Characterization of Cold-Pressed Key and Persian Lime Oils by Gas Chromatography, Gas Chromatography/Mass Spectroscopy, High-Performance Liquid Chromatography, and Physicochemical Indices

Paola Dugo,[†] Luigi Mondello,[‡] Giuseppe Lamonica,[‡] and Giovanni Dugo*,[‡]

Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.FF.NN., Università di Messina, Salita Sperone, 98166 Messina, Italy, and Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, Viale Annunziata, 98168 Messina, Italy

The physicochemical indices and the qualitative and quantitative composition of the volatile fraction and the oxygenated heterocyclic fraction of cold-pressed Key lime oil (types A and B) and Persian lime oil are reported. The volatile fraction of Persian lime oil is characterized by a higher content of limonene, γ-terpinene, esters, and monoterpene aldehydes and a lower content of β-pinene + sabinene, sesquiterpenes, and aliphatic aldehydes than Key lime oils. Oxypeucedanin was not detected in Key lime oil type A, while it is present in Key lime oil type B and Persian lime oil. This is probably due to the extraction technology used for Key lime oil type A, which allows the essential oil to come into contact with the juice. Under these conditions, the epoxy ring of oxypeucedanin is opened by hydrolysis to form oxypeucedanin hydrate.

INTRODUCTION

The volatile fraction of acid lime essential oil has been widely studied. Data reported in the literature refer to distilled lime oil (Guenther and Longenau, 1943; Slader, 1961a,b; Kovats, 1963; Scora et al., 1968; Guzman and Huet, 1970; Ziegler, 1971; Perez Zayas and Tapanes, 1974; Tapanes, 1974; Azzous and Reineccius, 1976; Moshonas and Shaw, 1980; McHale, 1980; Pino and Tapanes, 1983; Analytical Methods Committee, 1984; Alessandro et al., 1985; Haro and Faas, 1985; Chamblee et al., 1985; Khurdiya and Maheshwari, 1988; Pino and Rosado, 1988; Inoma et al., 1989; Edwards and Marr, 1990; Clark and Chamblee, 1992; Ohloff, 1994; Della Porta et al., 1995) and to cold-pressed Key (Slader, 1961a; Hunter and Brogden, 1965; Hunter and Moshonas, 1966; Calvarano and Gallino, 1975; Azzous and Reineccius, 1976; Huet et al., 1978; McHale, 1980; El-Samahy et al., 1982; Alessandro et al., 1985; Haro and Faas, 1985; Chamblee et al., 1985; Clark et al., 1987; Huet, 1991; Clark and Chamblee, 1992; Mondello et al., 1995a) and Persian (Shaw et al., 1971; Huet et al., 1978; Koketsu et al., 1983; Haro and Faas, 1985; Lancas et al., 1988; Lancas and Cavicchioli, 1990; Edwards and Marr, 1990; Huet, 1991; Olhoff, 1994) lime oils. Some papers report the composition of unknown cold-pressed lime oils (Ikeda et al., 1962; MacLeod and Buigues, 1964; Ashoor and Bernhard, 1967; MacLeod, 1968; Ziegler, 1971) and of solvent-extracted Key (Yang et al., 1992) and Persian (Shaw and Wilson, 1976) lime oils. Moreover, some papers distinguish between the composition of Key lime oil type A, obtained by centrifuga-

tion of the oil/juice emulsion produced by passing the whole fruit through a screw-press which crushes the fruit, and Key lime oil type B, obtained by rasping the peel to release the oil (Haro and Faas, 1985; Huet, 1991; Clark and Chamblee, 1992). The oxygen heterocyclic compounds of the nonvolatile fraction have been less extensively studied (Caldwell and Jones, 1945; Stanley and Vannier, 1957, 1967; Cieri, 1969; Latz and Madsen, 1968; Madsen and Latz, 1970; Calvarano and Gallino, 1975; Shu et al., 1975; Latz and Ernes, 1978; McHale, 1980; McHale and Sheridan, 1989; Huet, 1991; Nigg et al., 1993), and quantitative data are reported only by Stanley (1967), Cieri (1969), Shu (1975), McHale (1980), and McHale and Sheridan (1989). Only three papers report concurrent results on the composition of the volatile and nonvolatile fractions (Calvarano and Gallino, 1975; McHale, 1980; Huet, 1991). Most of the literature has been reviewed by Shaw (1979) and Lawrence (1976, 1978, 1980, 1981/82, 1982, 1982/83, 1985a,b, 1986, 1991, 1996).

This paper reports results on the chemical and physicochemical characterization of cold-pressed Key lime oils (types A and B) and cold-pressed Persian lime oils of different geographical origins.

MATERIALS AND METHODS

This research was carried out on 12 samples of industrial, cold-pressed Key and Persian lime oils, as described in Table 1. The following analyses were conducted on the oils: refractive index, optical rotation, evaporation residue, CD, GC, and GC/MS of the volatile fraction, and normal phase HPLC of the oxygen heterocyclic compounds of the nonvolatile fraction.

Physicochemical Indices. The physicochemical indices were determined following the ISO regulations.

CD. CD was determined according to the method of Sale (1953).

Gas Chromatographic Analysis (GC/FID). The volatile fraction was analyzed by HRGC/FID as described. Gas

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* Author to whom correspondence should be addressed (phone, +39-90-356022; fax, +39-90-6766532; e-mail, gdugo@pharma.unime.it).

† Dipartimento di Chimica Organica e Biologica.

‡ Dipartimento Farmaco-chimico.

Cold-Pressed Key and Persian Lime Oils

J. Agric. Food Chem., Vol. 45, No. 9, 1997 **3609**

Table 1. Analyzed Samples of Lime Oils

sample	type	origin	extraction technology
1	Key	Mexico	A
2	Key	Mexico	A
3	Key	Mexico	A
4	Key	unknown	A
5	Key	Mexico	B
6	Persian	Mexico	B
7	Persian	Mexico	B
8	Persian	Brazil	B
9	Persian	Florida	B
10	Persian	unknown	B
11	Persian	unknown	B
12	Persian	unknown	B

chromatograph: Fisons Mega Series 5160 equipped with a flame ionization detector, a split-splitless injector, and a Fisons data processor DP800. Fused silica capillary column: 30 m × 0.32 mm i.d., coated with SE-52 0.40−0.45 μm film thickness; column temperature, 45 °C (6 min) to 300 °C at 3 °C/min; injector and detector temperature, 280 °C; carrier gas, He at 100 kPa (3 mL/min); injection mode, split; split ratio, 100; injected volume, 0.4 μL of neat oil.

