Progress in Essential Oils

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Bergamot Oil

Bergamot oil, which is produced by cold-press of the fruit peels of *Citrus bergamia* Risso et Poit. (syn. *C. aurantium* L. subsp. *bergamia* [Risso et Poit.] Wight et Walker-Arn.) primarily in the Reggio Calabrian region along the Ionian coast of southern Italy.

Huang et al. (1986) examined the composition of an oil produced from the ripe fruits of *C. bergamia* grown in China. They reported that the oil contained the following constituents:

α-thujene (0.2%)
α-pinene (0.8%)
sabinene (0.8%)
β-pinene (4.8%)
myrcene (1.4%)
δ-3-carene (2.0%)
p-cymene (1.6%)
limonene (42.5%)
β-ocimene* (0.3%)
γ-terpinene (1.4%)
cis-linalool oxide[f] (0.1%)
cis-sabinene hydrate (0.1%)
terpinolene (0.1%)
linalool (17.9%)
dihydrolinalool[†] (0.1%)
terpinen-4-ol (0.2%)
α-terpineol (2.5%)
decanal (0.1%)
octyl acetate (0.1%)
nerol (0.5%)
neral (0.4%)
geraniol (5.7%)
linalyl acetate (11.4%)
geranial (0.5%)
methyl geranate (0.1%)
citronellyl acetate (0.2%)
neryl acetate (0.9%)
geranyl acetate (1.3%)
β-caryophyllene (0.1%)
(E)-β-farnesene (0.2%)
pentadecane (0.2%)

[f]furanoid form
*correct isomer not identified
[†]does not exist naturally; an impurity of synthetic linalool

In addition, trace amounts (<0.05%) of butyl acetate, (Z)-3-hexenol, hexanol, camphene, 6-methyl-5-hepten-2-one, α-phellandrene, 1,4-cineole, α-fenchyl alcohol, γ-heptalactone, citronellal, isopulegol, lavandulol, nonanol, dodecane, citronellol, pulegone, citronellyl formate, geranyl formate, nonyl acetate, α-terpinyl acetate, decyl acetate, α-humulene, an isomer of α-bergamotene, (Z)-nerolidol, (E)-nerolidol and β-bisabolene were also characterized in this oil.

Sawamura et al. (1999) reported that the composition of two samples of cold-pressed bergamot oil that were produced in Japan from fruit of Italian origin was found to be as follows:

α-pinene (1.3–1.6%)
β-pinene (6.8–8.9%)

T-1. Comparative percentage composition of bergamot oil analyzed either by conventional or fast GC (cont. on following page)

Compound	Conventional GC analysis	Fast GC analysis
tricyclene	t	t
α-thujene	0.33	0.34
α-pinene	1.27	1.30
camphene	0.04	0.05
sabinene	1.16	1.17
β-pinene	7.04	7.06
6-methyl-5-hepten-2-one	0.01	t
myrcene	0.98	1.16
octanal + α-phellandrene	0.08	0.09
δ-3-carene	t	t
α-terpinene	0.15	0.16
p-cymene + limonene[†]	42.66	42.37
(Z)-β-ocimene	0.02	0.07
(E)-β-ocimene	0.21	0.28
γ-terpinene	7.84	7.91
cis-sabinene hydrate	0.04	0.04
octanol	t	0.02
terpinolene	0.32	0.33
linalool	7.52	7.67
nonanal	0.03	0.04
cis-limonene oxide	t	t
trans-limonene oxide	t	0.01
camphor	0.01	-
citronellal	0.01	0.02
terpinen-4-ol	0.03	0.04
α-terpineol	0.09	0.10
decanal	0.06	0.07
octyl acetate	0.12	0.13
nerol	0.05	0.05
neral	0.24	0.26

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myrcene (0.7–0.9%)
α-terpinene (0.1–0.2%)
limonene (24.3–38.8%)
γ-terpinene (5.6–8.3%)
p-cymene (t–0.3%)
terpinolene (0.2–0.3%)
octanal (0–t)
decanal (t–0.1%)
linalool (4.2–18.2%)
linalyl acetate (32.1–39.0%)
terpinen-4-ol (0.1%)
neral (0.2%)
α-terpineol (0.1–0.2%)
geranial (0–0.3%)
geranyl acetate (0.2–0.3%)
decanol (0–t)
citronellol (t)
nerol (0–t)
geraniol (t)
nootkatone (t–0.1%)

t=trace (<0.1%)

A sample of Italian bergamot oil was found by Kubeczka and Formacek (2002) to possess the following composition:

α-pinene (1.0%)
α-thujene (0.3%)
β-pinene (5.9%)
sabinene (1.0%)
myrcene (0.9%)
α-terpinene (0.1%)
limonene (33.1%)
β-phellandrene (0.2%)
γ-terpinene (6.5%)
(E)-β-ocimene (0.3%)
p-cymene (0.3%)
terpinolene (0.3%)
octyl acetate (0.1%)
cis-sabinene hydrate acetate (0.1%)
linalool (14.6%)
linalyl acetate (32.5%)
trans-α-bergamotene (0.3%)
β-caryophyllene (0.3%)
undecanal (0.1%)
neral (0.2%)
α-terpinyl acetate (0.1%)
α-terpineol (0.1%)
neryl acetate (0.7%)
geranial (0.4%)
geranyl acetate (0.2%)
nerol (0.1%)
geraniol (0.1%)

T-1. Comparative percentage composition of bergamot oil analyzed either by conventional or fast GC (cont.)

