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## Gas Chromatographic Analysis of Volatile Components of Ginger Oil (*Zingiber officinale* Roscoe) Extracted with Liquid Carbon Dioxide

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Freeze-dried ginger powder was extracted with liquid carbon dioxide (600-700 psi) for 48 h. Volatile components in the oily extract were fractionated into one hydrocarbon fraction and two oxygenated hydrocarbon fractions by using silica gel column chromatography. Each fraction was analyzed by capillary GC and GC-MS. Out of 168 characterized compounds 90 were identified as far as possible by comparing GC retentions and mass spectral data. There were 58 tentatively identified compounds according to mass spectral data. Effects of liquid carbon dioxide extraction upon the volatile composition of ginger were discussed.

The volatile oil of ginger can be prepared by steam distillation, which is a simple and effective method to obtain essential oils from plants (Lawrence, 1984). However, the high temperature during steam distillation causes considerable problem to the flavor quality. Using supercritical or liquid carbon dioxide as an extractant (Krukonis, 1984; Moyler, 1984) is one of the recent efforts to overcome the thermal effect due to steam distillation.

Volatile ginger oil has been the subject of many research studies (Brooks, 1916; Varma et al., 1962; Nigam et al.,

1964; Connell and Sutherland, 1966; Connell, 1970; Connell and Jordan, 1971; Kami et al., 1972; Bednarczyk and Kramer, 1975; Bednarczyk et al., 1975; Masada, 1976; Sakamura and Hayashi, 1978; Chou et al., 1981; Smith and Robinson, 1981; MacLeod and Pieris, 1984). A recent study by Lawrence (1983) has shown the identification of 114 volatile components; many were reported for the first time in ginger. There were reports showing the profiles of gas chromatographic analyses of ginger oil extracted with supercritical carbon dioxide and solvent (dichloromethane) (Krukonis, 1984) and liquid carbon dioxide (Moyler, 1984). However, there were no specific indications of volatile compounds being identified. Our previous report has shown that, in ginger, not only the pungent principles (primarily gingerol compounds) but also the volatile components could be extracted by liquid carbon dioxide (Chen et al., 1986a).

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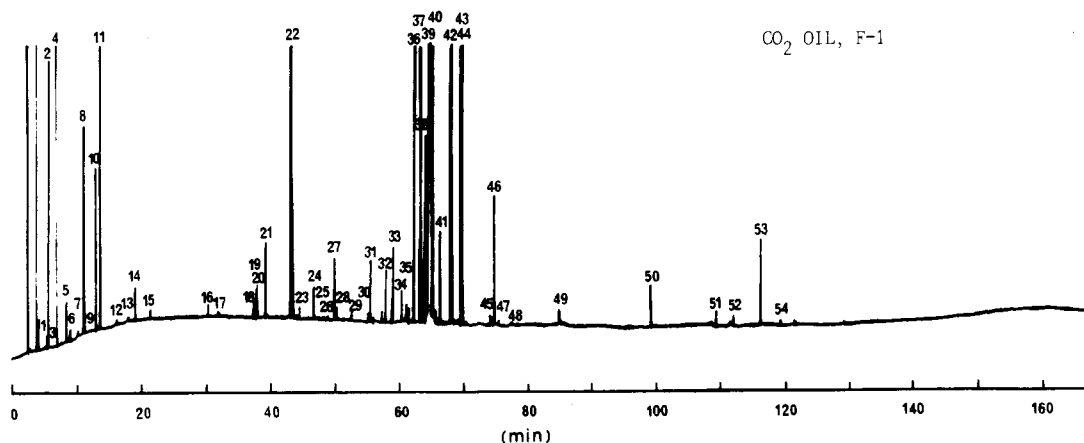


Figure 1. Capillary gas chromatogram of F-1 of ginger oil extracted with liquid carbon dioxide.

The present study reports the analysis of volatile components of ginger oil extracted with liquid carbon dioxide.

#### MATERIALS AND METHODS

**Reagents.** The solvents (*n*-pentane, diethyl ether) were reagent grade (E. Merck) and glass-distilled. *n*-Aldehydes ( $C_6$ – $C_{14}$ ) were obtained from Polyscience Corp. (Niles, IL). *n*-Paraffins ( $C_7$ – $C_{25}$ ) were obtained from Alltech Associates (Deerfield, IL). Authentic volatile samples were obtained from several chemical or flavor companies: 2-heptanone, 2-nonanone, and 2-undecanone were obtained from Aldrich (Milwaukee, WI); 2-tridecanone was obtained from Caro (Tokyo, Japan); 1,8-cineol, citronellal, 2-heptanol, 2-nonanol,  $\alpha$ -terpineol, citronellol, nerol, geraniol, citronellyl acetate, and geranyl acetate were obtained from H&R GmbH (Holzminden, West Germany); citral (geranial and neral) was obtained from Caro; eugenol was obtained from Wako (Tokyo, Japan); borneol and bornyl acetate were obtained from R. C. Treat (Suffolk, England); linalool was obtained from SCM Co. (Baltimore, MD); myrcene was obtained from Bush Boake Allen (London, England). Unless otherwise stated, all other chemicals were of reagent grade. Zingerone was obtained from ICN Biochemical (Plainview, NY).

