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RESEARCH ARTICLE

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Effect of Harvesting Time on Volatile Compounds Composition of Bergamot (*Citrus × Bergamia*) Essential Oil

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Abstract

Bergamot (*Citrus × bergamia*) is mainly cultivated in Calabria area (Italy) for its essential oil that is widely used in cosmetics, food and medicine. The harvest season (November to February) affects the quality of essential oil, consequently the aim of our study was to evaluate the composition of volatile fractions at different harvesting stages. Two different cultivars, Femminello and Fantastico, were collected in November, December and January and their volatile compounds composition was determined by SPME GC/MS. Fourty-two compounds were identified. Monoterpenes were the preponderant group; limonene was the most representative compound with similar concentrations in the varieties and at the three harvesting times. β-Ocimene, β-mircene and α-terpinene showed higher concentration in Femminello than Fantastico, while *trans*-α-bergamotene, α-pinene and citronellal were mostly concentrated in Fantastico. The concentration of β-pinene in Fantastico and Femminello was higher in December and January, respectively, and γ-terpinene concentration was higher in Femminello in November and January and in Fantastico in December. Linalool was the most preponderant monoterpene alcohol with a significantly higher concentration in Femminello than in Fantastico, but without significant differences during ripening. *cis*-γ-Bisabolene was the most preponderant sesquiterpene; it was more concentrated in Fantastico than in Femminello and showed a significantly higher concentration in December and January than in November. Linalyl and neryl acetate were the most abundant esters, with a significantly higher concentration in Fantastico. Fantastico seems to be the variety that produces higher volatile organic compounds in essential oil; moreover, the volatile compound concentration was higher at the second and third harvest time.

KEYWORDS

bergamot, Fantastico, Femminello, SPME GC/MS, volatile compounds

1 | INTRODUCTION

Bergamot (*Citrus × bergamia* Risso et Poit.), which belongs to the family Rutaceae[1], originated as the result of a chance crossing of *C. aurantium x C. medica* with lime[2] and it is mainly cultivated in the area of Reggio Calabria, in the South of Italy. Most of the worldwide bergamot cultivation is concentrated in this region thanks to its optimal soil properties (clay-silt soils with pH lower than 6.5–7.5) and the excellent meteorological conditions (low humidity and high temperature all over the year[3]). Rutaceae plants are widely used in

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medical treatments because of their high content of active compounds.[4] Bergamot is mainly used to extract an essential oil that has great commercial value as it is used in perfumery industry both for fixing the aromatic bouquets of perfumes and for blending all the other essences, exalting notes of freshness and fragrance.[3] Bergamot essential oil is also used in pharmaceutical industries,[3] since it is characterized by biological activity on the central nervous and cardiovascular systems[5]. Moreover, since it is recognized as safe,[6] bergamot oil is also used in food industry, as a flavouring of liqueurs, tea, coffee, candies, ice cream and drinks.[3]

The importance of medicinal plants for their beneficial biological activities in the human body is well known[7,8]; in particular essential oils exhibit antioxidant, antifungal, antimicrobial and antibacterial activities.[9]

Cold-pressed bergamot oil, obtained by rasping the fruit peel, is mainly composed of volatile compounds (93–96%); non-volatile substances that are responsible for toxic reactions at high concentration[1] represent the remaining 4–7%.

During fruit ripening, size, shape, colour, taste, flavour and aroma change; in particular, the emission of volatile components evolves in order to protect immature fruits from pests and herbivores.[10] Monoterpenes and sesquiterpenes are the main constituents of essential oil, followed by alcohols, aldehydes and esters originating from those hydrocarbons.[11]

Many previous studies have evaluated the volatile compounds composition of bergamot oil using different techniques. Dugo et al.[12] and Verzera et al.[13] studied the essential oil composition of Calabrian and Sicilian bergamot, respectively. The first study reported the composition of 432 samples of bergamot essential oils and demonstrated that quantitatively the composition showed peculiar and reproducible variations during the whole productive season. The second one, instead, reported the composition of bergamot oils of different cultivars, revealing differences in composition between the cultivars. Verzera et al.[3] studied the positive effect of different rootstocks on the volatile compound composition by high-resolution gas chromatography (HRGC) and HRGC/mass spectrometry (MS), identifying 78 components. Sawamura et al.[14] studied the volatile components of bergamot oil using GC, GC/MS and gas chromatography-olfactometry (GC-O), and found that the major compounds were limonene, linalyl acetate and linalool; but also that bergamot-like odour components were (*Z*)-limonene oxide, decanal, linalyl acetate and geraniol.

Bourgou et al.[15] investigated the effect of ripening stage on the volatile compounds composition of essential oil from four different Tunisian fruits of citrus, namely bitter orange, lemon, orange maltaise and mandarin, and found that green fruits (unripe) had the highest limonene concentration, suggesting that fruits could be harvested at immaturity if the aim is to obtain the highest level of limonene.