Compound	Conventional GC analysis	Fast GC analysis
linaly acetate	27.32	26.16
geranial	0.36	0.38
bornyl acetate	0.05	0.06
undecanal	0.01	0.03
nonyl acetate	0.02	0.02
α-terpinyl acetate	0.17	0.18
citronellyl acetate	0.03	0.04
neryl acetate	0.39	0.45
geranyl acetate	0.36	0.44
dodecanal	0.01	-
decyl acetate	0.03	0.04
sesquithujene	0.02	0.03
β-caryophyllene	0.03	0.34
trans-α-bergamotene	0.29	0.33
(Z)-β-farnesene	0.03	0.03
germacrene D	0.05	0.06
α-farnesene*	0.03	-
bicyclogermacrene	0.03	0.04
β-bisabolene	0.43	0.46
β-sesquiphellandrene	0.01	0.01
(E)-γ-bisabolene	0.01	0.02
(E)-nerolidol	0.02	0.03
trans-sesquisabinene hydrate	0.01	0.01
2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbonanol	0.01	0.02
campherenol	0.02	0.03
α-bisabolol	0.03	0.03
nootkatone	0.05	0.06

†major component of mixture
t=trace (<0.01%)
*correct isomer not identified

In addition, trace amounts (<0.05%) of camphene, 1,8-cineole, (Z)-β-ocimene, octanal, nonanal, *cis*-limonene oxide, trans-sabinene hydrate, decanal, terpinen-4-ol and citronellyl acetate were found in this commercial sample of Italian bergamot oil.

A comparison between conventional (ca. 46 min) and fast (9 min) GC analyses of bergamot oil was performed by Mondello et al. (2003). The results of this study can be found in **T-1**.

The same results were reported in a follow-up study (Mondello et al. 2004).

Gazea and Cicciarello (2003) determined the level of four monoterpene hydrocarbons in bergamot oils produced over three seasons from fruit collected from trees that were grafted on different rootstocks such as *Citrus macrophylla* Wester, *C. aurantium* L. and C. *volkameriana* Ten. et Pasq. The results revealed that there were some minor quantitative differences in the contents of the monoterpene hydrocarbons as can be seen in **T-2**.

The effect of rootstock on bergamot oil composition was also the subject of study by Verzera et al. (2003). Bergamot oil produced from fruits obtained from *C. bergamia* ('Castagnaro' cultivar) grafted on *C. aurantium* (bitter orange), *C. sinensis* (L.) Osbeck x *Poncirus trifolata* (L.) Raf (Carrizo orange), *P. trifoliata* (trifoliate orange), *C. macrophylla* (alemow), *C. volkameriana* (Volkamerian lemon), *C. sinensis* cv. 'Washington navel' x *P. trifoliata* (troyer orange). The cold-pressed oils, which were analyzed by GC-FID and GC/MS can be seen in **T-3**. In addition, trace amounts (<0.01%) of tricyclene, camphene, 6-methyl-5-hepten-2-one, octanal, α-phellandrene, hexyl acetate, δ-3-carene, 1,8-cineole, (Z)-β-ocimene, *cis*-sabinene hydrate, octanol, *cis*-linalool oxide (furanoid form), *trans*-linalool oxide (furanoid form), nonanal, heptyl acetate, *cis*-limonene oxide, *trans*-limonene oxide, isopulegol, camphor, citronellal, dodecane, geraniol, (E)-2-decenal, bornyl acetate, indole, undecanal, nonyl acetate, methyl geranate, dodecanal, decyl acetate, *cis*-α-bergamotene, (Z)-β-santalene, dodecanol, bicyclogermacrene, (Z)-α-bisabolene, (Z)-γ-bisabolene, germacrene B, 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol,

campherenol and α-bisabolol were characterized in the various bergamot oils.

Based on the quality of the oils, Verzera et al. (2003) reported that either Alemow or Volkamerian lemon could be substitute rootstocks for the normally used bitter orange rootstock.

Poiana et al. (2003) used GC/MS only to examine the composition of a cold-pressed bergamot oil, a distilled oil, alkali-treated cold-pressed oil and a number of supercritical CO_2 extracts of the cold-pressed oil over a range of conditions. A summary of the findings of this study is reported in **T-4**. The results revealed that alkali-treated and supercritical CO_2 extracts of C.P. bergamot oil contained low (0.01–0.05%) bergaptene contents.

Mandalari et al. (2006) reported that bergamot peel, which is an under-utilized byproduct of the cold-pressed bergamot oil industry could be used as a source of marketable pectins and flavonoids.

Pizzimenti et al. (2006) examined the composition of a bergamot processing byproduct known as 'Peratoner,' which is obtained from bergamot peel after it is almost depleted of oil. Analysis of the 'Peratoner' oil revealed that it possessed the following components in amounts of 0.1% or greater:

α-thujene (0.1%)
α-pinene (0.5%)
sabinene (4.3%)
myrcene (0.7%)
α-terpinene (0.1%)
limonene[a] + β-phellandrene (23.1%)
(Z)-β-ocimene (0.1%)
(E)-β-ocimene (0.3%)
γ-terpinene (6.0%)
terpinolene (0.3%)
linalool (36.9%)
terpinen-4-ol (0.4%)
α-terpineol (1.3%)
decanal (0.2%)
nerol + citronellol (0.1%)
linalyl acetate (22.8%)
geranial + perillaldehyde (0.1%)
α-terpinyl acetate (0.2%)
neryl acetate (0.4%)
geranyl acetate (0.4%)
β-caryophyllene (0.3%)
trans-α-bergamotene (0.3%)
β-bisabolene (0.4%)

[a]major component

The authors found via an in vivo test that 'Peratoner' possessed good antifungal properties against *Candida albicans* skin infections. A sample of bergamot oil

T-2. Comparative percentage composition of four selected monoterpene hydrocarbons in bergamot oil produced from fruit grown on trees of different rootstocks over three seasons

