

# Gas Chromatography, Mass Spectrometry, and Sniffing Port Analyses of Volatile Compounds of Fresh Bell Peppers (*Capsicum annuum*) at Different Ripening Stages

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Dynamic headspace gas chromatography, mass spectrometry, and sniffing port detection were used to analyze the volatile compounds of a Dutch commercial *Capsicum* cv. Mazurka at the ripening stages green, turning, and red. The samples were prepared by either cutting or blending of the fruits. The different bell pepper samples obtained had several odor compounds in common, i.e., 2,3-butanedione (caramel), 1-penten-3-one (chemical/pungent, spicy), hexanal (grassy), 3-carene (red bell pepper, rubbery), (*Z*)- $\beta$ -ocimene (rancid, sweaty), octanal (fruity), and 2-isobutyl-3-methoxypyrazine (green bell pepper). During bell pepper maturation the majority of volatile compounds, of which several had green-related odor notes, decreased or even disappeared. Only the levels of (*E*)-2-hexenal and (*E*)-2-hexenol, which have almond, fruity, sweet odors, were higher at the stages turning and red. Disruption of the cell structure favored lipid oxidation and the formation of related alcohols, aldehydes, and ketones.

## INTRODUCTION

The two main species of *Capsicum*, i.e., *Capsicum annuum* var. *grossum* Sendt (c.q. sweet bell pepper) and *Capsicum frutescens*, are widely used for their color, distinct taste, and aroma (Whitfield and Last, 1991). Bell peppers are consumed as immature (green) or mature (e.g., red, yellow) fruits due to their clear differences in flavor. The main quality parameters for *Capsicum* varieties are color and pungency (Govindarajan, 1986; Govindarajan et al., 1987). However, current research is also focusing on the flavor as an important parameter for the quality of fresh fruits and vegetables.

More than 125 volatile compounds have been identified (Van Straten and Maarse, 1991) in fresh and processed *Capsicum* fruits. The significance of these compounds for the aroma is not yet well-known, since the research on the odor evaluation of bell pepper volatile compounds has been very limited. Buttery et al. (1969) indicated that 2-methoxy-3-isobutylpyrazine, (*E,Z*)-2,6-nonadienal, and (*E,E*)-decadienal are important aroma compounds of bell peppers because of their low threshold values and distinct odors. Chitwood et al. (1983) suggested the relation between the volatile constituents 2-isobutyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, (*Z*)-3-hexenol and some sensory-perceived "green" aroma characteristics of three *Capsicum* cultivars grown in California. Wu and Liou (1986) indicated that tissue disruption increased the amount of volatile unsaturated C<sub>8</sub> aldehydes and alcohols. Some of these compounds have been sensorially described by green-related descriptors such as grassy and crushed leaves (Dravnieks, 1985). These aldehydes and alcohols might play an important role in determining the green bell pepper flavor. So far, the changes of volatile bell pepper constituents during maturation have not been investigated.

Several combinations of gas chromatography and column effluent sniffing techniques are in practice such as

Charm analysis (Acree et al., 1984), aroma extraction dilution analysis (Schieberle and Grosch, 1987), the Osme method (Miranda-Lopez et al., 1992), and the sensory sniffing-port panel method (Linsen et al., 1993).

In the present study, the composition of volatile compounds of a Dutch bell pepper cultivar, Mazurka, at three ripening stages was studied by using gas chromatography (GC), mass spectrometry (MS), and sniffing port analysis (GC-sniffing). The latter technique enables a direct correlation between GC-separated volatile compounds and their sensory perception.

## MATERIALS AND METHODS

**Bell Pepper Fruits.** *C. annuum* cv. Mazurka was grown on glass wool in a greenhouse with controlled temperature ( $\pm 20$  °C) and humidity (75%). The fruits were harvested at 6 (green stage), 8 (turning stage), and 10 (red stage) weeks after fruit setting, and individual weights of the whole bell peppers were measured. The fruits were subsequently stored at 13 °C (maximal 3 days) until volatile compounds were isolated by a dynamic headspace technique.

**Color Measurements.** Chlorophyll content was determined as described by Moran and Porath (1980) with minor modifications. Aliquots of 0.5 g of frozen bell peppers were homogenized with 10 mL of *N,N*-dimethylformamide (Merck, Amsterdam) and 10 mg of CaCO<sub>3</sub> in a Waring blender for 1 min at 4 °C and vacuum filtered using a sintered glass funnel (G3). The chlorophyll *a* concentration of the extracts was measured spectrophotometrically (Perkin-Elmer, UV-vis spectrometer Lambda 16) at 664.5 nm, using the specific absorption coefficient of chlorophyll *a* in *N,N*-dimethylformamide, 83.89 L g<sup>-1</sup>.

Carotenoid extraction was performed with hexane/acetone mixtures according to the AOAC (1984) method with some minor modifications. Aliquots of 5 g were homogenized with 60 mL of hexane, 40 mL of acetone, and 0.1 g of CaCO<sub>3</sub> in a Waring blender for 2 min and vacuum filtered using a sintered glass funnel (G3). The residue was dispersed in a mixture of 25 mL of hexane and 25 mL of acetone and filtered to extract residual carotenoids. The filtrates were pooled and washed with 100 mL of water. The final upper layer was separated, mixed with 9 mL of acetone, and diluted with hexane to 100 mL. Standard curves were made with  $\beta$ -carotene (97%, Merck), and the specific absorption coefficient was determined. The extracts were measured for carotenoids at 436 nm by using a spectrophotometer, and the absorbances were converted into grams of  $\beta$ -carotene per kilogram of dry matter.