**Liquid Carbon Dioxide Extraction.** Mature ginger (*Z. officinale* Roscoe) rhizomes were obtained from a supplier near Hsinchu (Taiwan). The rhizomes were washed, sliced, freeze-dried, ground, and sieved (200 mesh). About 110 g of freeze-dried ginger powder was placed in a glass Soxhlet extractor installed in a stainless steel liquid carbon dioxide extractor (Chen, 1987). Crushed dry ice (10 kg) was poured into the cylinder. The cylinder was closed tightly and heated with propylene glycol at  $45 \pm 2$  °C circulating in the lower outside jacket. The pressure inside the cylinder was increased and stabilized around 600–700 psi. The extraction time was 48 h. After extraction, the cylinder was cooled to  $-60$  °C by circulating precooled alcohol (95%) in both the lower and upper outside jackets. The oily material obtained (3.44% w/w dry-weight basis) after the evaporation of carbon dioxide was golden brown.

**Fractionation of Volatile Compounds by Column Chromatography.** A glass column (40 cm  $\times$  2 cm (i.d.)) packed with silica gel (50 g; C-200, Wako) was used. Ginger oil (836.7 mg) extracted by liquid carbon dioxide was dissolved in 5 mL of pentane and the resultant solution applied to the column. The column was eluted consecutively with 500 mL each of pentane (F-1), pentane/ethyl ether (9/1, F-2), and ether (F-3). Pentadecane (Alltech Associates; 7.136 mg), ethyl decanoate (Shiono; 5.632 mg), and dodecanol (H&R GmbH; 4.800 mg) were added to each fraction as internal standards. Each fraction was

dried over anhydrous sodium sulfate and concentrated in a spinning-band distillation apparatus (Kontes). Sensory judgement on each fraction was conducted by using blotters.

#### GC and GC-MS Analyses of Volatile Components.

The three fractions of ginger oil extracted by liquid carbon dioxide were subjected to gas chromatographic analyses on a Varian 3400 gas chromatograph. A fused silica column with a stationary phase equivalent to Carbowax 20M (DB-WAX+, 60 m  $\times$  0.32 mm; J&W Scientific) was installed. The oven temperature was programmed linearly from 50 to 225 °C at 1.5 °C/min and was held at 225 °C for 80 min. Other operating conditions were as follows: injector and detector temperatures, 250 °C; makeup helium flow, 30 mL/min; detector hydrogen flow, 30 mL/min; detector air flow, 30 mL/min. The samples were injected in the split mode with a split ratio of 1/100. The linear velocity of the helium carrier flow was 22 cm/s. Quantitative determinations were conducted on a Varian 4270 integrator. Linear retention indices for the volatile components were calculated with use of *n*-paraffins ( $C_7$ – $C_{25}$ ; Alltech Associates) as references (Majlát et al., 1974).

Capillary gas chromatography-mass spectrometry was carried out on a Hewlett-Packard 5985 B system equipped with a Hewlett-Packard 5840 A gas chromatograph. A fused silica capillary column (Carbowax 20M) was used. Analytical conditions were as follows: temperature program, 50–200 °C, 1.5 °C/min, isothermal at 200 °C, 50 min; injector temperature, 250 °C; helium carrier velocity, 30 cm/s; ion source temperature, 200 °C; ionization voltage, 70 eV; electron multiplier voltage, 2600 V.

#### RESULTS AND DISCUSSION

One of the advantages of using liquid or supercritical carbon dioxide as extractant is to avoid the undesirable components generated due to thermal processing such as steam distillation (Meyer-Warnod, 1984; Ohloff et al., 1985). In this study, the oily material extracted with liquid carbon dioxide displayed an odor reminiscent of fresh ginger. The extract was further fractionated by silica gel column chromatography with increasing polarity. The amount of volatile fraction in the liquid carbon dioxide extract was approximately 33%; the rest was made up by pungent components and some unidentified components (Chen et al., 1986a).

Volatile compounds in each fraction were analyzed by capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Figures 1–3 show the chromatograms of fractions 1–3, respectively.

Tables I–III list the volatile components identified in fractions 1–3. The identifications were accomplished by comparing the mass spectra and GC retentions with au-