Compound	1	2	3
α-pinene	1.1–1.7	1.6–1.7	1.5–1.9
sabinene	11.4–13.5	10.2–12.5	11.0–12.6
myrcene	1.6–1.7	1.4–1.8	1.7–1.8
γ-terpinene	10.3–12.1	8.1–8.8	8.0–10.2

1=*Citrus macrophylla* rootstock; 2=*C. volkameriana* rootstock; 3=*C. aurantium* rootstock

T-3. Comparative percentage composition of bergamot oils produced from different rootstocks

Compound	1	2	3	4	5	6
α-thujene	0.2	0.2	0.2	0.2	0.2	0.2
α-pinene	0.8	1.0	0.8	1.1	0.7	0.8
sabinene + β-pinene	5.7	5.7	6.3	6.7	4.7	5.4
myrcene	0.9	0.8	0.9	1.0	0.8	0.9
α-terpinene	0.1	0.1	0.1	0.1	0.1	0.1
p-cymene	0.1	0.1	0.2	0.1	0.1	0.1
limonene[a] + β-phellandrene	34.2	39.4	34.6	41.4	36.3	37.1
(E)-β-ocimene	0.2	0.1	0.2	0.1	0.1	0.1
γ-terpinene	4.3	5.5	5.4	7.1	3.6	4.4
terpinolene	0.2	0.2	0.2	0.3	0.2	0.2
linalool	13.7	11.0	11.6	8.2	14.8	13.5
terpinen-4-ol	t	t	0.1	t	t	t
α-terpineol	0.1	0.1	0.1	0.1	0.1	0.1
decanal	0.1	t	t	t	0.1	t
octyl acetate	0.1	0.1	0.1	0.1	0.1	0.1
nerol + citronellol	0.1	0.1	0.1	t	0.1	0.1
neral	0.2	0.2	0.2	0.2	0.2	0.2
carvone	t	t	t	t	t	0.1
trans-sabinene hydrate acetate	0.1	0.1	0.1	0.1	t	0.1
linalyl acetate	36.6	32.8	36.5	29.9	35.9	34.2
geranial + perillaldehyde	0.3	0.3	0.3	0.3	0.3	0.3
linalyl propionate	0.1	0.1	0.1	0.1	0.1	0.1
δ-elemene	0.1	t	t	t	0.1	t
α-terpinyl acetate	t	0.1	0.1	0.2	t	0.1
citronellyl acetate	0.2	t	0.2	t	t	t
neryl acetate	0.2	0.3	0.3	0.2	0.2	0.2
geranyl acetate	0.2	0.2	0.2	0.3	0.2	0.2
β-caryophyllene	0.2	0.2	0.2	0.3	0.2	0.3
trans-α-bergamotene	0.2	0.2	0.1	0.2	0.2	0.2
α-humulene	0.1	0.1	t	0.1	0.1	0.1
(Z)-β-farnesene	0.1	0.1	0.1	t	t	0.1
germacrene D	t	0.1	t	0.1	t	0.1
(E,E)-α-farnesene	t	t	0.1	t	t	t
β-bisabolene	0.3	0.3	0.3	0.4	0.3	0.3
(E)-nerolidol	t	t	0.1	t	t	t
nootkatone	0.1	t	t	t	t	t

t=trace (<0.0150); [a]major component
1=Bitter orange rootstock; 2=Carrizo orange rootstock; 3=Alemow rootstock;
4=Trifoliate orange rootstock; 5=Volkamerian lemon rootstock; 6=Troyer orange rootstock

originating from the bergamot consortium of Reggio Calabria (Italy) that was produced by the 'Pelatrice' process was analyzed by Sawamura et al. (2006) using GC-FID and GC/MS. The composition of the oil was found to be as follows:

α-pinene (1.3%)
β-pinene (6.2%)
sabinene (1.1%)
myrcene (0.8%)
α-terpinene (0.1%)
limonene (37.2%)
β-phellandrene (0.2%)
γ-terpinene (6.8%)
p-cymene (1.4%)
terpinolene (0.3%)
trans-sabinene hydrate (0.1%)
trans-linalool oxide[f] (0.1%)
trans-solanone[a†] (0.1%)
linalool (8.8%)
linalyl acetate (30.1%)
α-bergamotene[*] (0.3%)
β-caryophyllene (0.3%)
citronellyl acetate (0.1%)
β-farnesene[*] (0.1%)
neral (0.2%)
α-terpinyl acetate (0.2%)
α-terpineol (0.1%)
β-bisabolene (0.8%)
geranial (0.3%)
geranyl acetate (0.3%)
nerol (0.1%)
geraniol (0.1%)
nootkatone (0.1%)

[a]also known as 8-methyl-5-(1-methylethyl)-6,8-nonadien-2-one
[*]correct isomer not identified
[f]furanoid form
[†]incorrect identification based on GC elution order

In addition, the trace constituents characterized in this oil have all been identified before as trace constituents, except perrilyl acetate, octanoic acid and cedrol. Sawamura et al. also used GC-O (gas chromatography-olfactometry) to determine that the compounds characteristic of the bergamot-like aroma in the oil were *cis*-limonene oxide, decanal, linalyl acetate and geraniol.

Belsito et al. (2007) determined that the vacuum distillation of bergamot peels yields a bergapten-free high quality oil. A comparison between this oil and a typical cold-pressed oil can be seen in **T-5**.

Williams (2008) reported that the constituents of an Italian bergamot oil were as follows:

α-thujene (0.2%)
α-pinene (1.1%)
sabinene (1.1%)
β-pinene (6.5%)
myrcene (1.0%)
p-cymene (0.6%)
limonene (39.7%)
β-phellandrene (0.1%)
(E)-β-ocimene (0.1%)
γ-terpinene (5.5%)
terpinolene (0.2%)
linalool (12.9%)
neral (0.2%)
linalyl acetate (26.6%)
neryl acetate (0.5%) geranyl acetate (0.2%)
β-caryophyllene (0.3%)
trans-α-bergamotene (0.3%)
β-bisabolene (0.5%)
bergapten (0.1%)

In addition, camphene, α-terpineol, nerol, geranial and 1,8-cineole were noted as oil components; however, no quantitative data on them was reported.