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**Isolation of Volatiles.** Bell pepper samples were prepared by slicing 250 g of sample or by homogenization of 45 g of sample with 50 mL of water in a Waring blender for 10 s. The samples obtained were transferred to 500-mL glass bottles for dynamic headspace isolation of volatile compounds as described by Macleod and Ames (1986), using Tenax TA as an adsorbent. The homogenized samples were flushed with 30 mL/min purified nitrogen for 2 h at room temperature; the sliced samples were flushed for 6 h. The flushing times were arbitrarily chosen to obtain sufficient amounts of volatile components for flame ionization detection and sniffing port analysis. System control samples were made by using the same conditions as for the isolation procedures.

**Gas Chromatography Analysis.** Volatile compounds were desorbed from the Tenax tubes for 10 min at 200 °C (Chrompack TCT injector 16200, Chrompack, Bergen op Zoom, The Netherlands) and injected in a capillary CP-Wax CB52 column (50 m × 0.32 mm i.d., 1.5- $\mu$ m film thickness, Chrompack). The oven temperature was programmed from 40 to 150 °C at 2 °C/min and subsequently heated to 250 °C at 10 °C/min. The gas chromatograph (Carlo Erba GC vega 6000, Interscience, Breda, The Netherlands) was equipped with a flame ionization detector (FID) and two sniffing ports (SGE, Milton Keynes, Great Britain). FID response standard curves were made to calculate the quantitative amounts of bell pepper compounds such as hexanal, (*E*)-2-hexenal, octanal, (*Z*)-3-hexenol, (*E*)-2-hexenol, 2-isobutyl-3-methoxypyrazine, and linalool (Jansen Chimica, Tilburg, The Netherlands).

**GC-MS Analysis** Volatile compounds were identified by using a GC-MS (Carlo Erba, Mega 3600, QMD 8000, Interscience) which was equipped with a thermal desorption unit (Carlo Erba, Tekmar 5010, automatic desorber, Interscience). Thermal desorption (10 min at 200 °C) and GC column conditions were identical to those for gas chromatography. Electron impact mass spectral (EI-MS) analysis was carried out at an ionization energy of 70 eV. The calculated Kovats indices (KI) and MS fragmentation patterns of each component were compared with those of the authentic compound as reported in the literature (Kawakami and Kobayashi, 1991; Umano et al., 1992; Vernin et al., 1987).

To compare differences in ripening stages and cultivars, the peak areas (*V*s) of identified compounds (six replicates) were processed by analysis of variance (ANOVA).

**Sniffing Port Evaluation of Bell Pepper Volatiles.** The GC effluent was split in 20% for FID analysis, 40% for the left, and 40% for the right sniffing port. To prevent dehydration of the nasal membranes of the assessors, humidified air was added to the exit of the sniffing port.

An experienced GC-sniffing port panel was used for the evaluation of bell pepper odors. In five additional sessions, the assessors were trained to detect and describe the volatile compounds of bell peppers. A preliminary list of odor descriptors was created after the first session. Then, several reference samples such as crushed lettuce, cucumber, carrot, green and red bell pepper, and geranium (myrcene 97%, Jansen Chimica) were presented to the assessors to associate references with their odor descriptions. A list of 19 frequently used odor descriptors was compiled including almond, floral/soapy, mushroom, geranium-like, fruity, grassy/cut grass, chemical/pungent, cucumber/fresh green, caramel, musty, green bell pepper, red bell pepper, rubbery/burned rubber, lettuce, sweaty, spicy/herbal, rancid/oily, carrot, and sweet. The term "unknown" was added to the list for detectable smells missing a suitable descriptor. The duration of detection and the description of volatile odor compounds were registered with portable computers, which were programmed as described by Linssen et al. (1993). The background noise of the sniffing port panel was determined by analyzing clean Tenax tubes (Linssen et al., 1993). The raw data sets of each assessor were transferred into a database (Foxpro) for summarizing the individual sniffing responses of the 12 assessors, calculating the sniffing responses above the noise level, and summarizing the odor descriptors given to the total sniffing responses.

## RESULTS AND DISCUSSION

The composition of carbohydrates, organic acids, volatile flavor compounds, and color pigments changes during the

**Table 1. Fresh Weight, Dry Matter, and Color Pigment Contents of Bell Pepper Cv. Mazurka at the Green, Turning, and Red Stages**

analyses <sup>a</sup>	green	turning	red
fresh wt (g)	181.2 ± 15.6	199.2 ± 17.0	211.0 ± 26.9
dry matter (%)	6.1 ± 0.4	7.5 ± 0.2	8.7 ± 0.6
chlorophyll <i>a</i> (g/kg dm)	1.1 ± 0.4	0.4 ± 0.1	0.04 ± 0.04
carotenoids <sup>b</sup> (g/kg dm)	0.6 ± 0.1	0.6 ± 0.2	1.5 ± 0.2

<sup>a</sup> Mean value of 12 replicates ± standard deviation. <sup>b</sup> Expressed as  $\beta$ -carotene standard ( $E = 185.9 \text{ L g}^{-1}$ ).

maturation of bell peppers (Govindarajan, 1985, 1986). Some of these parameters are used by growers to determine the harvest time of bell peppers cultivated in greenhouses (Govindarajan, 1985). Both green and red bell peppers (commercial products) and an intermediate turning stage were chosen for the analysis of volatile compounds. The three ripening stages were evaluated by determining fresh weight, contents of dry matter, and color pigments. Table 1 presents the analytical data of green, turning, and red fruits from the cv. Mazurka. It appeared that fresh weight did not change during maturation, while dry matter content increased gradually. The chlorophyll *a* content was maximal at the green stage and decreased during ripening. In contrast, the carotenoid content increased upon maturation.

