

ESSENTIAL OIL COMPOSITION OF DIFFERENT CULTIVARS OF BERGAMOT GROWN IN SICILY

COMPOSIZIONE DELL'OLIO ESSENZIALE DI DIVERSE CULTIVAR
DI BERGAMOTTO COLTIVATE IN SICILIA

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ABSTRACT

Cold-pressed peel essential oils of different cultivars of bergamot (*Citrus bergamia*, Risso et Poiteau), "Castagnaro", "Feminello", "Fantastico" and a new clone of the Feminello cultivar, named "PCF", were analysed. The oils were obtained from bergamot plants of Calabrian origin cultivated in an experimental field in Sicily. The oils were analysed by HRGC and HRGC/MS. Seventy-five compounds, representing an average of 99.9% of the whole volatile fraction, were identified in each oil on polar and apolar capillary columns. The enantiomeric distribution of linalol and linalyl

RIASSUNTO

È stata studiata la composizione di oli essenziali di bergamotto di diverse cultivar coltivate in Sicilia, presso un campo sperimentale dell'Università di Catania. Gli oli appartenevano alle cultivar tradizionali: "Castagnaro", "Feminello" e "Fantastico" e ad un nuovo clone della cultivar "Feminello" denominato "PCF". Gli oli sono stati estratti in laboratorio ed analizzati tramite HRGC e HRGC/MS. In tutti gli oli analizzati sono stati identificati 75 componenti che, complessivamente, rappresentano oltre il 99% della frazione volatile. Il rapporto enantiomerico del

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acetate was studied by enantioselective capillary gas chromatography with modified cyclodextrins as chiral stationary phases. The oils were compared with each other and with previously analysed genuine Calabrian bergamot oils. The compounds identified were in accordance with the Calabrian bergamot oils and only some quantitative differences appeared. "Feminello" gave the best quality oil, as already assessed for the same cultivar grown in Calabria. The enantiomeric compositions of the chiral constituents analysed were in agreement with those reported in the literature for genuine cold-pressed bergamot oils.

linalolo e del linalile acetato è stato studiato tramite gascromatografia con fasi stazionarie chirali. La composizione di tali oli è stata confrontata con quella relativa ad oli di bergamotto Calabresi precedentemente analizzati. Gli oli di bergamotto Siciliani risultano abbastanza simili agli oli Calabresi. Gli oli della cultivar "Feminello" per la loro composizione sono gli oli di migliore qualità. I valori relativi ai rapporti enantiomerici del linalolo e linalile acetato sono in accordo con quelli riportati in letteratura per gli oli di bergamotto genuini.

INTRODUCTION

Citrus bergamia, Risso et Poiteau is mainly cultivated in Italy in a narrow strip of land on the Ionian and Thyrrean coasts, in the province of Reggio Calabria. Calabrian bergamot oil, considered a very high quality product, is widely used in the perfumery industry for its particular flavour notes, but is also used in flavourings, in Earl Grey Tea and tobacco. Because of its extensive use, the cultivation of bergamot has expanded to the Ivory Coast, Brazil, Argentina, Uruguay, Corsica, Guinea, Turkey and Cameroon. Numerous studies appear in the literature regarding the composition of the volatile fraction of Calabrian bergamot oil (CALVARANO, 1962; 1965; LA FACE, 1972; CALABRÒ and CURRÒ, 1975; SHAW, 1979; MAZZA, 1986; 1987; DUGO *et al.*, 1987; 1991; COTRONEO *et al.*, 1992; MONDELLO *et al.*, 1994; VERZERA *et al.*, 1996; 1998) and, some papers deal with bergamot oils from countries other than Italy (SCHWOB, 1953; 1955; HUET and DUPIUS, 1968; HUET, 1981; KOKETSU *et al.*, 1983; DEL-LACASSA *et al.*, 1997; HUANG *et al.*, 1987; BASER *et al.*, 1995; SAWAMURA *et al.*, 1999).