Mangiola et al. (2009) collected samples of 1 metric tonne lots of bergamot oil produced in Reggio Calabria. These oils were analyzed by GC-FID and the known constituents were characterized

T-4. Comparative percentage composition of some bergamot oils and supercritical CO_2 extracts of bergamot oil

Compound	1	2	3	4
α-thujene	0.4	0.4	0.4	0.4–1.2
α-pinene	1.5	1.4	1.4	1.3–3.5
camphene	t	t	t	t–0.1
sabinene	0.9	1.1	0.9	1.1–2.0
β-pinene	7.0	6.7	6.7	6.6–11.4
myrcene	1.0	0.9	0.9	1.0–1.8
octanal	t	t	t	t–0.1
α-phellandrene	t	t	t	t–0.1
α-terpinene	0.2	0.3	0.1	0.2–0.5
p-cymene	0.7	0.5	0.6	0.6–0.8
limonene	32.1	33.1	32.5	31.4–41.8
(Z)-β-ocimene	t	t	t	0.1–0.3
(E)-β-ocimene	0.3	0.3	0.2	0.3–0.6
γ-terpinene	8.5	8.7	8.5	8.6–11.3
cis-sabinene hydrate	0.1	t	t	0.1
octanol	t	t	t	t–0.1
terpinolene	0.4	0.5	0.4	0.5–0.7
linalool	12.1	11.9	11.8	9.3–13.9
terpinen-4-ol	t	0.1	t	t–0.1
α-terpineol	0.1	0.1	0.1	0.1–0.2
decanal	0.1	0.1	0.1	t–0.1
octyl acetate	0.1	0.1	0.1	0.1–0.2
nerol	0.1	0.1	0.1	t–0.1
neral	0.2	0.2	0.2	0.1–0.2
cis-sabinene hydrate aceate	0.1	0.1	0.1	0.1
linalyl acetate	29.7	30.6	31.3	15.0–30.1
geranial	0.3	0.3	0.3	0.1–0.3
linalyl propionate	0.1	0.1	0.1	t–0.1
α-terpinyl acetate	0.3	0.2	0.3	0.1–0.3
neryl acetate	0.5	0.4	0.4	0.1–0.6
geranyl acetate	0.4	0.3	0.4	0.1–0.6
β-caryophyllene	0.6	0.5	0.6	0.1–0.7
trans-α-bergamotene	0.6	0.5	0.5	0.1–0.6
β-bisabolene	0.8	0.5	0.7	0.1–0.8
nootkatone	0.1	t	0.1	t–0.1
bergapten	0.5	t	t	t

t=trace (<0.05%)
1=cold-pressed oil
2=distilled oil
3=alkali-treated cold-pressed oil
4=supercritical CO_2 extracts

by retention times. The oils were found to range in composition as follows:

α-thujene (0.3–0.4%)
α-pinene (1.3–1.4%)
sabinene (1.2%)
β-pinene (6.5–7.2%)
myrcene (1.0–1.1%)
p-cymene (0.1%)
limonene (39.6–43.7%)
γ-terpinene (7.4–8.7%)
linalool (3.9–9.9%)
α-terpineol (0.1%)
linalyl acetate (25.1–28.0%)
geranial (0.3%)
neryl acetate (0.3–0.4%)
geranyl acetate (0.2–0.4%)
β-caryophyllene (0.3%)
trans-α-bergamotene (0.3%)
β-bisabolene (0.4–0.5%)

Trace amounts (<0.05%) of δ-3-carene and terpinen-4-ol were characterized in this oil.

Menichini et al. (2010) examined the composition of a sample of Calabrian bergamot oil. Using GC/MS only, along with retention indices, the oil was determined to have the following composition:

α-thujene (0.4%)
α-pinene (1.5%)
sabinene (1.1%)
β-pinene (5.4%)
myrcene (1.1%)
α-terpinene (0.1%)
limonene (38.1%)
(E)-β-ocimene (0.1%)
γ-terpinene (7.3%)
cis-linalool oxide[f] (0.1%)
terpinolene (0.4%)
linalool (6.4%)
nonanal (0.1%)
terpinen-4-ol (0.2%)
α-terpineol (0.1%)
decanal (0.1%)
linalyl acetate (28.9%)
geranial (0.2%)
neryl acetate (0.8%)
geranyl acetate (0.7%)
α-bergamotene[*] (0.6%)
β-caryophyllene (0.5%)
(E)-β-farnesene (0.1%)
β-bisabolene (0.8%)
α-humulene (0.1%)
caryophyllene oxide (0.2%)

[f]furanoid form
[*]correct isomer not identified

In addition, most of the normally encountered trace constituents were characterized in this oil.

Five different bergamot oils (cold-pressed oil produced by Pelatrice process, terpeneless, colored oil with waxes obtained through distillation and fractionation, terpeneless colorless oil obtained through distillation and fractionation, furano-coumarin-free, colorless oil obtained through distillation and fractionation, and an oil treated with alkali to remove bergapten) were the subject of analysis by Costa et al. (2010) using GC-FID and GC/MS. The volatiles characterized in the five oils can be seen in **T-6**.

