

# Characterization of Some Volatile Constituents of Bell Peppers

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The steam volatile components of Californian green bell peppers (*Capsicum annuum* var. *grossum*, Sendt) have been qualitatively analyzed using conventional and capillary gas-liquid chromatographic separation with characterization by mass, infrared, and in some cases ultraviolet and proton magnetic resonance spectra. Major components identified in oil obtained by vacuum steam-distillation-continuous-extraction included 2-methoxy-3-isobutylpyrazine, *trans*- $\beta$ -ocimene, limonene, methyl salicylate, linalool, nona-*trans*,*cis*-2,6-dienal, deca-*trans*,*trans*-2,4-dienal, and hex-*cis*-3-enol. Additional components detected in small amounts in the vacuum isolated oil but in much larger amounts in oil isolated at atmospheric pressure were non-1-en-4-one, non-*trans*-2-en-4-one, nona-*trans*,*trans*-2,5-dien-

4-one, 2-pentylfuran, and benzaldehyde. The identities of 24 components were confirmed by direct comparison of their spectral and gas chromatographic retention data with those of authentic samples. Tentative identification was obtained for an additional 19 components from their mass spectral fragmentation patterns. Odor thresholds in water solution were determined for major components. 2-Methoxy-3-isobutylpyrazine was found to possess an extremely potent odor with an odor threshold of 2 parts per 10<sup>12</sup> parts of water. Generally about 70 to 80% of untrained sensory panelists (40) indicated that the odor of dilute water solutions of this compound was similar to that of fresh green bell peppers.

Green bell peppers are widely accepted both for use as food and as flavoring for other foods (Heiser and Smith, 1953). They differ from most other members of the genus *Capsicum* in that they generally do not have the "hot" taste associated with the Chili and Tabasco types. This "hot" taste has long been known (Nelson and Dawson, 1923) to be due to the presence of the essentially nonvolatile compound capsaicin (*N*-(4-hydroxy-3-methoxy-benzyl)-8-methyl-non-*trans*-6-enamide).

Green bell peppers possess a characteristic pleasant aroma, which enhances the flavor of many other fresh and cooked foods. This paper deals with the isolation, identification, and sensory characterization of some volatile compounds responsible for this aroma. Some preliminary results of part of this work were reported (Buttery *et al.*, 1969).

## EXPERIMENTAL

**Materials.** Fresh green bell peppers (*Capsicum annuum* var. *grossum*, Sendt) were obtained from a local wholesale market during the 1968 season.

Authentic samples of chemical compounds were obtained from reliable commercial sources or synthesized by well established methods. They were purified by gas chromatographic separation before use.

**Isolation of Vacuum Steam Distillable Oil.** The green bell peppers were cut open, the seeds removed, and the peppers macerated in a Waring blender. The resultant puree was treated in a vacuum steam-distillation-continuous-extraction apparatus (Nickerson and Likens, 1966; cf. Vogel, 1962, Wiberg, 1960) similar to that described previously for the isolation of the vacuum steam volatile oil of carrots (Buttery *et al.*, 1968). The apparatus was operated at 80 to 100 mm. Hg pressure with the product at 45° to 50° C. Hexane was used as the extracting solvent and the condensers were cooled with ice water. The extraction was carried out over a 3-hour period. The extract was dried over a small quantity of sodium sulfate, filtered, and the solvent carefully removed through low holdup fractional distillation columns to give the bell

pepper oil (1 to 2 p.p.m. of the whole pepper). In a typical isolation 5,100 grams of bell pepper puree was placed in a 12-liter flask together with 2 liters of odor free triple distilled water. Hexane (100 ml., 95%, purified further by fractional distillation through a 20-plate column) was used in the continuous extracting arm of the apparatus. The extracted oil, after removal of hexane by distillation, amounted to 10.5 mg.

**Isolation of Atmospheric Pressure Steam Distillable Oil.** This was carried out in the same apparatus as for the vacuum method except that atmospheric pressure was used, the puree was at 100° C., and pentane was the extracting solvent. Extraction periods of 1 to 5 hours gave a yield of volatile oil which varied from 10 to 20 p.p.m. of the whole pepper.