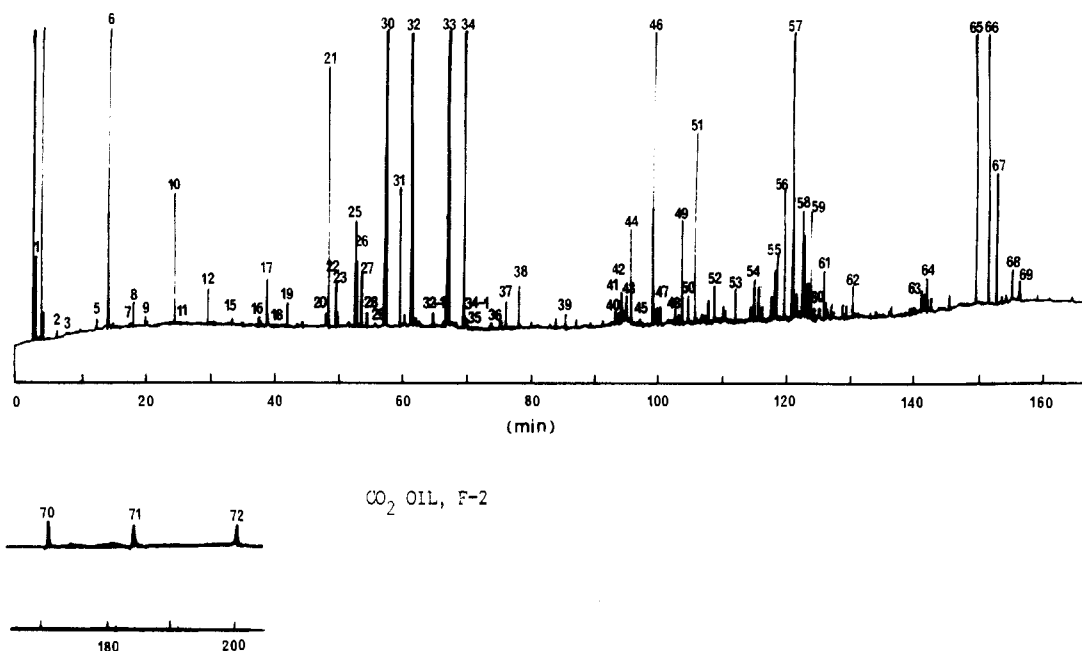


Figure 2. Capillary gas chromatogram of F-2 of ginger oil extracted with liquid carbon dioxide.

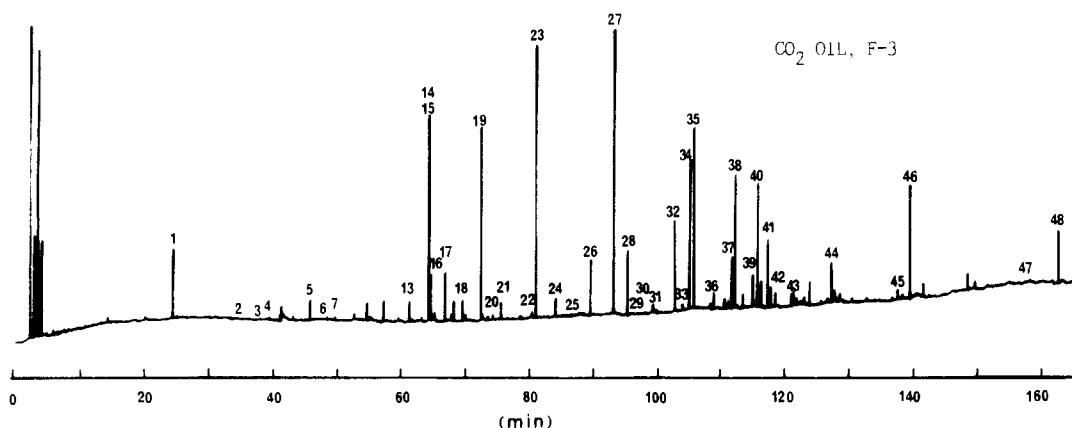


Figure 3. Capillary gas chromatogram of F-3 of ginger oil extracted with liquid carbon dioxide.

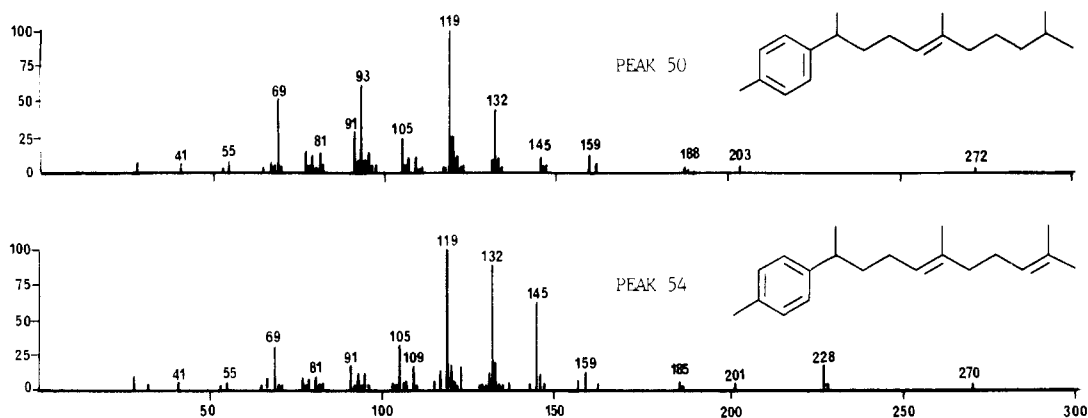


Figure 4. Mass spectra of peaks 50 and 54 identified in F-1.

thetic samples and published data (Minyard et al., 1968; Hashizume and Sakata, 1970; Bednarczyk and Kramer, 1975; Bednarczyk et al., 1975; Halim and Collins, 1975; Masada, 1976; Torii and Okamoto, 1976; EPA/NIH, 1980; Jennings and Shibamoto, 1980; TNO, 1980; Swigar and Silverstein, 1981; Smith and Robinson, 1981; Lawrence, 1983, 1984; Sakurai et al., 1983; MacLeod and Pieris, 1984).

**Volatile Components in F-1.** Table I and Figure 1 list 53 components identified in F-1. Compounds in this

fraction were in good agreement with those of Lawrence (1983, 1984). Quantitative estimation by using pentadecane (peak 22) as the internal standard indicated that F-1 comprised 71.4% of the volatile fractions eluted.

Monoterpenes and sesquiterpenes were two major categories of volatile components identified in this fraction. Higher percentages of sesquiterpene compounds as compared to monoterpene compounds in this fraction showed similar results as previously reported (Moyler, 1984).