**GC and GC-MS Analysis of Volatile Bell Pepper Compounds.** Wu and Liou (1986) observed that disruption of the cell structure affected the composition of volatile compounds of bell peppers. In this study, two methods for sample preparation, i.e., homogenization and cutting, were used; Figures 1 and 2 represent the gas chromatograms of the cv. Mazurka in the maturation stages green, turning, and red. Comparison of these stages shows that the total peak areas decreased during maturation. Both in homogenized and in sliced samples, the volatile compounds (numbered peaks) decreased or disappeared and only a few compounds increased upon ripening. Comparison of Figures 1 and 2 illustrates that the FID patterns of homogenized and sliced bell peppers differ very much. In general, the intensity of the FID response of homogenized samples was higher than that of sliced bell peppers, which indicates that the concentrations of volatile compounds in the headspaces above blended bell peppers are higher. Similar gas chromatograms were obtained for another Dutch bell pepper cultivar, Evident (Luning et al., 1994).

Identification of the different compounds from the bell pepper headspace concentrates was based upon mass spectral matching and comparison of calculated KI's with authentic samples. The identified volatile compounds are listed in Table 2 together with their average peak areas. Quantitative amounts (nanograms) were calculated using FID calibration curves of authentic compounds (Table 2). In total, 64 compounds were identified, of which 22 have not been detected before in *C. annuum* varieties, but they were often found in other fresh vegetables (Van Straten and Maarse, 1991).

The composition of volatile compounds of bell peppers differs clearly for the maturation stages and for the methods of sample preparation. Table 2 shows that the majority of volatile compounds from both homogenized and sliced samples decreased or even disappeared during ripening. The homogenized green bell peppers contain high levels of 1-penten-3-one (7), toluene (9), hexanal (12), (*Z*)- and (*E*)-2-pentenal (13, 17), (*Z*)-3-hexenal (22), (*E*)-2-hexenal (31), (*E*)- $\beta$ -ocimene (36), 1-hexanol (43), (*Z*)-3-hexenol (45), and 6-methylheptyl 2-propenoate (58), of which 9, 36, 43, 45, 58, (*Z*)- $\beta$ -ocimene (34), and (*E,Z*)-3,4-dimethyl-2,4,6-octatriene (46) were also the major peaks

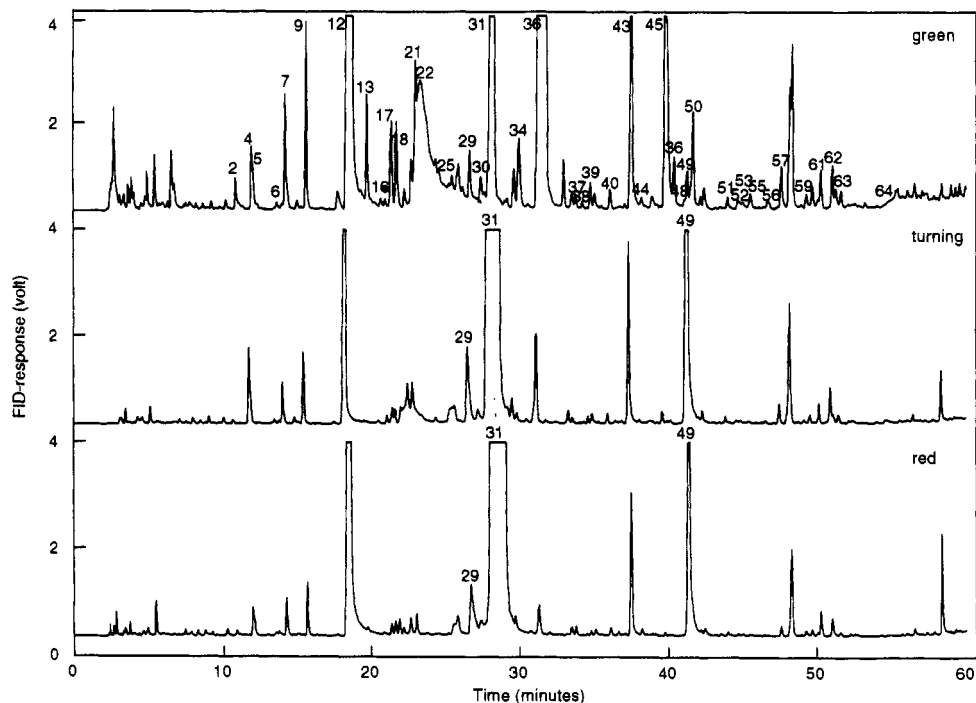


Figure 1. Dynamic headspace gas chromatograms of homogenized bell peppers of cv. Mazurka at the ripening stages green, turning, and red.

