

Evaluation of the Genuineness of Cold-pressed Bergamot Oil

Traditional analyses, high resolution gas chromatographic and chiral analyses

Carlo Mangiola, Enrico Postorino, Francesco Gionfriddo, Maurizio Catalfamo and Renato Manganaro, Stazione Sperimentale Essenze e Derivati dagli Agrumi, Reggio Calabria; and Giuseppe Calabrò, University of Messina

The evaluation of purity and quality of bergamot oil has always been one of the objectives of the Experimental Station for the Citrus Essences and Derivatives Industry in Reggio Calabria, Italy.¹ The results of studies carried out over the years have allowed determination of certain parameters of the variability intervals within which bergamot oils must fall, in order to be considered genuine. The parameters laid down by international regulations, such as the Association Française de Normalisation (AFNOR) and the International Organization for Standardization (ISO), have the same objective.^{2,3} Moreover, in regulating “Denominazione di Origine Controllata, Disciplinare per la Denominazione di Origine Protetta (DOP; Protected Designations of Origin)—Bergamot of Reggio Calabria—Essential Oil,” specific variability intervals are laid down within which the essential oil must fall in order to be sold with this name.⁴

The traditional physicochemical parameters of cold-pressed bergamot oil, set out by the above-mentioned authorities in order to establish the genuineness of the essential oil, are influenced by various factors, including the following:¹⁻⁴

- Relative density, optical activity and refractive index are influenced by cultivation techniques.
- Optical rotation is influenced by weather and/or seasonal conditions.
- Solubility in ethanol is influenced by the quantity of oxygenated compounds.
- Evaporation residue is influenced by the isolation method: indeed, if the fruits are processed drastically, the essential oil will contain higher levels of waxes, fatty acids, coumarins, etc.
- Acidity and peroxide index are indicators of the age and conservation of the essential oil.
- Ester and alcohol content, the main determinants of the peculiar olfactory characteristics of the oil, are influenced by the degree of ripeness of the fruits.
- CD, which numerically expresses the process of absorption of the essential oil in the UV wavelength zone between 370 nm and 280 nm, is proportional to the quantity of non-volatile residue and is negatively influenced by any dilutions carried out by the fraudulent addition of distilled oils. (According to ISO 4375, the letters “CD” are the alphabet letters in the graphic evaluation of the maximum UV absorption, where five letters of alphabet are used [A, B, C, D, E].)

At a Glance

In order to formulate an objective evaluation on the genuineness of bergamot oil, this article reports the analytical results obtained from six samples, taken from the same number of 1-metric-tonne lots of essential oil, isolated by peeling fruits with the so-called “special” machine, collected during the 2007–2008 season. In addition to the traditional analyses, high resolution gas chromatographic and chiral analyses were also carried out by the authors.

The traditional physicochemical parameters are no longer adequate to discover common adulterations, such as the addition of synthetic compounds. For this reason, techniques such as high resolution gas chromatography, two-dimensional gas chromatography and high pressure liquid chromatography (HPLC) are used to evaluate the genuineness and quality of bergamot oil.⁵⁻⁷

This article aims to provide elements for evaluation of the genuineness of bergamot oil. It presents recent data regarding the traditional physicochemical parameters, percentage composition of the volatile fraction and the distribution of the main chiral compounds determined in six samples collected from six genuine lots of cold-pressed oil each weighing 1 metric tonne.

Experimental

The six samples examined were collected from the same number of lots of bergamot oil, weighing one metric tonne each, and isolated by peeling with the so-called “special” machine, from bergamot fruits collected from various areas along the Ionian seacoast of the province of Reggio Calabria (Italy) during the 2007–2008 citrus fruit season. **T-1** and **T-2** show the results of the physicochemical analyses carried out on the six samples of bergamot oil and the variability intervals.