Nineteen Italian genuine cold-pressed bergamot oils (2008–2009 season), three 'Peratoner' oils produced by distillation, three Italian commercial oils of unknown harvest period, two commercially produced Ivory Coast (2008–2009 season) oils, three adulterated oils prepared in the laboratory by mixing varied amounts of one of the Italian commercial samples with a randomly selected oil from the 19 genuine oils and two other laboratory created adulterated oils by mixing a so-called commercial linalool oil at rates of 2% and 8%, respectively, with a randomly selected oil from the 19 genuine oils were analyzed using GC-FID, GC/MS, chiral GC and GC-C-IRMS (gas chromatography-combustion-isotope ratio mass spectrometry) by Schipilliti et al. (2011). The range in composition (components in amounts of >0.05%) of the 19 genuine oils, the two Ivory coast oils and the three Peratoner oils can be seen in **T-7**. Of the numerous trace constituents that are normally found in bergamot oils, only ascaridole, which was to the best of this reviewer's knowledge, the only newly characterized trace component.

T-5. Comparative percentage composition of a vacuum distilled and a cold-pressed bergamot oil

Compound	Vacuum-distilled oil	Cold-pressed oil
α-thujene	0.1	0.3
α-pinene	1.2	1.0
sabinene	0.9	0.9
β-pinene	5.5	5.2
myrcene	1.5	1.1
α-phellandrene + octanal	0.1	0.1
α-terpinene	0.1	0.1
limonene[t] + p-cymene	46.3	32.5
(Z)-β-ocimene	0.1	0.1
(E)-β-ocimene	0.3	0.2
γ-terpinene	6.0	5.5
cis-sabinene hydrate	0.1	0.1
terpinolene	0.3	0.2
linalool	7.8	14.4
terpinen-4-ol	t	0.1
α-terpineol	t	0.1
decanal	t	0.1
octyl acetate	0.1	0.1
nerol	t	0.2
neral	0.1	0.1
linalyl acetate	26.3	31.0
geranial	0.2	0.7
bornyl acetate	0.1	0.1
nonyl acetate	t	0.1
geranyl acetate	0.2	0.6
β-caryophyllene	0.3	0.6
(Z)-β-farnesene	0.1	0.5
β-bisabolene	0.3	0.5
5,7-dimethoxycoumarin	-	0.8
bergapten	-	0.2

t=trace (<0.05%)

Schipilliti et al. also compared the enantiomeric ratios of the Ivory Coast oils, the Italian oils and the Peratoner oils; however, the results were similar to those previously reported. In addition, the authors used GC-C-IRMS to establish some isotopic fingerprints of the above noted oils. From the fingerprint results obtained for 17 constituents, the authors were able to show that the Peratoner oils were the same as the cold-pressed oils, which demonstrates that the oil constituents possess the same thermodynamic and kinetic biosynthetic pathways, as would be expected from oils produced from the same geographic provenance and season. In contrast, the fingerprint data for the Ivory Coast samples were slightly different, as might be expected from a different geographic provenance, so the Ivory Coast oils could be differentiated from the Italian oils.

It was not possible to determine adulteration through examination of the compositional data on the adulterated oils (mixed bergamot oils); however, through the use of both chiral GC and GC-C-IRMS, these oils were found to be adulterated. Further, examination of the lab-prepared adulterated bergamot oil with 2% and 8% linalool could not be characterized as adulterated oils from the compositional data; however, as with the other adulterated oils, adulteration was readily determined using chiral GC and GC-C-IRMS.

A very detailed study and review of the composition of a bergamot oil was reported by Dugo et al. (2012). The study used a variety of analytical techniques, including GC-FID, GC/MS, chiral GC, GC-C-IRMS, HPLC and HPLC-MS-IT-TOF to examine Italian cold-pressed bergamot oils produced over seasons 2008–2009, 2009–2010 and 2010–2011, as well as some bergapten-free, colored and colorless, terpeneless, Peratoner, Fecce and a number of hand-pressed oils from the 'Castagnaro,' 'Femminello' and Fantastico cultivars produced in 2011. The composition data, enantiomeric distribution of some monoterpenoid constituents and the oxygen heterocyclic data for the oils produced over the three seasons, the bergapten-free oils, the colored and colorless terpeneless oils, and the Peratoner oils were all similar to the earlier published data of Costa et al. (2010) and Schipilliti et al. (2011). The two Fecce oils, which were produced by distillation from semi-fluid waxes of cold-extraction of bergamot, were reported to contain the following constituents in amounts of 0.05% or above:

α-thujene (0.17–0.33%)
α-pinene (0.64–1.38%)
sabinene (0.65–1.27%)
β-pinene (4.56–8.35%)
6-methyl-5-hepten-2-one (0.03–0.08%)
myrcene (1.00–1.14%)
α-terpinene (0.05–0.18%)
p-cymene (0.33–3.86%)
limonene (44.37–50.67%)
(Z)-β-ocimene (0.11–0.12%)
(E)-β-ocimene (0.13–0.23%)
γ-terpinene (3.68–8.25%)
cis-sabinene hydrate + octanol (0.03–0.05%)
terpinolene (0.19–0.40%)

T-6. Comparative percentage composition of five bergamot oils produced using different treatments