**Table I. Volatile Components of F-1 of Liquid Carbon Dioxide Extracted Ginger Oil**

peak no. <sup>a</sup>	compound	index <sup>b</sup> CW-20M	%	ID ref
1	$\alpha$ -thujene	1003	0.04	c
2	$\alpha$ -pinene	1014	0.71	c
3	toluene	1027	+	c
4	camphene	1053	2.23	c
5	$\beta$ -pinene	1100	0.13	c
6	sabinene	1110	0.03	c
7	$\delta$ -3-carene	1134	0.03	c
8	myrcene	1148	0.77	c, d
9	$\alpha$ -phellandrene	1152	+	c
10	limonene	1183	0.58	c
11	$\beta$ -phellandrene	1194	1.88	c
12	$\gamma$ -terpinene	1129	+	c
13	<i>p</i> -cymene	1249	+	c
14	terpinolene	1263	0.14	c
15	isomer of C <sub>11</sub> compd (69/150)	1292	0.42	f
16	isomer of C <sub>11</sub> compd (150/150)	1383	0.63	f
17	isomer of C <sub>11</sub> compd (69/150)	1397	+	f
18	$\delta$ -elemene	1448	0.09	c
19	$\alpha$ -cububene	1453	0.18	c
20	amorphene	1455	0.07	c
21	$\alpha$ -copaene	1467	0.47	c
22	int std (C <sub>15</sub> , 7.136 mg)	1503	3.50	
23	sesquiterpene compd (MW 204)	1514	0.07	f
24	<i>cis</i> - $\alpha$ -bergamotene	1536	0.20	c
25	$\beta$ -ylangene	1547	+	c
26	elemene compd (MW 204)	1555	+	f
27	$\beta$ -elemene	1565	0.38	c
28	$\beta$ -caryophyllene	1567	0.07	c
29	selina-4(10),11-diene	1588	0.07	c
30	$\gamma$ -elemene	1614	0.38	c
31	caryophyllene compd	1619	+	f
32	isomer of $\beta$ -farnesene	1637	0.32	f
33	isomer of $\beta$ -farnesene	1648	0.46	f
34	cadiene compd	1660	0.22	f
35	cadina-4,9-diene	1670	0.11	c
36	$\beta$ -gurjunene	1677	2.19	c
37	$\gamma$ -cadiene	1685	3.59	c
38	$\delta$ -cadiene	1692	1.38	c
39	zingiberene	1701	35.01	c
40	$\gamma$ -bisabolene	1705	6.40	c
41	cadinene compd	1714	0.69	f
42	$\beta$ -bisabolene	1732	13.35	f
43	$\beta$ -sesquiphellandrene	1748	14.19	c
44	<i>ar</i> -curcumene	1749	4.61	c
45	unknown sesquiterpene compd	1789	0.05	f
46	germacrene B	1795	0.81	c
47	calamenene	1803	0.03	c
48	calamenene compd	1821	+	f
49	unknown farnesene compd	1895	0.09	f
50	pentylcurcumene compd (MW 272)	2042	0.25	e
51	unknown	2154	0.09	
53	pentylcurcumene compd (MW 272)	2184	0.07	e
53	pentylcurcumene compd (MW 272)	2233	0.52	e
54	pentenylcurcumene compd (MW 270)	>2500	+	e
% uncharacterized compd			2.50	

<sup>a</sup>Numbers refer to Figure 1. <sup>b</sup>Calculated values. <sup>c</sup>Mass spectral data from the following sources: Masada, 1976; EPA/NIH, 1980; Jennings and Shibamoto, 1980; TNO, 1980; Swigar and Silverstein, 1981. <sup>d</sup>GC and GC-MS analyses of authentic compounds. <sup>e</sup>Tentative identified with mass spectral data matched one of the following references: Bednarczyk and Karmer, 1975; Bednarczyk et al., 1975; Halim and Collins, 1975; Hashizume and Sakata, 1970; Minyard et al., 1968; Sakurai, et al., 1983; Torii and Okamoto, 1976. <sup>f</sup>Tentative identification of structure proposal based upon mass spectra only.

Connell and Jordan (1971) noted that the conversion of zingiberene and  $\beta$ -sesquiphellandrene into *ar*-curcumene and the polymerization of zingiberene were due to the oxidative effect of steam distillation. In the present study, zingiberene was the most abundant component identified in F-1 (35.01%). The higher concentration of this com-

ponent in liquid carbon dioxide extract indicated that the present extraction was void of oxidative effects as may be caused by other processes.

Two new series of isomers were tentatively identified:

(1) Peaks 15–17, which could be three geometric isomers with molecular weights of 150, were tentatively identified.

Peak 15 (*m/z*): 69 (100), 41 (46), 81 (19), 79 (17), 39 (15), 53 (12), 107 (12), 135 (10), 150 (9).

Peak 16 (*m/z*): 150 (100), 135 (89), 91 (47), 95 (35), 107 (30), 79 (30), 39 (25), 41 (24), 77 (20).

Peak 17 (*m/z*): 69 (100), 81 (86), 41 (67), 150 (56), 53 (38), 82 (33), 39 (20), 135 (18), 51 (16).

Isomers of undecatriene with molecular weight of 150 were first found in the essential oil of odoriferous Hawaiian seaweed, *Dictyopteris* (Moore et al., 1974). Recently, isomers of undecatriene [(*E,Z*)-1,3,5-undecatriene] with significant odors were also found in celery, parsley, mandarin, apple, pear, passion fruit, kiwi, and pineapple (Berger et al., 1985). The three C<sub>11</sub> isomers found here did not have the same structure as those identified in the seaweed, vegetables, and fruits. The sensory characteristics of the present three C<sub>11</sub> components are not known.