Gas Chromatographic Analyses

The instrumental gas chromatographic conditions used were: gas chromatograph ThermoQuest Trace GC 2000 series; acquisition software Chromquest 2.52; injec-

Sample No.	Date of production	Relative density at 20°C	Refractive index at 20°C	Optical rotation at 20°C	Solubility in ethanol at 90°	Residue on evaporation (%)	Esters % (expressed as linalyl acetate)	CD	UV Max absorption
1	Nov 2007	0.8740	1.4685	+30.0	1:0.7	4.9	33.1	0.82	1.17
2	Dec 2007	0.8750	1.4676	+28.6	1:0.5	4.7	36.0	0.72	1.06
3	Jan 2008	0.8740	1.4686	+32.4	insoluble	4.9	33.2	0.78	1.12
4	Jan 2008	0.8750	1.4687	+32.4	insoluble	5.0	34.5	0.71	1.04
5	Feb 2008	0.8750	1.4684	+32.2	insoluble	5.0	35.6	0.77	1.15
6	Mar 2008	0.8750	1.4690	+32.2	insoluble	5.6	34.4	0.84	1.20

tion in split mode 1:100 of 1 μ L of bergamot oil sample diluted 1:10 with acetone; gas carrier 1.5 mL/min of constant flow helium; column Restek RTX-5: length 30 m, film thickness 0.25 μ m, internal diameter 0.25 mm, detector FID at 280°C; oven at programmed temperature: 70°C for 10 min; from 70–120°C with step of 3°C/min; from 120–170°C with step of 4°C/min; from 170–295°C with step of 10°C/min; isotherm at 295°C for 3 minutes. Total analysis time 54.5 minutes. The percentage composition of the volatile fraction of the samples examined is shown in **T-3**; gas chromatogram of one of the bergamot oils examined is shown in **F-1**.

Chiral Analysis

The gas chromatographic conditions used for this analysis were as follows:

Gas chromatograph 1: Gas chromatograph ThermoQuest Trace GC 2000 series; acquisition software Chromquest 2.52; injection 1 μ L in split mode 1:100 of sample diluted 1:10 with acetone; gas carrier 1.5 mL/min of constant flow helium; column Restek RTX-5: length 30 m, film thickness 0.25 μ m, internal diameter 0.25 mm, detector FID at 280°C; transfer line thermo-regulated by zone AUX of the gas chromatograph at 150°C; 6 port rotating VALCO valve, n° 6 cut-offs: min (7.93–8.55) β -pinene and sabinene, min (11.11–11.50) limonene, min (15.45–15.87) linalool, min (19.23–19.59) terpinen-4-ol, min (19.80–20.15) α -terpineol, min (22.24–22.62) linalyl acetate; Oven at programmed temperature: 70°C for 10 min; from 70–85°C with step of 3°C/min; from 85–175°C with step of 5°C/min; from 175–295°C with step of 7°C/min; isotherm at 295°C for 3 minutes. Total analysis time, 53.1 minutes.

Gas chromatograph 2: Gas chromatograph ThermoQuest Trace GC 2000 series; acquisition software Chromquest 2.52; carrier gas 1.5 mL/min of constant flow helium; column Mega DETBSBETA-086, length 25 m, film thickness 0.18 μ m, internal diameter 0.20 mm, detector FID at 220°C; oven at programmed temperature: 35°C for 22.75 min; from 35–160°C with step of 4°C/min; isotherm at 160°C for 1 minute. Total analysis time, 54.7 minutes.

T-4 shows the enantiomeric distribution of the main optically active compounds: sabinene, β -pinene,

Variability interval of the physicochemical parameters of the bergamot oils examined

T-2

	Minimum value	Maximum value
Relative density at 20°C	0.8740	0.8750
Refractive index at 20°C	1.4676	1.4690
Optical rotation at 20°C	+ 28.6	+ 32.4
Esters % (expressed as linalyl acetate)	33.10	35.96
Solubility in ethanol at 90°	insoluble	1:0.5
Residue on evaporation (%)	4.7	5.6
CD	0.71	0.84
UV Max absorption	1.04	1.20

limonene, linalool, linalyl acetate, α -terpineol, terpinen-4-ol. This analysis was carried out with a two-dimensional FID-FID gas chromatograph (instrument built at the Experimental Station), using the heart-cutting method (rotating 6 port Valco valve) already illustrated in a previous paper.⁶ Chiral gas chromatogram of one of the samples of bergamot oil examined in **F-2**.

T-5 shows the enantiomeric excess (*e/e*) of each of the optically active compounds calculated according to ISO regulations (22972, 2004).