A different study[11] on *Citrus aurantium* demonstrated that essential oil from ripe fruits showed the highest limonene concentration; on the other hand, unripe fruits had the highest linalool, myrcene and α-terpineol concentration.

Juice quality and volatile composition of red grapefruit[10] was evaluated at five different ripening stages and it was demonstrated that, as a consequence of fruits ripening, volatile emission decreased, with the exception of nootkatone (the main volatile organic compound [VOC] of grapefruit), which increased in ripe fruits.

Little is known on the effect of ripening stage on bergamot essential oil quality; consequently, an experiment with two varieties, Fantastico and Femminello, harvested at three different stages during the same season (2017/2018) was set up. The essential oil's composition of volatile compounds was measured with solid-phase microextraction (SPME) combined with gas chromatography/mass spectrometry (SPME-GC/MS), a recognized analytical chromatographic technique[16,17] suitable for this purpose, in order to evaluate the best ripening stage to enhance bergamot essential oil quality.

2 | EXPERIMENTAL

2.1 | Fruit harvest and sample preparation

Bergamot fruits of two different varieties, Fantastico and Femminello, were picked in four different areas (Saline Joniche, Croce Valanidi, Bova and San Gregorio) of Reggio Calabria province (approx. 38°06'41" N, 15°39'43" E). Fruits were harvested at three different harvesting stages: November 19th (fist harvest = 1H), December 3rd (second harvest = 2H) and January 7th (third harvest = 3H). At each sampling time eight fruits, four of each variety, from each area were picked and immediately sent to Cesena (north Italy) for oil extraction.

Extraction of oil was carried out in the laboratory by grating the rind and by pressing it in order to promote oil release. Oil was then transferred to a test tube and stored at -20 °C until analysis.

2.2 | SPME-GC/MS analysis

Before analysis, samples were centrifuged at 4500 rpm for 15 min. Headspace volatiles from each oil sample were measured by headspace solid-phase microextraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS), using a GCMS–QP2010 Plus (Shimadzu, Tokyo, Japan) equipped with an AOC 5000 autosampler (Shimadzu, Tokyo, Japan). About 10 mg of oil was diluted in 100 mL of water, and 1 mL of this solution was inserted into a 20 mL amber vial, sealed with aluminium crimp caps equipped with a septum. The samples were equilibrated at 40 °C for 30 min. A 2 cm x 0.11 µm (i.d.), 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) SPME fiber; Supelco, Bellefonte, PA, USA) was then inserted through the septum into the vial at 40 °C for another 10 min; vial penetration depth was 20 mm. Afterwards, the SPME fiber was desorbed at 240 °C for 7 min in the split mode. A ZB-Wax fused-silica capillary column (30 m × 0.25 mm i.d. × 1.0 µm f.t.; Phenomenex, Torrance, CA, USA) was used for the chromatographic separation.[18] The oven was programmed to warm from 40 °C (kept for 10 min) to 200 °C at 3 °C/min. After 3 min at 200 °C, the temperature was then increased by 10 °C/min up to 240 °C, and the

TABLE 1 Retention time, name, chemical class and retention index of the compounds identified in the volatile fraction of bergamot essential oil