**Capillary GLC Mass Spectral Analysis.** A 1,000-foot  $\times$  0.03-inch I.D. stainless steel capillary column coated with silicone SF 96-100 containing 5% Igepal CO-880 was used. The column was programmed from 70° to 170° C. at 1/2° per minute and held. Nitrogen at a 20-p.s.i. inlet pressure was the carrier gas. The effluent from the end of the column was split, about 10% going to a flame ionization detector and the rest to a silicone rubber membrane molecular separator of the Llewellyn type (Llewellyn and Littlejohn, 1966; Black *et al.*, 1969). The separator introduced components into a modified Consolidated 21-620 cycloidal type mass spectrometer. The outlet from the molecular separator to the atmosphere generally contained sufficient excess sample for informal sensory odor detection and evaluation. Compounds were identified by interpretation of their mass spectra followed by comparison with the mass spectra of an authentic sample measured on the same instrument. This identification was further verified by comparison of the capillary GLC retention time of the authentic sample with that of the unknown component in the pepper oil.

High resolution mass spectra were determined on some samples with a Consolidated 21-110B double focusing mass spectrometer, using batch introduction.

**Separation of Samples for Infrared (IR) and Proton Magnetic Resonance (PMR) Spectra.** For measurements of IR spectra of major components, samples were separated from the 1,000-foot  $\times$  0.03-inch I.D. capillary described above and collected in 150  $\times$  1 mm. borosilicate glass melting point tubes which were sealed at both ends immediately after collection and

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stored at  $-20^{\circ}\text{C}$ . until the spectra could be measured (less than 24 hours).

Larger amounts of samples were isolated using a 10-foot  $\times$   $\frac{1}{4}$ -inch O.D. Silicone SF 95-350 packed GLC column, and collecting the samples in  $150 \times 3$  mm. barosilicate glass tubes. Samples separated from the silicone column were repurified through a 5-foot  $\times$   $\frac{1}{4}$  inch O.D. Carbowax 20 M packed column.

IR spectra were recorded with a Perkin-Elmer 237 double beam grating instrument, generally using carbon disulfide or carbon tetrachloride solutions, with ultra-micro cavity cells and a reflecting beam condenser.

PMR spectra were recorded in carbon tetrachloride, using a Varian HA-100 at 100 MHz, generally with time averaging.

UV spectra were recorded in methanol, using a Cary 15.

**Odor Thresholds.** Thresholds of the GLC purified compounds were determined as described previously (Guadagni *et al.*, 1963) except that Teflon (Du Pont), instead of polyethylene, bottles and tubing were used as containers for the odor solutions.

## RESULTS AND DISCUSSION

The green bell pepper oil (*ca.* 2 p.p.m. of the whole pepper) obtained by vacuum steam-distillation-continuous-extraction had an aroma similar to that of the original peppers. During isolation the peppers were at a temperature no greater than  $45^{\circ}$  to  $50^{\circ}\text{C}$ . Figure 1 shows a capillary GLC analysis of the oil obtained. Table I lists the identities found for the constituents together with the type of evidence used for the identification. As can be seen, the major peaks are limonene, *trans*- $\beta$ -ocimene, 2-methoxy-3-isobutylpyrazine, and methyl salicylate.

Steam-distillation-continuous-extraction at atmospheric pressure (peppers at  $100^{\circ}\text{C}$ .) gave an oil (*ca.* 10 to 20 p.p.m. of the whole pepper) with relative amounts of some components quite different from those found in the vacuum isolated oil. Figure 2 shows a capillary GLC analysis of the oil extracted at atmospheric pressure, with the same peak numbering system as used in Figure 1 and Table I.

Relative percentages of components in both oils (as determined from GLC peak areas) are shown in Table I. These figures represent the percentages found in only one sample of oil for each isolation condition, although they were fairly representative of several samples studied by the authors. The values are dependent to some extent on the isolation method and gas chromatographic conditions used. They can only be expected to give an approximation of relative amounts of components as they occur in the pepper.

Spectral data found for the constituents listed in Table I (except for common food components whose spectra are well known) were as follows.

Mass spectral data (above  $m/e$  40, intensities in parentheses with base peak taken as 100) were as follows:

Hept-*trans*-3-en-2-one, molecular ion 112(28), major ions 55(100), 43(85), 97(55), 41(45), 69(11), 42(11), 71(5), 53(5), 56(5).

2-Pentylfuran, molecular ion 138(12), major ions, 81(100), 82(22), 53(15), 41(12), 95(5), 83(4), 68(3.5), 67(3.5), 94(3), 51(3), 43(2.5).

*trans*- $\beta$ -Ocimene, molecular ion 136(1.3), major ions, 93(100), 41(72), 80(37), 79(36), 91(29), 69(28), 77(28), 92(23), 53(22), 43(22), 121(2).

Non-1-en-4-one, molecular ion 140(1), major ions 43(100), 41(73), 69(60), 99(54), 71(41), 55(21), 84(20), 42(19), 44(8), 100(7), 125(2).