Residue Components

The concentration of the main components of the residue has already been studied by various authors as summarized in **T-6**.⁸⁻¹¹

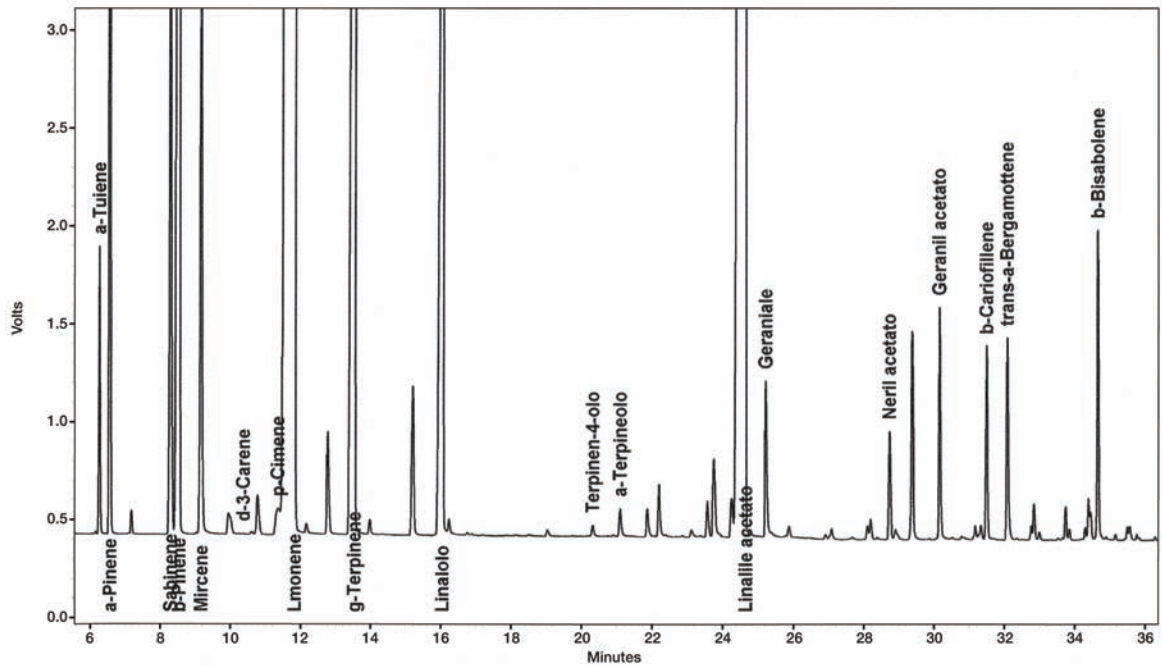
The content (mg/kg) of the residue main components in the samples examined was determined by HPLC using the following experimental conditions:

Chromatograph Thermo Finnigan surveyor with autosampler; detector PDA λ = 315 μ m; column Phenomenex Luna 5 μ (250 x 4.6) mm; eluent, hexane + ethyl acetate 90:10 in isocratic composition for 25 minutes; sample volume injected: 50 μ L of 1% bergamot oil in eluent mixture; data acquired by Ethernet Switch and Chromquest 4.1 software.

The identity of the eluted peaks was checked by scanning their ultraviolet spectra, by showing coincident