No.[a]	Compound name	RT (min)[b]	Chemical class	RI[c]	LRI[d]
1	α-Pinene	13.8	Monoterpene	1039	1028 ± 8
2	α-Thujene	14.3	Monoterpene	1003	–
3	β-Pinene	19.4	Monoterpene	1122	1112 ± 7
4	Sabinene	20.4	Monoterpene	1140	1124 ± 8
5	β-Myrcene	23.2	Monoterpene	1185	1161 ± 7
6	α-Terpinene	24.0	Monoterpene	1197	–
7	Limonene	25.2	Monoterpene	1220	1200 ± 7
8	1,8-Cineole	25.6	Monoterpene	1228	1211 ± 9
9	β-Phellandrene	25.7	Monoterpene	1230	1213 ± 7
10	(*E*)-β-Ocimene	27.3	Monoterpene	1260	1235 ± 8
11	γ-Terpinene	27.8	Monoterpene	1269	1246 ± 9
12	(*Z*)-β-Ocimene	28.2	Monoterpene	1276	1250 ± 6
13	p-Cymene	29.3	Monoterpene	1295	–
14	δ-3-Carene	29.9	Monoterpene	1306	–
15	Octanal	30.2	Aldehyde	1313	1289 ± 9
16	Nonanal	35.4	Aldehyde	1420	1391 ± 8
17	β-Terpineol	38.6	Monoterpene alohol	1489	–
18	Octyl acetate	39.0	Ester	1497	1474 ± 10
19	Citronellal	39.4	Monoterpene	1506	1478 ±10
20	Decanal	40.2	Aldehyde	1525	1498 ± 8
21	Linalool	42.1	Monoterpene alohol	1570	1547 ± 7
22	Linalyl acetate	42.7	Ester	1583	1555 ± 10
23	*cis*-α-Bergamotene	43.2	Monoterpene	1594	-
24	Nonyl acetate	43.4	Ester	1599	1581 ± 7
25	*trans*-α-Bergamotene	43.9	Monoterpene	1611	–
26	β-Caryophyllene	44.6	Sesquiterpene	1629	1595 ± 16
27	Linalyl propionate	44.8	Monoterpene	1634	1656 ± 10
28	*cis*-β-Santalene	46.6	Sesquiterpene	1678	–
29	Citronellyl acetate	46.9	Ester	1685	1660 ± 8
30	*cis*-β-Farnesene	47.0	Sesquiterpene	1687	1746 ± 9
31	α-Caryophyllene	47.6	Sesquiterpene	1702	–
32	Neral	48.0	Aldehyde	1712	1680 ± 13
33	α-Terpineol	48.5	Monoterpene alohol	1726	1697 ± 10
34	Germacrene D	49.1	Monoterpene	1741	1710 ± 14
35	Neryl acetate	49.4	Ester	1749	1724 ± 9
36	β-Bisabolene	49.5	Sesquiterpene	1752	–
37	Germacrene B	50.1	Monoterpene	1767	1819 ± 19
38	α-Farnesene	50.3	Sesquiterpene	1772	1746 ± 9
39	Geranyl acetate	50.6	Ester	1780	1752 ± 11
40	*cis*-γ-Bisabolene	51.4	Sesquiterpene	1800	–
41	*cis*-Geraniol	52.3	Monoterpene alohol	–	1847 ± 10
42	Nerol	53.9	Monoterpene alohol	–	1797 ± 11

[a]Order of elution.
[b]Retention time.
[c]Retention index calculated here on the basis of compound RT and RTs of an alkane (C_8–C_{20}) standard mixture.
[d]Retention index and corresponding uncertainty given on a polar column by identification Software NIST Mass Spectral Search Program on the basis of literature data.

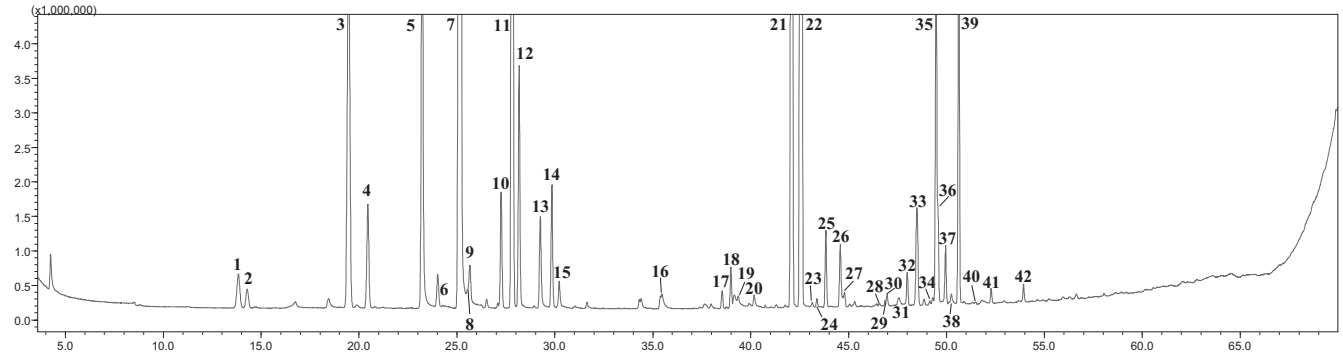

FIGURE 1 General chromatogram of volatile fraction of bergamot essential oil identified by GC-MS. Compounds are listed by number in Table 1