GC/MS Analysis. Some samples were analyzed by GC/MS (EI) on a Fisons MD800 (Milan, Italy) system coupled with Adams' library (Adams, 1995) and FFC banks (Mondello et al., 1995b); GC conditions: fused silica capillary column DB5-MS 30 m × 0.25 mm i.d., 0.25 μm film thickness; column temperature, 60−325 °C (10 min) at 3 °C/min; carrier gas, He delivered at a constant pressure of 83 kPa (39.7 cm/s); 1 μL of solution (1/10, v/v, essential oil/pentane) injected on a split-splitless injector; injector temperature, 250 °C; injection mode,

Table 2. Physicochemical Indices of the Cold-Pressed Key Lime Oils Analyzed

sample	optical rotation (deg)	refractive index	CD	residue (%)
1	+37.0	1.4859	6.4	13.4
2	+38.0	1.4855	6.7	13.4
3	+37.0	1.4844	6.4	10.1
4	+37.0	1.4842	6.6	12.3
5	+36.0	1.4860	7.0	14.0
average	+37.0	1.4852	6.6	12.6

Table 3. Physicochemical Indices of the Cold-Pressed Persian Lime Oils Analyzed

sample	optical rotation (deg)	refractive index	CD	residue (%)
6	46.2	1.4825	6.8	9.5
7	49.0	1.4810	5.8	8.1
8	48.0	1.4825	7.1	9.4
9	43.5	1.4808	5.2	8.9
10	50.0	1.4813	6.8	8.9
11	44.0	1.4822	6.2	9.9
12	50.2	1.4821	7.1	9.7
average	47.3	1.4818	6.4	9.2

split; split ratio, 20. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; *E* energy, 70 eV; mass scan range, 39−350 amu.

Separation of the Coumarin and Psoralen Components of the Nonvolatile Fraction. A 500 mL sample of Mexican lime oil was hydrodistilled to remove most of the volatile fraction; part of the residue (∼20 g) was fractionated
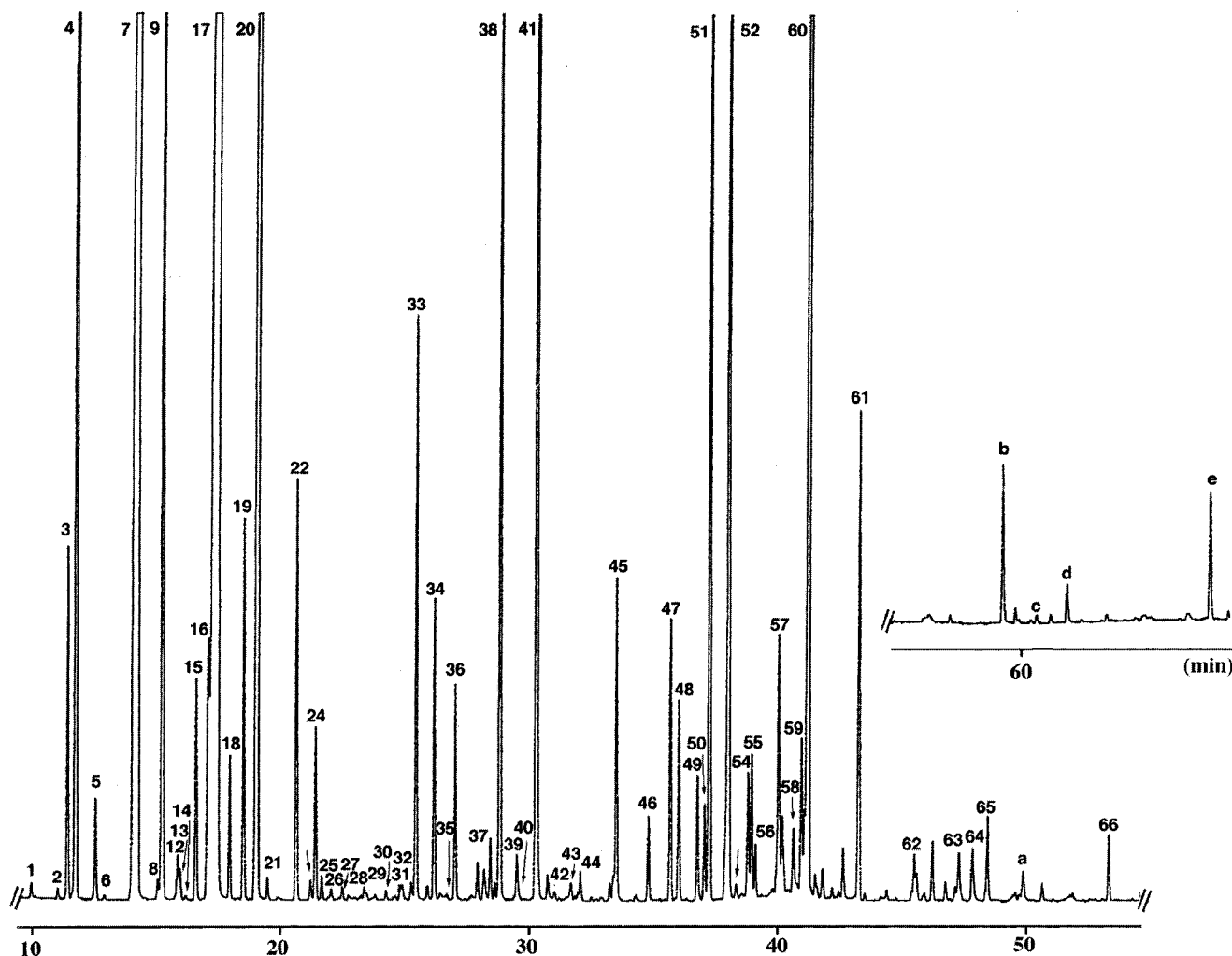

Figure 1. GC chromatogram of the volatile fraction of a cold-pressed Key lime oil: (a) 7-methoxycoumarin, (b) citropten, (c) isobergapten (tentative), (d) bergapten, (e) isopimpinellin. For identification of the other peaks, see Table 4.

3610 *J. Agric. Food Chem.,* Vol. 45, No. 9, 1997

Dugo et al.

