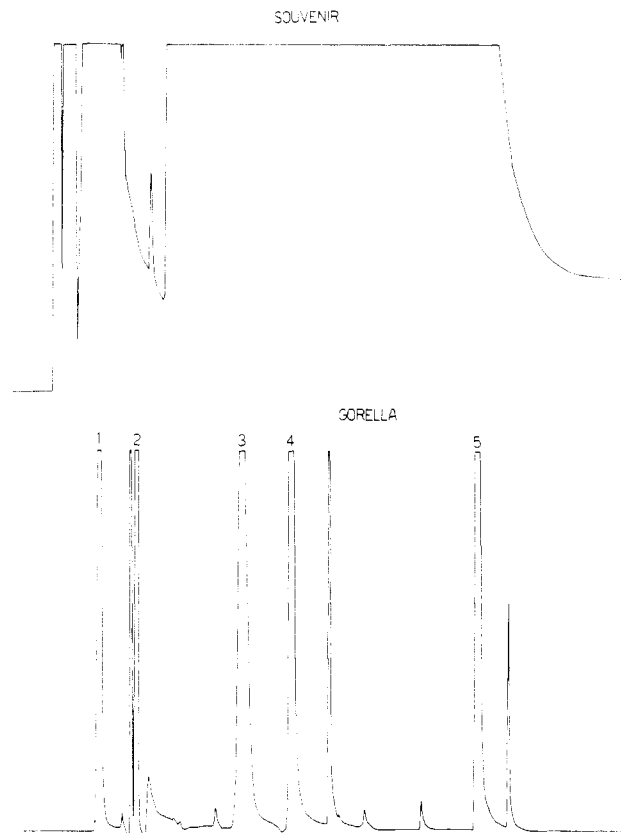


Figure 2. Specific analyses for sulfur-containing compounds. (1) Methanethiol; (2) dimethyl sulfide; (3) methylthiol acetate; (4) dimethyl disulfide; (5) methylthiol butyrate.

methanethiol, dimethyl sulfide, and dimethyl disulfide, methylthiol acetate and methylthiol butyrate have also been identified in strawberries. The latter products, which are identified for the first time in strawberries, are probably the precursors of the former. We also proved that objective flavor quality evaluations of strawberries are possible by measuring the total concentration of volatiles, released while macerating the fruits, and by specific analyses of the sulfur compounds. In both cases a representative isolation procedure, which imitates the mechanism of the mouth and the volatilization of the aroma components during maceration, is essential. For that purpose the headspace concentration on Tenax of disintegrated fruits is promising for quality evaluation of fresh fruits. From a practical point of view, it was established that important differences in flavor quality occur between

the different varieties, the flavor quality unfortunately not being in favor of the most commercially cultivated varieties.

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Determination of Chlorpyrifos and 3,5,6-Trichloro-2-pyridinol Residues in Peppermint Hay and Peppermint Oil

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Procedures are described for the determination of the insecticide *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (chlorpyrifos) and its metabolite 3,5,6-trichloro-2-pyridinol in mint hay and oil. After extraction, the chlorpyrifos residues are cleaned up on a silica gel column and quantitated by phosphorus-specific gas chromatography. The metabolite is separated from the extraction solvent by liquid-liquid partitioning with aqueous sodium carbonate, followed by chromatography on acid alumina. The column eluate is treated with *N,O*-bis(trimethylsilyl)acetamide and analyzed by electron capture gas chromatography. These methods are sensitive to 0.02 ppm in hay and to 0.1 ppm for the parent compound and 0.5 ppm for the metabolite in oil. Recoveries averaged 88%.

Field trials with Lorsban insecticide containing chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] as the active ingredient were encouraging for the control of red-backed cutworm and mint root borer in mint (Berry, 1978, 1979). As a result analytical methods for the analysis of this compound and its principal metabolite (3,5,6-trichloro-2-pyridinol) in mint hay and oil were needed. While a number of procedures for the analysis of chlorpyrifos in agricultural products have been described (Bowman and Beroza, 1967; Braun, 1974; McKellar, 1972, 1973; Maini and Collina, 1972), none were directly applicable to mint hay and oil and none provided for analysis of both chlorpyrifos and the pyridinol metabolite in a single sample extract. Struble and MacDonald (1973) and Dishburger et al. (1977) described column chromatographic procedures providing cleanup for chlorpyrifos in wheat and for the pyridinol metabolite in bovine tissues, respectively. This paper reports modifications of these methods to allow separation of sample extracts into chlorpyrifos and 3,5,6-trichloro-2-pyridinol fractions and to provide adequate removal of coextracted plant material.

EXPERIMENTAL SECTION

Apparatus and Reagents. A Varian 3740 gas chromatograph equipped with flame photometric and electron capture detectors was used. Standard laboratory glassware

was used throughout the procedure. Analytical-grade chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] and 3,5,6-trichloro-2-pyridinol were obtained from Dow Chemical Co., Midland, MI. *N,O*-Bis(trimethylsilyl)acetamide (BSA) was obtained from Pierce Chemical Co., Rockford, IL. All solvents were distilled in glass.

Gas Chromatography. A flame photometric detector operated with the 530-nm interference filter for phosphorus was used for the determination of chlorpyrifos. The detector temperature was 230 °C with gas flows to the detector of 140, 80, and 170 mL/min for hydrogen, air 1, and air 2, respectively. A 45 × 0.2 cm i.d. column packed with 5% OV-101 on 120-140-mesh Chromosorb WHP was used. Temperatures were column 175 °C and inlet 200 °C. The nitrogen carrier gas flow was 30 mL/min.

A ⁶³Ni electron capture detector and a 240 × 0.3 cm column packed with 10% OV-1 on 100-120-mesh Chromosorb WHP were used for the detection of 3,5,6-trichloro-2-pyridinol. The column, inlet, and detector temperatures were 175, 200, and 260 °C, respectively. The nitrogen carrier gas flow was 30 mL/min, and the makeup gas flow to the detector was 8 mL/min.

Quantitation was carried out by peak height comparison of at least three closely matched pairs of sample and standard injections for both chlorpyrifos and 3,5,6-trichloro-2-pyridinol.

Procedure. Chlorpyrifos in Hay. Hay samples were chopped in a mechanical food chopper and mixed thor-

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