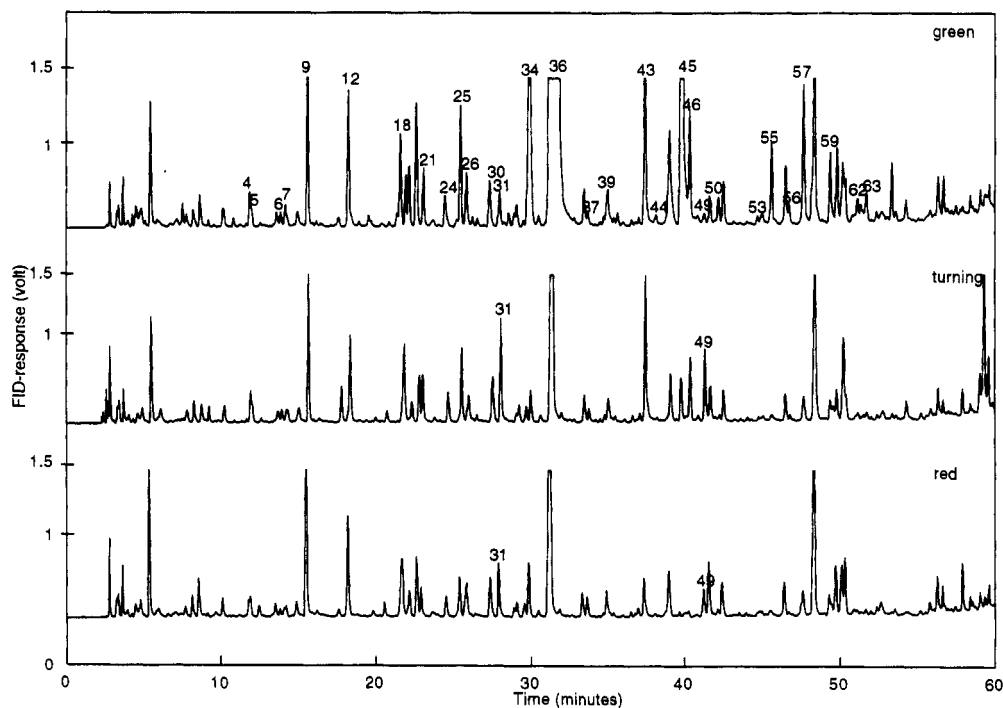


Figure 2. Dynamic headspace gas chromatograms of sliced bell peppers of cv. Mazurka at the ripening stages green, turning, and red.

from sliced samples. These compounds, except (*E*)-2-hexenal (31), decreased significantly ( $P < 0.05$ ) during maturation. However, significantly ( $P < 0.05$ ) higher average peak areas were observed in the homogenized samples at the turning and red stages for (*Z*)-2-hexenal (29), (*E*)-2-hexenal (31), and (*E*)-2-hexenol (49), of which 31 and 49 were also significantly ( $P < 0.05$ ) higher at these stages of sliced bell peppers. Buttery et al. (1969) reported that (*E*)- $\beta$ -ocimene, methyl salicylate, limonene, (*Z*)-3-hexenol, linalool, (*E*)-2-nonen-4-one, 2-isobutyl-3-methoxy-pyrazine, (*E,Z*)-2,6-nonadienal, and (*E,E*)-2,4-decadienal were the major compounds isolated from fresh green California bell peppers. They used a vacuum Likens-

Nickerson apparatus at  $\pm 50$  °C to extract the volatile compounds. Our experiments were performed at room temperature using a dynamic headspace method, which might explain why volatile compounds with higher KI's such as methyl salicylate and (*E,E*)-2,4-decadienal were not released. In addition, Wilkins and Madsen (1989) observed that headspaces of 20 g of heated (40 °C) Hungarian edelsüss paprika consisted mainly of hydrocarbons with limonene as the major component.

Comparison of the volatile composition of homogenized and sliced bell peppers in Table 2 reveals that a number of volatile compounds such as 2-ethylfuran (2), (*Z*)- and (*E*)-2-pentenal (13, 17), 3-penten-2-one (16), (*Z*)-3-hexenal

**Table 2. Identified Compounds and Their Average GC Peak Areas of Homogenized (HG) and Cut (CT) Mazurka Bell Peppers at the Ripening Stages Green, Turning, and Red**