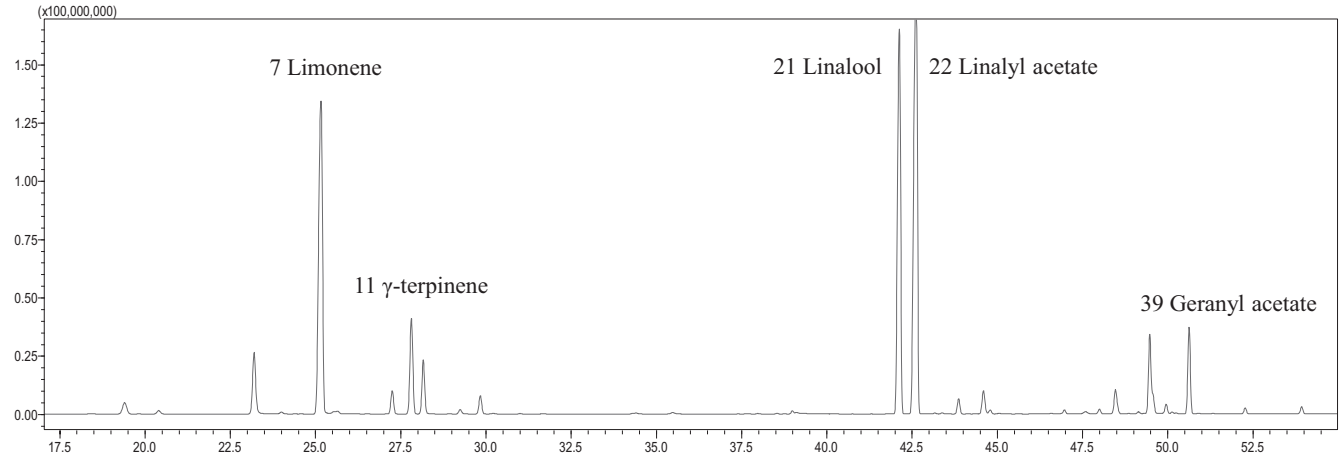

FIGURE 2 Five major constituents of essential oil sampled at the last harvest (January)

TABLE 2 Effect of variety and harvest time on the total concentrations (%) of different volatile compounds classes in bergamot oil

			Sesquiterpenes				
Variety	**Monoterpenes**	**Monoterpene alcohols**	**H1**	**H2**	**H3**	**Esters**	**Aldehydes**
Femminello	47.7	16.0	1.8	1.7	3.1	33.4	0.8
Fantastico	46.5	14.2	1.4	3.3	2.5	35.9	0.9
Significance	*n.s.*	*n.s.*	2*SEM* = 0.3			*	*n.s.*
Harvest time							
H1	44.9	16.3	-			36.4	0.70 b
H2	47.7	14.9	-			34.0	0.86 ab
H3	48.7	14.1	-			33.5	0.93 a
Significance	*n.s.*	*n.s.*	-			*n.s.*	*
Variety * harvest time	*n.s.*	*n.s.*	***			*n.s.*	*n.s.*

Data are expressed as mean of area percentage, $n = 8$. Abbreviations: H1, first harvest, November; H2, second harvest, December; H3, third harvest, January. *n.s.*: effect not significant; *: significant at $p \leq 0.05$. Values followed by the same letter are not statistically different according to a Student–Neuman–Keul test ($p \leq 0.05$). Values differing by 2*SEM* are statistically different.

final temperature was kept for 5 min. The injector, transfer line and the ion source temperatures were set at 240 °C, 240 °C and 200 °C, respectively. Helium was used as the carrier gas at an inlet pressure assuring a constant flow rate of 1.5 mL/min; the split ratio was 1:10.

The filament emission current was 70 eV. A mass range *m/z* from 30 to 250 was scanned from 3.5 to 70 min. The acquisition was carried out in Total Ion Current mode, using the GCMS solution software, version 2.50 SU1 (Shimadzu, Tokyo, Japan). Identification of

TABLE 3 Effect of variety and harvest time on monoterpene concentration (%) in bergamot oil

Variety	α-Pinene	α-Thujene	β-Pinene H1	β-Pinene H2	β-Pinene H3	Sabinene	β-Myrcene	α-Terpinene	Limonene	1,8-Cineole	β-Phellandrene	(*E*)-β-Ocimene
Femminello	0.20	0.1	1.7	1.5	2.2	0.4	3.13	0.17	28.1	0.06	0.25	0.8
Fantastico	0.24	0.1	1.6	2.8	1.8	0.4	2.96	0.16	27.6	0.06	0.27	0.7
Significance	*	*n.s.*	2*SEM* = 0.5			*n.s.*	*	*	*n.s*	*n.s.*	*n.s.*	*n.s.*
Harvest time												
H1	0.17 b	0.06 b	–			0.35	2.9 b	0.13 c	28.2	0.08 a	0.24 b	0.7
H2	0.26 a	0.09 a	–			0.44	3.1 a	0.16 b	26.9	0.08 a	0.25 ab	0.7
H3	0.24 a	0.09 a	–			0.43	3.1 a	0.21 a	28.5	0.03 b	0.28 a	0.8
Significance	**	**	–			*n.s.*	*	***	*n.s.*	***	*	*n.s.*
Variety * harvest time	**	*	***			**	*n.s.*	**	*n.s.*	***	**	*n.s.*

Data are expressed as mean of area percentage, $n = 8$. Abbreviations: H1, first harvest, November; H2, second harvest, December; H3, third harvest, January. *n.s.*: effect not significant; *, **, ***:, significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively. Values followed by the same letter are not statistically different according to a Student–Neuman–Keul test ($p \leq 0.05$). Values differing by 2*SEM* are statistically different.