Compound	1	2	3	4	5
α-thujene	0.3	t	-	0.3	0.3
α-pinene	1.0	t	t	1.0	1.0
sabinene	0.9	t	t	0.9	0.9
β-pinene	5.6	t	t	5.3	5.4
myrcene	0.9	0.1	t	0.9	1.0
octanal	0.1	t	t	t	0.1
α-terpinene	0.1	t	t	0.1	0.1
p-cymene	0.4	0.1	t	1.0	0.6
limonene	42.8	0.3	0.5	40.0	43.8
(Z)-β-ocimene	t	t	t	t	0.1
(E)-β-ocimene	0.2	0.1	t	0.2	0.2
γ-terpinene	6.2	0.2	0.7	5.2	6.2
cis-sabinene hydrate	t	0.1	t	t	t
terpinolene	0.2	t	0.1	0.2	0.2
linalool	5.6	22.2	18.6	10.0	10.7
terpinen-4-ol	t	t	0.1	t	t
α-terpineol	0.1	0.2	0.1	0.1	0.1
decanal	0.1	0.1	0.1	t	t
octyl acetate	0.1	0.2	0.2	0.1	0.1
nerol	t	0.1	0.1	0.1	0.1
neral	0.2	0.6	0.4	0.2	0.2
linalyl acetate	27.1	68.5	73.9	25.1	26.5
geranial	0.2	1.0	0.6	0.3	0.3
linalyl propionate	t	0.1	0.1	t	t
α-terpinyl acetate	0.1	0.3	0.3	0.1	0.1
citronellyl acetate	-	t	t	t	t
neryl acetate	0.3	0.9	0.8	0.3	0.3
geranyl acetate	0.3	0.9	0.6	0.3	0.3
decyl acetate	t	0.1	0.1	t	t
β-caryophyllene	0.3	0.8	0.7	0.2	0.3
trans-α-bergamotene	0.3	0.7	0.5	0.2	0.3
(E)-β-farnesene	t	0.1	0.1	t	0.1
α-humulene	t	0.1	t	t	t
germacrene D	t	0.1	0.1	t	t
(Z)-α-bisabolene	t	0.1	0.1	t	t
β-bisabolene	0.4	1.0	0.8	0.3	0.4
(E)-nerolidol	t	0.1	t	t	t
nootkatone	0.1	0.1	t	-	t

t=trace (<0.05%)
1=cold-pressed by Pelatice process
2=terpeneless, colored oil with waxes, obtained through distillation and fractionation
3=terpeneless, colorless oil, obtained through distillation and fractionation
4=furano-coumarin-free, colorless oil obtained through distillation and fractionation
5=cold-pressed oil treated with alkali to remove bergapten

linalool (13.89–27.52%)
nonanal (0.02–0.06%)
cis-limonene oxide (0–0.06%)
terpinen-4-ol (0.12–0.17%)
α-terpineol (0.72–1.67%)
octyl acetate (0.08–0.11%)
citronellol (t–0.05%)
neral (0.04–0.05%)
linalyl acetate (8.24–11.66%)
geranial (0.04–0.05%)
nonyl acetate (0.01–0.09%)
methyl geranate (t–0.06%)
α-terpinyl acetate (0.05–0.11%)
neryl acetate (0.12–0.29%)
geranyl acetate (0.10–0.33%)
β-caryophyllene (0.13–0.24%)
trans-α-bergamotene (0.14–0.17%)
β-bisabolene (0.15–0.19%)

t=trace (<0.05%)

Enantiomeric distribution of some selected monoterpenoid constituents in the Fecce oils revealed that their distribution was similar to those noted in the study by Costa et al. (2010).

Russo et al. (2012) showed that the electronic nose could be used to differentiate between bergamot oils of different cultivars such as 'Femminello,' 'Castagnaro' and 'Fantastico,' as well as process difference origins.

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T-7. Comparative percentage composition of Italian and Ivory Coast bergamot oil and Peratoner bergamot oils

Compound	Ivory Coast oil	Italian oils	Peratoner oils
α-thujene	0.12–0.25	0.12–0.27	0.11–0.18
α-pinene	0.57–1.09	0.51–1.15	0.46–0.72
sabinene	0.55–0.93	0.48–0.59	0.43–0.59
β-pinene	3.59–6.72	3.50–6.88	3.39–4.82
myrcene	0.71–0.84	0.68–0.99	0.62–0.82
α-terpinene	0.03–0.12	0.04–0.14	0.10–0.13
p-cymene	0.14–0.15	0.07–0.49	0.13–0.16
limonene	40.90–45.89	37.40–49.10	36.21–45.14
(E)-β-ocimene	0.08–0.15	0.16–0.24	0.19–0.21
γ-terpinene	3.78–8.03	6.36–8.10	6.20–7.65
cis-sabinene hydrate + octanol	0.02–0.03	0.02–0.05	0.01–0.03
terpinolene	0.12–0.25	0.18–0.28	0.23–0.27
linalool	6.81–19.39	18.38–33.11	18.38–33.11
terpinen-4-ol	0.01–0.03	0.02–0.16	0.13–0.15
α-terpineol	0.10–0.16	0.05–0.43	0.30–0.42
octyl acetate	0.04–0.08	0.05–0.09	0.06–0.09
nerol	0–0.10	0–0.08	0–0.07
neral	0.11–0.18	0.12–0.21	0.12–0.15
linalyl acetate	26.18–27.53	11.80–18.71	16.81–18.71
geranial	0.17–0.24	0.14–0.30	0.13–0.19
α-terpinyl acetate	0.05–0.12	0.05–0.16	0.05–0.09
neryl acetate	0.27–0.38	0.24–0.36	0.28
geranyl acetate	0.18–0.26	0.18–0.40	0.19–0.31
cis-α-bergamotene	0.01	0.01–0.27	0–0.01
β-caryophyllene	0.22–0.24	0.11–0.27	0.10–0.11
trans-α-bergamotene	0.18–0.22	0.01–0.25	0.07
β-bisabolene	0.26–0.34	0.10–0.39	0.09–0.10
nootkatone	0–0.05	0–0.07	0

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Non-volatiles in Bergamot Oil

Dugo et al. (1999) used HPLC to determine the coumarin and psoralen content of 451 genuine Italian bergamot oils of Calabrian origin. They determined that the four major non-volatile compounds were found to range slightly across the oils produced by the 'Pelatrice' process (1996–1997) in different areas in Calabria, as can be seen as follows:

bergamottin (1.37–1.60%)
5-geranyloxy-7-methoxycoumarin (0.14–0.18%)
citropten (0.18–0.26%)
bergapten (0.18–0.21%)

In addition, the authors also compared the percentage composition of these same four non-volatiles found in oils recovered from the solid-liquid residues of the cold extraction processes known as 'Torchiati,' 'Ricicli' or 'Pulixia dischi.' The results of this study are shown in **T-8**. Finally, the authors unequivocally characterized sinensetin and tetra-O-methyl-scutellarein as additional non-volatile components in bergamot oil.