no. <sup>a</sup>	KI <sup>b</sup>	compound	GC peak area <sup>c</sup>					
			green		turning		red	
			HG	CT	HG	CT	HG	CT
1	931	3-buten-2-one <sup>d</sup>	1.04	1.59	0.90	1.00	0.89	0.96
2	944	2-ethylfuran	3.38	e	0.53	e	0.81	e
3	962	2,3-butanedione <sup>d</sup>	0.04	0.16	0.04	0.07	0.03	0.05
4	965	3-pentanone <sup>d</sup>	8.49	1.91	7.02	1.62	4.41	1.28
5	967	pentanal	3.61	1.02	2.46	0.80	2.08	0.87
6	1005	2-butanol <sup>d</sup>	0.66	1.62	0.53	0.87	0.08	0.34
7	1009	1-penten-3-one <sup>d</sup>	17.34	1.25	5.24	0.58	5.54	0.57
8	1021	$\alpha$ -pinene	0.95	1.56	0.78	1.27	0.58	1.00
9	1032	toluene	15.14	14.72	7.09	10.70	7.46	10.22
10	1047	2,3-pentanedione <sup>d</sup>	e	0.17	e	0.17	e	0.14
11	1069	dimethyl disulfide <sup>d</sup>	1.77	1.45	0.35	1.23	0.29	0.41
12	1076	hexanal	7.83 <sup>f</sup>	0.28	4.88	0.17	5.31	0.19
13	1095	(Z)-2-pentenal <sup>d</sup>	14.87	e	0.19	e	0.09	e
14	1108	1-methoxy-2-propanol	1.01	0.86	0.74	0.54	0.86	0.63
15	1110	$\beta$ -pinene	0.05	0.06	0.06	0.05	0.05	0.03
16	1113	3-penten-2-one <sup>d</sup>	1.69	e	0.07	e	0.05	e
17	1118	(E)-2-pentenal <sup>d</sup>	14.73	e	1.06	e	1.42	e
18	1125	1-butanol <sup>d</sup>	7.94	4.41	5.29	4.55	3.12	2.18
19	1132	p-xylene	2.14	2.19	2.33	2.36	1.97	1.54
20	1138	m-xylene	1.72	4.84	3.00	5.22	2.73	3.39
21	1142	1-penten-3-ol <sup>d</sup>	6.98	3.22	2.58	2.24	2.44	2.60
22	1147	(Z)-3-hexenal	219.43	e	8.54	e	6.73	e
23	1152	3-carene	0.60	0.46	0.19	0.32	0.31	0.49
24	1167	myrcene	1.39	1.98	0.25	1.43	0.19	0.73
25	1177	2-heptanone	2.36	6.91	3.59	4.04	2.02	1.98
26	1181	heptanal	1.18	2.62	1.73	2.30	1.35	2.30
27	1183	o-xylene	2.42	2.17	2.50	4.82	2.60	2.53
28	1187	2,3-dihydro-4-methylfuran <sup>d</sup>	0.85	0.57	0.46	0.13	0.34	0.17
29	1194	(Z)-2-hexenal	9.61	e	11.71	e	13.78	e
30	1204	limonene	2.25	3.82	3.36	3.27	2.61	2.57
31	1212	(E)-2-hexenal	5.76 <sup>f</sup>	0.06	>13.9	0.21	>15.3	0.25
32	1232	2-pentylfuran	g	g	g	g	g	g
33	1233	1-pentanol	g	g	g	g	g	g
34	1237	(Z)- $\beta$ -ocimene	6.94	23.10	2.22	4.17	0.87	1.32
35	1253	3-octanone <sup>d</sup>	g	g	g	g	g	g
36	1254	(E)- $\beta$ -ocimene	180.14	379.63	44.79	67.62	10.83	28.44
37	1283	octanal	0.07 <sup>f</sup>	0.05	0.04	0.04	0.03	0.05
38	1288	(Z)-2-pentenol <sup>d</sup>	0.89	0.10	0.05	0.06	0.06	0.04
39	1297	(E)-2-pentenol <sup>d</sup>	3.57	1.10	0.67	0.74	0.62	0.45
40	1313	2-heptanal <sup>d</sup>	2.48	0.31	1.43	0.14	1.10	0.11
41	1322	4-nonanone	0.18	0.27	0.47	0.33	0.47	0.22
42	1327	6-methyl-5-hepten-2-one <sup>d</sup>	0.42	0.75	0.42	0.47	0.35	0.40
43	1332	1-hexanol	40.69	11.42	29.82	10.66	23.72	4.87
44	1342	(E)-3-hexenol	2.13	0.68	0.54	0.17	0.83	0.16
45	1364	(Z)-3-hexenol	2.88 <sup>f</sup>	1.74	0.05	0.06	0.02	0.01
46	1369	(E,Z)-3,4-dimethyl-2,4,6-octatriene	6.95	15.24	2.10	1.83	0.44	0.95
47	1377	dimethyl trisulfide <sup>d</sup>	0.12	0.32	0.07	0.25	0.09	0.14
48	1379	(E,E)-2,4-hexadienal <sup>d</sup>	0.58	e	e	e	e	e
49	1383	(E)-2-hexenol	0.11 <sup>f</sup>	0.02	2.34	0.15	1.95	0.09
50	1387	nonanal <sup>d</sup>	11.28	3.35	0.73	2.67	0.32	2.72
51	1418	(E)-2-octenal	1.30	e	1.04	e	0.60	e
52	1430	1-octen-3-ol	1.51	e	0.41	e	0.23	e
53	1435	1,2,4,5-tetramethylbenzene	1.35	0.86	0.41	0.32	0.35	0.19
54	1441	2-furancarboxyaldehyde	0.45	0.18	0.18	0.13	0.16	0.14
55	1442	1,3,8-p-menthatriene	1.12	4.33	0.44	0.63	0.30	0.27
56	1456	2-pentylthiophene <sup>d</sup>	1.42	1.16	0.67	0.53	0.27	0.13
57	1470	2-ethyl-1-hexanol <sup>d</sup>	4.87	5.88	2.52	1.76	2.24	1.46
58	1478	6-methylheptyl 2-propenoate	21.80	18.81	16.54	20.83	16.56	18.17
59	1492	decanal <sup>d</sup>	1.63	2.00	0.55	1.40	0.36	1.19
60	1500	2-sec-butyl-3-methoxypyrazine	<0.01	<0.01	e	e	e	e
61	1506	benzaldehyde	4.48	3.64	2.07	1.94	2.49	3.00
62	1510	2-isobutyl-3-methoxypyrazine	0.18 <sup>f</sup>	0.03	0.13	0.01	0.07	0.01
63	1527	linalool	0.15 <sup>f</sup>	0.09	0.06	0.01	0.03	0.01
64	1576	(E,Z)-2,6-nonadienal	<0.01	e	<0.01	e	e	e
total peak area			1234.6	608.9	992.8	190.2	985.9	123.7