Table 4. Composition of the Volatile Fraction of Key Lime Oil

peak no.	compounds	composition (%)					
		1[a]	2[a]	3[a]	4[a]	average[a]	5[b]
1	nonane	0.017	0.013	0.014	0.014	0.014	0.017
2	tricyclene	0.013	0.009	0.012	0.011	0.011	0.012
3	α-thujene	0.366	0.356	0.433	0.410	0.391	0.391
4	α-pinene	2.339	2.295	2.705	2.477	2.454	2.442
5	camphene	0.125	0.109	0.118	0.118	0.117	0.113
6	thuja-2,4(10)-diene[c]	0.006	0.005	0.005	0.005	0.005	0.005
7	sabinene + β-pinene	21.946	21.780	25.454	24.260	23.360	24.327
8	6-methyl-5-hepten-2-one	0.027	0.021	0.009	0.012	0.017	0.013
9	myrcene	1.257	1.259	1.297	1.242	1.264	1.179
10	dehydro-1,8-cineole[c]	tr	tr				tr
11	decane[c]	tr	tr				tr
12	octanal	0.065	0.062	0.054	0.052	0.058	0.042
13	α-phellandrene	0.034	0.024	0.038	0.034	0.033	0.027
14	δ-3-carene	0.005	0.005	0.003	0.005	0.005	tr
15	α-terpinene	0.354	0.251	0.242	0.173	0.255	0.091
16	p-cymene	0.301	0.393	0.229	0.408	0.323	1.947
17	limonene	49.281	49.317	49.422	49.387	49.352	49.380
18	(Z)-β-ocimene	0.145	0.142	0.131	0.137	0.139	0.128
19	(E)-β-ocimene	0.387	0.397	0.379	0.390	0.388	0.343
20	γ-terpinene	8.037	7.727	7.712	7.703	7.795	6.192
21	cis-sabinene hydrate	0.044	0.021	0.046	0.049	0.040	0.014
22	terpinolene	0.493	0.428	0.408	0.366	0.424	0.306
23	trans-sabinene hydrate[c]	0.042	0.020	0.042	0.043	0.037	0.015
24	linalool	0.181	0.178	0.158	0.166	0.171	0.170
25	nonanal	0.026	0.023	0.019	0.019	0.022	0.024
26	exo-fenchol	0.018	0.015	0.001	0.003	0.009	tr
27	cis-p-menth-2-en-1-ol[c]	0.017	0.019	0.010	0.014	0.015	0.015
28	trans-pinocarveol	0.012	0.015	0.014	0.012	0.013	tr
29	trans-p-menth-2-en-1-ol[c]	0.009	0.004	0.010	0.006	0.007	0.054
30	citronellal	0.008	0.009	0.012	0.013	0.010	0.029
31	borneol	0.037	0.030	0.024	0.015	0.027	0.018
32	cis-pinocamphone[c]	0.016	0.019	0.016	0.018	0.017	0.024
33	terpinen-4-ol	0.705	0.606	0.367	0.409	0.522	0.143
34	α-terpineol	0.347	0.318	0.221	0.237	0.281	0.213
35	dodecane[c]	0.008	0.003	0.004	0.008	0.006	0.034
36	decanal	0.223	0.220	0.199	0.201	0.211	0.137
37	nerol	0.047	0.046	0.016	0.035	0.036	0.095
38	neral	1.249	1.188	1.059	1.095	1.148	1.155
39	geraniol	0.068	0.066	0.030	0.031	0.049	0.037
40	piperitone	tr	tr	tr	tr	tr	tr
41	geranial + perilla aldehyde	2.000	1.974	1.710	1.759	1.861	1.967
42	bornyl acetate	0.010	0.008	0.006	0.009	0.008	0.019
43	tridecane[c]	0.022	0.027	0.026	0.015	0.023	0.013
44	undecanal	0.031	0.042	0.024	0.025	0.031	0.021
45	δ-elemene	0.337	0.371	0.247	0.273	0.307	0.068
46	neryl acetate	0.080	0.088	0.066	0.074	0.077	0.073
47	geranyl acetate	0.243	0.287	0.210	0.237	0.244	0.254
48	β-elemene	0.191	0.219	0.117	0.186	0.178	0.160
49	dodecanal	0.122	0.131	0.100	0.108	0.115	0.079
50	cis-α-bergamotene	0.095	0.102	0.076	0.084	0.089	0.089
51	β-caryophyllene	1.063	1.194	0.957	1.058	1.068	0.977
52	trans-α-bergamotene	1.433	1.581	1.132	1.298	1.361	1.353
53	(Z)-β-farnesene	0.010	0.016	0.008	0.013	0.012	0.013
54	α-humulene	0.124	0.139	0.102	0.122	0.122	0.112
55	(E)-β-farnesene	0.134	0.156	0.110	0.119	0.130	0.120
56	β-santalene	0.054	0.056	0.040	0.047	0.049	0.050
57	germacrene D	0.335	0.487	0.258	0.327	0.352	0.159
58	α-selinene	0.130	0.126	0.076	0.107	0.110	0.077
59	(Z)-α-bisabolene	0.174	0.205	0.059	0.175	0.153	0.153
60	(E,E)-α-farnesene + β-bisabolene	3.329	3.791	2.602	2.997	3.180	3.080
61	germacrene B	0.567	0.544	0.447	0.429	0.497	0.335
62	tetradecanal	0.052	0.057	0.063	0.045	0.054	0.050
63	2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	0.072	0.073	0.046	0.077	0.067	0.071
64	campherenol	0.073	0.072	0.048	0.066	0.065	0.073
65	α-bisabolol	0.099	0.105	0.070	0.088	0.091	0.096
66	hexadecanal	0.068	0.072	0.039	0.048	0.057	0.045
	hydrocarbons	93.112	93.547	94.863	94.398	93.980	93.693
	monoterpene hydrocarbons	85.089	84.517	88.588	87.126	86.330	86.883
	sesquiterpene hydrocarbons	7.976	8.987	6.231	7.235	7.607	6.746
	aliphatic hydrocarbons	0.047	0.043	0.044	0.037	0.043	0.064
	oxygenated compounds	5.991	5.789	4.688	4.963	5.358	4.946
	monoterpene aldehydes	2.257	3.171	2.781	2.867	2.769	3.151
	aliphatic aldehydes	0.587	0.607	0.499	0.498	0.548	0.398
	monoterpene alcohols	1.527	1.338	0.938	1.017	1.205	0.774
	sesquiterpene alcohols	0.244	0.250	0.164	0.231	0.222	0.240
	esters	0.333	0.383	0.292	0.320	0.330	0.346
	others	0.043	0.040	0.025	0.030	0.035	0.037

[a] Type A. [b] Type B; the content of p-cymene, fairly high in this oil, could be due to a nonadequate storage of the oil. [c] Identified for the first time in cold-pressed lime oil.

