Progress in Essential Oils

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Key, or Acid, Lime Oil

Citrus aurantifolia (Christm. et Panz.) Swingle is the lime species from which distilled lime, centrifuged Key lime type A, centrifuged Key lime type B, and expressed, or cold-pressed, acid lime oils are obtained. Distilled lime (the largest volume of the lime oils produced) is prepared by squeezing clean, whole limes through the broad end of a conical screw press that is fitted with 5 mm holes. The pressure within the press forces the crushed fruit peel and pulp to be expelled from an opening at the narrow end, during which time the oil glands are burst, releasing both the oil and the juice. The oil-juice emulsion is pumped into a stainless steel still, and the mixture is subjected to a distillation time of ca. 10 hr.

An oil known as centrifuged lime oil type A is produced from the oil-juice emulsion. In this instance, the emulsion is pumped into a finisher to remove the coarse detritus. The emulsion is broken and the oil is separated from the juice by two passes through a high-speed centrifuge.

The expressed, or cold-pressed, lime oil is produced using either a rasping process or a modified whole fruit processor. In both scenarios, the released oil is washed away from the process equipment with a spray of water. The oil/water emulsion is initially screened to remove any coarse peel fragments, after which the oil is isolated from this mixture by one or two passes through a high-speed centrifuge. After cooling the oil (a process known as winterization) the waxes and a portion of the non-volatile compounds precipitate out. Cold filtration to remove the precipitate yields the oil known as centrifuged lime oil type B.

All three oils—the distilled lime oil, the centrifuge lime oil type A and the centrifuged lime oil type B—are used in the flavor and fragrance industry, with the type B oil preferred in the fragrance industry.

Wright (1999) reported that the major quantitative and qualitatively important compounds of distilled lime were:

limonene (52.0%)
γ-terpinene (8.0%)
α-terpineol (7.0%)
terpinolene (5.0%)
p-cymene (5.0%)
1,4-cineole (3.0%)
1,8-cineole (2.0%)
β-pinene (2.0%)
β-bisabolene (1.0%)
α-fenchyl alcohol (0.7%)
terpinen-4-ol (0.7%)
borneol (0.5%)
2,6,6-trimethyl-2-vinyltetrahydropyran (0.2%)

Mondello et al. (2000) compared the analysis of a cold-pressed lime oil using conventional and fast GC. The results of this study are reported in **T-1**. A comparison between the analyses' times reveals that conventional GC analysis takes about 60 min, whereas the fast GC analysis was completed in less than 15 min. This latter type of analysis is of great value for the analysis of a large number of samples of the same oil from different geographical regions, or to follow the change in composition during the seasons.

Fresh mature fruits of *C. aurantifolia* 'Kazzi' cultivar were harvested from an experimental orchard in Bangalore at six ripening stages. Oils obtained from the peels by hydrodistillation were analyzed by Venkatseshwarlu and Selvaraj (2000), and their main constituents identified can be seen in **T-2**, where the effect of ripeness based on fruit color can be assessed.

Veriotti and Sacks (2001) analyzed samples of lime oil (unknown origin) and Mexican lime oil using GC/TOFMS (gas chromatography/time of flight mass spectrometry) although the authors proved the value of such a system to examine essential oils, the list of compounds identified will not be included in this review as many compounds were misidentified as could be seen from their GC elution order. It is assumed by this reviewer that they were using an inferior database and letting the computer decide on the peak identity.

Selvaraj et al. (2002) produced an oil by water distillation of the fruit peels obtained from mature fruit of 'Kazzi' lime grown in Bangalore. The oil was found to possess the following composition:

α-pinene (2.4%)
camphene (0.2%)
β-pinene (22.8%)
myrcene (1.5%)
limonene (46.1%)
γ-terpinene (9.8%)
terpinolene (0.6%)
linalool (0.5%)
α-fenchyl alcohol (0.6%)
terpinen-4-ol (1.4%)
α-terpineol (1.0%)
citronellol (0.6%)
geraniol (0.9%)
decanal (0.1%)
neral (2.0%)
geranial (0.8%)
neryl acetate (1.1%)
geranyl acetate (0.6%)

Chisholm et al. (2003) used both gas chromatography-olfactometry (GC-O) and GC/MS to examine the most intense aroma volatiles found in the lab-produced extracted and distilled Key lime (*C. aurantifolia*). Extraction of the lime was performed by removal of the zest of the fruit and its extraction by being stirred at 50 rpm with pentane-diethyl ether (1:1) for 1 hr. After filtration drying over anhydrous magnesium sulfate, the solvent was removed on a roto-vapor at

30°C. This yielded the so-called Key lime extract. To produce the the Key lime extract oil, the extract was refluxed in a 5% solution of citric acid for 0.5 hr, followed by conventional hydrodistillation. The most odor-active compounds found in the Key lime extract oil, in order of importance, were geranial, perillaldehyde, nonanal, linalool, nerol, citronellol, neral, dodecanal, decanal, neryl acetate, (E,E)-2,4-decadienal, undecanal, α-pinene, geraniol, terpinen4-ol, 7-methoxycoumarin, citronellal, limonene, 1,8-cineole, geranyl acetate, caryophyllene oxide, myrcene, tetradecanol, hexanal, germacrene B, borneol, citronellyl acetate, decyl acetate, α-terpineol and octanal.