Gas chromatogram of one of the bergamot oils examined

F-1



Percentage composition of the volatile fraction of the bergamot oils examined

T-3

Period of production	Limonene	Linalyl acetate	γ-Terpinene	Linalool	β-Pinene	α-Pinene	Sabinene	Myrcene	β-Bisabolene	α-Thujene	Neryl acetate	Geraniol	Geranyl acetate	trans-α-Bergamotene	β-Caryophyllene	p-Cymene	α-Terpineol	Terpinen-4-ol	δ-3-Carene	Other compounds
Nov 2007	41.78	25.98	7.38	9.94	6.52	1.28	1.15	1.05	0.43	0.33	0.32	0.31	0.28	0.29	0.28	0.12	0.08	0.02	traces	2.46
Dec 2007	39.57	28.04	7.37	9.73	7.01	1.32	1.23	0.99	0.39	0.40	0.32	0.32	0.22	0.27	0.25	0.12	0.08	0.02	0.003	2.35
Jan 2008	43.48	25.07	7.96	7.25	6.97	1.37	1.22	1.08	0.42	0.36	0.35	0.31	0.28	0.29	0.28	0.10	0.07	0.03	0.006	3.10
Jan 2008	42.71	27.04	8.71	5.57	6.93	1.36	1.20	1.06	0.45	0.36	0.39	0.31	0.31	0.31	0.30	0.10	0.06	0.03	0.004	2.80
Feb 2008	42.46	27.03	7.98	5.92	7.01	1.38	1.22	1.06	0.44	0.36	0.36	0.29	0.30	0.31	0.30	0.09	0.06	0.03	0.007	3.39
Mar 2008	43.74	27.99	8.36	3.92	7.18	1.37	1.20	1.07	0.49	0.36	0.34	0.29	0.39	0.34	0.31	0.08	0.06	0.02	0.007	2.48
Min. value %	39.57	25.07	7.37	3.92	6.52	1.28	1.15	0.99	0.39	0.33	0.32	0.29	0.22	0.27	0.25	0.08	0.06	0.02	traces	2.35
Max. value %	43.74	28.04	8.71	9.94	7.18	1.38	1.23	1.08	0.49	0.40	0.39	0.32	0.39	0.31	0.31	0.12	0.08	0.03	0.007	3.39

Enantiomeric distribution (%) of the main chiral compounds

T-4

Period of production	(+)- β -Pinene	(-)- β -Pinene	(+)-Sabinene	(-)-Sabinene	(+)-Limonene	(-)-Limonene	(+)-Linalool	(-)-Linalool	(+)-Linalyl acetate	(-)-Linalyl acetate	(+)- α -Terpineol	(-)- α -Terpineol	(+)-Terpinen-4-ol	(-)-Terpinen-4-ol
Nov 2007	9.4	90.6	16.2	83.8	98.0	2.0	0.6	99.4	0.4	99.6	32.0	68.0	48.7	51.3
Dec 2007	9.3	90.7	13.7	86.3	98.1	1.9	0.5	99.5	0.3	99.7	43.7	56.3	44.7	55.3
Jan 2008	8.7	91.3	15.6	84.4	98.0	2.0	0.5	99.5	0.3	99.7	34.1	65.9	45.7	54.3
Jan 2008	9.5	90.5	16.3	83.7	97.9	2.1	0.7	99.3	0.4	99.6	31.5	68.5	56.6	43.4
Feb 2008	9.3	90.7	16.2	83.8	97.9	2.1	0.6	99.4	0.4	99.6	33.5	66.5	59.2	40.8
Mar 2008	9.3	90.7	15.8	84.2	98.0	2.0	0.7	99.3	0.4	99.6	32.9	67.1	67.7	32.3
Min value %	8.7	90.5	13.7	83.7	97.9	1.9	0.5	99.3	0.3	99.6	31.5	56.3	44.7	32.3
Max value %	9.5	91.3	16.3	86.3	98.1	2.1	0.7	99.5	0.4	99.7	43.7	68.5	67.7	55.3

29

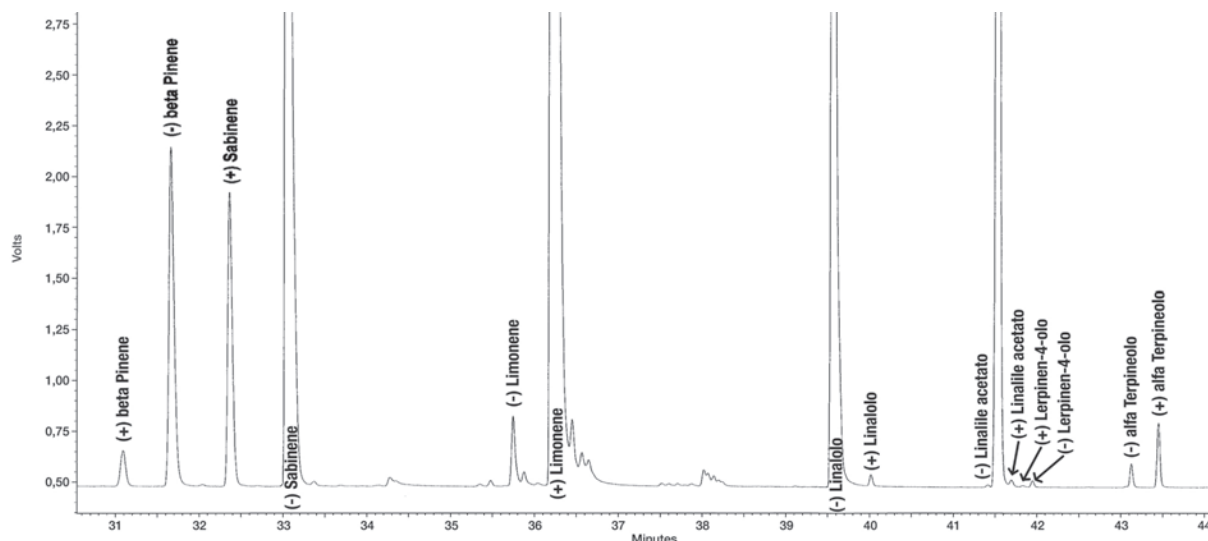
Enantiomeric excess (e/e %) of certain optically active isomers present in bergamot oil

