

Characteristics of the Steam-Distilled Oil and Carbon Dioxide Extract of *Zanthoxylum simulans* Fruits[†]

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The volatiles were isolated from fruits of *Zanthoxylum simulans* Hance using steam distillation and liquid carbon dioxide extraction, respectively, and analyzed by GC, GC–MS (both EI and CI modes), and GC–FTIR. Totally, 43 compounds were identified in the volatiles, including 20 terpenes, 10 alcohols, 8 esters, and 5 other components. Using chemical ionization, only terpenes became protonated ions, for which accurate molecular weights could be obtained. The yield in steam distillation was 1.69% (w/w), while that in liquid carbon dioxide extraction was 6.38% (w/w). The major volatile components (>10%) found in both volatiles were β -myrcene, limonene, 1,8-cineole, and (*Z*)- β -ocimene. Some minor components, including isobutyl acetate, isoamyl acetate, and α -terpinene, were not found in the extract of liquid carbon dioxide.

Keywords: *Zanthoxylum simulans*; characteristics; essential oil; carbon dioxide extract

INTRODUCTION

Chinese peppers, fruits of *Zanthoxylum simulans* Hance, are commonly cultivated in Guangdong, Henan, Shanxi, Jiangsu, and Hebei provinces in mainland China. Normally, the dried fruits are served as a spice for food flavoring, especially in stew meat cooking.

Chinese peppers are pungent but not as strong and specific as peppers (*Capsicum annuum*), in which piperine and chavicine are the major pungent components (Govindarajan, 1977). In fact, α - or β -sanshool was described as the character of pungency to Chinese peppers (Govindarajan, 1979). The distinct pungency is apparently due to the differences in the chemical structure of these pungent components. In the aroma profile from olfactory sensation, Chinese peppers should differ from peppers.

Although the chemical composition of the essential oils of *Zanthoxylum* species has been previously studied, especially *Zanthoxylum bungeanum* (Tirillini et al., 1991; Tirillini and Stoppini, 1994), the aroma constituents of Chinese peppers have not been reported in the literature. The research reported herein was designed to study the volatile constituents of the steam-distilled oil and carbon dioxide extract isolated from Chinese peppers. The physicochemical characteristics of these two different volatile isolates were also compared.

EXPERIMENTAL PROCEDURES

Materials. Chinese peppers (the dried fruits of *Z. simulans*) were purchased at a local market in Hsinchu City, Taiwan, and then ground to pass a 20-mesh sieve. Ground materials collected from the 20-mesh sieve were used for steam distillation and extraction.

Steam Distillation. Ground dried Chinese peppers (100 g) were placed into the steam distillation apparatus and distilled for 2 h.

Liquid Carbon Dioxide Extraction. Ground dried Chinese peppers were extracted using the method of Chen et al. (1986). Ground Chinese peppers (10 g) were weighed into a Soxhlet glass cell, and the cell was placed into the carbon dioxide extraction apparatus (J&W Scientific, Folsom, CA). After approximately 210 g of dry ice was weighed into the extraction vessel, the apparatus was closed and held in a water bath at 40 ± 1 °C. The extraction was carried out at the equilibrated pressure of 745–755 psig for 72 h.

Refractive Index Determination. The refractive index of the oil and extract was determined at 20 °C, using an Abbé refractometer (ATAGO, IT, Japan).

Gas Chromatographic (GC) Analysis. A Hewlett-Packard 5880A gas chromatograph equipped with a fused silica capillary column (50 m \times 0.23 mm i.d.; 0.12 μ m thickness, CP-Wax 52 CB, bonded polyethylene glycol phase, Chrompack) and a flame ionization detector was used to analyze the volatile components in the oil and extract. The operating conditions were as follows: injector temperature, 250 °C; detector temperature, 250 °C; hydrogen carrier flow rate, 1.5 mL/min; temperature program, 50 °C (10 min), 1.5 °C/min, 200 °C (10 min). The injection volume of sample was 0.2 μ L. A split ratio of 100:1 was used.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis. Mass spectra were collected using a Hewlett-Packard 5890A GC equipped with a Model TSQ-70 (Finnigan MAT, San Jose, CA) quadrupole mass spectrometer and the same column and temperature program as used for the gas chromatography except for the film thickness of 0.17 μ m. Helium was used as carrier gas at the flow rate of 1.5 mL/min. The injection volume of sample was 0.25 μ L. A split ratio of 60:1 was used. Mass spectra were obtained by electron-impact ionization (EI) at 70 eV and were compared with those of mass spectra libraries (NIST, U.S. National Institute of Standards and Technology). The operating conditions were as follows: injector temperature, 250 °C; ion source temperature, 120 °C; temperature of all connection parts, 210 °C; electron current, 200 A; electron multiplier voltage, 1200 V.