<sup>a</sup> Numbers refer to Figures 1 and 2. <sup>b</sup> Calculated Kovats indices on CP-Wax CB52 column. <sup>c</sup> Average peak areas of six replicates (Vs). <sup>d</sup> Not identified before in *C. annuum* varieties. <sup>e</sup> Not detected. <sup>f</sup> (Bold print) amount (ng) calculated from FID standard curves. <sup>g</sup> Peaks were insufficiently separated from neighboring compound.

(22), (Z)-2-hexenal (29), (E,E)-2,4-hexadienal (48), (E)-2-octenal (51), 1-octen-3-ol (52), and (E,Z)-2,6-nonadienal (64) were only detectable in homogenized samples. Fur-

thermore, the average peak areas of 1-penten-3-one (7), hexanal (12), (E)-2-hexenal (31), hexanol (43), (Z)-3-hexenol (45), and (E)-2-hexenol (49) were significantly (*P*

Table 3. Odor Compounds of Mazurka Bell Peppers Detected and Described by the Sniffing Port Panel

no.	KI <sup>a</sup>	component	odor descriptors	green		turning		red	
				HG <sup>b</sup>	CT <sup>b</sup>	HG	CT	HG	CT
1a	906	unidentified	chemical/pungent, rubbery	+ <sup>c</sup>	-	-	-	-	-
3	962	<i>2,3-butanedione</i> <sup>d</sup>	caramel, sweet	++	+++	++	++	++	++
7	1009	<i>1-penten-3-one</i>	chemical/pungent, spicy	+++	++	+++	++	+++	++
10	1047	<i>2,3-pentanedione</i>	fruity, sweet, caramel	-	-	-	+	-	+
12	1076	<i>hexanal</i>	grassy, herbal, green bell pepper (bp)	+++	++	+++	++	+++	++
12a	1092	unidentified	chemical/pungent, rubbery, spicy	++	++	++	++	++	++
13a	1104	unidentified	geranium, spicy, rotten	++	+++	+++	+++	++	+++
22	1147	<i>(Z)</i> -3-hexenal	grassy, green bp, fruity	+++	-	-	-	-	-
23	1152	<i>3-carene</i>	red bp, rubbery, green bp	++	++	+++	+++	+++	+++
27	1183	<i>o</i> -xylene	geranium, rubbery, spicy	+	-	-	+	+	+
31	1212	<i>(E)</i> -2-hexenal	fruity, almond, spicy, sweet	++	-	+++	-	+++	-
34	1237	<i>(Z)</i> - $\beta$ -ocimene	rancid, sweaty	++	++	++	++	++	+
36a	1255	unidentified	mushroom, herbal	++	++	-	++	-	+
37	1283	<i>octanal</i>	fruity	+++	+++	++	++	++	++
38a	1294	unidentified	mushroom	+++	++	+++	++	++	++
43	1332	<i>1</i> -hexanol	fruity, green bp, herbal	+	-	-	-	-	-
44a	1351	unidentified	geranium, spicy	++	-	-	-	-	-
45	1364	<i>(Z)</i> -3-hexenol	grassy, lettuce, cucumber	++	++	-	-	-	-
45a	1364	unidentified	geranium, spicy	-	-	+++	++	+++	++
47	1377	dimethyl trisulfide	rotten, musty	-	+++	++	+++	++	+++
49	1383	<i>(E)</i> -2-hexenol	almond, fruity, spicy	-	-	+	-	+	-
50	1387	nonanal	mushroom, herbal	+	+	-	-	-	-
51	1418	<i>(E)</i> -2-octenal	almond, sweet, herbal	++	-	+	-	-	-
51a	1427	unidentified	lettuce, green bp, cucumber	+++	+++	++	++	-	-
53	1435	<i>1,2,4,5-tetramethylbenzene</i>	rancid, sweet	++	++	+	-	-	-
60	1500	<i>2-sec-butyl-3-methoxypyrazine</i>	carrot, lettuce, grassy	+++	++	++	++	-	-
62	1510	<i>2-isobutyl-3-methoxypyrazine</i>	green bp, red bp, lettuce	+++	+++	+++	+++	+++	+++
63	1527	<i>linalool</i>	floral, green bp	++	++	++	++	-	-
63a	1554	unidentified	herbal, cucumber	++	-	++	-	-	-
64	1576	<i>(E,Z)</i> -2,6-nonadienal	cucumber	++	-	++	-	+	-

<sup>a</sup> Calculated Kovats index of sniffing response on CP-Wax CB52 column. <sup>b</sup> HG, homogenized; CT, cut bell pepper. <sup>c</sup> Number of assessors detecting a smell simultaneously; -, <6 assessors, below noise level; +, 6 assessors, above noise level; ++, 6 < assessors < 9, clear signal; +++, >9 assessors, clear signal. <sup>d</sup> Italicized compounds belong to basic odor profile investigated.