T-5

Prevalent isomer	Minimum value (e/e %)	Maximum value (e/e %)
(+)-Limonene	95.8	96.2
(-)-Linalyl acetate	99.2	99.4
(-)-Linalool	98.6	99.0
(-)- β -Pinene	81.0	82.6
(-)-Sabinene	67.4	72.6
(-)- α -Terpineol	12.6	37.0

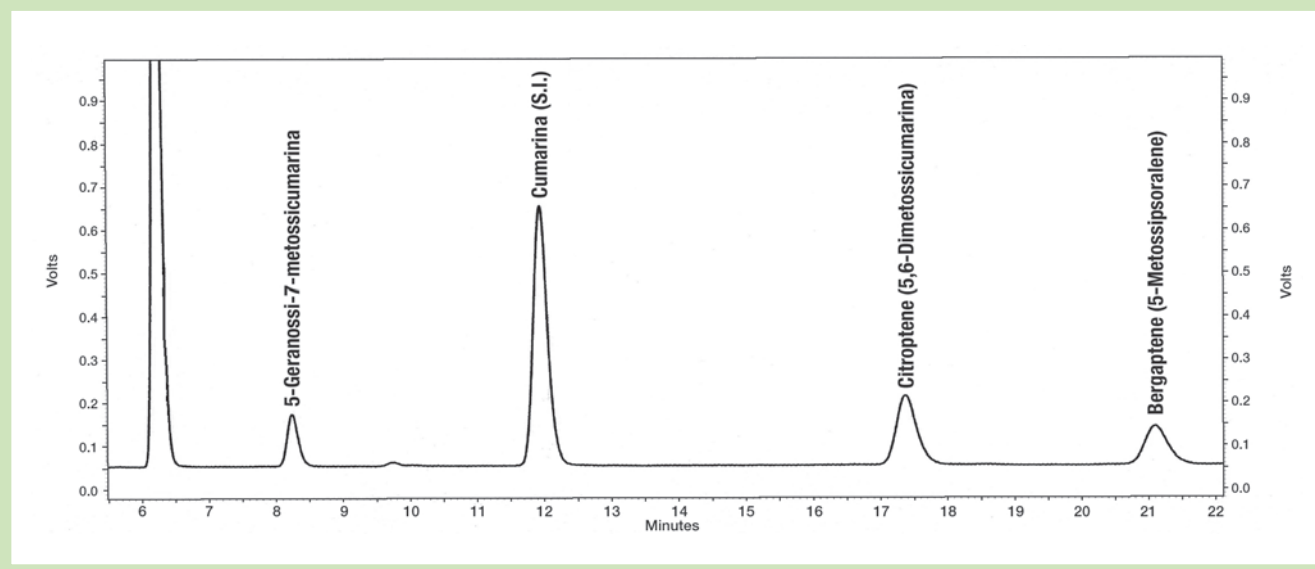
Chiral gas chromatogram of one of the samples of bergamot oil examined

F-2



Chromatogram of the main heterocyclic compounds in one of the samples of bergamot oil

F-3



Main heterocyclic compounds in bergamot oil (mg/kg)