Mass spectra were also obtained by chemical ionization (CI) using methane (reagent grade, Takachiho, Kagakokugyo KX, Japan) as the reagent gas. The pressure of the reagent gas was adjusted in such a way that the relative intensities of $[\text{CH}_3]^+$ and $[\text{C}_2\text{H}_5]^+$ produced were 100% and 70%, respectively.

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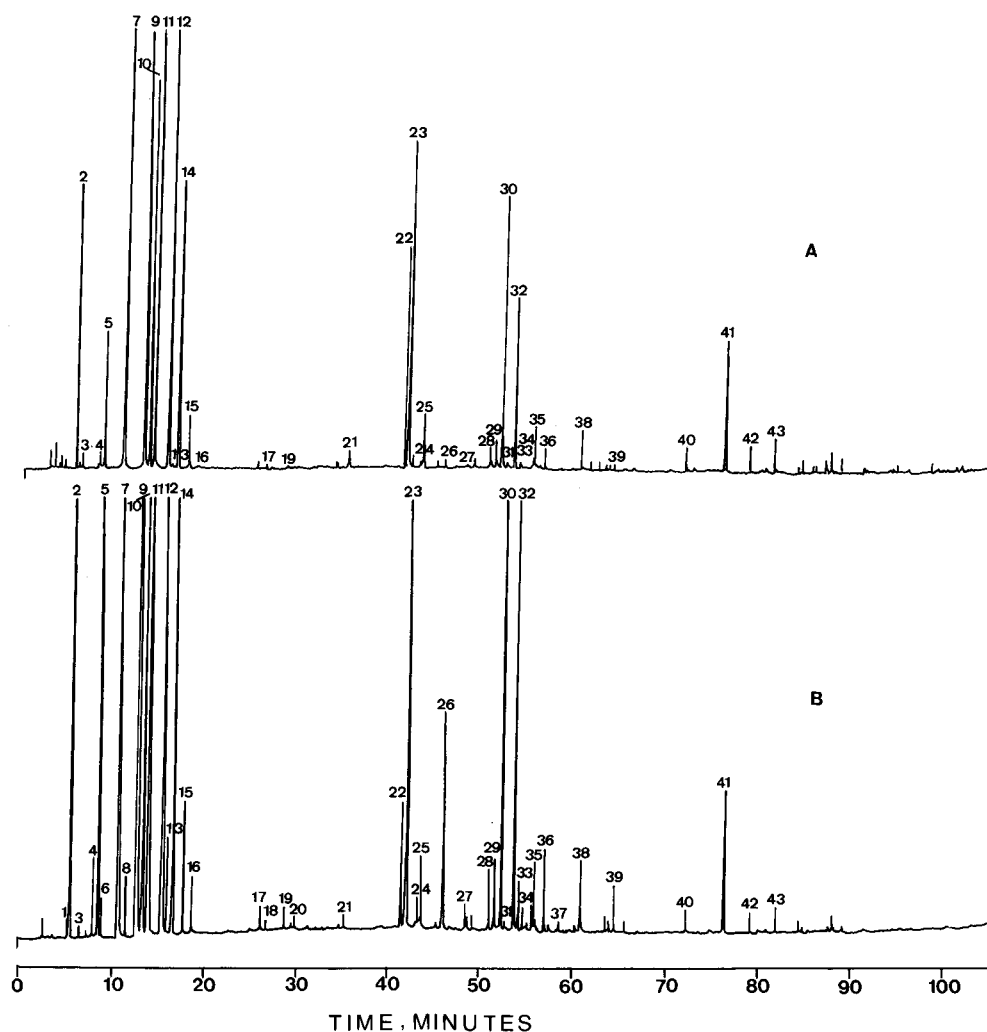


Figure 1. Capillary gas chromatograms of the volatile components from the fruits of *Z. simulans* using liquid carbon dioxide extraction (A) and steam distillation (B).