Cold-Pressed Key and Persian Lime Oils

J. Agric. Food Chem., Vol. 45, No. 9, 1997 **3611**

Table 5. Composition of the Volatile Fraction of Persian Lime Oil

		composition (%)							
peak no.	compounds	6	7	8	9	10	11	12	average
1	nonane	0.003	0.001	0.002	0.003	0.002	0.002	0.002	0.002
2	tricyclene	0.006	0.007	0.005	0.005	0.006	0.005	0.005	0.006
3	α-thujene	0.564	0.558	0.538	0.552	0.554	0.596	0.561	0.560
4	α-pinene	2.173	2.114	1.962	2.081	2.063	2.208	2.092	2.099
5	camphene	0.068	0.067	0.057	0.060	0.059	0.063	0.058	0.062
6	thuja-2,4(10)-diene[a]	tr	tr	tr	tr	tr	tr		tr
7	sabinene + β-pinene	13.644	13.418	12.406	12.036	12.181	14.555	12.282	12.931
8	6-methyl-5-hepten-2-one	0.023	tr	0.055		tr	0.010		
9	myrcene	1.491	1.469	1.371	1.430	1.452	1.344	1.498	1.436
10	dehydro-1,8-cineole[a]	tr	tr	tr	tr	tr	tr	tr	tr
11	decane[a]	tr	tr	tr	tr	tr	tr	tr	tr
12	octanal	0.054	0.051	0.044	0.049	0.044	0.052	0.053	0.050
13	α-phellandrene	0.015	0.032	0.022	0.020	0.012	0.012	0.014	0.018
14	δ-3-carene	0.006	0.006	0.003	0.012	0.010	0.005	0.009	0.007
15	α-terpinene	0.300	0.301	0.310	0.212	0.147	0.329	0.288	0.270
16	p-cymene	0.200	0.117	0.114	0.619	0.705	0.364	0.197	0.331
17	limonene	55.239	56.110	55.169	59.809	59.428	51.613	59.203	56.653
18	(Z)-β-ocimene	0.090	0.084	0.042	0.045	0.046	0.047	0.047	0.057
19	(E)-β-ocimene	0.168	0.169	0.084	0.093	0.092	0.102	0.095	0.115
20	γ-terpinene	13.008	12.979	14.511	12.549	12.768	15.647	13.323	13.541
21	cis-sabinene hydrate[a]	0.030	0.038	0.054	0.032	0.032	0.046	0.034	0.038
22	terpinolene	0.623	0.601	0.659	0.522	0.460	0.699	0.589	0.593
23	trans-sabinene hydrate	0.036	0.041	0.073	0.036	0.038	0.066	0.041	0.047
24	linalool	0.184	0.175	0.188	0.156	0.174	0.233	0.179	0.184
25	nonanal	0.008	0.013	0.013	0.010	0.008	0.012	0.010	0.011
26	exo-fenchol	0.002	tr	tr	tr	tr	tr	tr	tr
27	cis-p-menth-2-en-1-ol[a]	0.007	0.002	0.006	0.007	0.010	0.010	0.005	0.007
28	trans-pinocarveol	0.006	0.002	0.009	0.015	0.019	0.002	0.004	0.008
29	trans-p-menth-2-en-1-ol[a]	0.002	tr	tr	tr	tr	tr	tr	tr
30	citronellal	0.056	0.045	0.038	0.030	0.026	0.045	0.032	0.039
31	borneol	0.027	0.021	0.026	0.012	0.016	0.019	0.020	0.020
32	cis-pinocamphone[a]	0.004	tr	0.005	tr	0.004	0.005	0.004	0.003
33	terpinen-4-ol	0.110	0.039	0.079	0.052	0.098	0.124	0.079	0.083
34	α-terpineol	0.295	0.211	0.364	0.197	0.266	0.371	0.258	0.280
35	dodecane[a]	0.009	0.007	0.015	0.016	0.018	0.012	0.016	0.013
36	decanal	0.061	0.094	0.085	0.074	0.063	0.070	0.077	0.075
37	nerol	0.151	0.057	0.113	0.065	0.105	0.205	0.104	0.114
38	neral	1.568	1.720	1.404	1.112	1.056	1.659	1.119	1.337
39	geraniol	0.098	0.033	0.043	0.022	0.047	0.070	0.048	0.052
40	piperitone	tr	tr	tr	tr	tr	0.003	tr	tr
41	geranial + perilla aldehyde	2.557	2.770	2.325	1.907	1.808	2.672	1.845	2.269
42	bornyl acetate	0.005	0.004	0.005	0.010	0.007	0.005	0.003	0.006
43	tridecane[a]	0.002	0.004	0.005	tr	0.004	0.005	0.003	0.003
44	undecanal	0.012	0.014	0.017	0.010	0.015	0.011	0.013	0.013
45	δ-elemene	0.070	0.118	0.040	0.035	0.033	0.094	0.081	0.067
46	neryl acetate	1.150	0.984	1.030	0.929	0.832	1.260	0.805	0.999
47	geranyl acetate	0.279	0.312	0.256	0.193	0.177	0.275	0.175	0.238
48	β-elemene	0.074	0.088	0.048	0.068	0.060	0.086	0.061	0.069
49	dodecanal	0.034	0.042	0.053	0.031	0.031	0.031	0.044	0.038
50	cis-α-bergamotene	0.078	0.066	0.084	0.070	0.069	0.069	0.068	0.072
51	β-caryophyllene	0.718	0.671	0.605	0.476	0.495	0.549	0.510	0.575
52	trans-α-bergamotene	1.180	1.078	1.284	1.056	1.061	1.077	1.026	1.109
53	(Z)-β-farnesene	0.010	0.008	0.009	0.005	0.006	0.008	0.011	0.008
54	α-humulene	0.063	0.060	0.056	0.045	0.044	0.052	0.046	0.052
55	(E)-β-farnesene	0.116	0.102	0.122	0.096	0.098	0.097	0.097	0.104
56	β-santalene	0.046	0.039	0.049	0.040	0.039	0.040	0.037	0.041
57	germacrene D	0.099	0.115	0.116	0.071	0.062	0.101	0.086	0.093
58	α-selinene	0.041	0.051	0.041	0.031	0.032	0.071	0.032	0.043
59	(Z)-α-bisabolene	0.144	0.127	0.159	0.131	0.128	0.042	0.123	0.122
60	(E,E)-α-farnesene + β-bisabolene	1.993	1.817	2.254	1.782	1.780	1.853	1.710	1.884
61	germacrene B	0.146	0.168	0.188	0.108	0.098	0.162	0.125	0.142
62	tetradecanal	0.027	0.036	0.030	0.046	0.024	0.011	0.021	0.028
63	2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	0.058	0.048	0.067	0.050	0.049	0.052	0.046	0.053
64	campherenol	0.070	0.048	0.079	0.061	0.057	0.055	0.054	0.061
65	α-bisabolol	0.087	0.071	0.101	0.076	0.076	0.074	0.067	0.079
66	hexadecanal	0.082	0.091	0.120	0.062	0.074	0.064	0.078	0.082
	hydrocarbons	92.387	92.544	92.330	94.078	94.012	91.909	94.295	93.079
	monoterpene hydrocarbons	87.595	88.024	87.253	90.045	89.983	87.589	90.261	88.679
	sesquiterpene hydrocarbons	4.778	4.508	5.055	4.014	4.005	4.301	4.013	4.382
	aliphatic hydrocarbons	0.014	0.012	0.022	0.019	0.024	0.019	0.021	0.019
	oxygenated compounds	7.083	6.962	6.682	5.243	5.156	7.512	5.218	6.265
	monoterpene aldehydes	4.181	4.535	3.767	3.049	2.890	4.376	2.996	3.685
	aliphatic aldehydes	0.278	0.341	0.362	0.282	0.259	0.251	0.296	0.296
	monoterpene alcohols	0.948	0.619	0.955	0.594	0.805	1.146	0.772	0.834
	sesquiterpene alcohols	0.215	0.167	0.247	0.187	0.182	0.181	0.167	0.192
	esters	1.434	1.300	1.291	1.132	1.016	1.540	0.983	1.242
	others	0.027	tr	0.060	tr	0.004	0.018	0.004	