TABLE 4 Effect of variety and harvest time on monoterpene concentration (%) in bergamot oil

Variety	γ-Terpinene H1	γ-Terpinene H2	γ-Terpinene H3	(*Z*)-β-Ocimene	ρ-Cymene	δ-3-Carene	Citronellal	*cis*-α-Bergamotene	*trans*-α-Bergamotene	Linalyl propionate	Germacrene D	Germacrene B
Femminello	7.6	5.7	8.8	1.9	0.39	0.9	0.06	0.05	0.81	0.09	0.04	0.03
Fantastico	6.1	9.3	8.0	1.7	0.32	0.8	0.08	0.06	0.94	0.10	0.06	0.03
Significance	2*SEM* = 1.1			*	*n.s.*	*n.s.*	*	*	*	*n.s.*	*n.s.*	*n.s.*
Harvest time												
H1	–			1.6 b	0.36	0.68 c	0.07 b	0.04 b	0.61 b	0.10	0.04 b	0.02 b
H2	–			1.9 a	0.40	0.84 b	0.05 b	0.06 a	0.94 a	0.11	0.06 a	0.04 a
H3	–			1.9 a	0.31	0.97 a	0.09 a	0.07 a	1.08 a	0.09	0.06 a	0.04 a
Significance	–			**	*n.s.*	***	***	***	***	*n.s.*	*	*
Variety * harvest time	***			*n.s.*	*n.s.*	***	*n.s.*	*n.s.*	***	*n.s.*	***	**

Data are expressed as mean of area percentage, $n = 8$. Abbreviations: H1, first harvest, November; H2, second harvest, December; H3, third harvest, January. *n.s.*: effect not significant; *, **, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively. Values followed by the same letter are not statistically different according to a Student–Neuman–Keul test ($p \leq 0.05$). Values differing by 2*SEM* are statistically different.

TABLE 5 Effect of variety and harvest time on monoterpene alcohol concentration (%) in bergamot oil

Variety	β-Terpineol	Linalool	α-Terpineol H1	α-Terpineol H2	α-Terpineol H3	*cis*-Geraniol	Nerol
Femminello	0.04	15.6	1.1	1.1	1.4	0.15	0.2
Fantastico	0.05	13.0	0.7	1.3	1.0	0.11	0.1
Significance	*n.s.*	*	2*SEM* = 0.2			**	**
Harvest time							
H1	0.05	15.2	–			0.11	0.1 b
H2	0.05	14.2	–			0.15	0.2 a
H3	0.04	13.1	–			0.13	0.2 a
Significance	*n.s.*	*n.s.*	–			*n.s.*	**
Variety * harvest time	*n.s.*	*n.s.*	***			*n.s.*	*n.s.*

Data are expressed as mean of area percentage, $n = 8$. Abbreviations: H1, first harves, November; H2; second harvest, December; H3, third harvest, January. *n.s.*: effect not significant; *, **: significant at $p \leq 0.05$, $p \leq 0.01$, respectively. Values followed by the same letter are not statistically different according to a Student–Neuman–Keul test ($p \leq 0.05$). Values differing by 2*SEM* are statistically different.

volatile compounds was performed by comparing their mass spectra with those reported in the literature and the NIST Mass Spectral Database (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, USA). The analysis was repeated twice for each essential oil sample ($n = 2$).

2.3 | Statistical analysis

All the data were statistically analysed using the software SAS (SAS Institute Inc., Cary, NC, USA) in a factorial experimental design with variety (2 levels: Fantastico and Femminello) and harvest time (3 levels: H1, H2 and H3) as main factors. When analysis of variance showed a statistically significant effect of treatment ($p \leq 0.05$), means were separated by a Student–Newman–Keuls (SNK) test. When interaction between factors was significant, only for volatiles with a concentration higher than 1%, two times the standard error of means (SEM) was used as the minimum difference between two statistically different means for $p \leq 0.05$.

3 | RESULTS AND DISCUSSION

In the present experiment fourty-two volatile compounds were determined and grouped in five different classes: monoterpenes, monoterpene alcohols, sesquiterpenes, esters and aldehydes (Table 1 and Figure 1). Figure 2 shows the five most preponderant compounds of each characterized essential oil; they were limonene, γ-terpinene, linalool, linalyl acetate and geranyl acetate in order of elution. Monoterpenes were the most represented group; their concentration was not significantly influenced by the fruit variety or the sampling time (Table 2). This is in line with several studies[1,3,14,19,20] that assessed monoterpenes are the major group in bergamot essential oil. In agreement with our results, in a previous study Bourgou and collaborators[15] reported that monoterpenes reach a maximum concentration of 48.7% at full maturity. Esters were the second most represented group, followed by monoterpene alcohols, sesquiterpenes and aldehydes (Table 2). The concentration of monoterpene alcohols was not significantly influenced by the variety or the harvest time (Table 2). Sesquiterpene concentration was higher in Femminello than in Fantastico at H1 and H3, while the opposite was observed at H2 (Table 2). Sesquiterpene concentration in Femminello was similar at H1 and H2 and it increased at H3; Fantastico showed the highest concentration at H2, followed by H3 and H1 (Table 2). The concentration of esters was higher in Fantastico than in Femminello, but it was not influenced by sampling time (Table 2). Aldehyde concentration was not influenced by the variety, but it increased at the last harvest time in comparison with the first one; H2 showed intermediate values not significantly different from other sampling times (Table 2).

