The Composition of Bergamot Oil

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Bergamot oil is obtained by cold pressing the peel of the bergamot fruit (*Citrus bergamia*, Risso et Poiteau). The bergamot tree is cultivated mainly for its valuable oil, which constitutes an important raw material for the cosmetic and food industries.

In Italy, the cultivation of bergamot is limited approximately 2,000 hectares in a narrow strip of the Calabrian coast along the Tyrrhenian and Ionian seas.

Bergamot's harvest and industrial processing period is usually from December to March. Three cultivars are used for the production of the bergamot oil: Fantastico, Castagnaro and Femminello. Nowadays, Fantastico represents about 90% of bergamot oil production.

Bergamot oil, like other citrus oils, consists of a mixture of monoterpene and sesquiterpene hydrocarbons, oxygenated derivatives and a nonvolatile residue. Terpenes and oxygenated compounds make up 93-96% by weight of the oil, while the nonvolatile residue constitutes the remaining 4-7%. The nonvolatile residue is a natural odor fixative and therefore influences the olfactory properties of the oil.

In 1965, initial research on the composition of Italian bergamot oil was carried out on 50 samples using gas chromatography.[1] In 1979, Shaw extensively reviewed the literature on the quantitative analysis of bergamot oil, and found there were differences from the other citrus peel oils studied. The most striking differences were that bergamot oil was low in limonene (25-32%), high in linalool (16-41%) and high in linalyl acetate (11-41%).[2]

Some papers published on the composition of the volatile fraction of the Italian bergamot oil refer to a small number of samples[1,3] or only to qualitative aspects.[4] Other papers discuss the composition of bergamot oil from Corsica, the Ivory Coast, Brazil, China and Turkey.[5-8]

As regards the nonvolatile fraction, early studies on the composition of coumarins and psoralens were reviewed by Mossman and Bogert[9] and, later, by Di Giacomo and Calvarano[10] and by Lawrence.[11] However, due to the photosensitizing activity of bergapten, most of the studies carried out report only its content,[10,12-14] although many papers also report the level of citropten and bergamottin.[15-20] Only three report quantitative results for 5-geranyloxy-7-methoxycoumarin.[18-20]

In this paper, we report the results relative to the composition of the volatile fraction of 1,082 samples of bergamot oil produced between 1984 and 1993. Some of these results relative to the 1984-1985, 1985-1986 and 1987-1988 production season oils have been previously reported,[21,22] while those of 1991-1992 and 1992-1993 are reported here for the first time. Also previously reported are the composition of the nonvolatile fraction[23] and the enantiomeric distribution of linalool.[24]

Experimental

Our research was carried out on 1,082 samples of genuine Calabrian cold-pressed bergamot oil obtained at random dates during five harvest seasons between 1984 and 1993 (Table I). All the oils were obtained by using a "Pelatrice" machine, the only technology used in Italy today to obtain the bergamot oils. Each sample represented at least 50 kg of essential oils. For each sample we

Table I. 1,082 Bergamot oil samples analyzed

Production season	Number of samples
Dec 1984 - Mar 1985	406
Dec 1986 - Feb 1987	228
Dec 1987 - Feb 1988	195
Jan 1992 - Mar 1992	128
Dec 1992 - Jan 1993	125
All	1,082

Cultivar	Number of samples
Fantastico	1,036
Castagnaro	27
Femminello	19
All	1,082

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knew the production date (which often corresponded to the date the fruit was harvested), the cultivar and the provenance (the production area from which the fruits came). To examine the oil's characteristics during a season, we grouped each season's samples into fortnightly groups by production date.

All samples were analyzed by GC using an SE-52 column 30 m x 0.32 mm for quantitative results, as previously reported.[21,22] The component identification of the volatile fraction composition was carried out on three samples produced during the 1991-1992 season and on three samples produced during the 1992-1993 season. This identification was made by HPLC-GC/MS (ITD)[25,26] using SE-52 capillary columns 30 m x 0.32 mm and by GC/MS (quadrupole)[27,28] using SE-52 and Carbowax 20M capillary columns 60 m x 0.32 mm. The enantiomeric distribution of linalool

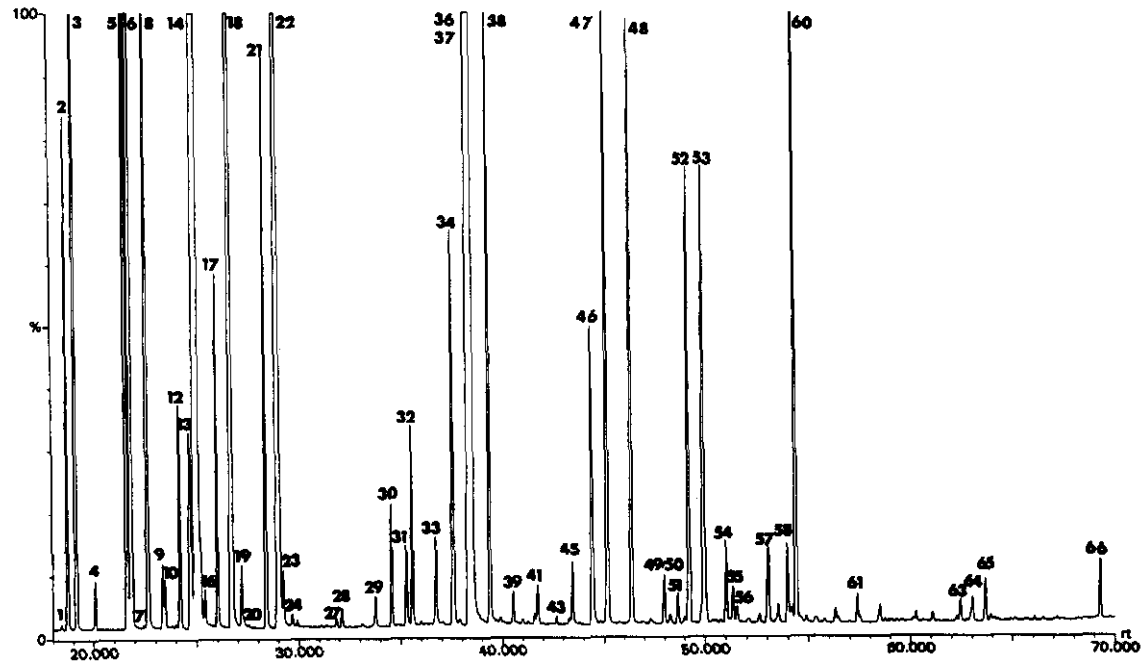


Figure 1. Gas chromatogram of bergamot oil on SE-52 column (For peak identification, see Table II.)