Non-*trans*-2-en-4-one, molecular ion 140(0.6), major ions 69(100), 41(65), 84(62), 43(23), 70(14), 55(11), 42(9), 85(8), 125(7).

Nona-*trans,trans*-2,5-dien-4-one, molecular ion 138(9), major ions 69(100), 41(98), 55(63), 97(26), 95(26), 109(21), 43(20), 123(14), 53(10), 81(10).

Phenylacetaldehyde, molecular ion 120(35), major ions 91(100), 65(34), 92(34), 51(15), 63(13), 50(9), 89(6), 62(5), 77(3).

Nona-*trans,cis*-2,6-dienal, molecular ion 138(0.6), major ions 41(100), 69(82), 70(68), 67(26), 53(19), 55(18), 68(18), 42(15), 81(11), 79(8), 109(7).

2-Methoxy-3-isobutylpyrazine, molecular ion 166(3.5), major ions 124(100), 41(21), 94(20), 151(16), 39(13), 43(11), 95(11), 53(11), 42(11), 93(10), 81(9), 125(8).

Deca-*trans,cis*-2,4-dienal, molecular ion 152(7), major ions 81(100), 41(26), 83(21), 67(21), 55(17), 95(14), 82(14), 68(11), 54(10), 79(10), 53(9).

Deca-*trans,trans*-2,4-dienal, molecular ion 152(8), major ions, 81(100), 41(42), 67(21), 55(21), 83(19), 82(17), 95(14), 54(13), 42(12), 53(10), 68(10).

Infrared absorption spectra found (in the region of 2.5 to 15 microns, absorption maxima in microns, S means strong, M means medium, and W means weak) were as follows:

*trans*- $\beta$ -Ocimene, ( $\text{CS}_2$ ), S(3.37, 3.44, 10.1, 11.1); M(3.25, 3.51, 6.1, 7.3, 9.1, 12.1); W(5.6, 7.9, 8.2, 8.7, 9.3, 10.4, 10.8, 11.6, 13.2).

Non-1-en-4-one, ( $\text{CCl}_4$ ), S(3.38, 3.41, 5.8, 10.8); M(3.48, 3.5, 6.1, 6.15, 6.8, 7.1, 7.3, 8.9, 9.4, 10.0); W(3.25, 3.30, 7.0, 7.6, 8.0, 8.3, 9.7, 11.8).

Non-*trans*-2-en-4-one, ( $\text{CCl}_4$ ), S(3.39, 3.41, 5.9, 5.96, 6.1, 10.3); M(3.5, 6.8, 6.9, 7.3, 7.3, 7.7, 8.4, 8.8, 9.4); W(3.3, 7.1, 7.6, 8.0, 8.2, 11.7).

Nona-*trans,cis*-2,6-dienal, ( $\text{CS}_2$ ), S(3.39, 3.43, 5.94, 10.3); M(3.33, 3.51, 3.57, 6.1, 8.5, 8.84); W(3.68, 5.8, 7.2, 7.3, 7.5, 7.7, 9.3, 9.9, 13.5, 14.3).

2-Methoxy-3-isobutylpyrazine, ( $\text{CS}_2$  and  $\text{CCl}_4$ ), S(3.39, 3.43, 3.50, 6.49, 6.85, 6.9, 7.2, 7.7, 8.6, 9.9); M(3.28, 3.46, 7.4, 8.4, 9.4, 11.8); W(3.33, 6.34, 7.9, 8.1, 9.2, 10.5, 10.8, 11.2, 13.3).

Nona-*trans,trans*-2,5-dien-4-one, ( $\text{CCl}_4$ ), S(3.39, 3.42, 6.0, 6.11, 6.19, 10.2); M(3.48, 5.95, 6.9, 7.3, 7.5, 7.7, 8.4, 8.7, 9.3); W(3.3, 3.33, 8.0, 8.2, 9.0, 9.6, 11.0, 11.7).

**Characterization of 2-Methoxy-3-isobutylpyrazine.** Some compounds were not easily identified from their mass and infrared absorption spectra alone, and it was necessary to obtain additional information on the samples. The most important of these was 2-methoxy-3-isobutylpyrazine (I). Its mass spectrum was initially difficult to interpret showing a molecular ion at  $m/e$  166, only 3.5% of the most intense ion at  $m/e$  124. The molecular ion undergoes a rearrangement, losing a propene fragment ( $m/e$  42) and giving the ion at  $m/e$  124. This is apparently characteristic of pyrazines that have a saturated aliphatic side-chain longer than 2 carbon atoms (Biemann, 1962). In the spectra published by Bondarovich *et al.* (1967) this type of fragmentation appeared only with 2-propylpyrazine and not with pyrazines having side-chains shorter than three carbons or with unsaturated propenyl side-chains.

