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Harvest timing and cultivar effects on phenolics and volatiles in bergamot juice and essential oil

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Abstract. *Gattuso A, Mafrica R, Mafrica D, De Bruno A, Poiana M. 2025. Harvest timing and cultivar effects on phenolics and volatiles in bergamot juice and essential oil. Asian J Agric 9: 889-897.* Bergamot (*Citrus bergamia*) is traditionally cultivated for essential oil production, but increasing interest in its health-promoting properties has enhanced the relevance of juice and derived products. However, the combined influence of harvest time and cultivar on the seasonal evolution of phenolic compounds in juice and volatile constituents in essential oil remains insufficiently defined. This study investigated these changes in the two main Protected Designation of Origin (PDO) cultivars, 'Fantastico' and 'Femminello'. Fruits were collected monthly from November to June, and phenolic compounds in juice and volatile constituents of essential oil were analyzed in parallel using UHPLC-DAD and GC-FID, respectively. Significant differences were observed both between cultivars and across ripening stages. Phenolic compounds in bergamot juice increased progressively during the harvest season, with higher late-season accumulation in 'Femminello,' reflecting both enhanced biosynthesis and dilution-concentration effects associated with fruit growth. In essential oil, ripening was characterized by a marked decrease in monoterpene alcohols and a concomitant increase in acetate esters, indicating a seasonal shift that strongly influences aromatic quality. Early harvests yielded oils richer in linalool and fresher aromatic notes, whereas mid- to late-season harvests were associated with higher ester content and smoother, more persistent fragrance profiles. These findings provide the first integrated, month-by-month characterization of phenolic and volatile dynamics in the two main bergamot cultivars. By linking biochemical evolution to harvest timing, the results offer practical guidance for defining cultivar-specific harvest windows aimed at maximizing functional phytochemicals in juice and tailoring the aromatic profile of essential oil, thereby supporting improved decision-making in bergamot production and processing.

Keywords: Bergamot cultivars, essential oil composition, juice quality, phenolic compounds, ripening dynamics

INTRODUCTION

Citrus fruits (Rutaceae) represent one of the most widely cultivated fruit crops worldwide due to their broad genetic diversity, adaptability to different pedo-climatic conditions, and high economic value. Their importance is linked not only to fresh fruit consumption but also to the production of processed derivatives, including juices and essential oils, which are appreciated for their sensory attributes and for their content of health-promoting compounds such as vitamins, dietary fiber, carotenoids, and polyphenols (Adenaike and Abakpa 2021). Among the citrus species, bergamot (*Citrus bergamia* Risso & Poiteau) is characterized by a highly restricted cultivation area, almost entirely limited to the coastal zone of Reggio Calabria in southern Italy. Three cultivars are recognized, Fantastico (FA), Femminello (FE), and Castagnaro (CA), with Fantastico and Femminello accounting for the greatest diffusion and economic yield (Strano et al. 2017).

Traditionally, bergamot has been grown for the extraction of its essential oil (Bergamot Essential Oil/BEO), which is obtained by cold pressing the flavedo and part of the mesocarp and has been protected by a European Protected Designation of Origin (PDO) since 1999. Bergamot essential oil is widely employed in perfumery, cosmetics, pharmaceuticals, and household products, and its use in food-related applications has also expanded in recent years (Sharmeen et al. 2021).

The yield and chemical composition of bergamot essential oil are influenced by several factors, including cultivar, harvest time, environmental conditions, and fruit physiological status (Masotti et al. 2003). Seasonal changes in volatile composition, particularly involving monoterpene hydrocarbons, oxygenated monoterpenes, and acetate esters, are known to affect aromatic quality and industrial suitability (Marzocchi et al. 2019). In addition to its aromatic value, bergamot essential oil has been associated with antimicrobial, antioxidant, and anti-inflammatory properties (Manzur et al. 2023), further supporting its relevance in multiple industrial sectors.

In parallel with essential oil production, increasing attention has been directed toward bergamot juice due to its high content of flavonoids and other phenolic compounds. Several studies have documented the antioxidant, anti-inflammatory, and antimicrobial activities of bergamot juice and its phenolic fraction (Filocamo et al. 2015; Giglio

et al. 2016; Impellizzeri et al. 2016). However, the concentration and profile of these compounds are strongly influenced by cultivar, ripening stage, and environmental conditions, which can markedly affect both nutritional value and sensory properties of the juice.