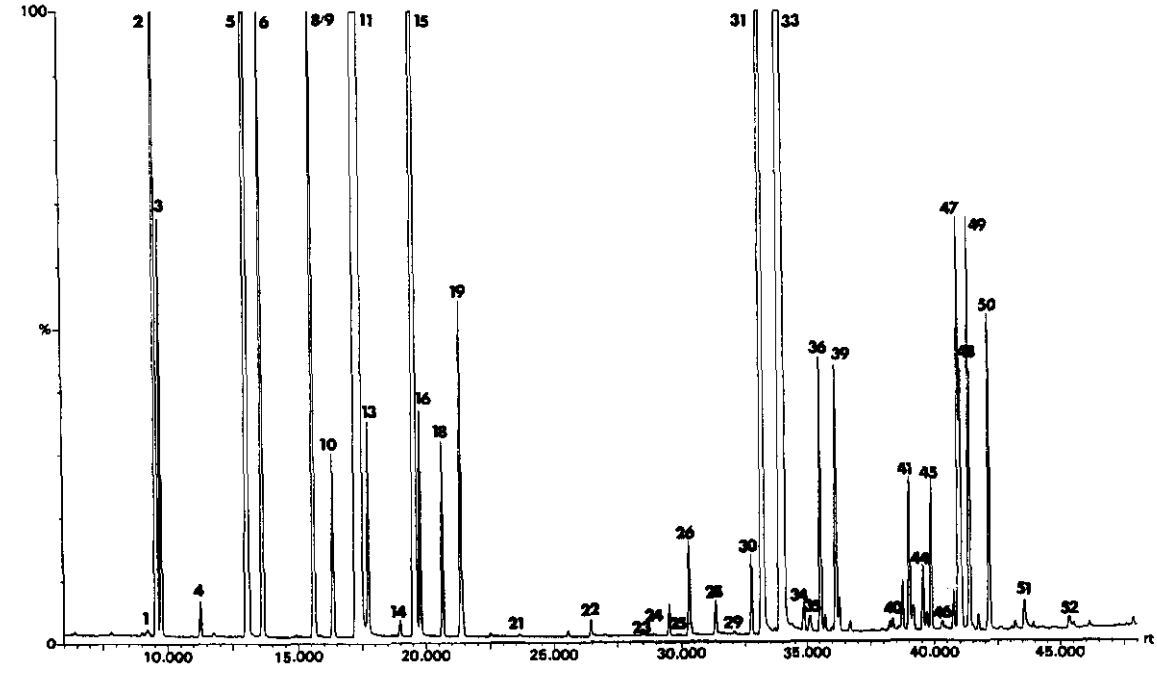


Figure 2. Gas chromatogram of bergamot oil on Carbowax 20M column (For peak identification, see Table II.)

in 50 samples produced during the 1991-1992 and 1992-1993 seasons was analyzed by GC using β-cyclodextrin capillary columns.[24] Coumarins and psoralens present in the nonvolatile residue of 128 oils produced during the 1991-1992 production season were analyzed by HPLC.[23]

Results and Discussion

Volatile fraction composition: Figures 1 and 2 are GC/MS (quadrupole) chromatograms of a bergamot oil, obtained on an SE-52 column and a Carbowax column, respectively. Table II reports peaks identified by GC/MS (quadrupole) on an SE-52 column, by GC/MS (quadrupole) on a Carbowax column and by LC-GC/MS (ITD).

For each sample we calculated the quantitative composition as a relative percentage of the peak area for each component, as well as the total amount of hydrocarbons, monoterpenes, sesquiterpenes, carbonyl compounds, alcohols and esters.

The ranges (minimum and maximum) for each component and for the classes of substances relative to the 1,082 samples of bergamot oil can be seen in Table IIIA. The data recorded in IIIA refer to the volatile part of the oil and do not include the nonvolatile residue that generally constituted approximately 4-7% of the oil. These data refer to the results obtained by GC with SE-52 columns and to the identification carried out by GC/MS (quadrupole) and by

Table II. Component identification by GC/MS (quadrupole) on SE-52 column, by GC/MS (quadrupole) on Carbowax column and by LC-GC/MS (ITD) on SE-52 column

Component*	A	B	C
tricyclene	1	1	1
α-thujene	2	3	2
α-pinene	3	2	3
camphene	4	4	4
sabinene	5	6	5
β-pinene	6	5	6
6-methyl-5-hepten-2-one	7	21	
myrcene	8	8	7
octanal	9	20	8
α-phellandrene	10	9	9
hexyl acetate		17	12
δ-3-carene	11	7	10
α-terpinene	12	10	11
p-cymene	13	18	13
limonene	14	11	14
β-phellandrene		13	
1,8-cineole	15	12	
(Z)-β-ocimene	16	14	15
(E)-β-ocimene	17	16	16
γ-terpinene	18	15	17
cis-sabinene hydrate	19		18
octanol	20	32	19
cis-linalool oxide (furanoid form)		23	
terpinolene	21	19	20
trans-linalool oxide (furanoid form)		25	
linalool	22	31	21
nonanal	23	22	22
heptyl acetate	24		23
cis-limonene oxide	25	24	24
trans-limonene oxide	26		25
isopulegol			26
camphor	27	29	27
citronellal	28	27	28
terpinen-4-ol	29	37	29
α-terpineol	30	44	30
decanal	31	28	31
octyl acetate	32	26	32
nerol	33	51	33
citronellol			34
neral	34	41	35
trans-sabinene hydrate acetate	35	30	36
linalyl acetate	36	33	37
geraniol	37	52	38
geranial	38	48	39
perillaldehyde	38		
bornyl acetate	39	35	40
undecanal	40	38	41
nonyl acetate	41	34	42
methyl geranate	42		43
linalyl propionate	43		44
δ-elemene	44		
α-terpinyl acetate	45	45	45
citronellyl acetate	46	40	46
neryl acetate	47	47	47
geranyl acetate	48	50	48
dodecanal	49		49
decyl acetate	50	43	50
cis-α-bergamotene	51		51
β-caryophyllene	52	39	52
trans-α-bergamotene	53	36	53
(Z)-β-farnesene	54		55
α-humulene	55	42	54
β-santalene	56		56
dodecanol		46	
germacrene D	57		57
bicyclogermacrene	58		
(E,E)-α-farnesene	59		
β-bisabolene	60	49	60
cis-γ-bisabolene			61
germacrene B			63
(E)-nerolidol	61		64
tetradecanal	62		
2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	63		65
campherenol	64	66	
α-bisabolol	65		67
nootkatone	66		

*the components are listed according to elution order on SE-52 column, 60 m
A = GC/MS on SE-52 column (see Figure 1); B = GC/MS on Carbowax 20M column (see Figure 2); C = LC-GC/MS (see Figures in References 25,26)

Table III. Composition (minimum and maximum percentages) of single components and classes of components in the volatile fraction of genuine Italian bergamot oils