The infrared and UV spectra of peak 39 showed some similarities to that of pyrazines published by Bondarovich *et al.* (1967). High resolution mass spectrometry showed a molecular weight of 166.1102, and therefore was consistent with the formula  $\text{C}_9\text{H}_{14}\text{N}_2\text{O}$ . The PMR spectrum of peak 39 was the most informative (Buttery *et al.*, 1969) and showed clearly an

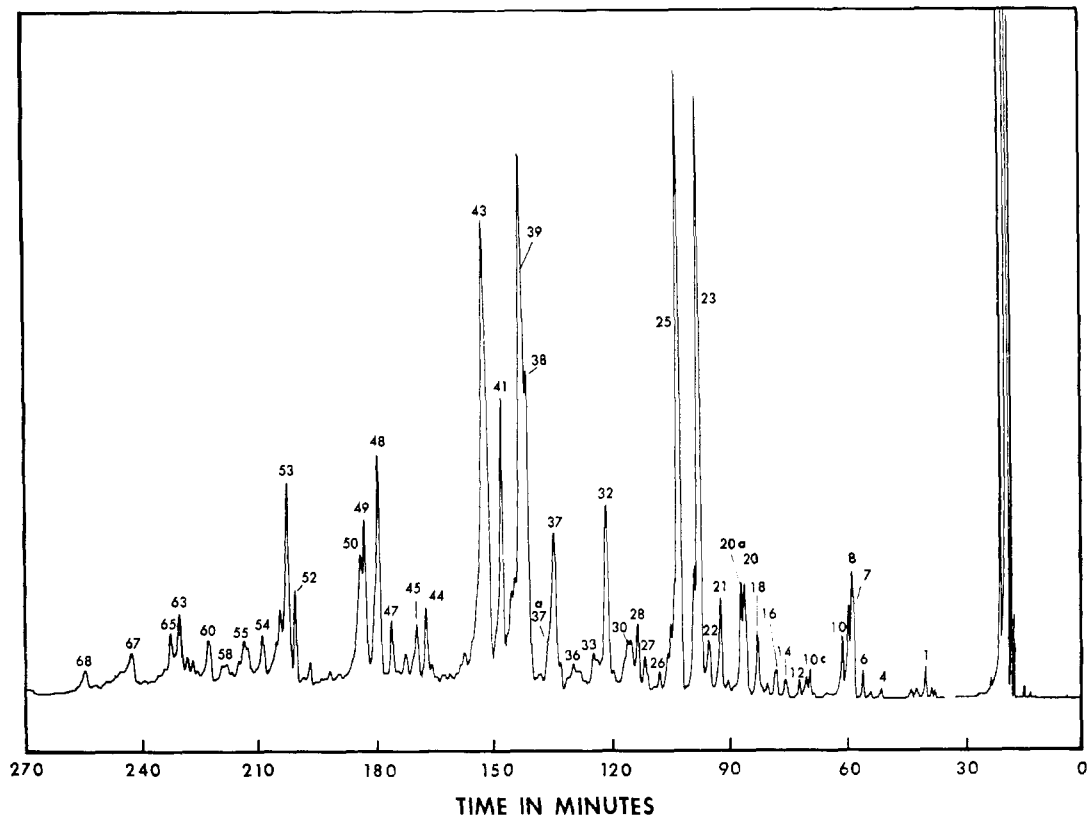


Figure 1. Temperature programmed capillary GLC analysis of Bell pepper oil obtained by 3 hour *vacuum* steam-distillation-continuous-extraction of bell peppers (peppers at 45° to 50° C.)

Column 1000-foot  $\times$  0.03-inch I.D. stainless steel capillary coated with silicone SF 96-100 containing 5% Igepal CO-880, programmed from 70° to 170° C. at  $\frac{1}{2}$ ° per minute and held