T-6

	Calabrò et al. (1975)		Calvarano et al. (1979)		Mondello et al. (1993)		Gionfriddo et al. (1997)	
	Min/Max values	Average value	Min/Max values	Average value	Min/Max values	Average value	Min/Max values	Average value
Bergamottin	14.000–22.000	18.200	11.400–27.300	20.300	10.200–27.500	18.700	6.300–8.300	7.400
Bergapten	1.500–3.300	2.200	1.600–4.000	2.500	1.100–3.200	2.100	1.900–3.300	2.500
Citropten	1.400–2.400	1.800	1.600–3.000	2.300	1.400–3.500	2.200	1.200–3.200	2.000
5-Geranyloxy-7-methoxy coumarin	1.200–1.500	1.200	-	-	800–2.200	1.300	500–1.100	720

Concentration of certain heterocyclic compounds in bergamot oil residue (mg/kg)

T-7

Period of production	Bergamottin	5 Geranoxy-7-methoxycoumarin	Citropten	Bergapten
Nov 2007	10.969	878	1.639	2.089
Dec 2007	12.173	795	2.124	2.005
Jan 2008	12.030	920	1.705	1.798
Jan 2008	13.848	1.007	1.718	1.718
Feb 2008	12.690	907	1.549	1.708
Mar 2008	14.089	1.035	1.340	1.384
Min. value	10.969	878	1.340	1.384
Max. value	14.089	1.035	2.124	2.089

retention time with the authentic compounds and concentration were estimated by comparison with a standard solution, taking into account response factors compared to an internal standard (coumarin, 40 mg/L) (see **T-7** and **F-3**).

Conclusion

The average percentage values in **T-4** show that the main components of the volatile fraction are: limonene, linalyl acetate, γ -terpinene, linalool and β -pinene. According to the observations made by Calvarano, the total content of the main monoterpene compounds (limonene, γ -terpinene, β -pinene) increased from 55.68% (November) to 59.28% (March)—in line with the ripening of the fruit.¹²

With reference to the oxygenated compounds, linalool decreased progressively (from 9.94% to 3.92%), while linalyl acetate increased slightly (from 25.98% to 28.00%).

As for the optically active components (see **T-5**), limonene predominated among the (+) isomers, while linalyl acetate, β -pinene, sabinene and α -terpineol predominated among the (-) isomers. The values illustrated in **T-5** mainly coincide with those reported by Gionfriddo et al.⁵

Finally, the authors observed that the Bergapten content decreased with ripening from 2.089 mg/kg to 1.384 mg/kg, the latter value being lower than the minimum value of 1.900 mg/kg obtained by Gionfriddo et al.⁶

Address correspondence to Giuseppe Calabrò, Dipartimento RIAM Università degli Studi di Messina, Italy; calabro@unime.it

References

1. A Di Giacomo, *Essenze Derivati Agrumari* 3, 232–242, (1972)
2. Association Francaise de Normalisation, NFT75-215, 170, Settembre (1989)
3. International Standard Organization, Edizioni, (1978, 1981, 1983, 1998, 2001)
4. Disciplinare per la Denominazione di Origine Protetta “Bergamot di Reggio Calabria—Olio Essenziale” G.U.R.I. 25/05/2001, n. 120 (2001)
5. F Gionfriddo et al., *Essenze Derivati Agrumari*, 73, 29–39, (2003)
6. F Gionfriddo et al., *Essenze Derivati Agrumari*, 74, 151–152, (2004)
7. F Gionfriddo et al., *Essenze Derivati Agrumari*, 70, 133–145, (2000)
8. G Calabrò and P Currò, *Essenze Derivati Agrumari*, 45, 246 (1975)
9. I Calvarano, A Ferlazzo and A Di Giacomo, *Essenze Derivati Agrumari*, 49, 12 (1979)
10. L Mondello, I Stagno D’Alcontres, R Del Duce and F Crispo, *Flav Frag*, 8, 17 (1993)
11. F Gionfriddo, E Postorino and F Bovalo, *Essenze Derivati Agrumari*, 67, 342 (1997)
12. M Calvarano, *Essenze Derivati Agrumari*, 38, 3–20 (1968)

To purchase a copy of this article or others, visit www.PerfumerFlavorist.com/ magazine. 