Limonene, (*Z*)-β-ocimene, β-mircene and β-pinene were the most preponderant monoterpenes, in both varieties (Tables 3 and 4); similar to our results, other authors[19,20] observed that, together with linalool and linalyl acetate, limonene is one of the most important compounds in bergamot essential oil. Limonene is characterized by a cool, fresh and minty odour,[14] but in the present experiments its concentration was not influenced by variety and harvest time (Table 3). In comparison with the values reported in literature[1,14,19], the concentrations we measured (27.6–28.1%) were lower. β-Mircene and α-terpinene concentrations were significantly higher in Femminello than in Fantastico (Table 3). It is well known that β-mircene induces a grassy and metallic aroma,[5] indicating Femminello as the cultivar with a more pungent bouquet. The concentration of α-pinene was significantly higher in Fantastico than in Femminello (Table 3); while α-thujene, sabinene, 1,8-cineole, β-phellandrene, and (*E*)-β-ocimene were not influenced by the variety (Table 3). α-Pinene, α-thujene, and β-mircene concentration increased from first to second harvest, while it was steady in the third (Table 3). β-Mircene, γ-terpinene and δ-3-carene showed an increase of their concentration with

TABLE 6 Effect of variety and harvest time on sesquiterpene concentration (%) in bergamot oil

	β-Caryophillene						β-Bisabolene				
Variety	**H1**	**H2**	**H3**	***cis*-β-Santalene**	***cis*-β-Farnesene**	**α-Caryophillene**	**H1**	**H2**	**H3**	**α-Farnesene**	***cis*-γ-Bisabolene**
Femminello	0.9	0.9	1.6	0.03	0.11	0.1	0.60	0.54	1.03	0.01	0.01
Fantastico	0.7	1.4	1.1	0.03	0.15	0.1	0.41	1.36	0.99	0.02	0.02
Significance	*2SEM* = 0.3			*n.s.*	*	*n.s.*	*2SEM* = 0.1			*n.s.*	**
Harvest time											
H1	–			0.01 b	0.10 b	0.1 b	–			0.02	0.01 b
H2	–			0.03 a	0.15 a	0.2 a	–			0.02	0.03 a
H3	–			0.04 a	0.15 a	0.2 a	–			0.01	0.01 b
Significance	–			***	**	**	–			*n.s.*	**
Variety * harvest time	***			***	***	**	***			*n.s.*	***

Data are expressed as mean of area percentage, $n = 8$. Abbreviations: H1, first harvest, November; H2, second harvest, December; H3, third harvest, January. *n.s.*: effect not significant; *, **, ***: significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively. Values followed by the same letter are not statistically different according to a Student–Neuman–Keul test ($p \leq 0.05$). Values differing by 2*SEM* are statistically different.

harvesting time (Tables 3 and 4). 1,8-Cineole showed its highest concentration at the first two sampling times; while β-phelladrene showed the highest values in January, not different from December but higher than in November (Table 3). Sabinene and (*E*)-β-ocimene did not show any effect of sampling time (Table 3). β-Pinene gives a green, smoky and woody aroma[14]; its concentration was similar in the two varieties, at H1 and H3; while at H2 Fantastico showed an increase of this compound concentration compared to Femminello (Table 3). In Femminello, β-Pinene concentration was similar at H1 and H2 and increased at the end of the season; while in Fantastico β-pinene concentration was lower at H1 and H3 in comparison to H2 (Table 3). γ-Terpinene, gives an oily and smoky aroma[14], and its concentration in bergamot oil harvested in November and January was higher in Femminello than in Fantastico; while in December Fantastico displayed an increase of this compound concentration in comparison to Femminello (Table 4). (*Z*)-β-Ocimene (responsible for a green and spicy aroma[5]) concentration was significantly higher in Femminello than in Fantastico (Table 4). The concentration of citronellal, *cis*-α-bergamotene and *trans*-α-bergamotene was significantly higher in Fantastico than Femminello (Table 4); while ρ-cimene, δ-3-carene, linalyl propionate, germacrene D and germacrene B were not influenced by the variety (Table 4). (*Z*)-β-Ocimene, *cis*-α-bergamotene, *trans*-α-bergamotene, germacrene D and germacrene B concentration increased from first to second and third harvest, the latter not being significantly different (Table 4). ρ-Cymene and Linalyl proprionate were not influenced by sampling time (Table 4). δ-3-carene increased with harvest time (Table 4); while Citronellal concentration was higher at H3 than at H2 and H1, which showed similar values (Table 4).