In contrast, the most odor-active compounds found in the so-called distilled oil in order of importance were geranial, perillaldehyde, nonanal, linalool, nerol, citronellol, neral, limonene, 1,8-cineole, decanal, hexanal, dodecanal, terpinen-4-ol, geraniol, (E,E)-2,4-decadienal, undecanal, 2,6,6-trimethyl-2-ethenyl-tetrahydropyran, caryophyllene oxide, borneol, α-terpineol, hexadecanal, 2,2-dimethyl-5-(1-methyl-1-propenyl)-tetrahydrofuran, *trans*-limonene oxide, an isomer of β-terpineol, (Z)-3-hexenol, tetradecanal, germacrene B, 1,4-cineole, dehydro-1,8-cineole, *cis*-limonene oxide, terpinen-1-ol and α-fenchyl alcohol.

Chisolm et al. also analyzed the major constituents of the so-called distilled oil and a commercial sample of distilled lime oil. These results are shown in **T-3**.

Selvaraj et al. (2004) showed that the process of degreening 'Kazzi' lime with either ethylene or acetylene did not affect the peel oil content but it did not affect the peel oil content, but it did affect the peel oil composition as can be seen in **T-4**.

A lab-distilled oil produced from the peels of mature fruit of the 'Kazzi' lime collected from the Chittagong region of Bangladesh was analyzed by Chowdhury et al. (2006) using GC/MS. The oil composition was characterized as follows:

α-pinene (2.2%)
sabinene (5.2%)
β-pinene (18.7%)
myrcene (1.4%)
limonene (39.6%)
1,8-cineole (0.1%)
δ-3-carene† (0.1%)
β-ocimene° (0.2%)
linalool (0.5%)

T-1. Comparative lime oil analysis using conventional and fast GC analyses

Compound	Conventional GC	Fast GC
tricyclene	t	t
α-thujene	0.60	0.59
α-pinene	2.31	2.13
camphene	0.06	0.06
sabinene + β-pinene	12.04	12.13
myrcene	1.66	1.55
octanal + α-phellandrene	0.06	0.06
δ-3-carene	0.01	0.01
α-terpinene	0.25	0.27
p-cymene + limonene + (Z)-β-ocimene	55.24	56.55
(E)-β-ocimene	0.11	0.10
γ-terpinene	14.21	13.26
cis-sabinene hydrate	0.04	0.03
terpinolene	0.38	0.40
linalool	0.22	0.20
nonanal	t	0.01
citronellal	0.02	0.03
terpinen-4-ol	0.11	0.09
α-terpineol	0.31	0.30
decanal	0.10	0.09
nerol	0.12	0.11
neral	1.22	1.20
geraniol + piperitone	0.06	0.06
geranial	1.98	1.88
undecanal	0.05	0.04
δ-elemene	0.10	0.10
neryl acetate	0.99	0.90
geranyl acetate	0.21	0.20
β-elemene	0.09	0.09
dodecanal	0.04	0.04
cis-α-bergamotene	0.08	0.07
β-caryophyllene	0.54	0.50
trans-α-bergamotene	1.13	1.14
α-humulene	0.05	0.05
(E)-β-farnesene	0.11	0.10
β-santalene	0.04	0.04
germacrene D	0.07	0.08
α-selinene	0.03	0.03
(Z)-α-bisabolene	0.13	0.13
(E,E)-α-farnesene + β-bisabolene	1.86	1.84
germacrene B	0.11	0.11
tetradecanal	0.03	0.03
2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	0.05	0.05
campherenol	0.07	0.06
α-bisabolol	0.08	0.08
herniarin	0.23	0.22
hexadecanal	0.10	0.10
citropten	0.25	0.23
bergapten	0.13	0.12
isopimpinellin	0.08	0.08

t=trace (<0.01%)

neral + geranial (18.3%)
citronellal (0.1%)
sabinene hydrate* (<0.1%)
cis-verbenol (0.7%)
nonanal (<0.1%)
camphene† (0.8%)
α-pinene epoxide† (0.1%)
decanal (0.6%)
neryl acetate (2.1%)
geraniol (1.1%)
terpinen-4-ol (0.2%)
cis-piperitol (0.1%)
hexadecanal† (0.1%)
carvone (0.1%)
β-caryophyllene (0.2%)
α-bergamotene (1.1%)
(Z)-α-bisabolene† (1.8%)
α-humulene (1.4%)
α-farnesene* (0.7%)
perillaldehyde (0.1%)
β-bisabolene (0.3%)
undecanal (0.1%)
caryophyllene oxide (0.1%)
neryl propionate (0.5%)
β-elemene† (0.1%)

*correct isomer not identified
†incorrect identification based on GC elution order

Afolayan and Asekun (2008) purchased both ripe and rotten fruits of *C. aurantifolia* in South Africa, removed the peels and separately macerated and hydrodistilled them for 3 hr. The oils isolated from ripe and rotten fruit were analyzed by GC/MS only, and their results can be seen summarized in **T-5**.