A distilled bergamot oil was analyzed by Buiarelli et al. (2002) using HPLC to determine the level of non-volatiles. The results of this study can be seen as follows:

citropten (2.30)[a]
bergapten (0.16)
bergamottin (13.0)
5-methoxy-7-hydroxycoumarin (70.0)
[a]mg/L (ppm)

Gionfriddo et al. (2004) discussed the methods used to remove furocoumarins (psoralens) from bergamot oil. They reported that there were three chemical or physical methods: (a) a cold process that involves the aqueous alkaline bergapten (lactonic ring) degradation while leaving the lactonic ring of bergamottin and 5-geranyloxy-7-methoxycoumarin unchanged, (b) a hot process that uses vacuum fractional distillation and (c) a physical process that uses supercritical fluid extraction. Bergapten-free bergamot oil can also be obtained from fruit that have been genetically modified to contain no bergapten. This means that users of bergapten-free bergamot oil need to determine whether their oil is obtained from a GMO (genetically modified organism), because numerous consumer companies do not permit the use of GMO products.

Finally, Gionfreddo et al. reported that treatment of bergamot oil with a 6% aqueous solution of KOH over a 12 hour period resulted in a reduction of the bergapten content from 2310 to <1 mg/kg of oil. The furanocoumarin content of an oil of bergamot that had been treated with alkali to remove bergapten was determined by Frerot and Decorzant (2004). The authors used HPLC and a UV diode array detector to characterize the following furocoumarins in the oil:

bergapten (8.0 ppm)
oxypeucedanin (53.5 ppm)
epoxybergamottin (70.3 ppm)
bergamottin (16,312.0 ppm)

Using HPLC as their method of analysis and standards of the furanocoumarins, Mangioli et al. (2009) examined the oxygen heterocyclic components of a Reggio Calabrian bergamot oil. The oxygen heterocyclic components that were characterized in this oil were as follows:

bergamottin (1.097–1.409%)
5-geranyloxy-7-methoxycoumarin (0.080–0.104%)
citropten (0.134–0.212%)
bergapten (0.138–0.209%)

Costa et al. (2010) determined the non-volatile constituents in five different bergamot oils using reversed phase HPLC. The results of this study are shown in **T-9**.

Menichini et al. (2010) examined the photo-induced cytotoxic activity of Calabrian bergamot oil. They found that the oil contained citropten (0.4%) and bergapten (1.7%). Furthermore, the authors reported that a dose-response photo-induced cytotoxic activity of bergapten was found after 20 min exposure to UV irradiation, but it could be found that could not be found with citropten. They noted from a structure-activity relationship that the antiproliferative activity of bergapten depended on the presence of the furano-ring. Skin treated with bergapten and exposure to UVA light (known as PUVA) is used to treat psoriasis. The treatment causes skin redness and inflammation, which in turn inhibits skin cell proliferation, which shows a potential positive for the maligned bergapten. Examination of the oxygen heterocyclic compounds in the Fecce oils by Dugo et al. (2012) using HPLC revealed the following data

citropten (0–14)[a]
bergapten (0–268)
bergamottin (0–37)
5-geranyloxy-7-methoxycoumarin (0–3)

[a]mg/L (ppm)

Dugo et al. (2012) also noted that although herniarin, which was detected in the oils obtained from the 'Femminello' and 'Castagnaro' cultivars, was not found in oils of the 'Fantastico' cultivar; however, the fact that it was not detected could be a seasonal effect.

The non-volatile oxygen heterocyclic compounds found in a genuine cold-pressed oil of bergamot by Russo et al. (2012) by HPLC were as follows:

herniarin (67±3.2)[a]
citropten (2,232±26.3)
bergapten (2,474±28.4)
sinesetin (103±2.8)
tetra-O-methyl-scutellarein (195±3.6)
bergamottin (19,605±73.2)
5-geranyloxy-7-methoxycoumarin (1,065±7.5)

[a]mg/L of oil (ppm)

T-8. Percentage composition of the major four non-volatiles found in bergamot oils isolated by the 'Torchiati,' 'Ricicli' and 'Pulzia dischi' processes

Non-volatile compound	'Torchiati' oil	'Ricicli' oil	'Pulizia dischi' oil
bergamottin	1.17–1.66	1.18–1.57	0.93–1.35
5-geranyloxy-7-methoxycoumarin	0.12–0.17	0.16–0.18	0.11–0.14
citropten	0.14–0.19	0.09–0.22	0.13–0.17
bergapten	0.14–0.20	0.13–0.29	0.15–0.24

T-9. Comparative analysis by HPLC of the coumarins and psoralens in five different bergamot oils

Compound	1	2	3	4	5
herniarin	-	0.251	-	-	-
sinensetin	-	0.372	-	-	0.183
citropten	1.927[a]	6.134	-	t	t
bergapten	2.070	4.215	-	t	t
bergamottin	21.685	39.203	-	0.020	18.194
5-geranyloxy-7-methoxycoumarin	1.423	2.827	-	t	1.299

[a]mg/L (ppm); t=trace (<0.001 ppm); see **T-6** footnote for origin of 1–5 oils

T-10. Comparative enantiomeric ratios of selected monoterpenoid compounds in five bergamot oils produced using different treatments