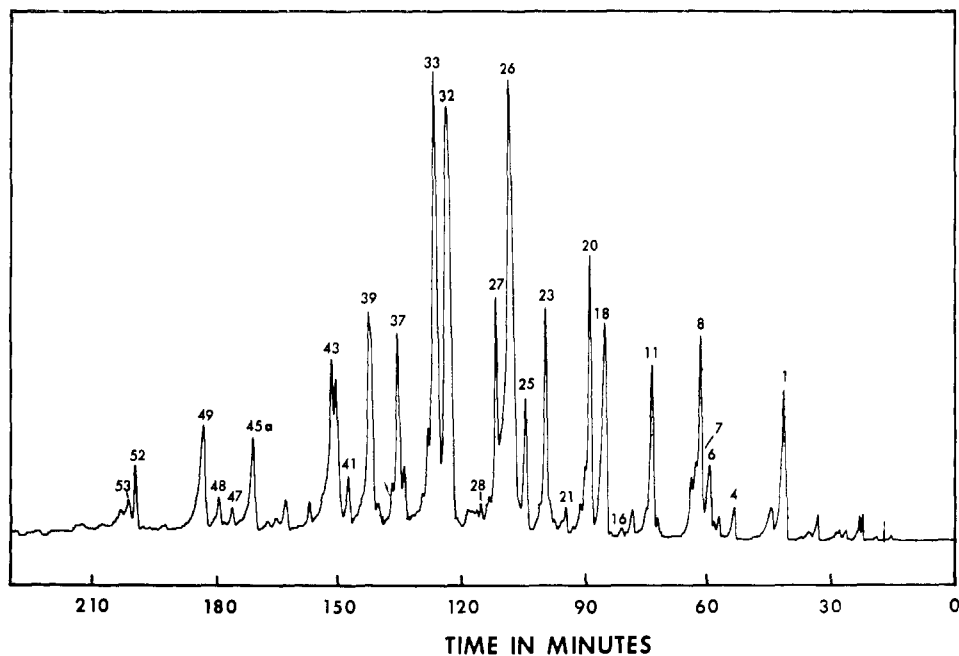


Figure 2. Temperature programmed capillary GLC analysis of Bell pepper oil obtained by 5 hour *atmospheric* steam-distillation-continuous-extraction of bell peppers (peppers at 100° C.)

Peak numbering system as for Figure 1 and Table I. GLC conditions as for Figure 1

isobutyl and a methoxy group attached in adjacent positions on a heterocyclic aromatic ring such as pyrazine.

2-Methoxy-3-isobutylpyrazine had not been reported in the literature until the authors' recent communication (Buttery *et al.*, 1969). It was synthesized from the amino acid leucine by the following steps: (1) conversion of leucine to its

methyl ester by reaction with methanol and anhydrous HCl; (2) conversion of the methyl ester to leucine amide by reaction with anhydrous ammonia in methanol; (3) condensation of the leucine amide with glyoxal to give 2-hydroxy-3-isobutylpyrazine; (4) reaction of 2-hydroxy-3-isobutylpyrazine with diazomethane to give 2-methoxy-3-isobutylpyrazine.

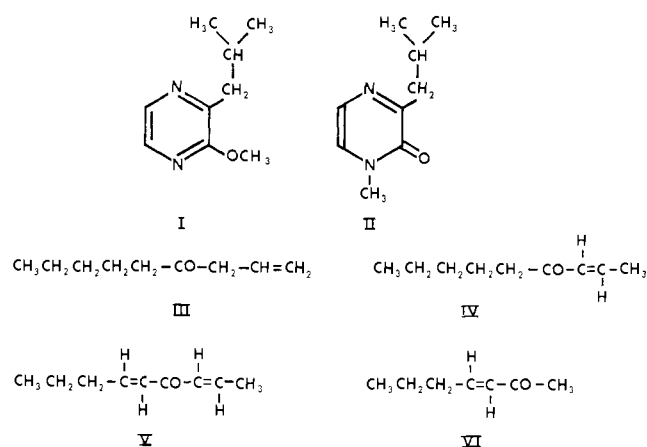
Table I. Identities of Constituents of Steam Volatile Bell Pepper Oil