<0.05) larger for the homogenized samples. These results confirm the findings of Wu and Liou (1986) that tissue disruption of green bell peppers favored the formation of 2-ethylfuran, hexanal, *(Z)*-3-hexenal, *(E)*-2-hexenal, hexanol, *(Z)*-3-hexenol, and *(E)*-2-hexenol. They suggested the activity of oxidative and hydroperoxide-cleavage enzyme systems, because the addition of stannous chloride caused a significant reduction of these compounds. For tomatoes, lipoxygenase and specific cleavage enzymes yielded mainly hexanal and *(Z)*-3-hexenal (Gaillard et al., 1977; Gaillard and Matthew, 1977). These compounds were subsequently converted into their corresponding alcohols by an alcohol oxidoreductase (Schormüller and Grosch, 1965). Gaillard and Matthew (1977) postulated that tomatoes have very low or no isomerase activity because the concentration of the more stable *(E)*-2-hexenal was relatively low. Table 2 shows that the levels of *(Z)*-3-hexenal (22) and *(Z)*-3-hexenol (45) decreased during bell pepper maturation while the levels of *(E)*-2-hexenal (31) and *(E)*-2-hexenol (49) increased. As stated by Gaillard et al. (1977) for tomatoes, the activities of several enzymes seem to be changed during ripening of bell peppers, especially the ones involved in the formation of lipid degraded products.

**Sniffing Port Evaluation of Volatile Bell Pepper Compounds.** The volatile compounds of the GC column effluents were also detected by a GC-sniffing panel. Sniffing of blank Tenax revealed that detection of an odor by fewer than 6 of 12 assessors could be considered as background "noise" for our panel. Twenty odor descriptors were obtained from the training sessions and were used for odor evaluation of the volatile compounds by the GC-sniffing panel. The odor compounds were identified by comparing calculated KI's of sniffing responses with these indices of GC-MS-identified compounds. Moreover, the

odor descriptors used by the panel were compared with descriptors reported in the literature (Dravnieks, 1985; Sheen et al., 1991). Table 3 shows the volatile odor compounds detected by the GC-sniffing panel of the cv. Mazurka and their major odor descriptions. In this table, 9 of 30 odor compounds could not be identified by GC-MS or detected by FID due to extremely low concentrations or masking by other (odorless) compounds. For instance, *(E)*- $\beta$ -ocimene (36) was associated with a mushroom, herbal odor (36a) because of their similar KI's. However, the mass spectrum revealed additional masses that do not belong to *(E)*- $\beta$ -ocimene, which is indicative of an inadequate GC separation. A minor compound probably contributed to the mushroom, herbal odor. Furthermore, the odor descriptors of the sniffing response at KI = 1364 changed from grassy, lettuce, green bell pepper (45) in the green maturation stage to geranium, spicy (45a) in the turning and red maturation stages. The KI value of *(Z)*-3-hexenol (45) corresponds with the KI value of the observed sniffing response (Table 3). *(Z)*-3-Hexenol (45), which is known as a green, grassy odor (Dravnieks, 1985), disappears upon maturation (Table 2). Since a sniffing response (45a) was still observed, another unidentified compound might be responsible for the detected geranium, spicy odor (45a).

**Basic Odor Profile.** Our GC-sniffing port results indicate that a kind of basic odor profile was present in each of the investigated bell pepper samples and that this profile was independent of the ripening stage and sample preparation method (Table 3). This basic odor profile consisted of 2,3-butanedione (caramel, sweet), 1-penten-3-one (chemical/pungent, spicy), hexanal (grassy, spicy, green bell pepper), 3-carene (red, green bell pepper, rubbery), *(Z)*- $\beta$ -ocimene (rancid, sweaty), octanal (fruity), 2-isobutyl-3-methoxypyrazine (green, red bell pepper,

lettuce), and three unidentified compounds (12a, 13a, and 38a); all are listed as italicized compounds in Table 3. From these compounds, the characteristic green bell pepper odor of 2-isobutyl-3-methoxypyrazine is well-known (Buttery et al., 1969) and the alkylmethoxypyrazines are found in many vegetables (Murray and Whitfield, 1975). However, a volatile bell pepper compound with a red/green bell pepper and rubbery odor has not yet been reported. The mass spectrum,  $m/z$  (relative intensity) 93 (100), 91 (40), 92 (29), 79 (28), 41 (22), 136 (19), 121 (18), 105 (12), and KI value (1152) imply that this compound might be 3-carene. Interestingly, Keller et al. (1981) detected the same compound ( $\delta$ -3-carene) as one of the major monoterpene hydrocarbons (13.4%) in the neutral solvent fraction of mild red chili (*C. annuum*). This fraction had an odor threshold of 11 ppb in water.