Component* or class of components	A - Overall		B - Harvest period					
	1,082 samples 1984-1993		829 samples 1984-1988		128 samples 1991-1992		125 samples 1992-1993	
	Min	Max	Min	Max	Min	Max	Min	Max
tricyclene	0.002	0.007	nd		0.002	0.005	0.003	0.007
α-thujene	0.190	0.491	0.237	0.491	0.190	0.441	0.217	0.423
α-pinene	0.731	1.844	0.927	1.844	0.731	1.654	0.872	1.680
camphene	0.022	0.053	0.022	0.053	0.023	0.046	0.026	0.051
sabinene	0.777	1.778	0.783	1.778	0.777	1.524	0.783	1.627
β-pinene	4.374	11.024	4.413	11.024	4.374	9.240	4.878	10.810
6-methyl-5-hepten-2-one	0.002	0.010	nd		0.002	0.010	0.002	0.010
myrcene	0.656	1.575	0.709	1.575	0.710	1.237	0.656	1.173
octanal	0.025	0.056	0.026	0.056	0.026	0.042	0.025	0.045
α-phellandrene	0.025	0.056	0.026	0.056	0.026	0.042	0.025	0.045
hexyl acetate	-	trace	nd		-	trace	-	trace
δ-3-carene	0.002	0.009	nd		0.002	0.005	0.002	0.009
α-terpinene	0.081	0.282	0.081	0.282	0.085	0.226	0.084	0.202
p-cymene	0.060	0.893	0.060	0.793	0.138	0.583	0.109	0.893
limonene + β-phellandrene	25.626	53.187	27.384	53.187	27.227	49.319	25.626	46.051
1,8-cineole	0.010	0.021	nd		0.010	0.18	0.012	0.021
(Z)-β-ocimene	0.017	0.068	0.017	0.063	0.019	0.035	0.021	0.068
(E)-β-ocimene	0.104	0.361	0.104	0.361	0.190	0.315	0.194	0.322
γ-terpinene	5.727	11.378	5.727	11.378	5.817	10.485	5.766	9.583
cis-sabinene hydrate	0.024	0.063	0.025	0.053	0.024	0.057	0.034	0.063
octanol	0.002	0.021	0.002	0.021	0.002	0.012	0.002	0.021
cis-linalool oxide (furanoid form)	-	trace	nd		-	trace	-	trace
terpinolene	0.210	0.475	0.210	0.475	0.245	0.558	0.233	0.417
trans-linalool oxide (furanoid form)	-	trace	nd		-	trace	-	trace
linalool	1.745	20.260	1.745	20.260	2.696	17.300	4.541	18.587
nonanal	0.012	0.076	0.012	0.067	0.013	0.060	0.019	0.076
heptyl acetate	trace	0.008	nd		trace	0.007	trace	0.008
cis-limonene oxide	0.002	0.019	nd		0.002	0.011	0.002	0.019
trans-limonene oxide	0.002	0.015	nd		0.003	0.11	0.002	0.015
isopulegol	-	trace	nd		-	trace	-	trace
camphor	0.002	0.012	nd		0.002	0.007	0.002	0.012
citronellal	0.004	0.030	0.004	0.030	0.009	0.024	0.006	0.020
terpinen-4-ol	0.013	0.042	0.013	0.041	0.016	0.042	0.013	0.038
α-terpineol	0.030	0.098	0.030	0.098	0.044	0.095	0.045	0.098
decanal	0.037	0.086	0.037	0.086	0.038	0.072	0.046	0.079
octyl acetate	0.071	0.185	0.071	0.180	0.078	0.145	0.086	0.185
nerol + citronellol	0.010	0.111	0.010	0.111	0.015	0.097	0.027	0.105
neral	0.122	0.344	0.122	0.344	0.168	0.282	0.189	0.326
trans-sabinene hydrate acetate	0.057	0.127	0.057	0.124	0.063	0.116	0.060	0.127
linalyl acetate	15.616	40.375	15.616	40.375	19.835	38.403	20.154	40.285
geraniol	0.004	0.010	nd		0.004	0.008	0.006	0.010
geranial + perillaldehyde	0.247	0.494	0.247	0.494	0.270	0.445	0.300	0.492

C - Cultivar						D - Provenance/Fantastico			
1,036 samples Fantastico		27 samples Femminello		19 samples Castagnaro		356 samples Area 5		88 samples Area 10	
Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
0.002	0.007	0.003	0.004	0.003	0.004	0.002	0.007	0.003	0.006
0.190	0.491	0.230	0.399	0.285	0.405	0.190	0.491	0.262	0.412
0.731	1.844	0.906	1.491	1.080	1.560	0.731	1.844	1.047	1.680
0.022	0.053	0.023	0.040	0.029	0.045	0.023	0.053	0.028	0.048
0.783	1.778	0.777	1.364	1.030	1.547	0.872	1.769	0.971	1.778
4.413	11.024	4.374	8.340	5.988	9.448	4.995	11.024	5.487	9.447
0.002	0.010	0.002	0.010	0.002	0.002	0.002	0.009	0.002	0.009
0.656	1.575	0.745	1.194	0.779	1.134	0.749	1.575	0.718	1.138
0.025	0.056	0.030	0.048	0.036	0.053	0.025	0.054	0.027	0.056
0.25	0.056	0.030	0.048	0.036	0.053	0.025	0.054	0.027	0.056
-	trace	nd		nd		-	trace	-	trace
0.002	0.009	0.003	0.005	0.003	0.003	0.002	0.007	0.003	0.008
0.081	0.282	0.122	0.206	0.100	0.207	0.081	0.282	0.082	0.198
0.060	0.893	0.060	0.840	0.084	0.459	0.076	0.818	0.060	0.813
25.626	53.187	28.672	45.672	31.418	44.782	30.263	53.187	26.819	46.370
0.012	0.018	0.010	0.015	0.014	0.021	0.010	0.018	0.012	0.021
0.017	0.068	0.022	0.061	0.019	0.037	0.017	0.068	0.020	0.040
0.104	0.361	0.207	0.359	0.201	0.320	0.104	0.358	0.179	0.310
5.727	11.378	5.839	9.522	6.791	9.321	5.727	11.378	5.843	9.008
0.024	0.063	0.025	0.048	0.029	0.046	0.024	0.059	0.028	0.053
0.002	0.021	0.002	0.013	0.002	0.008	0.002	0.021	0.002	0.018
-	trace	nd		nd		-	trace	-	trace
0.210	0.475	0.256	0.413	0.244	0.384	0.210	0.475	0.232	0.381
-	trace	nd		nd		-	trace	-	trace
1.745	20.260	5.444	19.110	4.015	18.303	1.745	17.300	3.187	17.366
0.012	0.076	0.024	0.054	0.028	0.058	0.013	0.075	0.022	0.076
trace	0.007	0.002	0.008	trace	0.006	trace	0.008	trace	0.006
0.002	0.019	0.003	0.016	0.005	0.006	0.002	0.017	0.003	0.017
0.002	0.015	0.004	0.012	0.005	0.006	0.003	0.014	0.003	0.014
-	trace	nd		nd		-	trace	-	trace
0.002	0.012	0.002	0.007	0.003	0.004	0.002	0.011	0.002	0.012
0.004	0.030	0.007	0.023	0.007	0.021	0.005	0.028	0.006	0.019
0.013	0.042	0.013	0.028	0.015	0.034	0.013	0.042	0.015	0.034
0.030	0.098	0.039	0.084	0.041	0.095	0.030	0.095	0.035	0.098
0.037	0.086	0.038	0.071	0.048	0.082	0.040	0.082	0.044	0.080
0.073	0.185	0.071	0.155	0.076	0.139	0.083	0.185	0.073	0.149
0.010	0.111	0.023	0.101	0.014	0.069	0.011	0.101	0.011	0.094
0.122	0.344	0.196	0.296	0.173	0.294	0.122	0.304	0.159	0.282
0.059	0.127	0.057	0.095	0.065	0.099	0.060	0.128	0.063	0.111
15.616	40.375	19.979	37.367	22.155	33.541	15.616	37.705	20.545	38.215
0.004	0.010	0.006	0.008	0.004	0.010	0.004	0.010	0.006	0.008
0.247	0.494	0.306	0.467	0.270	0.464	0.247	0.283	0.247	0.492