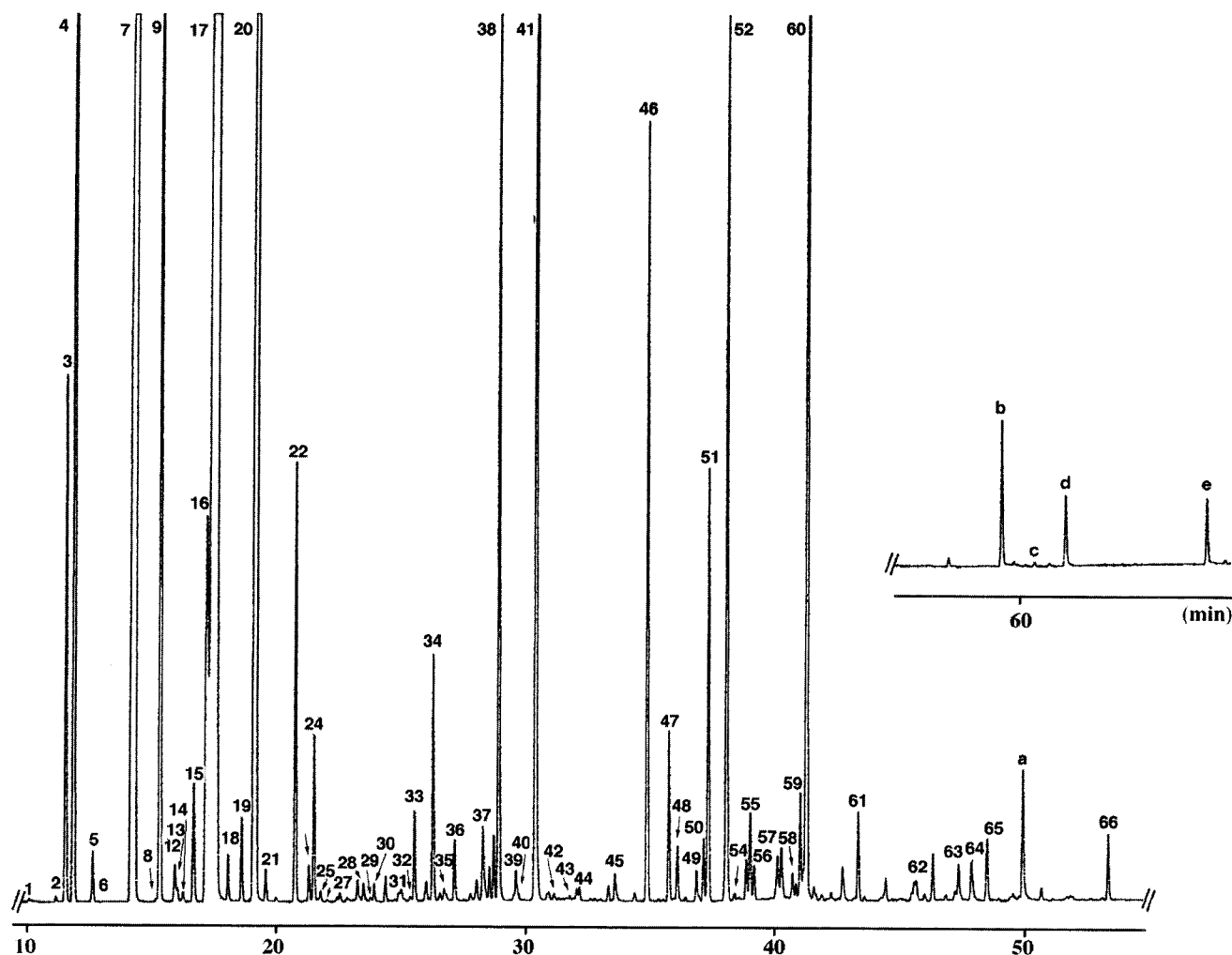[a] Identified for the first time in cold-pressed lime oil.

Figure 2. GC chromatogram of the volatile fraction of a cold-pressed Persian lime oil: (a) 7-methoxycoumarin, (b) citropten, (c) isobergapten (tentative), (d) bergapten, (e) isopimpinellin. For identification of the other peaks, see Table 5.

on a glass column (30 × 6 cm i.d.) filled with 400 g of silica gel (0.063–0.200 mm; Baker Analyzed) with a mixture of petroleum ether and ethyl acetate (80:20) as eluent. The fractions were monitored by TLC (5 × 10 cm plates coated with 0.25 mm SIL 254 UV 254 silica gel (Aldrich); eluent, petroleum ether:ethyl acetate (70:30)) and HPLC under the conditions already mentioned. The fractions were gathered according to their composition into seven groups that contained the following compounds. Fraction 1: bergamottin, 45%; 5-(geranyloxy)-7-methoxycoumarin, 49%; 5-(geranyloxy)-8-methoxypsoralen, 5%. Fraction 2: bergamottin, 9%; isoimperatorin, 2%; 5-(geranyloxy)-7-methoxycoumarin, 63%; 5-(geranyloxy)-8-methoxypsoralen, 22%; 5-(isopentenyloxy)-7-methoxycoumarin, 2%; 5-(isopentenyloxy)-8-methoxypsoralen, 1%. Fraction 3: bergamottin, 5%; isoimperatorin, 4%; 5-(geranyloxy)-7-methoxycoumarin, 26%; 5-(geranyloxy)-8-methoxypsoralen, 17%; 5-(isopentenyloxy)-7-methoxycoumarin, 8%; 5-(isopentenyloxy)-8-methoxypsoralen, 27%. Fraction 4: 5-(isopentenyloxy)-8-methoxypsoralen, 42%; citropten, 11%; 8-(geranyloxy)psoralen, 9%. Fraction 5: 5-(isopentenyloxy)-8-methoxypsoralen, 5%; citropten, 57%; 8-(geranyloxy)psoralen, 20%; herniarin, 8%. Fraction 6: citropten, 35%; 8-(geranyloxy)psoralen, 1%; herniarin, 10%; bergapten, 15%. Fraction 7: isopimpinellin, 100%.