Limonene and β-pinene concentrations measured in the present experiment were slightly lower than those reported in the literature; while (*Z*)-β-ocimene and β-mircene concentrations were higher than in previous reports.[1,3,14,19] In a study by Kirbaşlar et al.[21] on Turkish bergamot oil, a lower concentration of limonene (23.7%) and a similar concentration of β-pinene (3%) were found, compared to our experiment.

In bergamot oil, linalool, which gives a flowery, fruity, lavender and sweet aroma,[3,14] was the preponderant alcohol. Among monoterpene alcohols, linalool, *cis*-geraniol and nerol showed a significantly higher concentration in Femminello than in Fantastico; β-terpineol was the only alcohol not influenced by the variety (Table 5). α-Terpineol concentration was higher in Femminello than in Fantastico at first and third harvest, while at the second sampling time there was not significant difference between the two varieties (Table 5). In Femminello, α-terpineol concentration was similar in the first and second harvest and increased in the last one (Table 5). On the other hand, Fantastico showed the highest α-Terpineol concentration in December, followed by January and November (Table 5). Nerol concentration was significantly higher in essential oil sampled in the last two harvests (December and January) than in the first one; meanwhile, no significant differences between sampling times were observed for other compounds (Table 5). Nerol is an important compound for

TABLE 7 Effect of variety and harvest time on esters concentration (%) in bergamot oil

		Linalyl acetate						Geranyl acetate		
Variety	Octyl acetate	H1	H2	H3	Nonyl acetate	Citronellyl acetate	Neryl acetate	H1	H2	H3
Femminello	0.22	27.0	28.3	22.1	0.04	0.02	3.6	3.5	2.8	4.8
Fantastico	0.30	33.6	24.9	28.3	0.07	0.03	3.1	2.3	4.2	3.7
Significance	**	2*SEM* = 3.1			**	*n.s.*	**	2*SEM* = 0.7		
Harvest time										
H1	0.22	-			0.04 b	0.01 b	2.9 b	-		
H2	0.23	-			0.06 ab	0.02 b	3.6 a	-		
H3	0.31	-			0.07 a	0.05 a	3.5 a	-		
Significance	*n.s.*	-			*	**	**	-		
Variety*harvest time	**	***			***	**	*n.s.*	***		

Data are expressed as mean of area percentage, $n = 8$. Abbreviations: 1H, first harvesting, November; 2H, second harvesting, December, 3H, third harvesting, January. n.s., *, **, ***: effect not significant, significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively. Values followed by the same letter are not statistically different according to a Student–Neuman–Keul test ($p \leq 0.05$). Values differing by 2SEM are statistically different.

TABLE 8 Effect of variety and harvest time on aldehyde concentration (%) in bergamot oil

Variety	Octanal	Nonanal	Decanal	Neral
Femminello	0.10	0.05	0.1	0.6
Fantastico	0.16	0.09	0.1	0.5
Significance	*	**	*n.s.*	*n.s.*
Harvest time				
H1	0.10b	0.05 a	0.05 b	0.5 b
H2	0.14 a	0.08 a	0.10 ab	0.6 a
H3	0.15 a	0.07 a	0.13 a	0.6 a
Significance	**	*n.s.*	**	**
Variety*harvest time	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Data are expressed as mean of area percentage, $n = 8$. Abbreviations: H1, first harvest, November; H2, second harvest, December; H3, third harvest, January. *n.s.*: effect not significant; *, **:, significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. Values followed by the same letter are not statistically different according to a Student–Neuman–Keul test ($p \leq 0.05$)

aroma because it gives the sour citrusy flavour[14]. Linalool concentration was is in line with that in some previous studies[3,19-21] and higher than in others[1,14]; α-terpineol and nerol concentrations in this study were higher than those reported in the literature.[1,3,14,19] Earlier reports[11,15] showed that a lower linalool concentration corresponded to a higher limonene concentration; in our study a similar response was observed at the last harvesting time; however, the effect of sampling time was not significant.

The sesquiterpenes measured in bergamot essential oil were, in decreasing order: β-caryophillene, β-bisabolene, α-caryophillene, *cis*-β-farnesene, *cis*-β-santalene, α-farnesene and *cis*-γ-bisabolene (Table 6). *cis*-β-Farnesene and *cis*-γ-bisabolene concentrations were significantly higher in Fantastico than Femminello; while *cis*-β-santalene, α-farnesene and α-caryophillene concentrations were not influenced by the variety. β-Caryophyllene, was the most important sesquiterpene, it is responsible for a citrusy and flowery aroma.[14] Although at the first harvest (November) no significant differences were found between the two varieties, at the second harvest (December) a higher concentration was registered in Fantastico than in Femminello; at the end of ripening (January), however, the opposite was found (Table 6). Femminello showed the highest concentration in January, while no significant differences were observed in the first sampling (Table 6). Fantastico showed higher concentration in December and January than in November (Table 6). Femminello showed a higher concentration of β-Bisabolene than Fantastico in the first harvest (November); while the opposite was found at H2. In January no significant differences between two varieties were observed (Table 6). Concentration of β-bisabolene in Femminello essential oil was significantly higher in January, than at the other two harvest times. In Fantastico the highest β-bisabolene concentration was measured in December followed by November and January (Table 6).