Using an original microwave-hydrodiffusion process on lime peel (*C. aurantifolia*) to isolate the oil, Bousbia et al. (2009) compared the results of the analysis using GC-FID and GC/MS of this oil with oils obtained by both hydrodistillation and cold-pressing. A summary of this comparative analysis is presented in **T-6**.

Bosquez-Molina et al. (2010) have demonstrated that the incorporation of a combination of distilled lime oil and thyme oil in a mesquite gum-based coating was highly successful in the control of fruit decaying fungi like *Colleototricum gloeosporioides* and *Rhizopus stolonifer*. The composition of the lime oil used in this study was as follows:

α-pinene (2.1%)
β-pinene (20.5%)
δ-3-carene (0.5%)
limonene (45.1%)
γ-terpinene (10.6%)
neral (2.1%)
geranial (2.8%)
α-terpineol (0.8%)
nerol (0.7%)
geraniol (0.6%)
β-elemene (0.3%)
β-caryophyllene (1.8%)
γ-elemene (0.8%)
trans-α-bergamotene (1.4%)
α-humulene (0.2%)
α-farnesene* (2.1%)
germacrene D (0.5%)
(Z)-α-bisabolene (0.2%)
β-bisabolene (1.9%)

*correct isomer not identified

The authors also misidentified five other components of the oil.

Two Mexican commercial samples of the distilled Key lime oil type A were analyzed using GC-FID and GC/MS by Bonaccorsi et al. (2011). They found that the oil possessed the following composition:

α-thujene (0.3%)
α-pinene (2.0–2.1%)
camphene (0.1%)
sabinene (2.4–2.5%)

T-2. Percentage composition of 'Kazzi' lime peel oils obtained from different ripening stages

Compound	DG	LG	CT	HY	MY	FY
α-pinene	1.2	1.7	1.9	2.3	2.4	2.5
camphene	0.1	0.2	0.2	0.2	0.2	0.2
β-pinene	13.5	16.7	18.4	20.8	21.7	23.8
myrcene	0.6	0.7	0.9	1.5	1.5	1.6
α-phellandrene	0.4	0.6	0.5	-	-	-
limonene	33.0	37.7	42.8	43.7	45.1	47.0
γ-terpinene	7.9	8.8	10.0	10.1	9.6	9.9
terpinolene	0.7	0.3	0.8	0.8	0.7	0.5
decanal	0.4	-	0.2	0.1	-	0.1
linalool	1.5	1.6	1.0	0.8	0.5	0.6
α-fenchyl alcohol	1.0	1.3	0.9	0.8	0.6	0.5
terpinen-4-ol	3.9	2.8	2.1	1.9	1.5	1.2
α-terpineol	1.9	2.1	1.6	1.3	1.1	0.7
neral	4.2	3.0	2.6	2.6	2.2	1.8
neryl acetate	1.7	1.4	1.3	1.3	1.2	0.9
citronellol	1.2	1.2	0.7	0.4	0.6	0.4
geranial	3.6	2.5	1.4	0.8	0.9	0.7
geranyl acetate	3.0	2.0	1.2	0.7	0.7	0.6
geraniol	7.3	4.5	2.4	1.0	1.4	0.9

Fruit ripeness (color): DG=dark green; LG=light green; CT=green color turning yellow; HY=half yellow; MY=75% yellow; FY=full yellow

T-3. Comparative percentage composition of a so-called distilled oil and a commercial sample of distilled lime oil

Compound	So-called distilled oil	Commercial distilled oil
α-pinene	1.7	1.6
β-pinene	6.3	2.7
myrcene	1.5	1.2
2,2,6-trimethyl-2-ethenyltetrahydropyran	0.1	0.2
limonene	32.6	29.9
neral	1.8	1.3
geranial	0.9	0.4
α-terpineol	12.5	10.1
γ-terpineol†	0.7	2.5
terpinen-1-ol	0.2	1.3