This paper reports the results on the composition of the volatile fraction of bergamot oils of four cultivars grown in Sicily. In order to evaluate the quality of the oils, their composition was compared with that of previously analysed Calabrian bergamot oil (VERZERA *et al.*, 1996; 1998). The enantiomeric distribution of linalol and linalyl acetate was studied to confirm the authenticity of the bergamot oils and the results were compared with those reported in the literature (KONIG *et al.*, 1997; JUCKELKA and MOSANDL 1996; MOSANDL and JUCKELKA 1997; MONDELLO *et al.*, 1998). Moreover, the oil composition is reported and compared to Calabrian bergamot oil.

MATERIALS AND METHODS

In Sicily, in an experimental field of the "Istituto di Coltivazione Arborea" of the University of Catania, bergamot plants of different cultivars were planted in 1987 using planting stock of Calabrian origin. The cultivars were "Fantastico", "Castagnaro", "Feminello" (the typical Calabrian cultivars) and a new

clone of the Feminello cultivar, named "PCF".

Fruit was picked in February 1999 and two samples of each cultivar were analysed. Extraction of the essential oil was carried out in a laboratory by applying manual pressure to the rind so as to cause breaking of the utricles and release of the oil which was collected on a watch glass. The oil was transferred to a test tube, centrifuged and analysed. The qualitative and quantitative composition of the volatile fraction was studied by HRGC and HRGC/MS.

HRGC analysis

Each oil was analysed by HRGC on a 5160 Mega series Carlo Erba Gas Chromatograph (Fisons Instrument, Milan, Italy) equipped with a C-R3A Shimadzu data processor using the following experimental conditions: SE-52 fused silica capillary column, 30 m x 0.32 mm i.d., film thickness, 0.40-0.45 μm (Mega, Legnano (MI), Italy); column temperature, 45°C (6 min) to 250°C at 3°C/min; injection mode, split; split ratio, 1:100; detector, FID; injector and detector temperature, 250°C; carrier gas, He 100 kPa; injected volume, 1 μL of net whole oil. The quantitative composition was obtained by peak area normalization and the response factor for each component was considered to equal one.

HRGC-MS analysis

Samples were analyzed by HRGC/MS (EI) on a Fisons MD 800 (Fisons Instrument, Milan, Italy) system coupled with Adams' library (ADAMS, 1995). Two different columns were used: 1) Mega 5 MS fused silica capillary column, 30 m x 0.25 mm i.d., film thickness, 0.25 μm (Mega, Legnano (MI) Italy); carrier gas, He, 90 kPa; linear velocity, 42.7 cm/sec at 40°C; column temperature, 40°C (2 min) to 240°C at 3.0°C/min. 2) Megawax fused silica capillary column, 30 m x 0.32 mm;

film thickness, 0.40-0.45 μm (Mega, Legnano (MI), Italy); carrier gas, He 90 kPa; linear velocity, 34.7 cm/sec at 40°C; column temperature, 40°C (6 min) to 220°C at 2°C/min; for both columns: injector temperature, 250°C; injection mode, split; split ratio, 1:30; volume injected, 1 μL of a solution of oil in pentane in a 1:100 ratio; interface temperature, 250°C; acquisition mass range, 41-300 amu; solvent cut, 2 min.

Linear retention indices of the compounds were determined on the basis of homologue n-alkane hydrocarbons analysed under the same GC conditions. Compound identification was confirmed by comparing the mass spectra of the compounds with published spectra and of retention indices with published index data.

Chiral analysis

Enantiomeric ratios of linalol and linalyl acetate were obtained by gas chromatography with a fused silica capillary column 25 m x 0.25 mm i.d., coated with a diethyl 2,3-di-O-(tert-butyl)dimethylsilyl- β -cyclodextrin) 30% in PS 086, 0.25 μm film thickness (Mega, Legnano, (MI) Italy); column temperature, 45° (6 min), to 180°C, at 2°C/min; injection mode, split; split ratio, 1:30; detector, FID; injector and detector temperature, 250°C; carrier gas, He 100 kPa; injected volume, 1 μL of an oil-in-pentane solution, 1:100.