Table III. Composition (minimum and maximum percentages) of single components and classes of components in the volatile fraction of genuine Italian bergamot oils (continued)

Component* or class of components	A - Overall		B - Harvest Period					
	1,082 samples 1984-1993		829 samples 1984-1988		128 samples 1991-1992		125 samples 1992-1993	
	Min	Max	Min	Max	Min	Max	Min	Max
bornyl acetate	0.010	0.037	0.010	0.031	0.012	0.027	0.013	0.037
undecanal	0.004	0.024	0.004	0.024	0.004	0.017	0.004	0.024
nonyl acetate	0.004	0.053	0.004	0.035	0.007	0.027	0.006	0.053
methyl geranate	0.002	0.015	nd		0.002	0.014	0.003	0.015
linalyl propionate	0.013	0.066	0.013	0.066	0.016	0.058	0.021	0.066
δ-elemene	-	trace	-	trace	-	trace	-	trace
α-terpinyl acetate	0.093	0.263	0.093	0.255	0.104	0.240	0.102	0.263
citronellyl acetate	0.012	0.053	0.012	0.053	0.012	0.053	0.017	0.053
neryl acetate	0.145	0.670	0.145	0.662	0.191	0.582	0.178	0.670
geranyl acetate	0.175	0.799	0.175	0.799	0.180	0.723	0.181	0.633
dodecanal	0.002	0.047	nd		0.015	0.041	0.002	0.047
decyl acetate	0.013	0.053	nd		-	trace	0.013	0.053
cis-α-bergamotene	0.020	0.048	nd		0.020	0.45	0.021	0.048
β-caryophyllene	0.225	0.524	0.225	0.524	0.225	0.404	0.245	0.423
trans-α-bergamotene	0.211	0.437	0.230	0.437	0.211	0.367	0.212	0.367
(Z)-β-farnesene	0.038	0.089	0.004	0.009	0.005	0.009	0.005	0.010
α-humulene	0.017	0.045	0.017	0.045	0.018	0.031	0.017	0.043
β-santalene	0.005	0.009	0.039	0.078	0.043	0.075	0.045	0.088
dodecanol	-	0.009	nd		-	0.008	-	0.009
germacrene D	0.039	0.106	0.039	0.106	0.042	0.093	0.048	0.099
bicyclogermacrene	0.023	0.076	0.023	0.076	0.028	0.075	0.025	0.76
(E,E)-α-farnesene	-	trace	nd		-	trace	-	trace
β-bisabolene	0.298	0.648	0.306	0.648	0.298	0.503	0.300	0.532
cis-γ-bisabolene	-	trace	nd		-	trace	-	trace
germacrene B	-	0.006	nd		-	0.006	-	0.005
(E)-nerolidol	0.012	0.36	nd		0.012	0.027	0.015	0.036
tetradecanal	0.004	0.010	nd		0.004	0.010	0.004	0.010
2,3-dimethyl-3-(4-methyl-3-pentenyl) 2-norbornanol	0.007	0.024	nd		0.007	0.021	0.008	0.024
campherenol	0.010	0.025	nd		0.010	0.025	0.011	0.024
α-bisabolol	0.011	0.030	nd		0.011	0.026	0.011	0.030
nootkatone	0.012	0.068	nd		0.012	0.068	0.012	0.068
hydrocarbons	42.195	78.258	44.532	78.258	42.616	74.458	42.195	69.959
monoterpenes	40.748	76.811	43.352	76.811	41.394	73.121	40.748	68.707
sesquiterpenes	0.954	1.894	1.019	1.894	0.954	1.488	1.004	1.556
oxygenated compounds	21.540	57.261	21.540	53.712	25.339	57.063	29.566	57.261
carbonyl compounds	0.379	1.033	0.379	1.000	0.669	0.954	0.698	1.033
alcohols	1.850	20.491	1.850	20.491	2.919	17.599	4.765	18.864
esters	17.167	41.484	17.167	41.411	21.370	39.285	21.719	41.4844

*the components are listed according to the elution order on SE-52 column, 60 m; nd = not detected

C - Cultivar						D - Provenance/Fantastico			
1,036 samples Fantastico		27 samples Femminello		19 samples Castagnaro		356 samples Area 5		88 samples Area 10	
Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
0.010	0.037	0.012	0.028	0.010	0.023	0.013	0.037	0.010	0.029
0.004	0.024	0.005	0.020	0.006	0.011	0.004	0.024	0.004	0.021
0.004	0.053	0.010	0.036	0.012	0.023	0.006	0.039	0.007	0.046
0.002	0.015	0.004	0.015	0.004	0.005	0.003	0.014	0.002	0.011
0.013	0.066	0.019	0.053	0.020	0.050	0.013	0.066	0.013	0.061
-	trace	-	trace	-	trace	-	trace	-	trace
0.094	0.263	0.093	0.223	0.095	0.217	0.104	0.225	0.098	0.227
0.012	0.053	0.012	0.042	0.012	0.037	0.012	0.053	0.012	0.053
0.150	0.662	0.211	0.670	0.145	0.394	0.223	0.658	0.150	0.539
0.180	0.799	0.189	0.493	0.175	0.610	0.180	0.799	0.184	0.585
0.004	0.047	0.002	0.034	0.027	0.029	0.004	0.042	0.005	0.047
0.013	0.053	0.015	0.052	0.013	0.044	0.013	0.053	0.017	0.053
0.025	0.046	0.027	0.048	0.020	0.044	0.024	0.046	0.020	0.048
0.225	0.524	0.282	0.458	0.266	0.478	0.243	0.520	0.248	0.490
0.212	0.437	0.211	0.352	0.232	0.398	0.215	0.433	0.224	0.348
0.004	0.010	0.005	0.008	0.005	0.008	0.004	0.009	0.005	0.009
0.017	0.045	0.022	0.035	0.21	0.037	0.017	0.039	0.019	0.038
0.039	0.88	0.048	0.070	0.046	0.078	0.039	0.080	0.045	0.080
-	0.009	nd		nd		-	0.008	-	0.007
0.039	0.106	0.047	0.099	0.040	0.077	0.043	0.096	0.039	0.091
0.023	0.076	0.030	0.045	0.028	0.055	0.027	0.075	0.027	0.064
-	trace	-	trace	-	trace	-	trace	-	trace
0.300	0.648	0.298	0.491	0.306	0.616	0.304	0.648	0.306	0.514
-	trace	nd		nd		-	trace	-	trace
-	0.006	nd		nd		-	0.006	-	0.005
0.012	0.036	0.016	0.023	0.015	0.017	0.012	0.032	0.014	0.030
0.004	0.010	0.005	0.010	0.005	0.007	0.004	0.010	0.005	0.010
0.007	0.023	0.009	0.024	0.009	0.011	0.008	0.022	0.008	0.023
0.010	0.024	0.011	0.021	0.015	0.025	0.010	0.023	0.010	0.024
0.011	0.030	0.012	0.023	0.013	0.017	0.011	0.026	0.011	0.026
0.012	0.068	0.029	0.064	0.023	0.027	0.012	0.068	0.012	0.068
42.195	78.258	44.151	69.560	49.420	67.352	45.693	78.258	46.475	71.181
40.748	76.811	43.148	68.007	48.251	65.864	44.739	76.811	45.354	70.006
0.954	1.894	1.004	1.553	1.018	1.709	0.954	1.877	0.991	1.633
21.540	57.261	30.315	55.689	32.361	50.478	21.540	54.144	28.800	53.358
0.379	1.033	0.661	0.972	0.622	0.957	0.521	0.971	0.546	0.989
1.850	20.491	5.569	19.347	4.136	18.526	1.850	17.599	3.307	17.679
17.167	41.484	21.170	38.282	23.243	34.432	17.167	38.758	21.512	39.353

LC-GC/MS (ITD). As can be seen from Tables II and IIIA and Figures 1 and 2, 76 components were identified. They represent an average of 99.9% of the volatile fraction.