(2) Isomers of pentyl- and pentenylcurcumene compounds (peaks 50 and 52–54) were also tentatively identified in the extract. Structural assignments and mass spectra of peaks 50 and 54 are shown in Figure 4. The mass spectrum of peak 54 was almost identical with that of 2,6-dimethyl-10-*p*-tolylundeca-2,6-diene as proposed by Halim and Collins (1975).

Sensory analysis of F-1 indicated the presence of "green" and "spicy" characteristics.

**Volatile Components in F-2.** There were 69 volatile components characterized in F-2 (Table II; Figure 2). Ethyl decanoate (peak 30) was used as an internal standard. Quantitative estimation indicated F-2 comprised ca. 17.95% of the volatile fractions eluted. Aldehydes and esters of monoterpenes and sesquiterpenes were the major components identified. It is interesting to note that the presence of various tentative identified esters of monoterpenes and sesquiterpenes in ginger is being reported for the first time.

Detection of alcohol compounds such as linalool (peak 21), 4-terpineol (peak 27), zingiberenol (peak 51), selinen-4-ol (peak 49), and  $\beta$ -10-cadinol (peak 52) in F-2 was probably due to carryover during fractionation. The detection of two dehydration products of zingiberenol [zingiberene (peak 32-1) and  $\beta$ -sesquiphellandrene (peak 34-1)] during GC analysis was indirect evidence to confirm the presence of zingiberenol in this fraction (Terhune et al., 1975).

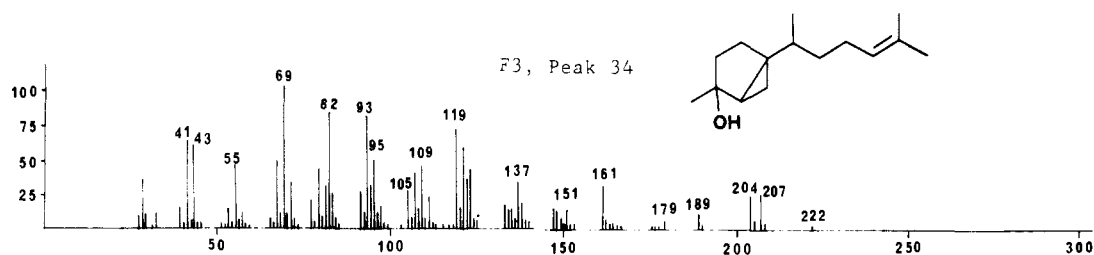
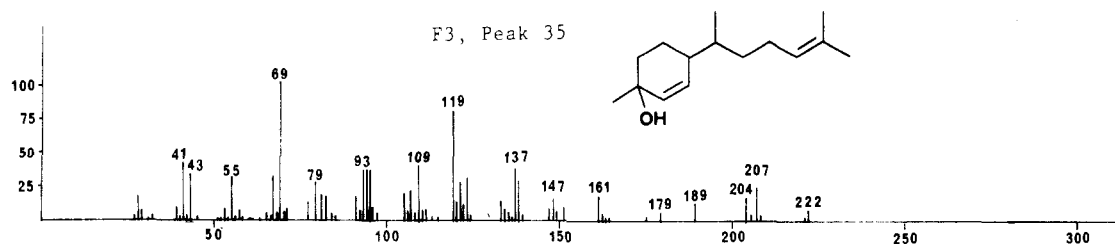
Only trace amounts of thermal degradative products of nonvolatile gingerol compounds were detected: aldehydes [hexanal (peak 3), octanal (peak 9), decanal (peak 18)] and 2-alkanones [2-heptanone (peak 5), 2-nonanone (peak 12), 2-undecanone (peak 25), 2-tridecanone (peak 36)]. The previous findings of hexanal, octanal, decanal, 2-heptanone, 2-nonanone, and 2-undecanone in steam-distilled volatiles of ginger were reported by several groups (Masada, 1976; Smith and Robinson, 1981; Lawrence, 1984). According to our recent reports (Chen et al., 1986b; Chen and Ho, 1987), the contents of these aldehydes and ketones in ginger products were closely related to thermal processing. The presence of only trace amounts of these degradative products in liquid carbon dioxide extract confirmed again its character of low-temperature operation (Moyler, 1984).

The sensory analysis of F-2 indicated that this fraction had the "spicy", "ginger-like", "floral", and "pungent" characteristics of ginger. Bauer and Garbe (1985) noted