†the natural occurrence of this compound is unsubstantiated

β-pinene (20.2–21.2%)
myrcene (1.0–1.1%)
octanal (t–0.1%)
α-terpinene (0.2%)
p-cymene (0.1–0.2%)
limonene (50.3–51.1%)
(Z)-β-ocimene (0.1%)
(E)-β-ocimene (0.2–0.3%)
γ-terpinene (7.7–9.3%)
terpinolene (0.3–0.4%)
linalool (0.2%)
isogeranial (0.3–0.4%)
terpinen-4-ol (t–0.1%)
α-terpineol (0.2–0.3%)
decanal (0.2%)
neral (1.1–1.4%)
geraniol (t–0.1%)
geranial (1.9–2.3%)
δ-elemene (t–0.6%)
neryl acetate (0.1–0.2%)
geranyl acetate (0.2%)
β-elemene (0.2–0.3%)
dodecanal (0–0.1%)
decyl acetate (0–0.1%)
cis-α-bergamotene (0–0.1%)
β-caryophyllene (0.8–1.0%)
trans-α-bergamotene (1.1%)
(E)-β-farnesene (0.1%)
α-humulene (0.1%)
germacrene D (0.1–0.3%)
trans-β-bergamotene (0–0.1%)
valencene (0–0.1%)
bicyclogermacrene (0–0.1%)
α-selinene (0–0.1%)
(Z)-α-bisabolene (0–0.1%)
(E,E)-α-farnesene (1.0–1.3%)
β-bisabolene (1.8–1.9%)
germacrene B (0.5–0.6%)
α-bisabolol (0.1%)

t=trace (<0.05%)

In addition, trace amounts of tricyclene, α-fenchene, thuja-2,4(10)-diene, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, decane, α-phellandrene, δ-3-carene, *cis*-sabinene hydrate, p-cymenene, *trans*-sabinene hydrate, nonanal, 4,8-dimethyl-1,3E,7-nonatriene, α-fenchyl alcohol, *trans*-p-mentha-2,8-dien-1-ol, *cis*-p-menth-2-en-1-ol, (E,E)-allo-ocimene, *cis*-p-mentha-2,8-dien-1-ol, *trans*-limonene oxide, *trans*-pinocarveol, citronellal, pinocarvone, borneol, isopinocamphone, *trans*-piperitol, nerol, citronellol, piperitone, decanol, perillaldehyde, bornyl acetate, tridecane, undecanal, α-terpinyl acetate, citronellyl acetate, γ-elemene, β-santalene, γ-curcumene, β-selinene, β-sesquiphellandrene, (E)-α-bisabolene, *cis*-sesquisabinene hydrate, *trans*-sesquisabinene hydrate, caryophyllene oxide, tetradecanal,

T-4. Percentage composition of 'Kazzi' lime peel oils obtained from green and yellow lime and ethylene and acetylene-induced degreening

Compound	DG	FY	E-IND.Y	A-IND.Y
α-pinene	1.4+0.22	2.4+0.07	1.8+0.06	1.6+0.09
camphene	0.2+0.02	0.2+0.01	0.1+0.02	0.1+0.03
β-pinene	15.1+1.60	22.8+1.01	15.5+2.19	17.4+0.56
myrcene	0.7+0.05	1.5+0.07	0.8+0.26	1.1+0.05
α-phellandrene	0.5+0.06	-	0.4+0.04	1.1+0.02
limonene	35.3+2.36	46.1+0.92	37.5+4.46	44.1+1.97
γ-terpinene	8.4+0.43	9.8+0.12	9.8+0.94	6.8+0.43
terpinolene	0.5+0.20	0.6+0.10	0.7+0.09	0.9+0.29
decanal	0.4+0.01	0.1+0.01	0.1+0.05	0.3+0.05
linalool	1.6+0.03	0.6+0.03	1.3+0.38	1.1+0.11
α-fenchyl alcohol	1.2+0.16	0.5+0.04	0.6+0.21	0.1+0.03
terpinen-4-ol	3.3+0.57	1.3+0.16	3.8+0.09	2.3+0.94
α-terpineol	2.0+0.11	0.9+0.19	2.1+0.21	2.6+1.32
neral	3.6+0.57	2.0+-0.16	2.3+0.37	1.0+0.32
neryl acetate	1.6+0.15	1.1+0.16	1.3+0.16	0.3+0.18
citronellol	1.2+0.01	0.5+0.12	1.3+0.27	-
geranial	3.0+0.56	0.8+0.08	0.8+0.13	1.0+0.48
geranyl acetate	2.5+0.46	0.7+0.07	1.6+0.12	0.3+0.15
geraniol	5.9+1.37	1.2+0.23	1.8+0.09	4.4+0.70

DG=dark green fruit; FY=full yellow fruit; E-IND.Y=ethylene-induced degreening of DG fruit; A-IND.Y=acetylene-induced degreening of DG fruit

T-5. Comparative percentage composition of the distilled oils of the peels of ripe and rotten fruits

Compound	Ripe fruit oil	Rotten fruit oil
butyl acetate	0.6	0.8
nonane	-	0.3
α-pinene	1.9	1.4
camphene	0.2	0.3
β-pinene	13.1	8.4
myrcene	1.6	1.4
α-phellandrene	0.3	0.3
α-terpinene	0.9	0.9
limonene	21.0	21.3
(Z)-β-ocimene	-	0.6
(E)-β-ocimene	-	0.2
γ-terpinene	8.3	8.9
terpinolene	2.5	8.5
linalool	-	5.5
p-menth-3-en-1-ol†	0.2	-
p-mentha-3,4,8-triene†	0.1	-
α-fenchyl alcohol	0.5	0.7
citronellal	0.5	-
β-terpineol*†	0.8	0.8
myrtenol	-	0.2
terpinen-4-ol	2.7	3.3
terpineol*	-	0.2
α-terpineol	11.7	14.1
γ-terpineol[a]	0.6	0.5
trans-linalool oxidef	0.7	-
decanal	1.7	0.6

2,3-dimethyl-3-(4-methyl-3-pentenyl-2-nor-bornanol, campherenol and (E,E)-farnesal in one or both of the type A lime oils.