RESULTS AND DISCUSSION

Seventy-five compounds were identified in each oil. Table 1 reports the compounds identified and their linear retention indices calculated on Mega 5 MS and Megawax capillary columns.

For each sample, the quantitative composition (as a relative percentage of peak area) for each compound, as well as the total amount of hydrocarbons,

monoterpenes, sesquiterpenes, carbonyl compounds, alcohols, esters and the linalol/linalyl acetate ratio was calculated. Table 2 reports the average composition of single compounds and of classes of substances for the oils of the different cultivars together with the mini-

imum and maximum values of the Calabrian bergamot oils previously analysed (VERZERA *et al.*, 1998). The compounds are reported according to their retention times on an SE 52 column. The data obtained for the two samples of each cultivar were in agreement. The values

Table 1 - Linear retention indices calculated on Mega 5MS and Megawax capillary columns for the compounds identified in Sicilian bergamot oils.

Compound	Retention indices		Compound	Retention indices	
	Mega 5MS	Megawax		Mega 5MS	Megawax
tricyclene	913	-	neral	1231	1645
α -thujene	920	1012	<i>trans</i> -sabinene hydrate acetate	1246	1501
α -pinene	925	1008	geraniol	1251	1783
camphene	936	1043	linalyl acetate	1251	1542
sabinene	964	1105	perillaldehyde	1257	1729
β -pinene	964	1085	geranial	1262	1697
6-methyl-5-hepten-2-one	981	-	bornyl acetate	1275	-
myrcene	987	1152	undecanal	1300	1580
α -phellandrene	997	1146	nonyl acetate	1308	-
octanal	997	1270	methylgeranate	1316	-
hexyl acetate	1000	-	δ -elemene	1328	1446
δ -3-carene	1002	1128	linalyl propanoate	1333	-
α -terpinene	1009	1160	α -terpinyl acetate	1340	1663
<i>p</i> -cymene	1016	-	citronellyl acetate	1349	-
limonene	1021	1181	neryl acetate	1360	1705
β -phellandrene	1021	1186	geranyl acetate	1379	1738
1,8-cineole	1021	-	dodecanal	1402	-
(<i>Z</i>)- β -ocimene	1036	-	decyl acetate	1404	1662
(<i>E</i>)- β -ocimene	1045	1239	β -caryophyllene	1404	1555
γ -terpinene	1051	1225	<i>cis</i> - α -bergamotene	1405	-
<i>cis</i> -sabinene hydrate	1058	1447	<i>trans</i> - α -bergamotene	1427	1560
octanol	1071	1546	α -humulene	1437	1624
terpinolene	1079	1260	β -santalene	1449	-
linalol	1094	1537	<i>cis</i> - β -farnesene	1452	1649
nonanal	1098	1372	dodecanol	1460	-
heptyl acetate	1111	-	germacrene D	1466	1664
<i>cis</i> -limonene oxide	1122	1403	bicyclogermacrene	1482	1688
<i>trans</i> -limonene oxide	1127	1423	β -bisabolene	1500	1701
isopulegol	1132	-	(<i>E,E</i>)- α -farnesene	1502	1730
camphor	1127	-	<i>cis</i> - γ -bisabolene	1504	-
citronellal	1146	1457	germacrene B	1539	1778
terpinen-4-ol	1165	1575	(<i>E</i>)-nerolidol	1556	-
α -terpineol	1179	1670	tetradecanal	1605	-
decanal	1199	1476	2,3-dimethyl-3-(4-methyl- 3-pentenyl)-2-norbarnanol	1638	-
octyl acetate	1210	1461	campherenol	1654	-
nerol	1222	1830	β -bisabolol	1657	-
citronellol	1222	-	nootkatone	1780	2434
carvone	1229	1684			

Table 2 - Percentage composition (mean values X of two samples) of single compounds and of classes of substances for Sicilian "Castagnaro", "Fantastico", "Feminello" and "PCF" oils and minimum and maximum values for Calabrian oils (VERZERA *et al.*, 1998).