Isopimpinellin was isolated directly by crystallization from fraction 7. Fractions 2, 4, and 5 required fractional crystallization. Bergamottin was isolated as previously described for lemon oil (Dugo et al., 1997). Isoimperatorin and 5-(isopentenyloxy)-7-methoxycoumarin were not obtained at the required degree of purity, and identification was made according to Dugo et al. (1997) and McHale and Sheridan (1989) and by means of the retention times and the UV spectra.

Separation of Components of Fraction 2. 5-(Geranyloxy)-7-methoxycoumarin was crystallized by addition of petroleum ether to a solution of ethyl acetate. After separation of crystals, the solution showed the following composition: bergamottin, 19%; isoimperatorin, 6%; 5-(geranyloxy)-7-methoxycoumarin, 37%; 5-(geranyloxy)-8-methoxypsoralen, 35%; 5-(isopentenyloxy)-7-methoxycoumarin, 3%; 5-(isopentenyloxy)-8-methoxypsoralen, 1%. The compounds of the mixture were separated by semipreparative HPLC, in the recycle mode, with a Waters Associates setup composed of a 519 pump with 225 μL heads, a gradient controller (600E), a manual injector (U6K), a spectrophotometric detector (model 484), a 25 × 100 mm PrepPak cartridge (Porasil; 15–20 μm, 125 Å) inserted in a Waters RCM 25 × 100 module, compression solvent isopropyl alcohol at 1400 psi, and a three-port recycle valve (Valco). The mobile phase was hexane:ethyl acetate, 95:5; flow rate, 20 mL/min; the injection volume was 2 mL of a hexane: ethyl acetate (50:50) solution containing ca. 40 mg of the mixture. Five fractions were obtained, which showed the following composition: (2a) bergamottin, 87%; isoimperatorin, 11%; (2b) isoimperatorin, 71%; 5-(geranyloxy)-7-methoxycoumarin, 27%; (2c) 5-(geranyloxy)-7-methoxycoumarin, 88%; 5-(geranyloxy)-8-methoxypsoralen, 10%; (2d) 5-(geranyloxy)-7-methoxycoumarin, 17%; 5-(geranyloxy)-8-methoxypsoralen, 80%; (2e) 5-(isopentenyloxy)-7-methoxycoumarin, 97%.

Separation of Components of Fraction 4. 5-(Isopentenyloxy)-8-methoxypsoralen was crystallized by addition of petroleum ether to a solution of ethyl acetate. After separation of crystals, the solution contained the other components of the fraction.

Separation of Components of Fraction 5. Citropten was crystallized by addition of petroleum ether to a solution

Cold-Pressed Key and Persian Lime Oils

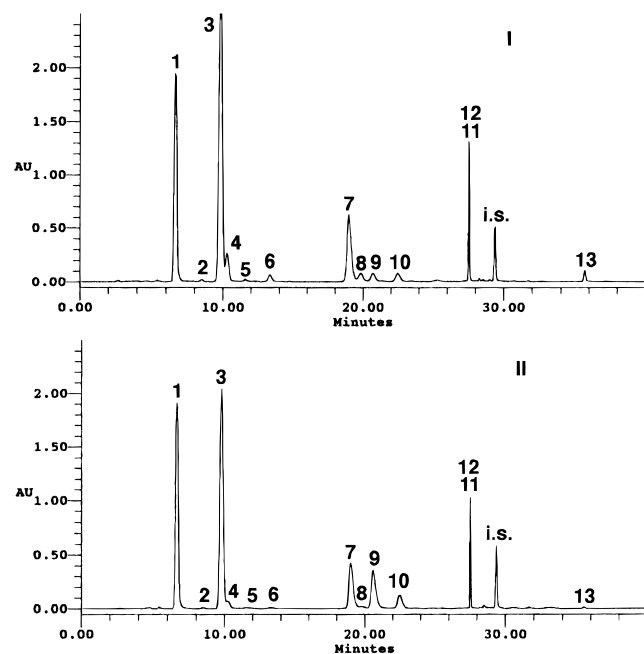*J. Agric. Food Chem.,* Vol. 45, No. 9, 1997 **3613**

Figure 3. HPLC chromatograms of a cold-pressed Key lime oil type A (I) and a cold-pressed Persian lime oil (II) obtained with method A. i.s., internal standard, tangeretin. For identification of the other peaks, see Tables 6 and 7.

of ethyl acetate. After separation of crystals, the solution showed the following composition: 5-(isopentenyloxy)-8-methoxypsoralen, 12%; citropten, 42%; 8-(geranyloxy)psoralen, 26%; herniarin, 19%. The compounds of fraction 5 were separated by semipreparative HPLC, in the recycle mode, using the equipment above described. The mobile phase was hexane:ethyl acetate, 90:10; flow rate, 20 mL/min; the injection volume was 2 mL of a hexane:ethyl acetate (50:50) solution containing ca. 100 mg of the fraction. Two fractions were isolated, the first containing 5-(isopentenyloxy)-8-methoxypsoralen, 11%, and citropten, 76%, and the second containing citropten, 10%, 8-(geranyloxy)psoralen, 45%, and herniarin, 45%. Even though 8-(geranyloxy)psoralen and herniarin were not obtained separately, the identity of these two components was confirmed by electron-impact (EI) mass spectrometry, direct introduction of the sample, and increasing the temperature of the probe during the analysis so that the two components were volatilized one by one.

Purity was monitored by HPLC, under the same experimental conditions mentioned for the analysis of lime oils. The spectral contrast technique of the photodiode array detector, which makes it possible to detect coelution by matching all spectra within a peak (Millenium 2010, 1993), was used. All the compounds isolated were spectrally pure. The identity of each compound isolated was confirmed by ^{1}H-NMR (300 MHz, Varian) and EI mass spectrometry (70 eV; Finnigan, Mat90). Structural assignment was obtained by comparison with data from authentic samples or with literature data.

Normal Phase HPLC. The oxygen heterocyclic fraction was analyzed by HPLC using a Waters Associates instrument, with two different methods, as described: model 519 pump, 600E gradient controller, Rheodyne 9125 injector, and photodiode array detector (PDA), model 996. Peak integration and quantitative calculations were performed with the Millenium 2010 system and a calibration curve that was obtained for each previously isolated standard component against a tangeretin standard or a coumarin standard.