Three Mexican commercial samples of type B lime oil were analyzed by Bonaccorsi et al. (2011) using GC-FID and GC/MS. The constituents characterized in this oil were as follows:

α-thujene (0.3%)
α-pinene (1.9–2.0%)
camphene (0.1%)
sabinene (2.3–3.0%)
β-pinene (18.0–20.0%)
6-methyl-5-hepten-2-one (t–0.1%)
myrcene (1.0–1.1%)
α-phellandrene (t–0.1%)
α-terpinene (0.1–0.2%)
p-cymene (0.1–0.3%)
limonene (49.3–51.0%)
(Z)-β-ocimene (0.1%)
(E)-β-ocimene (0.3%)
γ-terpinene (8.7–9.9%)
cis-sabinene hydrate (0–0.1%)
octanol (0–0.1%)
terpinolene (0.3–0.5%)
linalool (0.2%)
α-fenchyl alcohol (0–0.1%)
isogeranial (0–0.3%)
terpinen-4-ol (0.1%)
α-terpineol (0.3–0.8%)
decanal (0.2%)
neral (1.6–1.8%)
geraniol (t–0.1%)
geranial (2.6–2.9%)
δ-elemene (0.4–0.5%)
neryl acetate (0.1%)
geranyl acetate (0.2–0.3%)
β-elemene (0.2–0.3%)
dodecanal (0–0.1%)
decyl acetate (0–0.1%)
cis-α-bergamotene (0–0.1%)
β-caryophyllene (0.7–1.0%)
γ-elemene (0–0.1%)
trans-α-bergamotene (0.9%)
(Z)-β-farnesene (0–0.1%)
(E)-β-farnesene (0–0.1%)
α-humulene (0.1%)
germacrene D (0–0.3%)
trans-β-bergamotene (0–0.1%)
valencene (0–0.1%)
bicyclogermacrene (0–0.1%)
α-selinene (0–0.1%)
(Z)-α-bisabolene (0–0.1%)
(E,E)-α-farnesene (1.1–1.2%)
β-bisabolene (1.3–1.5%)
germacrene B (0.5–0.6%)
α-bisabolol (0.1%)

t=trace (<0.05%)

Trace amounts of tricyclene, α-fenchene, thuja-2,4(10)-diene, decane, octanal, δ-3-carene, p-cymenene, *trans*-sabinene hydrate, nonanal, p-mentha-1,3,8-triene, *trans*-p-mentha-2,8-dien-1-ol, *cis*-p-menth-2-en-1-ol, (E,E)-allo-ocimene, *cis*-limonene oxide, *trans*-limonene oxide, *trans*-pinocarveol, isopulegol, citronellal, (Z)-isocitral, borneol, isopinocamphone, p-cymen-8-ol, octyl acetate, nerol, citronellol, carvone, piperitone, perillaldehyde, bornyl acetate, isobornyl acetate, 2,3-benzopyrrole, *trans*-pinocarvyl acetate, tridecane, undecanal, methyl geranate, citronellyl acetate, β-sesquisabinene, β-santalene, γ-curcumene, β-selinene, (Z)-γ-bisabolene, β-sesquiphellandrene, (E)-γ-bisabolene, (E)-α-bisabolene, 7-epi-α-selinene, *cis*-sesquisabinene hydrate, *trans*-sesquisabinene hydrate, caryophyllene oxide, dodecyl acetate, tetradecanal, 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-nor-bornanol, (Z)-nerolidol acetate, campherenol and (E,E)-farnesal were found in one or more of the Key lime type B commercial oils.

A hydrodistilled lime oil that was produced in 1.3% yield from *C. aurantifolia* fruit collected in Imphal (Manipur, India) was analyzed using GC-FID and

T-5. Comparative percentage composition of the distilled oils of the peels of ripe and rotten fruits (Cont.)