	Sicilian oils				Calabrian oils	
	"Castagnaro"	"Fantastico"	"Feminello"	"PCF"	min	max
	X	X	X	X		
tricyclene	tr	tr	tr	tr	tr	tr
α -thujene	0.30	0.29	0.19	0.22	0.19	0.46
α -pinene	1.18	1.14	0.75	0.90	0.72	1.76
camphene	0.03	0.03	0.02	0.02	0.02	0.05
sabinene	7.09	6.63	3.70	5.90	4.81	12.69
β -pinene						
6-methyl-5-hepten-2-one	tr	tr	tr	tr	tr	0.01
myrcene	1.20	1.07	0.96	0.96	0.63	1.23
octanal	0.03	0.03	0.02	0.02	0.03	0.08
α -phellandrene	0.02	0.04	0.03	0.02	0.02	0.04
hexyl acetate	tr	tr	tr	tr	tr	tr
δ -3-carene	tr	tr	tr	tr	tr	tr
α -terpinene	0.17	0.16	0.10	0.12	0.10	0.24
<i>p</i> -cymene	0.04	0.04	0.02	0.03	0.01	0.72
limonene + β -phellandrene	51.32	43.80	40.48	40.89	25.38	45.41
1.8-cineole	tr	tr	tr	tr	0.01	0.02
(<i>Z</i>)- β -ocimene	0.01	0.01	0.01	0.01	0.01	0.05
(<i>E</i>)- β -ocimene	0.13	0.15	0.13	0.16	0.17	0.32
γ -terpinene	7.63	7.07	4.68	6.03	5.27	11.15
<i>cis</i> -sabinene hydrate	0.04	0.05	0.03	0.04	0.01	0.05
octanol	tr	tr	tr	tr	tr	0.01
terpinolene	0.32	0.31	0.21	0.26	0.22	0.45
linalol	1.32	4.93	9.27	4.23	3.63	22.68
nonanal	0.04	0.02	0.01	0.03	0.02	0.06
heptyl acetate	tr	tr	tr	tr	tr	0.02
<i>cis</i> -limonene oxide	tr	tr	tr	tr	tr	tr
<i>trans</i> -limonene oxide	tr	tr	tr	tr	tr	tr
isopulegol	tr	tr	tr	tr	tr	tr
camphor	tr	tr	tr	tr	tr	0.08
citronellal	0.02	0.02	0.02	0.02	tr	0.02
terpinen-4-ol	0.02	0.02	0.01	0.02	0.01	0.03
α -terpineol	0.03	0.05	0.05	0.03	0.04	0.13
decanal	0.07	0.04	0.03	0.06	0.05	0.10
octyl acetate	0.16	0.07	0.04	0.16	0.07	0.20
nerol + citronellol	0.01	0.02	0.03	0.02	0.02	0.08
neral	0.13	0.26	0.29	0.20	0.10	0.28
<i>trans</i> -sabinene hydrate acetate	0.08	0.08	0.05	0.07	0.05	0.10
linalyl acetate	26.01	30.91	36.43	36.67	21.84	41.36
geraniol	tr	tr	tr	tr	tr	tr

tr = <0.01.