The concentrations of both prevalent sesquiterpenes, β-caryophyllene and β-bisabolene, identified in this study were higher than those reported in the literature[1,3,14,19,20]; while in Turkish bergamot oil the concentration of β-bisabolene was similar to that in ours (1.2%).[21]

The esters in bergamot essential oil were linalyl acetate, geranyl acetate, neryl acetate, octyl acetate, nonyl acetate and citronellyl acetate in decreasing order (Table 7). Linalyl acetate was the most preponderant ester and its concentration was significantly higher, at H1 and H3, in Fantastico than in Femminello; on the other hand, at H2, the opposite was registered (Table 7). In Femminello, linalyl acetate concentration was similar at H1 and H2, while it decreased at H3; in Fantastico its concentration decreased about 26% between the second and first harvests and increased again by about 14% in January (Table 7). Linalyl acetate is one of the most important compounds for the flavour because its aroma is defined as bergamot-like and flowery.[14] Geranyl acetate, which gives citrusy, fruity, floral and herbal aromas,[3,14] was significantly higher at H1 and H3 in Femminello than in Fantastico;

at H2 it was higher in Fantastico than in Femminello (Table 7). In Femminello the highest concentration was recorded at the end of the season; intermediate values were found in November, while they were lowest in December (Table 7). In Fantastico, geranyl acetate concentration increased about 78% between the first and second harvest and decreased about 10% in January compared to the second one (Table 7). Octyl acetate and nonyl acetate concentrations were significantly higher in Fantastico than Femminello; on the contrary, neryl acetate was higher in Femminello than Fantastico (Table 7). Finally, for citronellyl acetate no significant differences between varieties were observed. The concentration of neryl acetate was similar at H2 and H3 and higher than at H1 (Table 7). The concentration of nonyl acetate was higher in January than November, while in December there were similar concentrations (Table 7). At the third harvest, the citronellyl acetate concentration was higher than at H1 and H2, which showed similar values (Table 7).

The concentrations of linalyl acetate determined in this study are in accordance with those in other reports in the literature on Italian bergamot oil,[1,3,14,19] but lower than those in Turkish bergamot[21]; results for geranyl acetate and neryl acetate were higher than those reported in the same studies.[1,3,14,19,21]

Octanal was the preponderant aldehyde, followed by neral, decanal and nonanal (Table 8). Octanal and nonanal concentrations were significantly higher in Fantastico than in Femminello; no significant differences between varieties were observed for the other aldehydes (Table 8). Concentrations of octanal and neral, which contribute to lemon aroma,[3] increased from first to second and third harvest, which were not different (Table 8). Nonanal was the only aldehyde whose concentration was not influenced by harvest time (Table 8). Decanal increased from first to third harvest; the values measured at the second sampling time were intermediate and not different from H1 and H3 (Table 8). Several authors investigated aldehyde concentrations in bergamot oil[1,3,14,19,21]; all of them found values lower than those reported in this study.

The ratio of linalool to linalyl acetate is one of the authenticity and quality indices of bergamot essential oil. According to Statti et al.[22] if this ratio is lower the quality of the essential oil is better, because during ripening linalool tends to become linalyl acetate, following a declining trend. In this study, Fantastico and Femminello showed different ratios that, averaged over the three harvest times, were 0.455 and 0.566, respectively, which is slightly higher than those in the literature.[12] The different values obtained in this study could be due, beside harvest time, to extraction techniques, climatic and agronomic conditions.

4 | CONCLUSION

During the ripening stages of bergamot, five different groups of volatile compounds were found in the essential oil. The ripening stage affects significantly the volatile compounds composition of bergamot oil; monoterpenes and aldehydes showed a significant increase during ripening, balanced by a decrease of monoterpene alcohols and esters; sesquiterpenes, instead, showed an unstable trend during ripening.

Knowledge of the influence of ripening stage on VOC production has important consequences for industrial output, as harvest could be programmed in those periods when VOCs are most abundant.

Fantastico seemed to produce a higher concentration of volatiles than Femminello and, in addition, had a lower linalool/linalyl acetate ratio, indicating a higher product quality. Consequently, Fantastico may be preferred at the industrial level, also taking into consideration that it produces for a longer period and with a higher yield than Femminello. The comparisons between the two varieties and the results obtained are very important for farmers since they can consciously choose the best variety to plant from a production and quality point of view. Moreover, industries can adjust the prodution process in relation to the variety in order to optimize essential oil extraction.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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