Compound	Ripe fruit oil	Rotten fruit oil
cyclofenchol	-	0.9
3-cyclohexen-1-acetaldehyde†	0.1	0.2
citral*	0.9	1.4
geraniol	0.7	1.2
tridecane	0.3	0.2
δ-elemene	0.9	0.4
citronellyl acetate	-	t
neryl acetate	0.1	0.2
geranyl acetate	0.1	0.2
tetradecanal†	0.4	-
trans-β-bergamotene†	2.8	-
α-copaene†	0.1	-
β-caryophyllene	0.1	0.4
β-elemene	-	0.4
dodecanal	-	0.8
trans-α-bergamotene	-	4.2
(E)-β-farnesene	0.7	0.3
β-selinene	-	0.1
β-santalene	0.1	0.2
δ-cadinene	0.2	0.2
(E)-γ-bisabolene	-	0.1
aromadendrene†	-	0.1
germacrene D	0.5	0.2
(E)-α-bisabolene†	0.4	0.2
(E,E)-α-farnesene	6.4	4.8
α-gurjunene†	0.2	0.2
(Z)-α-bisabolene	0.2	0.1
δ-elemene†	0.3	0.2
hexadecane	-	0.3
hexadecanal	0.2	0.1
α-bisabolol	0.3	0.2
octadecanal	-	0.1
hexadecanoic acid	0.4	1.2
heptadecane	0.2	0.1
eicosane	0.1	0.2
linoleic acid	0.2	-
tricosane	1.0	-

t=trace (<0.05%); ffuranoid form; *correct isomer not identified; †incorrect identification; ªdoes not occur naturally

GC/MS by Rana et al. (2012). The oil composition was determined to be as follows:

α-pinene (0.5%)
β-pinene (1.3%)
myrcene (0.7%)
limonene (46.4%)
(Z)-β-ocimene (0.2%)
(E)-β-ocimene (0.3%)
γ-terpinene (0.3%)
terpinolene (0.2%)
trans-linalool oxide[f] (0.1%)
linalool (1.5%)
nonanal (0.5%)
trans-p-mentha-2,8-dien-1-ol (0.1%)
cis-p-mentha-2,8-dien-1-ol (0.1%)
β-terpineol[*a] (0.2%)
citronellal (0.2%)
isomenthone (0.2%)
terpinen-4-ol (0.7%)
α-terpineol (2.6%)
decanal (0.9%)
trans-carveol (0.2%)
citronellol (2.2%)
neral (9.1%)
geraniol (1.4%)
geranial (12.1%)
citronellyl formate (0.5%)
undecanal (0.4%)
δ-elemene (0.2%)
neryl acetate (0.3%)
geranyl acetate (0.3%)
β-caryophyllene (0.5%)
tetradecane (0.1%)
dodecanal (0.4%)
β-bisabolene (0.8%)
hexadecane (0.1%)
dodecyl acrylate[b] (7.2%)

[*]correct isomer not identified
[f]furanoid form
[a]does not occur in CP fruit oil
[b]probable contaminant or artefact

Trace amounts (<0.05%) of δ-3-carene, terpinolene, *cis*-limonene oxide and 6-methyl-5-hepten-2-one were also found in this oil.

T-6. Comparative percentage composition of lime oil produced by three different methods

Compound	MHD	HD	CP
α-pinene	1.9	1.9	1.6
β-pinene	11.6	13.1	14.0
myrcene	1.4	1.5	1.4
limonene	60.6	63.4	68.8
γ-terpinene	11.9	11.2	8.9
linalool	0.3	0.4	0.2
citronellal	0.1	0.1	0.1
terpinen-4-ol	0.1	0.2	t
α-terpineol	0.4	0.4	0.2
nerol	0.2	0.1	0.1
neral	1.7	1.6	1.1
geraniol	0.1	0.1	0.1
geranial	2.2	2.1	1.8
citronellyl acetate	t	t	t
neryl acetate	0.9	0.6	0.2
β-caryophyllene	0.6	0.3	0.4
trans-α-bergamotene	1.0	0.5	0.6
α-humulene	0.1	0.1	0.1
(E)-β-farnesene	t	0.1	t
(Z)-α-bisabolene	0.1	0.1	0.1
β-bisabolene	1.5	0.8	0.9
α-bisabolol	t	t	t

t=trace (<0.1%); MAD=microwave-hydrodiffusion; HD=hydrodistillation; CP=cold-pressing

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Chiral Constituents

Bonaccorsi et al. (2011) used chiral GC analysis to determine the enantiomeric ratios of three samples of Mexican Key lime oils (type A). Their results are summarized as follows:

(4R)-(+)-α-thujene (0.9–1.1%):(4S)-(-)-α-thujene (98.9–99.1%)
(1R,5R)-(+)-α-pinene (21.8–22.5%):(1S,5S)-(-)-α-pinene (77.5–78.2%)
(3R)-(+)-camphene (7.1–7.2%):(3S)-(-)-camphene (92.8–92.9%)
(1R,5R)-(+)-β-pinene (3.5–4.5%):(1S,5S)-(-)-β-pinene (95.5–96.5%)
(1R,5R)-(+)-sabinene (15.2–15.4%):(1S,5S)-(-)-sabinene (84.6–84.8%)