	Sicilian oils				Calabrian oils	
	"Castagnaro" X	"Fantastico" X	"Feminello" X	"PCF" X	min	max
geranial + perillaldehyde	0.19	0.38	0.43	0.29	0.20	0.44
bornyl acetate	0.02	0.02	0.01	0.02	0.01	0.02
undecanal	0.01	0.01	tr	0.01	tr	0.02
nonyl acetate	0.04	0.03	0.01	0.04	0.01	0.03
methylgeranate	0.01	0.01	0.01	tr	tr	0.01
δ -elemene	tr	tr	tr	tr	tr	tr
linalyl propanoate	0.04	0.04	0.04	0.04	0.02	0.06
α -terpinyl acetate	0.16	0.15	0.10	0.16	0.07	0.22
citronellyl acetate	0.03	0.02	0.03	0.03	0.01	0.03
neryl acetate	0.25	0.31	0.30	0.39	0.13	0.64
geranyl acetate	0.34	0.20	0.16	0.17	0.11	0.51
dodecanal	0.05	0.03	0.02	0.04	0.02	0.05
decyl acetate	0.02	0.02	0.02	0.02	0.01	0.03
<i>cis</i> - α -bergamotene	tr	tr	tr	tr	0.02	0.05
β -caryophyllene	0.28	0.23	0.23	0.27	0.15	0.43
<i>trans</i> - α -bergamotene	0.30	0.27	0.26	0.28	0.16	0.36
α -humulene	0.02	0.06	0.01	0.01	0.01	0.04
β -santalene	0.01	tr	tr	tr	tr	0.02
<i>cis</i> - β -farnesene	0.05	0.02	tr	tr	0.03	0.09
dodecanol	tr	tr	tr	tr	-	tr
germacrene D	0.05	0.05	0.04	0.08	0.03	0.11
bicyclogermacrene	0.03	0.02	0.03	0.04	0.01	0.04
(<i>E,E</i>)- α -farnesene	0.01	0.02	tr	tr	tr	tr
β -bisabolene	0.41	0.39	0.37	0.41	0.21	0.51
<i>cis</i> - γ -bisabolene	tr	tr	tr	tr	tr	0.01
germacrene B	0.01	0.01	0.01	0.01	tr	0.01
(<i>E</i>)-nerolidol	0.01	tr	0.01	0.01	0.01	0.03
tetradecanal	0.01	0.01	tr	0.01	-	tr
2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol [†]	0.01	0.01	0.01	0.01	0.01	0.02
campherenol	0.02	0.02	0.02	0.02	0.01	0.02
α -bisabolol	0.02	0.02	0.02	0.02	0.01	0.02
nootkatone	0.06	0.07	0.06	0.08	0.01	0.09
Hydrocarbons	70.59	61.76	52.21	56.63	39.81	71.48
Monoterpenes	69.44	60.74	51.27	55.54	38.75	70.48
Sesquiterpenes	1.15	1.03	0.94	1.09	0.68	1.44
Oxygenated compounds	29.21	37.82	47.52	42.91	28.43	59.84
Carbonyl compounds	0.60	0.85	0.89	0.75	0.58	1.07
Alcohols	1.47	5.11	9.44	4.39	3.81	22.85
Esters	27.14	31.85	37.19	37.77	22.80	42.21
linalol/linalyl acetate	0.05	0.16	0.25	0.12	0.16	0.50

tr = <0.01;
t = tentative identification.

obtained for each single compound and, therefore for the classes of substances, were very close for both samples of the same cultivar.

For all samples, the main compounds were limonene, linalyl acetate, linalol, γ -terpinene, sabinene and β -pinene. Monoterpenes were the main class of substances. Moreover, the oils were characterised by a high amount of alcohols and esters, and about 99% of these classes were made up of linalol and linalyl acetate. There were only small amounts of carbonyl compounds and of sesquiterpenes.

The Sicilian and Calabrian bergamot oils had a similar composition. In fact the compounds identified were the same and, the amount of almost all the compounds and classes of substances for Sicilian bergamot oils was included in the range reported for the Calabrian bergamot oils. There were some quantitative differences, however; the average amount of limonene in "Castagnaro" oils was higher than the maximum value reported for the Calabrian oils (VERZERA *et al.*, 1998); the opposite occurred for linalol, alcohols, and the ratio between linalol and linalyl acetate. "Fantastico" and "PCF" oils had a linalol/linalyl acetate ratio lower than the minimum value reported for the Calabrian oils

(VERZERA *et al.*, 1998). The average amount of sabinene, β -pinene, γ -terpinene in "Feminello" oils was lower than the minimum value reported for the Calabrian oils (VERZERA *et al.*, 1998).