**Maturation Odor Profile.** Table 3 demonstrates that differences in sniffing patterns were observed between the ripening stages and between sample preparation methods. In general, a larger number of volatile odor compounds was detected in the green bell peppers than in the turning and red ones. In fact, only one additional compound, peak 45a (geranium, spicy), was observed in the turning and red ripening stages of both sliced and homogenized samples. Likewise, (*E*)-2-hexenol (49), which has an almond, fruity, and spicy odor, was detected as an additional compound in the turning and red homogenized bell peppers. Table 3 shows also that several odor compounds associated with green descriptors, such as mushroom, herbal [unidentified (36a)], grassy, lettuce, cucumber [(*Z*)-3-hexenol (45)], mushroom, herbal [nonanal (50)], lettuce, green bell pepper, cucumber [unidentified (51a)], carrot, lettuce, grassy [2-*sec*-butyl-3-methoxypyrazine (60)], and floral, green bell pepper [linalool (63)] were detected mainly in the green bell peppers and disappeared or decreased upon maturation. Some green related compounds, such as (*Z*)-3-hexenal (22) (grassy, green bell pepper, fruity), 1-hexanol (43) (fruity, green bell pepper, herbal), and two unidentified compounds, (44a) (geranium, spicy) and (63a) (herbal, cucumber), were observed only in homogenized samples of green bell peppers. These results are in agreement with the study of Chitwood et al. (1983), who suggested that (*Z*)-3-hexenol, 2-*sec*-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine might be responsible for the frequent use of green descriptors in the aroma descriptive analysis of three investigated *C. annuum* cultivars (Anaheim, Jalapeño, and Fresno). They reported also that other compounds with rose, floral, and apple odors may be important contributors to *Capsicum* aroma. Keller et al. (1981) observed that volatiles of fresh red Jalapeño pepper extracts had a pleasant floral aroma besides a characteristic bell pepper odor. Table 3 shows the presence of the volatile compounds linalool (63), (*E*)-2-hexenal (31), and (*E*)-2-hexenol (49), which have floral, almond, fruity, and sweet aroma notes.

**Sampling Odor Profile.** Comparison of the sniffing patterns of the two preparation methods demonstrates that a larger number of odor compounds were detected in the homogenized samples. In fact, (*Z*)-3-hexenal (22), (*E*)-2-hexenal (31), (*E*)-2-hexenol (49), (*E*)-2-octenal (51), and (*E,Z*)-2,6-nonadienal (64) were detected only in the homogenized bell peppers. These compounds have distinct odor characteristics such as grassy, almond, fruity, and cucumber. Therefore, it is likely that tissue disruption will influence the fresh bell pepper flavor drastically. Buttery et al. (1969) reported (*E,Z*)-2,6-nonadienal as an important contributor to fresh bell pepper flavor because

of its relatively low threshold (0.01 ppb) and its typical cucumber smell.

**Conclusion.** The results of this study indicate that differences in flavor of fresh green and red bell peppers are mainly caused by a decrease of volatile compounds during maturation, of which several have green related odors. Furthermore, tissue disruption increased the number of odor-active compounds.

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- Registry No. Supplied by Author:** 3-Buten-2-one, 78-94-4; 2-ethylfuran, 3208-16-0; 2,3-butanedione, 431-03-8; 3-pentanone, 96-22-0; pentanal, 110-62-3; 2-butanol, 78-92-2; 1-penten-3-one, 1629-58-9;  $\alpha$ -pinene, 80-56-8; toluene, 108-88-3; 2,3-pentanedione, 600-14-6; dimethyl disulfide, 624-92-0; 1-hexanal, 66-25-1; (*Z*)-2-pentenal, 1576-86-9; 1-methoxy-2-propanol, 107-98-2;  $\beta$ -pinene, 127-91-3; 3-penten-2-one, 625-33-2; (*E*)-2-pentenal, 1576-87-0; 1-butanol, 71-36-3; *p*-xylene, 106-42-3; *m*-xylene, 108-38-3; 1-penten-3-ol, 616-25-1; (*Z*)-3-hexenal, 6789-80-6; 3-carene, 13466-78-9; myrcene, 123-35-3; 2-heptanone, 110-43-0; heptanal, 111-71-7; *o*-xylene, 95-47-6; 2,3-dihydro-4-methylfuran, 34314-83-5; (*Z*)-2-hexenal, 16635-54-4; limonene, 138-86-3; (*E*)-2-hexenal, 6728-26-3; 2-pentylfuran, 3777-69-3; 1-pentanol, 71-41-0; (*Z*)- $\beta$ -ocimene, 3338-55-4; 3-octanone, 106-68-3; (*E*)- $\beta$ -ocimene, 3779-61-1; octanal, 124-13-0; (*Z*)-2-pentenol, 1576-95-0; (*E*)-2-pentenol, 1576-96-1; (*E*)-2-heptenal, 18829-55-5; 4-nonanone, 4485-09-0; 6-methyl-5-hepten-2-one, 110-93-0; 1-hexanol, 111-27-3; (*E*)-3-hexenol, 928-97-2; (*Z*)-3-hexenol, 928-96-1; (*E,Z*)-3,4-dimethyl-2,4,6-octatriene, 7216-56-0; dimethyl trisulfide, 3658-80-8; (*E*)-2-hexenol, 928-95-0; (*E,E*)-2,4-hexadienal, 142-83-6; nonanal, 124-19-6; (*E*)-2-octenal, 2548-87-0; 1-octen-3-ol, 3391-86-4; 1,2,4,5-tetramethylbenzene, 95-93-2; 2-furancarboxyaldehyde, 98-01-7; 1,3,8-menthatriene, 21195-59-5; 2-pentylthiophene, 4861-58-9; 2-ethyl-1-hexanol, 104-76-7; 6-methylheptyl 2-propenoate, 54774-91-3; decanal, 112-31-2; 2-*sec*-butyl-3-methoxypyrazine, 24168-70-5; benzaldehyde, 100-52-7; 2-isobutyl-3-methoxypyrazine, 24683-00-9; linalool, 78-70-6; (*E,Z*)-2,6-nonadienal, 557-48-2.

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