Table 1. Physicochemical Characteristics of the Steam-Distilled Oil and Liquid Carbon Dioxide Extract from the Fruits of *Z. simulans*^a

	steam-distilled oil	liquid CO ₂ extract
yield (% w/w)	1.69	6.30
refractive index	1.48	1.49
appearance	golden yellow	brown
λ_{\max} (nm)	266	268

^a Duplicate experiment.

Gas Chromatography–Fourier Transform Infrared Spectrometry (GC–FTIR) Analysis.

The oil and extract from ground Chinese peppers were analyzed using the method of Chyau and Wu (1989). A Hewlett-Packard 5890A GC equipped with a Hewlett-Packard 5965A infrared detector (IRD) and the same column and stationary phase as used for the gas chromatography were used. The operating conditions were as follows: injector temperature, 250 °C; detector temperature, 250 °C; helium carrier flow rate, 1.5 mL/min; temperature program, 50 °C (3 min), 3 °C/min, 200 °C (30 min). The injection volume of sample was 0.2 μ L. A split ratio of 50:1 was used.

RESULTS AND DISCUSSION

The extracts were isolated from the Chinese peppers (*Z. simulans* fruits) using steam distillation and liquid carbon dioxide extraction, and their characteristics are shown in Table 1. The refractive indices of two products

are similar, while their yields and appearance were greatly affected by the method of volatile isolation employed. The yield of oil was 1.69% (w/w), while that of the liquid carbon dioxide extract was 6.38% (w/w). However, the absorption maxima in the UV spectrum are similar.

The two volatile isolates from Chinese peppers were subjected to gas chromatography analysis, and their volatile components are shown in Figure 1. Generally, the two chromatograms exhibited the same volatile profile, except for some quantitative differences. These volatile components were primarily identified by their Kovats indices using standard C₈–C₂₅ alkanes and further analyzed using GC–MS (both EI and CI modes) and GC–FTIR. From the results shown in Tables 2 and 3, totally 43 compounds were identified in the volatiles, including 20 terpenes, 10 alcohols, 8 esters, and 5 other components.

A comparison of the quantitative differences of volatiles between the oil and extract (Table 2) shows that the effects of hydrolysis on components of the oil might be the reason for quantitative differences. Linalool, one of the high-level components, was found in a high amount in the oil. It was postulated that it might be formed from the hydrolysis of linalyl acetate. Similar results were also observed when the CO₂ extract of *Lavandin grosso* flowers was compared with the oil (Pellerin, 1991). However, some minor components, including isobutyl acetate, isoamyl acetate, and α -ter-

Table 2. Constituents of the Steam-Distilled Oil and Liquid Carbon Dioxide Extract from the Fruits of *Z. simulans*