(4R)-(+)-α-phellandrene (46.3–49.1%):(4S)-(-)-α-phellandrene (50.9–53.7%)
(4R)-(+)-β-phellandrene (34.4–36.1%):(4S)-(-)-β-phellandrene (63.9–65.6%)
(4R)-(+)-limonene (97.1–97.2%):(4S)-(-)-limonene (2.8–2.9%)
(3S)-(+)-linalool (26.3–31.3%):(3R)-(-)-linalool (68.7–73.7%)
(3R)-(+)-citronellal (33.0%):(3S)-(-)-citronellal (77.0%)
(4S)-(+)-terpinen-4-ol (28.8–29.2%):(4R)-(-)-terpinen-4-ol (70.8–71.2%)
(4R)-(+)-α-terpineol (16.4–15.9%):(4S)-(-)-α-terpineol (84.1–89.6%)

Three samples of Mexican Key lime type B were examined by Bonaccorsi et al. (2011) for their enantiomeric ratios of 12 constituents. These results are summarized as follows:

(4R-(+)-α-thujene (0–1.2%):(4S)-(-)-α-thujene (98.8–100%)
(1R,5R)-(+)-α-pinene (22.3–23.3%):(1S,5S)-(-)-α-pinene (76.7–77.9%)
(3R)-(+)-camphene (5.4–8.2%):(3S)-(-)-camphene (9.8–94.6%)
(1R,5R)-(+)-β-pinene (3.7–4.5%):(1S,5S)-(-)-β-pinene (95.5–96.3%)
(1R,5R)-(+)-sabinene (15.2–15.4%):(1S,5S)-(-)-sabinene (84.6–84.8%)
(4R)-(+)-α-phellandrene (41.6–47.9%):(4S)-(-)-α-phellandrene (52.1–58.4%)
(4R)-(+)-β-phellandrene (26.3–37.5%):(4S)-(-)-β-phellandrene (68.5–73.7%)
(4R)-(+)-limonene (97.0–97.2%):(4S)-(-)-limonene (2.8–3.0%)
(3S)-(+)-linalool (33.9–34.8%):(3R)-(-)-linalool (65.2–63.9%)
(3R)-(+)-cintronellal (19.0–27.2%):(3S)-(-)-citronellal (72.8–81.0%)
(4S)-(+)-terpinen-4-ol (20.8–29.1%):(4R)-(-)-terpinen-4-ol (70.9–79.2%)
(4R)-(+)-α-terpineol (16.2–20.5%):(4S)-(-)-α-terpineol (79.5–83.8%)

Bonaccorsi et al. (2012) used chiral GC in a multidimensional GC system to examine the enantiomeric ratio of 10 constituents of Key lime oils (*C. aurantifolia*). The ratios of these 10 constituents were as follows:

(4R)-(+)-α-thujene (0–2%):(4S)-(-)-α-thujene (98–100%)
(3R)-(+)-camphene (11–16%):(3S)-(-)-camphene (84–89%)
(1R,5R)-(+)-α-pinene (6–9%):(1S,5R)-(-)-α-pinene (91–94%)
(1R,5R)-(+)-sabinene (28–32%):(1S,5S)-(-)-sabinene (68–72%)
(4R)-(+)-α-phellandrene (83–>99%):(4S)-(-)-α-phellandrene (<1–17%)
(4R)-(+)-β-phellandrene (51–70%):(4S)-(-)-β-phellandrene (30–49%)
(4R)-(+)-limonene (94–96%):(4S)-(-)-limonene (4–6%)
(3S)-(+)-linalool (53–70%):(3R)-(-)-linalool (30–47%)
(4S)-(+)-terpinen-4-ol (42–59%):(4R)-(-)-terpinen-4-ol (41–58%)
(4R)-(+)-α-terpineol (29–44%):(4S)-(-)-α-terpineol (56–71%)

In addition, the authors described the use of gas chromatography-combustion isotope ratio mass spectrometry to confirm the genuineness of the Key lime oils examined.

I. Bonaccorsi, P. Dugo, L. Mondello, D. Sciarrone, G. Dugo and L. Haro-Guzman, *Analytical characterization of industrial essential oils from fruits and leaves of* Citrus aurantifolia *Tan. and* C. latifolia *Swing*. J. Essent. Oil Res., **23**, 68–79 (2011).

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Non-volatile Constituents

Buiarelli et al. (2002) used HPLC to examine the composition of a commercial distilled lime oil that was procured in Italy. Although the authors did not quantify the volatile components they did determine that the following non-volatiles were found:

methylumbelliferone (9.3)[a]
citropten (141.1)
5-geranyloxy-7-methoxycoumarin (15.0)

[a]mg/L or ppm

The authors also determined that the methyl anthranilate level in this distilled oil was 3 ppm.