In order to better evaluate the quality of the oil analysed, the oil of each cultivar was then compared with the Calabrian bergamot oils of the same cultivar, previously analysed (VERZERA *et al.*, 1996). The comparison was limited to the main compounds: limonene, linalol, linalyl acetate and to the different classes of substances (Table 3). Particular attention was paid to linalol and linalyl acetate, as these compounds characterise the olfactory quality of bergamot oil; linalol supplies it with fresh and lavender olfactory notes, while linalyl acetate gives fruity notes. A limited amount of these compounds would reduce the quality of the oil.

Sicilian "Castagnaro" oils were very different from Calabrian "Castagnaro" oils especially for the lower amount of alcohols and linalol and the higher amount of monoterpenes and limonene. Esters, linalyl acetate, carbonyl compounds and sesquiterpenes were similar in both oils. Sicilian "Fantastico" oils were similar to Calabrian "Fantastico" oils except for the lower amount of alcohol and linalol. Sicilian "Feminello"

Table 3 - Comparison between Sicilian and Calabrian (VERZERA *et al.*, 1996) "Castagnaro", "Fantastico" and "Feminello" bergamot oils. Each value is the average of two different samples.

	"Castagnaro"		"Fantastico"		"Feminello"	
	Sicilian oils	Calabrian oils	Sicilian oils	Calabrian oils	Sicilian oils	Calabrian oils
limonene	51.32	37.77	43.80	38.38	40.48	37.36
linalol	1.32	10.82	4.93	8.57	9.27	11.72
linalyl acetate	26.01	27.70	30.91	28.12	36.43	29.27
monoterpenes	69.94	57.98	60.74	59.72	51.27	55.49
sesquiterpenes	1.15	1.32	1.03	1.34	0.94	1.26
carbonyl compounds	0.60	0.78	0.85	0.79	0.89	0.80
alcohols	1.47	10.99	5.11	8.73	9.44	11.88
esters	27.14	28.70	31.85	29.25	37.19	30.25
linalol/linalyl acetate	0.05	0.39	0.16	0.30	0.25	0.40

oils differed from Calabrian "Feminello" oils only in the higher amount of esters and linalyl acetate (VERZERA *et al.*, 1996).

Regarding the "PCF" oils, due to the lack of information to evaluate their quality, they were compared with the oils of other Sicilian cultivars. The average amount of alcohols and linalol was similar to that of "Fantastico" oils, while the average amount of esters and linalyl acetate was similar to that of "Feminello" oils.

The enantiomeric ratio of linalol and linalyl acetate was determined in each oil because these compounds are good indicators of genuineness. The results are reported in Table 4. The enantiomeric ratios of the compounds analysed were in agreement with those reported in the literature for Italian bergamot oils. In fact, the results confirmed that (R)-linalyl acetate as well as (R)-linalol occur in high enantiomeric purity in bergamot oil (KONIG *et al.*, 1997; JUCKELKA and MOSANDL 1996; MOSANDL and JUCKELKA 1997; MONDELLO *et al.*, 1998).

In conclusion, the Sicilian bergamot oils analysed had a composition similar to genuine Calabrian bergamot oils, with only some quantitative differences being observed. The major difference was the lower amount of linalol for "Castagnaro", "Fantastico" and "PCF" oils. The results confirm "Feminello" oil as having the best quality, as already assessed for the Calabrian grown cultivar.

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Table 4 - Enantiomeric ratios for linalol and linalyl acetate in the oils analysed*.

		"Castagnaro"	"Feminello"	"PCF"	"Fantastico"
linalol	3R (-)	99.4	99.5	99.4	99.4
	3S (+)	0.6	0.5	0.6	0.6
linalyl acetate	3R (-)	99.7	99.8	99.7	99.7
	3S (+)	0.3	0.2	0.3	0.3

*Identical values were obtained for the two samples of the same cultivar.

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