Method A: The columns used were two 150 × 3.9 mm i.d. NovaPak silica with a particle size of 4 μm (Waters Associates). Two mobile phases were used: eluent A (hexane:ethyl acetate, 93:7) and eluent B (hexane:ethyl alcohol, 90:10). The HPLC analyses of lime oil samples were performed according to the following program: 0−15 min, 100% A; 15−30 min, from 100% A to 5% A + 95% B, with a linear gradient; 30−35 min, 5% A + 95% B; 35−40 min, from 5% A + 95% B to 100% A. The
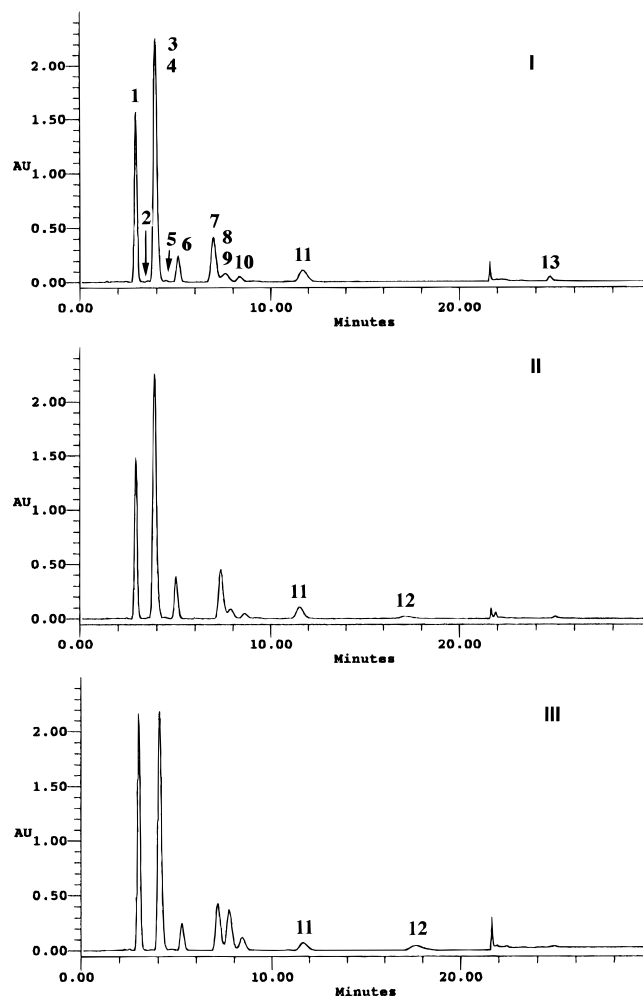

Figure 4. HPLC chromatograms of a cold-pressed Key lime oil type A (I), a cold-pressed Key lime oil type B (II), and a cold-pressed Persian lime oil (III) obtained with method B. For identification of the other peaks, see Tables 6 and 7.

flow rate was 1.0 mL/min, the pressure was 600 psi, and the column temperature was 30 °C.

Method B: The column used was a 150 × 3.9 mm i.d. μ-Porasil with a particle size of 10 μm (Waters Associates). Two mobile phases were used: eluent A (hexane:ethyl acetate, 92:8) and eluent B (hexane:ethyl alcohol, 90:10). The HPLC analyses of lime oil samples were performed according to the following program: 0−15 min, 100% A; 15−20 min, from 100% A to 100% B, with a linear gradient; 20−30 min, 100% B; 30−35 min, from 100% B to 100% A. The flow rate was 1.25 mL/min, the pressure was 204 psi, and the column temperature was 30 °C.

With both methods, the injection volume was 20 μL of a solution obtained by diluting ∼20 mg of the essential oil and 50 μL of a tangeretin solution (method A) or ∼20 mg of the essential oil and 100 μL of a coumarin solution (method B) of known concentration (∼1 mg/mL) to 1 mL of hexane:ethyl acetate (75:25). Detection was by UV absorbance at 315 nm. The UV spectra of eluting peaks were monitored with the PDA detector in the region 200-400 nm.

RESULTS AND DISCUSSION

Tables 2 and 3 report the values of the physicochemical indices of Key lime and Persian lime oils, respectively. Tables 4 and 5 report the composition of the volatile fraction of the two oils, and Tables 6 and 7 report the composition of the coumarin and psoralen fractions.

Physicochemical Indices. All the values reported in Tables 2 and 3 are included in the ranges expected

3614 *J. Agric. Food Chem.,* Vol. 45, No. 9, 1997

Dugo et al.

Table 6. Coumarins and Psoralens in the Key Lime Oils Analyzed (mg/100 g of Oil)

peak no.	compounds	1[a]	2[a]	3[a]	4[a]	average	5[b]
1	bergamottin (5-(geranyloxy)psoralen)	3823	3364	3157	3287	3408	3154
2	isoimperatorin (5-(isopentenyloxy)psoralen)	+	+	+	+		+
3	5-(geranyloxy)-7-methoxycoumarin	3678	3065	4045	4026	3703	4093
4	5-(geranyloxy)-8-methoxypsoralen	+	+	+	+		+
5	5-(isopentenyloxy)-7-methoxycoumarin	+	+	+	+		+
6	cnidilin (5-(isopentenyloxy)-8-methoxypsoralen)	35	33	25	30	31	24
7	citropten (5,7-dimethoxycoumarin)	632	491	580	622	581	484
8	8-(geranyloxy)psoralen	+	+	+	+		+
9	herniarin (7-methoxycoumarin)	86	92	90	96	91	74
10	bergapten (5-methoxypsoralen)	124	114	100	114	113	89
11	isopimpinellin (5,8-dimethoxypsoralen)	365	353	357	350	356	331
12	oxypeucedanin (5-[(2′,3′-epoxyisopentyl)oxy]psoralen)						144
13	oxypeucedanin hydrate (5-[(2′,3′-dihydroxyisopentyl)oxy]psoralen)	+	+	+	+		+

[a] Type A. [b] Type B.

Table 7. Coumarins and Psoralens in the Persian Lime Oil Analyzed (mg/100 g of Oil)

peak no.	compounds	6	7	8	9	10	11	12	average
1	bergamottin	3918	3140	2221	3030	3101	3002	3058	3067
2	isoimperatorin	+	+	+	+	+	+	+	
3	5-(geranyloxy)-7-methoxycoumarin	3450	3002	3781	1943	3359	2462	3780	3111
4	5-(geranyloxy)-8-methoxypsoralen	+	+	+	+	+	+	+	
5	5-(isopentenyloxy)-7-methoxycoumarin	+	+	+	+	+	+	+	
6	cnidilin	7	7	6	8	8	5	6	7
7	citropten	504	326	535	363	405	400	569	443
8	8-(geranyloxy)psoralen	+	+	+	+	+	+	+	
9	herniarin	415	439	496	415	346	594	339	435
10	bergapten	221	169	250	158	205	165	205	196
11	isopimpinellin	212	169	293	196	211	228	213	217
12	oxypeucedanin	286	328	257	210	274	233	313	272
13	oxypeucedanin hydrate	+	+	+	+	+	+	+	

by the *Food Chemical Codex* (1981). Key lime oils show values of optical rotation lower than those of Persian lime oils; this agrees with the content of limonene, β-pinene, and sabinene of the two oils (see Tables 4 and 5) and with the enantiomeric ratios of these components. For both oils, in fact, the ratio between (−)- and (+)-limonene is about 2:98; the ratio between (−)- and (+)-β-pinene is 97:3 in Key lime oil and 90:10 in Persian lime oil; the ratio between (−)- and (+)-sabinene is 85:15 in Key lime oil and 80:20 in Persian lime oil. These values have been obtained by using an automatic GC−GC system, developed in our laboratory, using a precolumn coated with SE-52 and a main column coated with a chiral phase (Mondello et al., 1997). The nonvolatile residue shows higher values for Key lime oil (average value ~13%) than Persian lime oil (average value ~9%). As can be seen from Tables 2, 3, 6, and 7, the CD values and the total amount of coumarins and psoralens in the two oils are about the same. So, this would suggest that the differences in the amount of nonvolatile residues are due to compounds that do not absorb in the UV region.