Feger (2006) used high-speed countercurrent chromatography, HPLC and spectroscopic characterization of the non-volatiles found in cold-pressed lime oil of Mexican origin. The non-volatiles identified were as follows:

1. heraclenol, or 8-(2',3'-dihydroxy-isopentyloxy)-psoralen
2. oxypeucedanin hydrate, or 5-(2',3'-dihydroxy-isopentyloxy)-psoralen
3. herniarin, or 7-methoxycoumarin
4. 5,7,8-trimethoxycoumarin
5. xanthotoxin, or 8-methoxypsoralen
6. citropten, or 5,7-dimethoxycoumarin
7. pabulenol, or 5-(2'-hydroxy-3'-methyl-but-3-enyloxy)-psoralen
8. oxypeucedanin methanolate, or 5-(2'-hydroxy-3'-methoxyisopentenyloxy)-psoralen
9. isopimpinellin, or 5,8-dimethoxypsoralen
10. bergapten, or 5-methoxypsoralen
11. heraclenin, or 8-(2',3'-epoxy-isopentyloxy)-psoralen
12. oxypeucedanin, or 5-(2',3'-epoxy-isopentyloxy)-psoralen
13. nobiletin, or 3',4',5,6,7,8-hexamethoxyflavone
14. tetra-O-methylscutellarein, or 4',5,6,7-tetramethoxy flavone
15. 3,3',4',5,6,7,8-heptamethoxyflavone
16. iso-oxypeucedanin, or 5-isopentyl-2'-ocy)-psoralen
17. isopentenyloxycoumarin
18. imperatorin, or 8-isopentenyloxypsoralen
19. phellopterin, or 5-methoxy-8-isopentenyloxypsoralen
20. osthol, or 7-methoxy-8-isopentenylcoumarin
21. 5-isopentenyloxy-7-methoxycoumarin
22. cnidiun, or 5-isopentenyloxy-8-methoxycoumarin
23. isoimperatorin, or 5-isopentenyloxypsoralen
24. 8-geranyloxypsoralen
25. 6,7-dimethoxy-5-geranyloxycoumarin
26. auraptene, or 7-geranyloxycoumarin
27. 5-geranyloxy-8-methoxypsoralen
28. and 29. two isomers of neral oxypeudaninyl acetal
30. and 31. two isomers of geranial oxypeucedaninyl acetal
32. bergamottin, or 5-geranyloxypsoralen
33. 5-geranyloxy-7-methoxycoumarin

It is worth noting that oxypeucedanin methanolate was not a true constituent, but an artefact produced during countercurrent chromatography.

Furthermore, Bonaccorsi et al. (2011) used reversed phase HPLC to measure the amounts of oxygen heterocyclic components found in three samples of the type B oils. Their results are shown in **T-7**.

Bonaccorsi et al. (2011) determined the oxygen heterocyclic components of two samples of type A lime oils, as can be seen in **T-8**.

Russo et al. (2012) used a modified solvent system in their HPLC analysis of the non-volatile oxygen heterocyclic compounds found in a genuine cold-pressed Key lime oil (ex: *C. aurantifolia*). The compounds characterized were as follows:

T-7. Oxygen heterocyclic constituents of three Mexican type B lime oils

Compound	1	2	3
herniarin	3,880[a]	4,670	3,350
oxypeucedanin hydrate	1,690	1,710	1,620
citropten	10,950	9,230	5,940
isopimpinellin	7,300	6,540	3,010
bergapten	3,000	3,920	2,160
byakangelicol	1,020	460	80
oxypeucedanin	10,720	6,660	7,560
isoimperatorin	70	210	410
imperatorin	380	430	660
5-isopentenyloxy-7-methoxycoumarin	2,790	2,670	2,100
cnidicin	250	110	70
8-geranyloxypsoralen	4,470	4,540	3,800
bergamottin	36,400	41,590	25,320
5-geranyloxy-7-methoxycoumarin	43,140	45,350	27,770

[a]mg/L (ppm)

T-8. Oxygen heterocyclic components of two commercially available type A lime oils

Compound	1	2
herniarin	1,460[a]	2,970
oxypeucedanin hydrate	780	1,160
citropten	7,350	11,740
isopimpinellin	5,670	10,210
bergapten	2,000	3,450
byakangelicol	90	-
oxypeucedanin	260	-
isopimperatorin	370	390
imperatorin	830	900
5-isopentenyloxy-7-methoxycoumarin	4,170	4,830
cnidicin	340	90
8-geranyloxypsoralen	6,520	8,100
bergamottin	37,300	56,130
5-geranyloxy-7-methoxycoumarin	41,550	63,320

[a]mg/L

heniarin[a] (3,880+45.8)
oxypeucedanin hydrate (1,690+203)
citropten (10,950+92.8)
isopimpinellin (7,300+46.9)
bergapten (3,000+31.1)
byakangelicol (92+9.9)
oxypeucedanin (10,600+85.1)
isoimperatorin (88+5.9)
imperatorin (39.0+10.3)
cnidilin[a] (249+7.6)
5-isopentenyloxy-7-methoxycoumarin (2,790+15.0)
cnidicin (250+62)
8-geranyloxypsoralen (4,470+28.7)
bergamottin (36,401+150.9)

[a]mg/L of oil

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