The analysis carried out by HPLC-GC/MS (ITD)[25,26] allowed the LC pre-separation of the bergamot oil into four fractions: hydrocarbons (fraction 1); aliphatic aldehydes and esters (fraction 2); monoterpenic aldehydes, sesquiterpene alcohols, some monoterpene alcohols (fraction 3); other monoterpene alcohols (fraction 4). It also allowed the transfer of each fraction into a GC capillary column so that we could do an MS identification of the components, even those present only as traces.

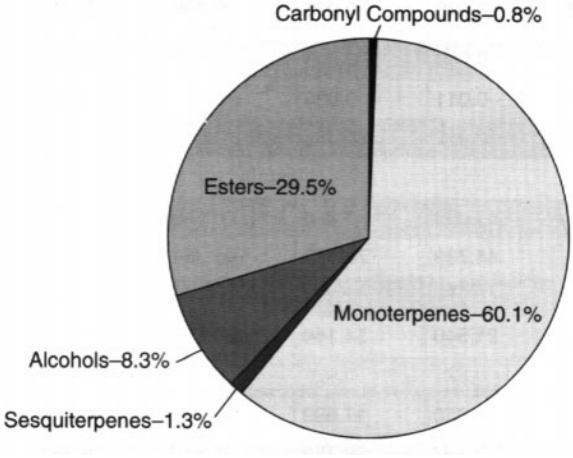
This technique allowed certain identification of some peaks present as traces, as well as of peaks which were coeluted in GC analyses of the whole oil. For example, hexyl acetate-octanal and geraniol-linalyl acetate coeluted, while nonanal overlapped the linalool peak.

The analyses carried out using Carbowax 20M allowed the separation of components, such as β-phellandrene-limonene and geraniol-linalyl acetate, that co-eluted on SE-52. Unfortunately, the techniques used did not allow for the detection of citronellol, which coeluted with nerol on SE-52 and with geranyl acetate on Carbowax 20M. Furthermore, nerol and citronellol were in the same LC fraction.

Figure 3 shows the average composition for the classes of substances of all bergamot oils analyzed. This Figure points out the large amounts of alcohols and esters—together they constitute approximately 40% of the whole oil—and the small amount of carbonyl compounds.

The main components were limonene, linalyl acetate, linalool, β-pinene and γ-terpinene, which altogether constitute more than 90% of the whole oil. Bergamot oil is marked by a lower amount of limonene (25.6-53.0%) and a higher amount of linalool (1.7-20%) and linalyl acetate (15.6-40.4%) than the other citrus peel oils.

Bergamot oils present wide ranges for each component



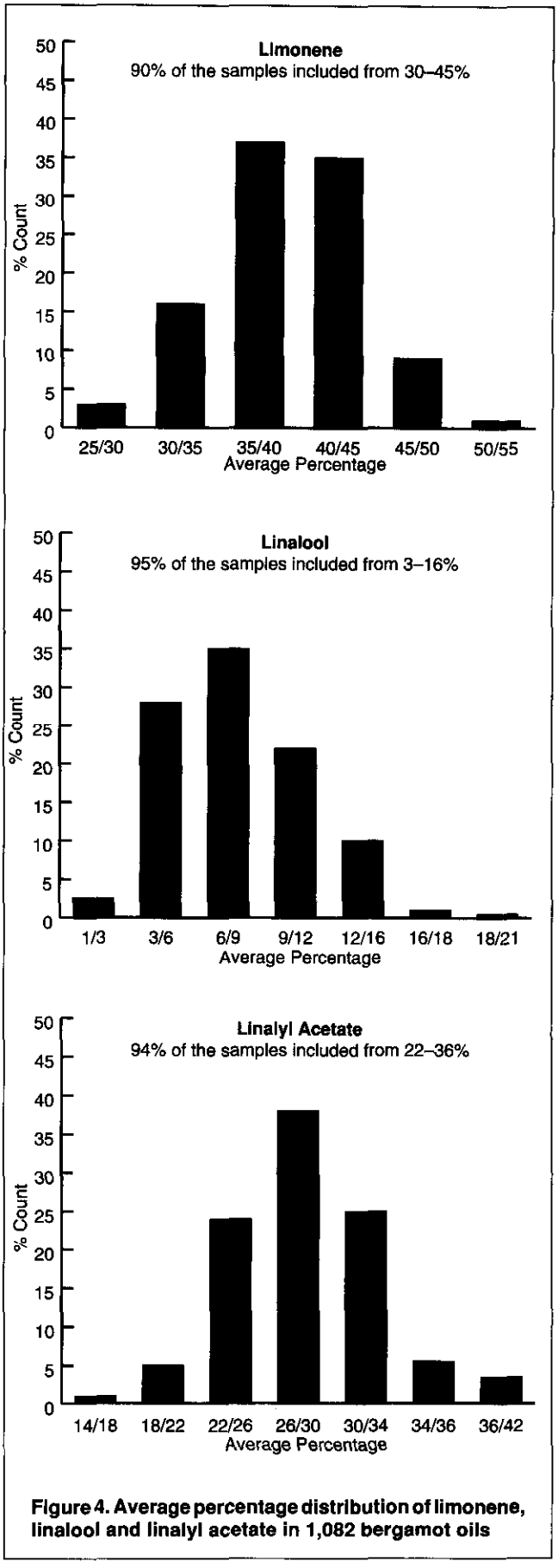


Figure 4. Average percentage distribution of limonene, linalool and linalyl acetate in 1,082 bergamot oils

and, obviously, for the classes of substances. These ranges are wider than those observed either for lemon or mandarin.[29,30] Such quantitative variations in the oil composition make the detection of genuineness and quality more difficult than with other citrus oils. These wide ranges are often due to a small number of samples analyzed. The bar graphs in Figure 4 show that 90% of the samples had a limonene content of 30-45%, 95% of the samples possessed a linalool content of 3-16%, and 94% of the samples had a linalyl acetate content of 22-36%.

Variations in the oil composition during a harvest season and among harvest seasons: To examine the variations in oil composition from season to season and from week to week within a season, we broke the data into three harvest periods (new data from 1992 to 1993, new data from 1991 to 1992 and previously analyzed data from 1984 to 1988), as well as into fortnightly data groups within each harvest period. Table IIIB shows each period's range of content percentages for each component and each class of components. As can be seen from Table IIIB, the results for the three harvest periods are in agreement with each other. Moreover, the hydrocarbon content for the 1991-1992 and 1992-1993 periods is lower than that observed in the previous years, while the opposite is true for the oxygenated content.