**Table II. Volatile Components of F-2 of Liquid Carbon Dioxide Extracted Ginger Oil**

peak no. <sup>a</sup>	compound	index <sup>b</sup> CW-20M	%	ID ref	peak no. <sup>a</sup>	compound	index <sup>b</sup> CW-20M	%	ID ref
1	acetone	811	0.10	<i>c, d</i>	38	unknown terpene compd	1825	0.30	<i>f</i>
2	unknown ester	1035	+	<i>f</i>	39	unknown sesquiterpene compd	1894	0.10	<i>f</i>
3	hexanal	1701	+	<i>c, d</i>	40	caryophyllene oxide compd	1976	0.11	<i>f</i>
5	2-heptanone	1171	0.06	<i>c, d</i>	41	$\beta$ -ionone compd	1982	0.07	<i>f</i>
6	1,8-cineol	1203	7.51	<i>c, d</i>	42	unknown	1984	0.17	
7	<i>p</i> -cymene	1249	0.05	<i>c</i>	43	$\beta$ -caryophyllene oxide	1993	0.16	<i>e</i>
8	2-heptyl acetate	1250	0.13	<i>c</i>	44	butylated $\beta$ -ionone compd	1999	0.73	<i>f</i>
9	octanal	1274	0.06	<i>c, d</i>	45	2-pentadecanone	2016	+	<i>c</i>
10	6-methyl-5-hepten-2-one	1324	0.71	<i>c</i>	46	sesquiterpene ester (caryophyllene-like)	2039	3.98	<i>f</i>
11	2,6-dimethyl-5-heptenal	1330	+	<i>c</i>	47	unknown	2050	0.14	
12	2-nonanone	1377	0.21	<i>c, d</i>	48	$\beta$ -ionone compd	2075	0.11	<i>f</i>
15	2-nonenal	1412	0.06	<i>c</i>	49	selinen-4-ol	2086	0.89	<i>c</i>
16	2-nonyl acetate	1452	0.06	<i>c</i>	50	unknown	2097	0.21	
17	citronellal	1461	0.63	<i>c, d</i>	51	zingiberenol	2109	1.43	<i>f</i>
18	decanal	1485	+	<i>c, d</i>	52	$\beta$ -10-cadinol	2144	0.30	<i>c</i>
19	camphor	1491	0.17	<i>c</i>	53	propenyldimethoxybenzene	2181	0.27	<i>c</i>
20	limonene epoxide F1	1542	+	<i>c</i>	54	unknown zingiberenol ester	2215	0.33	<i>f</i>
21	linalool	1546	1.63	<i>c, d</i>	55	unknown sesquiterpene compd (187/220)	2256	0.45	<i>f</i>
22	limonene epoxide F2	1554	+	<i>c</i>	56	farnesyl aldehyde	2268	0.97	<i>c</i>
23	bornyl acetate	1559	0.31	<i>c, d</i>	57	unknown curcumenyl ester	2283	3.87	<i>f</i>
25	2-undecanone	1586	0.71	<i>c, d</i>	58	unknown	2303	0.79	
26	bornyl acetate	1587	0.63	<i>c, d</i>	59	unknown	2317	0.91	
27	4-terpineol	1594	0.36	<i>c</i>	60	eugenol	2332	0.08	<i>c, d</i>
28	propenyl benzyl ether	1602	0.11	<i>c</i>	61	unknown curcumenyl ester	2342	0.42	<i>f</i>
29	isopinocamphe	1627	+	<i>c</i>	62	unknown sesquiterpene ester (base peak 203)	2399	0.25	<i>f</i>
30	int std (ethyl decanoate, 5.632 mg)	1629	10.32		63	unknown bisabolene ester	>2500	0.18	<i>f</i>
31	citronellyl acetate	1648	0.96	<i>c, d</i>	64	unknown	>2500	0.27	
32	neral	1666	12.16	<i>c, d</i>	65	unknown sesquiterpene ester (base peak 203)	>2500	2.73	<i>f</i>
32-1	zingiberene	1696	0.09	<i>c</i>	66	unknown bisabolene ester	>2500	2.73	<i>f</i>
33	geranial	1720	24.81	<i>c, d</i>	67	geranyl ester (dodecyl ?)	>2500	0.96	<i>f</i>
34	geranyl acetate	1742	2.31	<i>c, d</i>	68	unknown sesquiterpene (136/218)	>2500	0.27	<i>f</i>
34-1	$\beta$ -sesquiphellandrene	1743	+	<i>c</i>	69	unknown sesquiphellandryl ester	>2500	0.17	<i>f</i>
35	<i>ar</i> -curcumene	1745	0.05	<i>c</i>	70	unknown geranyl ester	>2500	0.34	<i>f</i>
36	2-tridecanone	1799	+	<i>c, d</i>	71	unknown citronellyl ester	>2500	0.34	<i>f</i>
37	anethole	1803	0.17	<i>c</i>	72	unknown geranyl ester	>2500	0.41	<i>f</i>
						% uncharacterized compd		10.14	

<sup>a</sup>Numbers refer to Figure 2. <sup>b</sup>Calculated values. <sup>c</sup>Mass spectral data from the following sources: Masada, 1976; EPA/NIH, 1980; Jennings and Shibamoto, 1980; TNO, 1980; Swigar and Silverstein, 1981. <sup>d</sup>GC and GC-MS analyses of authentic compounds. <sup>e</sup>Tentative identified with mass spectral data matched one of the following references: Bednarczyk and Karmer, 1975; Bednarczyk et al., 1975; Halim and Collins, 1975; Hashizume and Sakata, 1970; Minyard et al., 1968; Sakurai et al., 1983; Torii and Okamoto, 1976. <sup>f</sup>Tentative identification or structure proposal based upon mass spectra only.

**Figure 5.** Mass spectrum of peak 34 (sesquisabinene hydrate) identified in F-3.**Figure 6.** Mass spectrum of peak 35 (zingiberenol) identified in F-3.

that long-chain fatty acid esters of monoterpenes are indispensable in creating certain specific nuances. It may be possible that some of those tentatively identified esters of monoterpenes and sesquiterpenes with higher GC re-

tentions (e.g., peaks 60–72) may relate to some of the sensory properties described above.