peak no. ^a	compound	Kovats index ^b	peak area ^c (%)		MW	CI ^d [M + H] ⁺	mode of identification ^e
			A	B			
1	isobutyl acetate	1012		0.03	116		GC, EI
2	α -pinene	1017	2.70	4.16	136	137	GC, EI, CI
3	camphene	1057	0.13	0.04	136	137	GC, EI, CI
4	β -pinene	1103	0.21	0.27	136	137	GC, EI, CI
5	sabinene	1114	1.71	1.60	136	137	GC, EI, CI
6	isoamyl acetate	1118		0.11	130		GC, EI
7	β -myrcene	1153	9.39	11.98	136	137	GC, EI, CI
8	α -terpinene	1167		0.23	136	137	GC, EI, CI
9	limonene	1192	18.66	18.61	136	137	GC, EI, CI
10	β -phellandrene	1201	5.75	6.87	136	137	GC, EI, CI
11	1,8-cineole	1210	10.50	14.23	154	155	GC, EI, CI
12	(<i>Z</i>)- β -ocimene	1228	12.74	15.42	136	137	GC, EI, CI
13	γ -terpinene	1234	0.08	0.37	136	137	GC, EI, CI
14	(<i>E</i>)- β -ocimene	1242	3.94	6.13	136	137	GC, EI, CI
15	<i>p</i> -cymene	1258	0.76	0.53	134	135	GC, EI, CI
16	terpinolene	1270	0.04	0.24	136	137	GC, EI, CI
17	(<i>Z</i>)-3-tridecen-1-yne	1363	0.03	0.11	178		GC, EI
18	heptyl acetate	1392	0.03	0.12	158	159	GC, EI, CI
19	2-methyl-2-heptenal	1393	0.07	0.09	126		GC, EI
20	3,4-dimethyl-2,4,6-octatriene	1408		0.15	136		GC, EI
21	(<i>E</i>)-sabinene hydrate	1473	0.28	0.18	154		GC, EI
22	linalyl acetate	1553	3.51	0.52	196		GC, EI
23	linalool	1559	4.76	5.90	154	155	GC, EI, CI
24	<i>n</i> -octanol	1573	0.12	0.17	130		GC, EI
25	linalyl formate	1579	0.96	0.35	182		GC, EI
26	4-terpineol	1610	0.13	0.93	154		GC, EI
27	β -caryophyllene	1642	0.18	0.16	204	205	GC, EI, CI
28	sabina ketone	1675	0.38	0.32	138		GC, EI
29	α -humulene	1684	0.54	0.41	204	205	GC, EI, CI
30	α -terpineol	1693	4.95	2.65	154		GC, EI
31	3-thujen-2-ol	1707	0.11	0.15	152		GC, EI
32	α -terpinyl acetate	1712	2.69	2.62	196		GC, EI
33	germacrene D	1720	0.22	0.26	204	205	GC, EI, CI
34	neryl acetate	1727	0.08	0.13	196		GC, EI
35	zingiberene	1743	0.69	0.32	204	205	GC, EI, CI
36	geranyl acetate	1758	0.31	0.34	196		GC, EI
37	δ -cadinene	1773		0.11	204	205	GC, EI, CI
38	allethrolone	1814	0.68	0.39	152		GC, EI
39	geraniol	1868	0.08	0.21	154		GC, EI
40	sesquiterpene alcohol	1986	0.47	0.11	220		GC, EI
41	farnesol ^f	2054	2.01	0.58	222		GC, EI
42	elemol	2098	0.40	0.10	222		GC, EI
43	spathulenol	2145	0.57	0.12	220		GC, EI

^a The peak numbers correspond to Figure 1. ^b Linear indices determined on CP-Wax 52 CB column. ^c A, liquid carbon dioxide extract; B, steam-distilled oil. (Both averaged from duplicate experiment). ^d Determined in methane positive chemical ionization. ^e Identified by GC, electron-impact and chemical ionization MS techniques, respectively. ^f Correct isomer not identified.

pinene, were not found in the CO₂ extract. We believe that these components, which were found only in the steam-distilled oil, might have been formed during the distillation process.

The major components (>10%) found in both volatile concentrates were β -myrcene, limonene, 1,8-cineole, and (*Z*)- β -ocimene (Table 2). These results are similar to those of the essential oil from *Z. bungeanum* dried fruits using hydrodistillation (Tirillini et al., 1991), which contained 16.62% β -myrcene, 26.98% limonene, and only 1.51% 1,8-cineole. Tirillini et al. (1994) analyzed the essential oil from *Z. bungeanum* fresh fruits and found that its major volatile components were 25.26% β -pinene, 14.07% β -phellandrene, 20.47% limonene, and 12.20% (*Z*)- β -ocimene. Tirillini et al. (1994) also found that the fruit secretory gland contained 9.23% β -pinene, 36.68% β -phellandrene, 9.29% piperitone, and 19.51% hydroxy- α -sanshool. However, in this study the oil and CO₂ extract from *Z. simulans* fruits contained higher percentages of 1,8-cineole (10.50% and 14.23%) and smaller percentages of limonene (18.66% and 18.61%) and β -myrcene (9.39% and 11.98%), respectively.