Peak No. (Figures 1 and 2)	Confirmed Identity <sup>a,b</sup>	Predicted Identity	Approx. Relative % in Oil	
			Vacuum isolated	Atmospheric isolated
1	Hexanal (MS, RT)		0.3	2
4	Hex- <i>trans</i> -2-enal (MS, IR, RT)		0.2	0.5
6	Furfural (MS, RT) plus	Xylene (MS)	0.3	1
7	Hex- <i>cis</i> -3-enol (MS, IR, RT)			
8	Heptan-2-one (MS, RT)		1.5	4
10		Styrene (MS)	0.6	...
10C		Terpene hydro- carbon (MS)	0.3	...
11	Hept- <i>trans</i> -3-en-2-one (MS, RT)		0.3	2
12		Terpene hydro- carbon (MS)	0.2	...
14		Trimethyl-ben- zene (MS)	0.2	...
16		Ethyltoluene (MS)	0.3	...
18	Benzaldehyde (MS, IR, RT)		1	5
20	2-Pentylfuran (MS, RT)		1	4
20a		Trimethylbenzene (MS)	1	...
21		Terpene hydro- carbon (MS)	1	0.04
22		Diethylbenzene (MS)	0.5	...
23	Limonene (MS, IR, RT)		10	3
25	<i>trans</i> , $\beta$ -Ocimene (MS, IR, RT)		11	2
26	Non-1-en-4-one (MS, IR, RT)		0.2	15
27	Phenylacetaldehyde (MS, RT)		0.6	2
28		Alkylbenzene MW 134 (MS)	0.7	...
30		Terpenoid alco- hol (MS)	1	...
32	Linalool (MS, IR, RT)		3	12
33	Non- <i>trans</i> -2-en-4-one (MS, IR, RT)		0.5	12
36		Alkylbenzene MW 134 (MS)	0.5	...
37	Nona- <i>trans</i> , <i>cis</i> -2,6- dienal (MS, IR, RT)		3	4
38		Alkylmethoxy- pyrazine MW 166 (MS)	16	6
39	2-Methoxy-3-isobutyl- pyrazine, (MS, IR, PMR, UV, RT) plus Nona- <i>trans</i> , <i>trans</i> -2,5- dien-4-one (MS, IR, RT)			
41	Naphthalene (MS, RT)		3	1
43	Methyl salicylate (MS, IR, RT)		11	2
44		Triethylbenzene (MS)	1	...
47	Deca- <i>trans</i> , <i>cis</i> -2,4-dienal (MS, RT)		0.7	0.5
48	2-Methylnaphthalene (MS, RT)		4	0.6
49	Deca- <i>trans</i> , <i>trans</i> -2,4-dienal (MS, RT)		2	4
50	1-Methylnaphthalene (MS, RT)		2	...
52		Alkylbenzene MW 162 (MS)	1	1
53	Biphenyl (MS, IR, RT)		3	0.6
54		Dimethyl naph- thalene (MS)	1	...
55		Dimethylnaph- thalene (MS)	1	...
60		Methoxynaph- thalene (MS)	1	...
63		Acenaphthene (MS)	1.5	...

<sup>a</sup> MS, IR, PMR, UV, RT = mass, infrared absorption, proton magnetic resonance, and ultraviolet absorption, spectral and GLC retention evidence, respectively.

<sup>b</sup> Evidence cited under this column is consistent with that of authentic compound.

Steps 1 through 3 proceed in good yield. The last step gives the 2-methoxy-3-isobutylpyrazine in about 25% yield, which is sufficient for the purpose. The major product formed in this last step is 1-N-methyl-2-keto-3-isobutyl-1,2-dihydropyrazine (II). This keto compound, however, has a much higher boiling point than the 2-methoxy-3-isobutylpyrazine and is readily separated by GLC or distillation.



**C<sub>9</sub> Unsaturated Ketones.** The C<sub>9</sub> ketones non-1-en-4-one (III), non-*trans*-2-en-4-one (IV), and nona-*trans,trans*-2,5-dien-4-one (V) were also unusual compounds and had not previously been reported, either as food components or in their synthetic form. They are, however, somewhat related to oct-1-en-3-one, reported as a metallic- or mushroom-flavored component of dairy products (Stark and Forss, 1962). Their structure was deduced largely from their IR and mass spectra. Peak 34 was the first characterized of the group. Its IR spectrum with maxima at 5.9, 5.96, and 6.1 microns indicated a carbonyl group conjugated with a double bond. The IR absorption at 10.3 microns indicated that the double bond was *trans*. The mass spectrum showed that the molecular weight was 140 and that the major ion had *m/e* 69, which was thought could correspond to the ion CH<sub>3</sub>-CH=CH-CO<sup>+</sup>. Assuming that the molecule was a straight chain, this then fixed the position of both the double bond and carbonyl group. Other ions at *m/e* 84, 125, and 97 were consistent with this. The combined data indicated that peak 34 was non-*trans*-2-en-4-one (IV). This compound was synthesized by chromic acid oxidation of non-*trans*-2-en-4-ol, itself formed from the condensation of the Grignard reagent from 1-bromopentane with but-*trans*-2-enal. The synthetic compound, after purification by GLC, had IR, mass spectra, and GLC retention time identical with that of peak 34.