**Volatile Components in F-3.** There were 46 volatile components characterized in F-3 (Table III; Figure 3).

**Table III. Volatile Components of F-3 of Liquid Carbon Dioxide Extracted Ginger Oil**

peak no. <sup>a</sup>	compound	index <sup>b</sup> CW-20M	%	ID ref
1	2-heptanol	1323	1.51	c, d
2	cis-linalool oxide	1435	+	c
3	trans-linalool oxide	1464	+	c
4	6-methyl-5-hepten-2-ol	1465	+	c
5	2-nonanol	1524	+	c, d
6	linalool	1547	+	c, d
7	1-octanol	1560	+	c
8	sabinene hydrate	1562	+	c
9	isopulegol	1578	+	c
11	4-terpineol	1595	+	c
12	citronellyl formate	1605	+	c
13	neral	1665	0.60	c, d
14	$\alpha$ -terpineol	1694	5.28	c, d
15	borneol	1695	4.80	c, d
16	zingiberene	1696	1.40	c
17	geranial	1718	1.49	c, d
18	$\beta$ -sesquiphellandrene	1743	0.20	c
19	citronellol	1769	5.71	c, d
20	myrtenol	1787	0.20	c
21	nerol	1799	0.54	c, d
22	unknown alcohol	1845	0.20	f
23	geraniol	1851	8.30	c, d
24	isomer of cubebol	1882	0.55	e
25	$\beta$ -bisabolol	1908	+	e
26	isomer of cubebol	1936	1.73	e
27	int std (dodecanol, 4.80 mg)	1973	14.12	
28	sesquisabinene hydrate	1997	1.93	f
29	ethylmethoxyphenol	2022	+	c
30	nerolidol	2030	+	c
31	curcumenyl alcohol	2038	0.23	f
32	elemol	2076	2.78	c
33	unknown sesquiterpene alcohol	2087	0.20	f
34	sesquisabinene hydrate compd	2102	4.41	f
35	zingiberenol	2109	5.34	f
36	$\beta$ -10-cadinol	2144	0.53	c
37	unknown sesquiterpene alcohol	2177	1.44	f
38	unknown (base peak 150)	2182	4.13	f
39	guaiol	2214	1.10	c
40	$\beta$ -eudesmol	2222	4.12	c
41	unknown naphthalenol compd (MW 222)	2241	2.51	f
42	unknown (base peak 150)	2254	0.52	f
43	o-octylanisole	2282	0.36	c
44	trans- $\beta$ -sesquiphellandrol	2358	1.31	e
45	cis- $\beta$ -sesquiphellandrol	2396	0.15	e
46	unknown sesquiphellandrol compd	2499	3.60	f
47	coniferyl alcohol	>2500	+	c
48	zingerone	>2500	+	c, d
	% uncharacterized compd		18.71	

<sup>a</sup> Numbers refer to Figure 3. <sup>b</sup> Calculated values. <sup>c</sup> Mass spectral data from the following sources: Masada, 1976; EPA/NIH, 1980; Jennings and Shibamoto, 1980; TNO, 1980; Swigar and Silverstein, 1981. <sup>d</sup> GC and GC-MS analyses of authentic compounds. <sup>e</sup> Tentative identified with mass spectral data matched one of the following references: Bednarczyk and Karmer, 1975; Bednarczyk et al., 1975; Halim and Collins, 1975; Hashizume and Sakata, 1970; Minyard et al., 1968; Sakurai et al., 1983; Torii and Okamoto, 1976. <sup>f</sup> Tentative identification or structure proposal based upon mass spectra only.

Quantitative estimation using dodecanol (peak 27) as internal standard indicated that F-3 comprised about 10.65% (w/w) of the volatile fractions eluted.

The mass spectra of two tentatively identified sesquiterpene alcohols sesquisabinenehydrate (and isomers) and zingiberenol are shown in Figures 5 and 6. These compounds were reported previously by Terhune et al. (1975) in ginger. However, because reference mass spectra were unavailable, these components can only be categorized as being tentatively identified. Indirect evidence proving the presence of these components was the identification of two dehydration products—zingiberene and  $\beta$ -sesquiphel-

landrene. Terhune et al. (1975) proposed that  $\beta$ -sesquiphellandrene and zingiberenol underwent thermal dehydration during GC analysis to produce zingiberene and  $\beta$ -sesquiphellandrene.

The present finding of trace amounts of zingerone (peak 48), a pungent compound also derived from thermal degradation of gingerol compounds (Connell and Sutherland, 1969; Chen et al., 1986b; Chen and Ho, 1987), confirmed again the absence of severe thermal treatment of the present extraction method. Sensory analysis of F-3 indicated "green", "fatty green", and a faint "floral" characteristic, typical flavor notes reported for monoterpene and sesquiterpene alcohols (Arctander, 1969).