Volatile Fraction. Figures 1 and 2 show the GC chromatograms of the volatile fraction of a Key lime oil and a Persian lime oil. Tables 4 and 5 report the composition for each component and for classes of components of the volatile fraction of the two oils; 69 components were identified, which constitutes more than 99% of the volatile fraction. As can be seen from the chromatograms, in addition to the components reported in Tables 4 and 5, 7-methoxycoumarin, citropten, bergapten, and isopimpinellin were identified by GC/MS. The identification of these components has been confirmed by comparison of their linear retention indices and their mass spectra with those of standard compounds. Moreover, isobergapten was tentatively identified. Research is in progress to unambiguously isolate and characterize this component.

Data reported in Tables 4 and 5 are expressed as relative percentages of the peak areas, without taking into account the nonvolatile residue. In the Key lime oils (Table 4) the monoterpene hydrocarbons range from 85% to 88% of the volatile fraction, and the sesquiterpene hydrocarbons go from 6.2% to about 9.0%. The oxygenated compounds represent about 4.7−6.0%, among which monoterpene aldehydes were the most abundant, ranging from 2.3% to 3.2%, followed by monoterpene alcohols (0.8−1.5%). Aliphatic aldehydes represent 0.4−0.6%, esters 0.3−0.4%, and sesquiterpene alcohols about 0.2%. Limonene is the main component, representing a little less than 50% in all the samples, followed by β-pinene + sabinene, which range from 22% to 25%, and γ-terpinene (about 6−8%).

The type A oils analyzed generally showed a higher content of monoterpene alcohols than the type B oils, probably due to the technology used for extraction of the type A oil. This allows the oil to come in contact with the juice, which has an acid pH and can cause hydration of the monoterpene hydrocarbons with formation of alcohols.

Our qualitative and quantitative results generally agree with those reported by Chamblee et al. (1985), Clark et al. (1987), and Clark and Chamblee (1992), with the following exceptions: our samples showed a higher content of δ-elemene; we detected thuja-2,4(10)-diene, (Z)-α-bisabolene, (E,E)-α-farnesene, (Z)-β-farnesene, β-santalene, germacrene D, α-selinene, *cis*-sabinene hydrate, *exo*-fenchol, *cis*- and *trans-p*-menth-2-en-1-ol, *trans*-pinocarveol, *cis*-pinocamphone, dehydro-1,8-cineole, and some aliphatic hydrocarbons; we did not find β-copaene and citronellyl acetate.

In Persian lime oil (Table 5) monoterpene hydrocarbons represent 88−90% of the volatile fraction, and sesquiterpene hydrocarbons range from 4% to 5%. Oxygenated compounds represent 5.2−7.5%, and monoterpene aldehydes are the most abundant among these

Cold-Pressed Key and Persian Lime Oils

J. Agric. Food Chem., Vol. 45, No. 9, 1997 **3615**

components, representing 2.9–4.5%, followed by esters, 1.0–1.5%. Monoterpene alcohols range from 0.6% to 1.1%, and aliphatic aldehydes show values of about 0.3%, while sesquiterpene alcohols represent about 0.2%.

Persian lime oil shows lower values of sesquiterpene hydrocarbons and aliphatic aldehydes and higher values of monoterpene hydrocarbons, monoterpene aldehydes, and esters than Key lime oil. More specifically, esters are 3 or 4 times more abundant in Persian lime oil than in Key lime oil. Moreover, Persian lime oil presents higher contents of limonene and γ-terpinene and a sharply lower content of sabinene + β-pinene than Key lime oil.

Coumarins and Psoralens. Figure 3 shows the HPLC chromatograms of a Key lime type A oil and a Persian lime oil obtained with HPLC method A. Under these conditions, the separation of isoimperatorin and 8-(geranyloxy)psoralen is achieved, in contrast with the method proposed by McHale and Sheridan (1989), but isopimpinellin and oxypeucedanin were coeluted. The quantitative determination of these two components was carried out analyzing the samples with HPLC method B. Figure 4 shows the HPLC chromatograms of a Key lime type A oil, a Key lime type B oil, and a Persian lime oil obtained with HPLC method B. Tables 6 and 7 report the composition of the coumarin and psoralen fraction of Key and Persian lime oils, respectively.

Four coumarins (5-(geranyloxy)-7-methoxycoumarin, 5-(isopentenyloxy)-7-methoxycoumarin, citropten, herniarin) and nine psoralens (bergamottin, isoimperatorin, 5-(geranyloxy)-8-methoxypsoralen, 5-(isopentenyloxy)-8-methoxypsoralen, 8-(geranyloxy)psoralen, bergapten, isopimpinellin, oxypeucedanin, oxypeucedanin hydrate) have been identified in the oxygen heterocyclic fraction of lime oils.

Bergamottin and 5-(geranyloxy)-7-methoxy coumarin were the main components of all the samples analyzed. Both components were present in amounts higher than 3 g/100 mL of oil. Key lime oil type A did not contain oxypeucedanin, which was present in Key lime oil type B and Persian lime oil.

As previously shown for bitter orange oil (Dugo et al., 1996) and lemon oil (Radford and Olansky, 1994), the absence of oxypeucedanin is due to contact between the oil and the juice during the extraction. Under these conditions, the epoxy ring of oxypeucedanin is opened by hydrolysis to form oxypeucedanin hydrate, which is water soluble.

Key lime oils showed a higher content of (isopentenyloxy)-7-methoxypsoralen, citropten, and isopimpinellin and a lower content of herniarin and bergapten than Persian lime oils.

Comparing our results to those reported by McHale and Sheridan (1989), we did not detect the presence of heraclenin, byakangelicol, and isobyakangelicol, while isoimperatorin and oxypeucedanin hydrate were detected. The presence of isoimperatorin was reported in literature only by Stanley (1967). Moreover, both our Key lime oil and Persian lime oil samples showed higher values for the compounds analyzed, especially for bergamottin and 5-(geranyloxy)-7-methoxy psoralen, than those reported by McHale and Sheridan. On the whole, the reported data can be a useful reference for the evaluation of the authenticity of cold-pressed lime oils.

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3616 *J. Agric. Food Chem.,* Vol. 45, No. 9, 1997

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