In the identification of the volatile components, the mass spectrometry technique provided the advantage

of high sensitivity and rapid elucidation of the compound of interest with the help of a mass spectral data bank. In addition, FTIR spectra of the compound of interest could supplement detailed structure elucidation (Bicchi and Frattini, 1987), especially for the determination of isomers. In the case of γ -terpinene and terpinolene, both of their spectra contained fragment ions 93, 121, and 136, while they absorbed differently in the infrared region (Table 3).

Using methane positive chemical ionization, only monoterpenes, with the exception of 3,4-dimethyl-2,4,6-octatriene, became protonated ions, thereby allowing us to accurately obtain their molecular weights. These results were consistent with the findings of Vernin and Metzger (1984), who used ammonia positive chemical ionization. However, for other types of components similar correlation was not observed.

In addition, the extract of liquid carbon dioxide might also contain nonvolatile components, which may contribute most to the pungent taste of Chinese peppers, or chemical and bioactive constituents as found in the roots and bark of *Z. simulans* (Wu and Chen, 1993; Chen et al., 1994a,b). However, the composition of these nonvolatile components and their significance in the

Table 3. Characteristic Infrared Bands of Some Major Volatile Components in the Steam-Distilled Oil from the Fruits of *Z. simulans*

peak no. ^a	compound	IR band position ^b (cm ⁻¹)
2	α-pinene	787, 1213, 1452, 2927 (s)
5	sabinene	871, 1030, 1148, 1463, 1657, 2964 (s,d), 3076
7	β-myrcene	901 (s), 990, 1106, 1447 (d), 1596, 1602, 2931 (s,d), 3092
8	α-terpinene	795, 1166, 1462 (s), 2964 (s,d)
9	limonene	893, 987, 1080, 1221, 1379, 2931 (s,d), 3083
10	β-phellandrene	907, 967, 1383, 1446, 1595, 2741, 2929 (s), 2979, 3095
11	1,8-cineole	884, 987, 1081, 1221, 1377, 1466, 2943 (s,d)
12	β-ocimene	900, 989, 1103, 1284, 1388, 1611, 1798, 2742, 2928 (s,d), 3093
13	γ-terpinene	950, 1463, 2902 (s), 2970
15	p-cymene	815, 1516, 2989 (s)
16	terpinolene	795, 1211, 1445, 1710, 2916 (s,d)
18	heptyl acetate	1051, 1295 (s), 1373, 1462, 1762, 2939 (d)
20	3,4-dimethyl-2,4,6-octatriene	792, 956, 1236, 1383, 1649, 1756, 2927 (s)
22	linalyl acetate	930, 1016, 1242 (s), 1374, 1750 (s), 2933 (d)
23	linalool	833, 923 (s), 996, 1103, 1310, 1378, 1452, 2931 (s), 2979 (s), 3637
24	n-octanol	925, 1050, 1227, 1280, 1712, 2933 (s,d)
26	4-terpineol	1035, 1242 (s), 1376, 1755, 2963 (s)
28	sabina ketone	897, 1009, 1139, 1258, 1377, 1752 (s), 2938 (s,d), 3082
30	α-terpineol	798, 927, 1163, 1379, 2973 (s,d), 3642
32	α-terpinyl acetate	1018, 1136, 1259 (s), 1374, 1792 (s), 2933
36	geranyl acetate	1028, 1235 (s), 1377, 1759, 2937 (s)
41	farnesol	925, 1105, 1362, 1652, 2929 (s,d), 3635
42	elemol	907, 1005, 1169, 1381, 1636, 2874, 2973 (s), 3086, 3643

^a The peak numbers correspond to Figure 1. ^b s, strong; d, double.

pungent characteristics of Chinese peppers need to be investigated further.

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