Peak 26 was found to rearrange to non-*trans*-2-en-4-one under acidic and high temperature conditions. Its mass spectrum indicated a molecular weight of 140. IR absorption spectrometry showed maxima at 5.8 microns, indicating a carbonyl not conjugated with any double bonds, and maxima at 3.25, 6.1, 10.0, and 10.8 microns, indicating a terminal vinyl group. Non-1-en-4-one (III) was therefore suspected and subsequently synthesized by mild chromic acid oxidation of non-1-en-4-ol formed from the condensation of the Grignard reagent from allyl bromide with hexanal. The GLC purified synthetic compound had IR, and mass spectra and GLC retention time identical to that of peak 26.

Peak 40 had an odor similar to non-*trans*-2-en-4-one and non-1-en-4-one indicating that it might be a related compound, although this is not a reliable basis for structure determination. The mass spectrum with a major ion at *m/e* 69 also showed similarities to non-*trans*-2-en-4-one. The molecular ion at

138, however, indicated a diunsaturated ketone. The IR spectrum was rather unusual and not typical of the commonly encountered diunsaturated carbonyl compounds. PMR spectra with two doublets at 4.75 and 4.9 p.p.m. (*ca.* 1H each) and complex overlapping multiplets between 5.1 and 5.5 p.p.m. (*ca.* total of 2H) together with the absence of any evidence for a saturated CH<sub>2</sub> group next to the "carbonyl" (no maxima between 2.0 and 2.5 p.p.m.) indicated that the two double bonds were attached each side of the carbonyl. By analogy with the other C<sub>9</sub> unsaturated ketones the carbonyl was expected in the 4 position. This was supported by a doublet in the PMR spectrum at 1.25 p.p.m. (*ca.* 3H, each peak of the doublet showing further splitting on higher resolution indicating the *trans* configuration) and also the major mass spectral ion at *m/e* 69, both suggesting the arrangement —CH=CH—CH<sub>3</sub>. Nona-*trans,trans*-2,5-dien-4-one (V) was synthesized by the condensation of the Grignard reagent from *trans*-1-bromoprop-1-ene with *trans*-2-hexenal. The ketone was formed directly in the condensation. No intermediate alcohol could be isolated, apparently it is so readily oxidized that it is not stable in air. The purified synthetic ketone had IR, mass spectra, and GLC retention time identical to peak 40.

All three C<sub>9</sub> ketones possessed aromas which in dilute solution could be described as mushroom-like and in more concentrated form as sweet.

It is interesting that two C<sub>7</sub> ketones, 2-heptanone (peak 4), and hept-*trans*-3-en-2-one (VI), also occur in the oil. The latter compound is somewhat related to the diunsaturated C<sub>9</sub> ketone. It was synthesized in a similar way by oxidation of hept-*trans*-3-en-2-ol formed from the condensation of the Grignard reagent of methyl bromide with hex-*trans*-2-enal.

The concentration of the C<sub>9</sub> ketones is very small in the oil obtained using the steam-distillation-extraction procedure under vacuum. However, they are apparently formed by heating and increase in concentration rapidly on heating so that after an hour's steam-distillation-continuous-extraction at atmospheric pressure the monounsaturated C<sub>9</sub> ketones together with linalool form the bulk of the oil, and compounds that were major in the vacuum-distilled oil become relatively minor. Other compounds that also increase in concentration but to a lesser extent are hexanal, furfural, 2-heptanone, benzaldehyde, 2-pentylfuran, phenylacetaldehyde, nona-*trans,trans*-2,6-dienal, and deca-*trans,trans*-2,4-dienal. Some of these lesser-increased compounds are known as products of fatty acid autoxidation. As the C<sub>9</sub> ketones are straight chain, it seems possible that they may also arise from fatty acids by some kind of controlled oxidation. However, during the steam-distillation-extraction at atmospheric pressure, it is doubtful whether there is much contact of the product with air because the container would be filled with steam. Lipid autoxidation also would not explain the formation of linalool, a terpenoid, on heating.

In an experiment aimed at learning which compounds were formed by air oxidation a quantity (5000 grams) of bell peppers was macerated in a Waring blender and allowed to stand at room temperature in contact with the air for 24 hours before isolation of volatiles by vacuum steam-distillation-continuous-extraction. The oil obtained gave a very similar GLC pattern to that in Figure 1, except for a *ca.* 10-fold increase for peaks at the retention times of the *trans,trans*- and *trans,cis*-deca-2,4-dienal. There was also a several-fold increase in the relative size of the peak corresponding to the retention time of nona-*trans,cis*-2,6-dienal. There was no apparent increase in the relative size of the peaks corresponding to the retention times of the C<sub>9</sub> unsaturated ketones.