Figure 5 shows fortnightly averages of content percentages for the component classes during each harvest period. The behavior of each class is almost the same from period to period (the curves have a consistent shape within a component class), as is the average value from period to period (the curves lie close together on the vertical axis).

An overall observation is that the sesquiterpenes showed a fairly constant content throughout the season, the carbonyl compounds decreased slightly, while the alcohols showed a clear-cut decrease that was balanced by the small increase in esters and monoterpene hydrocarbons.

The 1992-1993 samples showed a higher average content of oxygenated compounds (mainly alcohols) and a lower content of monoterpenes. We observed the same differences in lemon and mandarin oils produced during the same time period (1991-1993) as compared to those produced in previous seasons.[31,32] This behavior was probably due to the peculiar meteorological conditions experienced during these two years.

Variations in oil composition relative to fruit cultivar: The samples were also grouped by fruit cultivar (Fantastico, Femminello and Castagnaro) independent of production year. Table IIIC shows each cultivar's range of percentages for each component and each class of components. Figure 6 shows each cultivar's average content of monoterpenes, esters, alcohols, sesquiterpenes and carbonyl compounds.

Fantastico cultivar oils had a higher content of monoterpenes and sesquiterpenes and a lower content of alcohols and esters than the other two cultivar oils; Femminello had the highest content of alcohols and esters. From Table IIIC and Figure 6, one can see that bergamot oil composition is certainly influenced by cultivar.

Variations in oil composition relative to the fruit provenance: The oils analyzed were obtained from fruits that originated from approximately 70 villages. All the villages were grouped into ten areas (Figure 7) in order to determine any possible influence of the production area on oil composition.

Table IIID contains data for two of the ten areas and shows each area's range of percentages for each component and for each class of components.

Figure 8 shows each area's average content of monoterpenes, sesquiterpenes, esters, carbonyl compounds and alcohols. Only Fantastico oils are reported, and the production year is ignored. The Fantastico oils from the two areas had different monoterpene, alcohol and ester contents. These differences are more evident in Figure 9, which shows the behavior of the fortnightly average content of limonene, linalool and linalyl acetate during the season. From this information, one can see that the production area probably influenced the oil composition more than did the cultivar or the production year.

Coumarins and psoralens content: Figure 10 shows an HPLC chromatogram of the coumarin fraction of a genuine bergamot oil. The bergamottin, 5-geranyloxy-7-methoxycoumarin, citropten and bergapten contents are summarized in Table IV.

During the season, the average content of bergamottin and of 5-geranyloxy-7-methoxycoumarin did not vary, while that of citropten and bergapten decreased considerably. From this data, it was concluded that no correlation existed between the production areas of the fruits and the coumarin composition. However, the nonvolatile fraction composition proved useful in being able to distinguish a genuine bergamot oil from a reconstituted one.[23]

Linalool enantiomeric ratio: In genuine bergamot oils, (+)-linalool is not present or is present only as a trace constituent not exceeding 0.5% of the total linalool content.[24] All samples of commercial linalool examined were racemic mixtures. Linalool's enantiomeric (+)/(–) ratio in the genuine bergamot oil is obviously modified by the addition of commercial linalool.

The addition of small amounts of reconstituted oils to the genuine oils can be detected by the presence of (+)-linalool.[24] The chromatograms of a genuine and a reconstituted bergamot oil are shown in Figure 11.

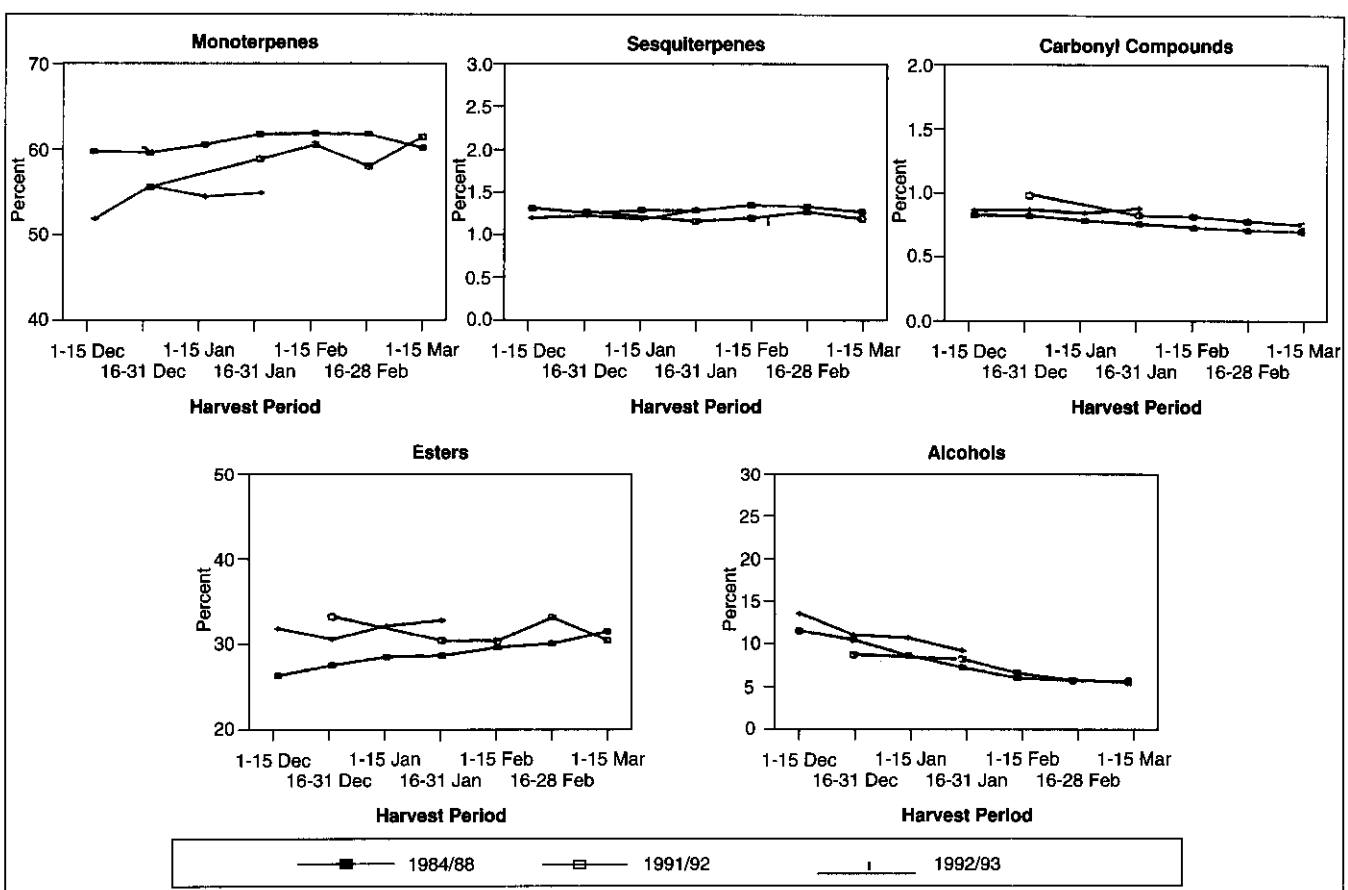


Figure 5. Fortnightly average percentage content of monoterpenes, sesquiterpenes, carbonyl compounds, esters and alcohols in 1,082 bergamot oils