Compound	1	2	3	4	5
(1R,5R)-(+)-β-pinene	9.5	-	-	9.5	9.6
(1S,5S)-(-)-β-pinene	90.5	-	-	90.5	90.4
(4S)-(+)-sabinene	85.4	-	-	85.4	85.7
(4R)-(-)-sabinene	14.6	-	-	14.6	14.3
(4R)-(+)-limonene	98.2	-	-	98.2	98.2
(4S)-(-)-limonene	1.8	-	-	1.8	1.8
(3S)-(+)-linalool	0.6	0.6	0.5	0.5	0.5
(3R)-(-)-linalool	99.4	99.4	99.5	99.5	99.5
(3S)-(+)-linalyl acetate	0.3	0.3	0.2	0.3	0.2
(3R)-(-)-linalyl acetate	99.7	99.7	99.8	99.7	99.8

See **T-6** footnote for origin of 1–5 oils

Forlot and Pevet (2012) reviewed the biological properties of bergamot oil and in particular bergapten (the major phototoxic component). They reported that although bergapten has been used in the photo-therapeutic treatment of dermatoses such as psoriasis and vitiligo, the melatonergic potential has become a more recent area of study.

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Chiral Analysis of Bergamot Oil

An oil of bergamot was subjected to chiral GC analysis by Hara et al. (1999). The authors found that, for selected constituents, the enantiomeric ratios were as follows:

(1R,5R)-(+)-α-pinene (32.8%):(1S,5S)-(-)-α-pinene (67.2%)
(1R,5R)-(+)-β-pinene (8.5%):(1S,5S)-(-)-β-pinene (91.5%)
(4R)-(+)-limonene (98.3%):(4S)-(-)-limonene (1.7%)
(3S)-(+)-linalool (0.2%):(3R)-(-)-linalool (99.8%)
(4R)-(+)-α-terpineol (70.5%):(4S)-(-)-α-terpineol (29.5%)
(3R)-(+)-citronellal (1.0%):(4S)-(-)-citronellal (99.0%)

The enantiomeric distribution of four monoterpene hydrocarbons in a Japanese-produced cold-pressed bergamot oil was determined by Mitiku et al. (2001) to be as follows:

(4R)-(+)-limonene (98.30%):(4S)-(-)-limonene (1.70%)
(1R,5R)-(+)-α-pinene (30.52%):(1S,5S)-(-)-α-pinene (69.48%)
(1R,5R)-(+)-sabinene (17.85%):(1S,5S)-(-)-sabinene (82.15%)
(1R,5R)-(+)-β-pinene (7.40%):(1S,5S)-(-)-β-pinene (92.60%)

Mangiola et al. (2009) used chiral GC to determine the enantiomeric ratios of a number of constituents of Reggio Calabrian bergamot oil. Their results are summarized as follows:

(1R,5R)-(+)-β-pinene (8.7–9.5%):(1S,5S)-(-)-β-pinene (90.5–91.3%)
(1R,5R)-(+)-sabinene (13.7–16.3%):(1S,5S)-(-)-sabinene (83.7–86.3%)
(4R)-(+)-limonene (97.9–98.1%):(4S)-(-)-limonene (1.9–2.1%)
(3S)-(+)-linalool (0.5–0.7%):(3R)-(-)-linalool (99.3–99.5%)
(3S-(+)-linalyl acetate (0.3–0.4%):(3R)-(-)-linalyl acetate (99.6–99.7%)
(4R)-(+)-α-terpineol (31.5–43.7%):(4S)-(-)-α-terpineol (56.3–68.5%)
(4S)-(+)-terpinen-4-ol (44.7–67.7%):(4R)-(-)-terpinen-4-ol (32.3–55.3%)

Using chiral GC, Costa et al. (2010) also measured the enantiomeric ratio of the selected monterpenoid compounds in five different bergamot oils. The results of the study can be seen in **T-10**.

Using either direct enantiomeric selective (chiral) GC or multidimensional chiral GC, Bonaccorsi et al. (2011) determined the enantiomeric ratios of 13 constituents found in eight genuine samples of Italian bergamot oil. Their results can be seen as follows:

(1R)-(+)-α-thujene (0.9–1.4%):(1S)-(-)-α-thujene (98.6–99.1%)
(1R,5R)-(+)-α-pinene (30.2–32.6%):(1S,5S)-(-)-α-pinene (67.4–69.8%)
(3R)-(+)-camphene (10.7–12.1%):(3S)-(-)-camphene (87.9–89.3%)
(1R,5R)-(+)-β-pinene (8.5–9.6%):(1S,5S)-(-)-β-pinene (90.4–91.5%)
(1R,5R)-(+)-sabinene (16.1–17.2%):(1S,5S)-(-)-sabinene (82.8–83.9%)
(4R)-(+)-α-phellandrene (46.0–50.5%):(4S)-(-)-α-phellandrene (49.5–54.0%)
(4R)-(+)-β-phellandrene (66.3–72.4%):(4S)-(-)-β-phellandrene (27.6–33.7%)
(4R)-(+)-limonene (98.1–98.3%):(4S)-(-)-limonene (1.7–1.9%)
(1R)-(+)-camphor (25.3–42.1%):(1S)-(-)-camphor (57.9–74.7%)
(3S)-(+)-linalool (0.4–0.5%):(3R)-(-)-linalool (99.5–99.6%)
(3S)-(+)-linalyl acetate (0.2–0.3%):(3R)-(-)-linalyl acetate (99.7–99.8%)
(4S)-(+)-terpinen-4-ol (15.0–21.7%):(4R)-(-)-terpinen-4-ol (78.3–85.0%)
(4R)-(+)-α-terpineol (45.8–78.8%):(4S)-(-)-α-terpineol (21.2–54.2%)

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