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**Registry No.**  $\alpha$ -Thujene, 2867-05-2;  $\alpha$ -pinene, 80-56-8; toluene, 108-88-3; camphene, 79-92-5;  $\beta$ -pinene, 127-91-3; sabinene, 3387-41-5;  $\delta$ -3-carene, 13466-78-9; myrcene, 123-35-3;  $\alpha$ -phellandrene, 99-83-2; limonene, 138-86-3;  $\beta$ -phellandrene, 555-10-2;  $\gamma$ -terpinene, 99-85-4; *p*-cymene, 99-87-6; ferdipolene, 586-62-9;  $\delta$ -elemene, 20307-84-0;  $\alpha$ -cubebene, 17699-14-8; amorphene, 65323-72-0;  $\alpha$ -copaene, 3856-25-5; *cis*-bergamotene, 18252-46-5;  $\beta$ -ylangene, 20479-06-5; elemene, 11029-06-4;  $\beta$ -elemene, 33880-83-0;  $\beta$ -caryophyllene, 87-44-5; selina-4(10),11-diene, 17066-67-0;  $\gamma$ -elemene, 29873-99-2;  $\beta$ -farnesene, 18794-84-8; cadina-4,9-diene, 523-47-7;  $\beta$ -gurjunene, 73464-47-8;  $\gamma$ -cadinene, 39029-41-9;  $\delta$ -cadinene, 483-76-1; zingiberene, 495-60-3;  $\gamma$ -bisabolene, 495-62-5;  $\beta$ -bisabolene, 495-61-4;  $\beta$ -sesquiphellandrene, 20307-83-9; ar-curcumene, 644-30-4; germacrene B, 15423-57-1; calamenene, 483-77-2; acetone, 67-64-1; 2-heptanone, 110-43-0; hexanal, 66-25-1; 1,8-cineol, 470-82-6; 2-heptyl acetate, 5921-82-4; octanal, 124-13-0; 6-methyl-5-hepten-2-one, 110-93-0; 2,6-dimethyl-5-heptenal, 106-72-9; 2-nonanone, 821-55-6; 2-nonyl acetate, 14936-66-4; 2-nonanal, 2463-53-8; citronellal, 106-23-0; decanal, 112-31-2; camphor, 76-22-2; limonene epoxide (isomer 1), 28098-68-2; limonene epoxide (isomer 2), 28098-67-1; linalool, 78-70-6; bornyl acetate, 76-49-3; undecanone, 112-12-9; 4-terpineol, 562-74-3; propenyl benzyl ether, 15895-87-1; isopinocamphe, 80-56-8; citronellyl acetate, 150-84-5; neral, 106-26-3; geranial, 141-27-5; geranyl acetate, 105-87-3; 2-tridecanone, 593-08-8; anethole, 104-46-1;  $\beta$ -ionone, 79-77-6;  $\beta$ -caryophyllene oxide, 1139-30-6; 2-pentadecanone, 2345-28-0; zingiberenol, 58334-55-7;  $\beta$ -10-cadinol, 36564-42-8; propenyldimethoxybenzene, 73105-29-0; farnesyl aldehyde, 19317-11-4; eugenol, 97-53-0; 2-heptanol, 543-49-7; *cis*-linalool oxide, 11063-77-7; *trans*-linalool oxide, 11063-78-8; 6-methyl-5-hepten-2-ol, 1569-60-4; 2-nonanol, 628-99-9; 1-octanol, 111-87-5; sabinene hydrate, 546-79-2; isopulegol, 89-79-2; citronellyl formate, 105-85-1;  $\alpha$ -terpineol, 98-55-5; borneol, 507-70-0; citronellol, 106-22-9; myrtenol, 515-00-4; nerol, 106-25-2; geraniol, 106-24-1;  $\beta$ -bisabolol, 15352-77-9; sesquisabinene, 58319-04-3; ethylmethoxyphenol, 80652-16-0; nerolidol, 7212-44-4; curcumenyl alcohol, 19431-84-6; elemol, 639-99-6; guaial, 489-86-1;  $\beta$ -eudesmol, 473-15-4; o-octylanisole, 20056-59-1; *trans*- $\beta$ -sesquiphellandrol, 56144-27-5; *cis*- $\beta$ -sesquiphellandrol, 56144-26-4; coniferyl alcohol, 458-35-5; zingerone, 122-48-5; carbon dioxide, 124-38-9; cubebol, 23445-02-5.

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## Uptake of Selenium and Mutagens by Vegetables Grown in Fly Ash Containing Greenhouse Media

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Broccoli, endive, lettuce, onions, spinach, tomatoes, and perennial ryegrass were cultured in pots or pillow packs containing growth media that had been amended with increasing percentages of soft coal fly ash. The crops absorbed selenium in proportion to the percentage of fly ash in the growth media. Broccoli, onions, and lettuce absorbed the highest concentrations of selenium. The levels of selenium in ryegrass were maintained through five successive cuttings of the plants. Endogenous mutagens were found in endive, lettuce, and ryegrass. Endive and lettuce absorbed and translocated mutagens from the fly ash amended soil whereas ryegrass did not have this ability. A rat microsomal enzyme preparation was able to partially deactivate these mutagens to innocuous compounds.

Dietary selenium, at controlled levels above minimum requirements, has been correlated with anticarcinogenic properties in both laboratory animal studies and human epidemiologic investigations (Chortyk and Schlotzhauer, 1984). This property of selenium may be due to two of its known functions including its role in the active site of the enzyme glutathione peroxidase (Rotruk et al., 1973)

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and its interaction with toxic heavy metals. Glutathione peroxidase inactivates hydroperoxides and lipoperoxides, thus protecting cell constituents against free-radical damage (Tappel, 1978). Although the mechanism is not understood, selenium counteracts the toxicity of cadmium and mercury, possibly by promoting their accumulation in nontoxic forms (Frost and Lisk, 1975).

The minimum requirement for selenium in man is estimated to range from 0.05 to 0.1 ppm in the diet. In many areas of the world, soils and also crops cultivated in these areas are deficient in selenium. Human selenium deficiency has been associated with Keshan disease in China where the soils have been shown to be deficient in selenium. The heart myopathy that develops in children raised in this area can be remedied in its early stages by