Detection of Genuineness

The quantitative composition of the volatile fraction of bergamot oil depends on the site of cultivation, the fruit cultivar and the fruit harvest period. For these reasons, bergamot oil showed wide ranges in the percentage composition of each component. It was difficult, therefore, to determine the genuineness of an unknown sample of bergamot oil by comparing only the percentage content of

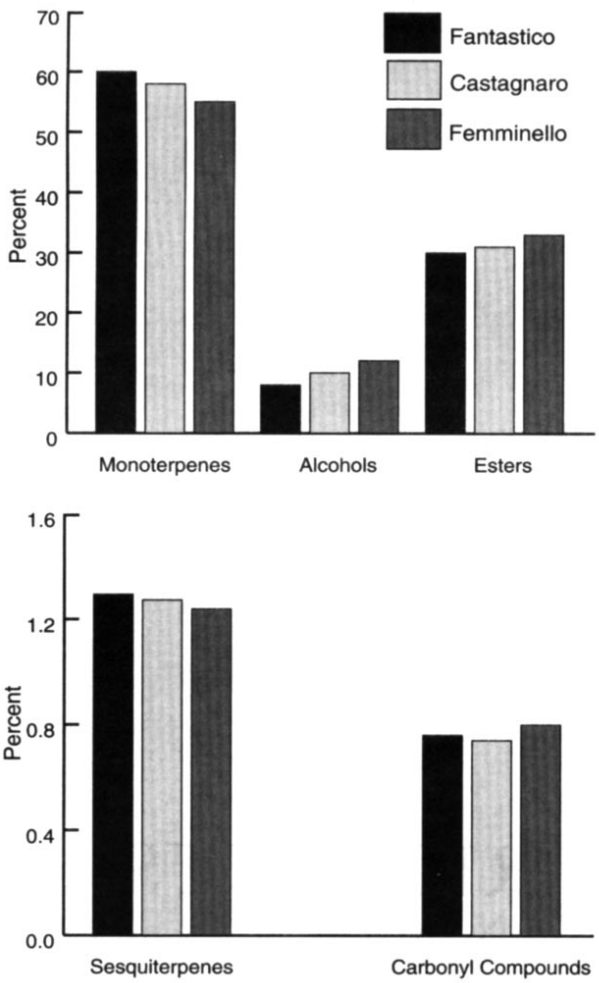


Table IV. Coumarin and psoralen content (%) in 128 genuine Italian bergamot oils from the 1991-1992 season

	Average	Minimum	Maximum
bergamottin	1.87	1.02	2.75
5-geranyloxy-7-methoxycoumarin	0.13	0.08	0.22
citropten	0.22	0.14	0.35
bergapten	0.21	0.11	0.32

Table V. Coumarin and psoralen content percentages, main volatile fraction component percentages, (–)/(+)-linalool enantiomeric ratio for two well-reconstituted oils

Coumarin and psoralen content percentages	Oil 1	Oil 2
bergamottin	1.21	1.11
5-geranyloxy-7-methoxycoumarin	0.42	0.52
citropten	0.20	0.18
bergapten	0.20	0.17
Main volatile fraction component percentage		
limonene	33.43	30.66
linalool	15.11	16.08
linalyl acetate	31.39	33.38
(–)/(+)-Linalool enantiomeric ratio		
(–)-linalool	99.30	99.50
(+)-linalool	0.70	0.50

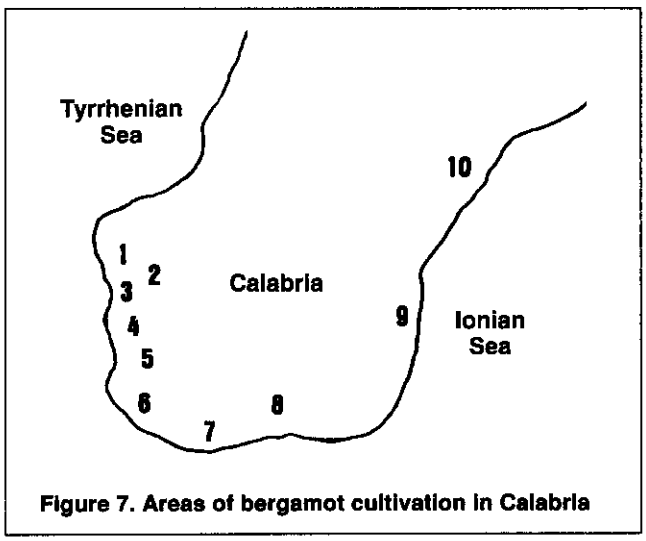
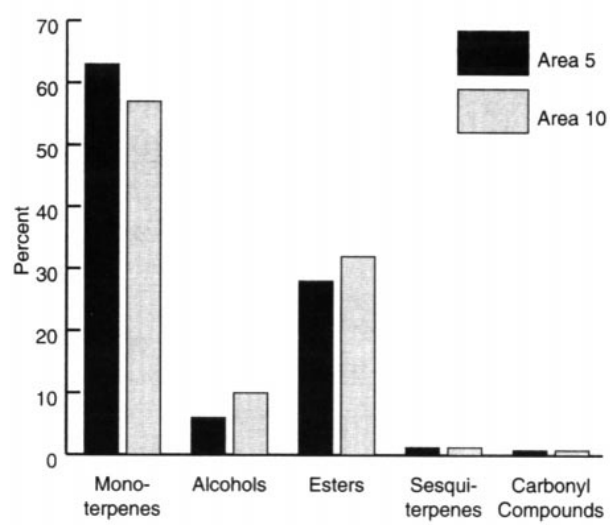


Figure 7. Areas of bergamot cultivation in Calabria



each of its components with the range of percentage contents for the genuine bergamot oil components.

Reconstituted bergamot oils are generally obtained by mixing monoterpenes and distilled oils of different origins, citrus oils other than bergamot oil, linalool, linalyl acetate and, at times, small amounts of natural bergamot oils.

Foreign markets, such as the USA, use limits for limonene (max 40%), linalool (min 8%) and linalyl acetate (min 22%, max 36%) to define the quality of a bergamot oil. This method is not always able to characterize an unknown oil because some genuine samples could have limonene, linalool and linalyl acetate content outside those limits