This formation of unsaturated aldehydes probably results from some type of enzyme-catalyzed (and directed) oxidation of the lipid, and is somewhat similar to the increase in *nona-trans,cis-2,6-dienal* concentration in macerated cucumbers exposed to air (Fleming *et al.*, 1968). The character of the aroma of several samples of macerated bell peppers was somewhat similar to that of cucumber after the macerated pepper was allowed to stand several hours. *Nona-trans,cis-2,6-dienal*, of course, is well known to possess a very potent cucumber odor (Forss *et al.*, 1962).

**Other Components.** Most components listed in Table I are known to occur in other foods and were generally readily identified from their mass spectral patterns. Hexanal and *hex-cis-3-enol* occur in most green vegetables. The concentration of *hex-cis-3-enol* in the pepper may be greater than that indicated by Table I because the steam-distillation-continuous-extraction procedure used with hydrocarbon solvent is inefficient in removing low boiling compounds which are relatively water soluble.

The terpenoids, limonene, linalool, and *trans-β-ocimene* occur in a number of essential oils although *trans-β-ocimene* is considerably less common. Benzaldehyde, furfural, heptan-2-one, phenylacetaldehyde, and methyl salicylate occur in a number of foods.

The large variety of aromatic hydrocarbons may arise from a possible petroleum-based spray used on the pepper plants by the growers. However, it is rather surprising that the aromatic compounds are not accompanied by the common petroleum aliphatic hydrocarbons. Pippen, Mecchi, and Nonaka (1969) have proposed that a similar series of aromatic hydrocarbons found in chicken might arise from degradation of carotenoids. It is possible that the aromatic compounds in bell peppers have a related origin.

**Aroma Significance of Bell Pepper Components.** In informal preliminary sensory evaluation of components as they left the end of the capillary GLC column, it was readily apparent to the authors that the odor from peak 39, 2-methoxy-3-isobutylpyrazine, was very similar to that of bell pepper. Other odors readily recognized in the vacuum isolated oil were those from peak 37 (*nona-trans,cis-2,6-dienal*) as cucumber odor, peaks 47 and 49 (*deca-2,4-dienals*) as fried chicken, peak 23 (limonene) as citrus, and peak 43 (methyl salicylate) as oil of wintergreen. In the oil obtained at atmospheric pressure, the  $C_9$  ketones had sweet mushroom-like odors.

Table II lists the odor thresholds in water solution found for most of the components identified. 2-Methoxy-3-isobutylpyrazine, with a threshold of 2 parts in  $10^{12}$ , had the lowest threshold of any compound examined over the last several years by the authors. Its air/water partition coefficient at 25° C., as determined by the authors, was  $1.8 \times 10^{-3}$  (wt. compd. per ml. air/wt. compd. per ml. water), which gives a calculated odor threshold in air of  $3.6 \times 10^{-15}$  gram/ml. or  $1.3 \times 10^7$  molecules per ml. The fact that it is also a major component of the vacuum distilled oil indicates that it must be important to bell pepper aroma. About 70 to 80% of 40 untrained panelists who were asked to characterize the aroma of dilute water solutions of 2-methoxy-3-isobutylpyrazine (100 to 300 p.p.b.) described it as being like that of green bell peppers. A preliminary capillary GLC mass spectral analysis of the vacuum steam volatile oil of chili peppers showed that 2-methoxy-3-isobutylpyrazine was present in the same order of concentration as found in bell peppers, and it is likely that this compound is also important to the aroma of chili and other varieties of peppers.

**Table II. Odor Thresholds of Some Bell Pepper Constituents**

Component	Threshold in P.P.B.
Hexanal	4.5
Furfural	3000
Hex- <i>trans</i> -2-enal	17
Heptan-2-one	140
Hex- <i>cis</i> -3-enol	70
Hept- <i>trans</i> -3-en-2-one	56
Benzaldehyde	350
2-Pentylfuran	6
Limonene	10
Non-1-en-4-one	0.4
Phenylacetaldehyde	4
Linalool	6
Non- <i>trans</i> -2-en-4-one	0.9
<i>Nona-trans,cis-2,6-dienal</i>	0.01
2-Methoxy-3-isobutylpyrazine	0.002
<i>Nona-trans,trans-2,5-dien-4-one</i>	4
Methyl salicylate	40
<i>Deca-trans,trans-2,4-dienal</i>	0.07

Other important aroma components are *nona-trans,cis-2,6-dienal* with a threshold of 0.01 p.p.b. and *deca-trans,trans-2,4-dienal* with a threshold of 0.07 p.p.b. The  $C_9$  ketones, because of their relatively high concentration in the heated bell pepper oil and their reasonably low thresholds probably contribute to the cooked bell pepper aroma.

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