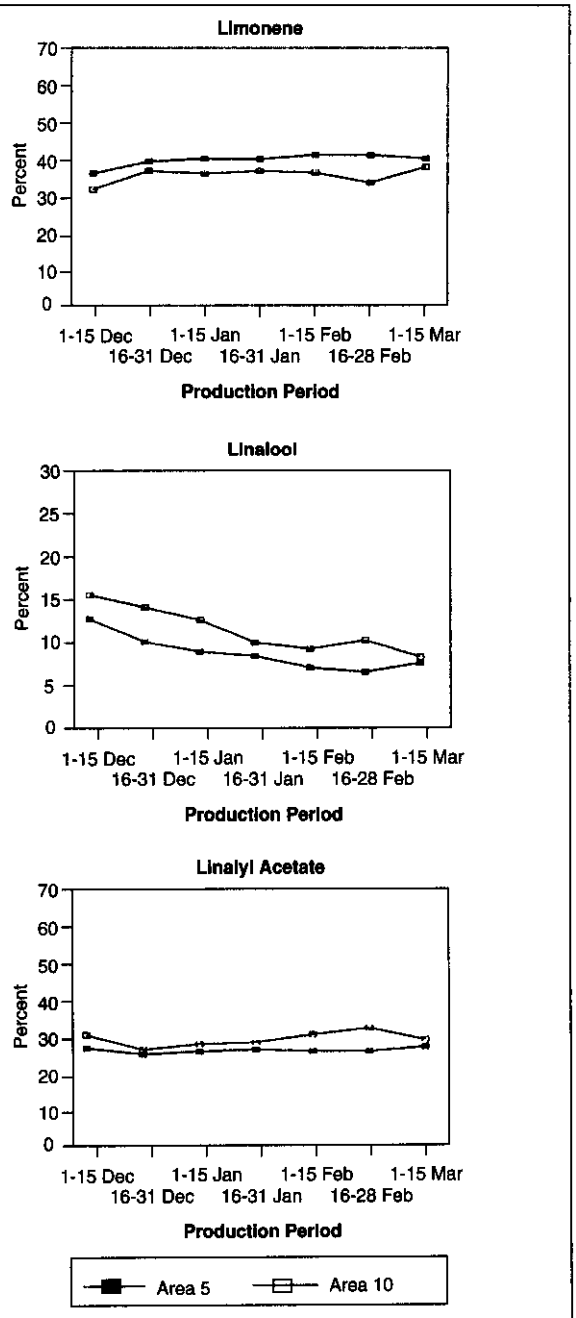


Figure 9. Fortnightly average content of limonene, linalool and linalyl acetate during the production season for Fantastico oils from areas 5 and 10

Table VI. Ratios between some components of the volatile fraction for genuine and reconstituted bergamot oils

Components	1,082 Genuine oils		Reconstituted commercial oils	
	Min	Max	1	2
citronellal/terpinen-4-ol	0.167	1.875	0.119	0.136
octyl acetate/α-terpineol	0.842	4.742	0.561	0.500
γ-terpinene/sabinene + β-pinene	0.661	1.279	0.733	0.670
trans-sabinene hydrate acetate/α-terpineol	0.704	3.323	0.354	0.303

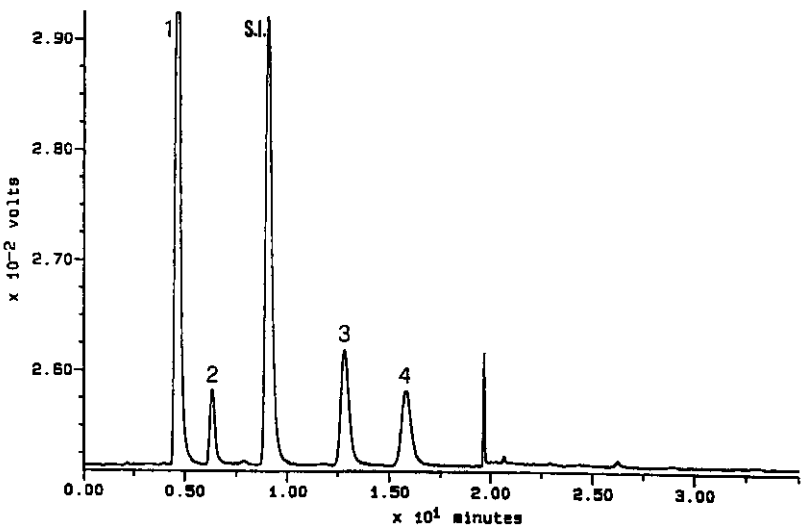


Figure 10. HPLC chromatogram of coumarin fraction of a genuine bergamot essential oil; 1) bergamottin, 2) 5-geranyloxy-7-methoxycoumarin, 3) citropten, 4) bergapten, S.I.) Internal Standard

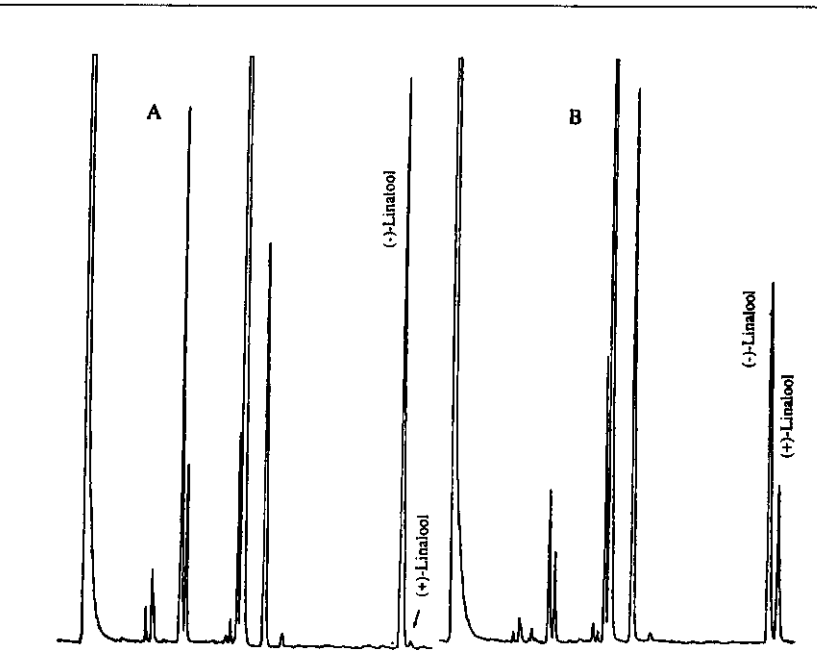


Figure 11. Chromatograms of a genuine (A) and a reconstituted (B) bergamot oil

(Table IIIA and Figure 1). Furthermore, it is easy to make a reconstituted bergamot oil within those limits.

In addition, one can find on the market some partially reconstituted oils which show both a) an enantiomeric ratio of linalool similar to that of genuine oils, and b) coumarin and psoralen component values that are primarily within the range of valid values shown in Table IV for genuine bergamot oils. An example of analytical data for two "well-reconstituted" bergamot oils is given in Table V.

Here we propose a method based upon the ratio between certain components of the oil. This ratio allows for the detection of these well-reconstituted oils. Our method uses the ratios between citronellal/terpinen-4-ol, octyl-acetate/α-terpineol, γ-terpinene/sabinene + β-pinene and trans-sabinene hydrate acetate/α-terpineol.

Table VI reports the values of the above-indicated ratios for genuine bergamot oils (our 1,082 samples) together with the ratios obtained for the two well-reconstituted commercial oils. The ratios relative to the commercial bergamot oils are quite different from those reported for the genuine oils (or, in a few cases, near their minimum values), even though the ranges of the ratios reported in Table IV were very wide for the genuine oils. Certainly the values of these ratios help to define an unknown bergamot oil; however, we intend to further verify the usefulness of these ratios by analyzing a large number of reconstituted oils.

In conclusion, the enantiomeric distribution of some components, the ratios between some components and the composition of coumarins and psoralens, taken together, tell us a great deal about the genuineness and quality of unknown bergamot oil, even if it is a well-reconstituted oil.

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