Despite the economic and industrial importance of bergamot, a relevant knowledge gap persists. Previous studies have generally focused either on the phenolic composition of bergamot juice or on the volatile profile of the essential oil, often considering single cultivars or limited harvest periods (Giuffrè 2019; Marzocchi et al. 2019). To date, no research has provided a systematic, parallel assessment of phenolic and volatile profiles across the entire harvest season while directly comparing the two main PDO cultivars. Such integrated information is essential for optimizing harvest management, improving product standardization, and supporting value-oriented decision-making along the bergamot production chain.

Building on recent work that identified cultivar-specific ripening dynamics and optimal harvest timing for Fantastico and Femminello based on bio-agronomic parameters (Mafrica et al. 2025), the present study aims to address this gap by providing the first month-by-month, season-long characterization of phenolic compounds in bergamot juice and volatile constituents in bergamot essential oil. Fruits were sampled from November to June to capture the full range of ripening-related biochemical changes.

The objectives of this study were therefore to evaluate: (i) the seasonal evolution of phenolic compounds in bergamot juice; (ii) the dynamics of major volatile constituents in bergamot essential oil; and (iii) the implications of these biochemical changes for defining cultivar-specific harvest strategies aimed at maximizing nutritional, functional, and aromatic quality. By integrating juice and essential oil profiling across the complete harvest window, this work provides new insights into the biochemical development of bergamot fruit and provides practical guidance for growers and industry stakeholders within the PDO production system. We hypothesized that harvest timing and cultivar significantly influence the seasonal evolution of phenolic compounds in bergamot juice and the volatile composition of bergamot essential oil, such that phenolic accumulation in juice increases with ripening while volatile profiles shift from monoterpene alcohols toward acetate esters in a cultivar-dependent manner.

MATERIALS AND METHODS

Fruits sampling and processing

Bergamot fruits of the Fantastico (FA) and Femminello (FE) cultivars, both grafted onto sour orange (*Citrus aurantium* L.), were harvested from an experimental orchard located in Melito di Porto Salvo, in the province of Reggio Calabria (PDO-designated area; 15°48′22″ E, 37°55′22″ N). The orchard was established in 2016, and at the time of sampling, the trees were 5-6 years old and in full productive phase. Management practices were uniform across the orchard: irrigation was supplied via drip lines every three days from April to October; fertilization followed standard regional guidelines with annual applications of nitrogen, phosphorus, and potassium; trees were hand-pruned after harvest to maintain canopy balance; and pest and disease control complied with integrated management protocols. Sampling was conducted monthly from November 2021 to June 2022, following a fully crossed factorial design consisting of two cultivars (FA, FE) and eight harvest dates (2 × 8 design). For each cultivar and time point, three biological replicates were collected. Each replicate consisted of 12 fruits harvested from different trees to ensure independence and avoid pseudoreplication. The twelve fruits belonging to each biological replicate were processed together and treated as a single experimental unit for both juice extraction (phenolic profiling) and essential oil extraction (volatile profiling). Meteorological data for the entire sampling period were obtained from ARPACAL and are reported in Table 1. Although the ARPACAL meteorological station is located approximately 30 km from the experimental orchard, it lies within the same coastal belt of southern Calabria and is characterized by comparable altitude, maritime influence, and prevailing wind patterns; therefore, the recorded temperature and rainfall data are considered representative of the microclimatic conditions at the study site.

Bergamot Juice (BJ) was obtained by manually squeezing the fruits using a commercial juicer. Essential oil (BEO) was extracted manually by scraping the flavedo of twenty fruits. The peel was mixed with cold distilled water and pressed to facilitate the release of oil from the glandular cavities. The resulting emulsion was centrifuged at 9,000 rpm for 10 min at 4°C (NF 1200R, Nüve, Ankara, Turkey). The supernatant was then transferred into 5 mL Eppendorf tubes and centrifuged again at 13,000 rpm to remove residual water and solid impurities.

Table 1. Meteorological data (monthly mean temperature and rainfall) for the period January 2021 to June 2022, recorded by the ARPACAL station at Capo Spartivento, Italy, located approximately 30 km from the experimental site

Year	Month	Mean temperature	Rainfall
2021	Jan	12.0	46.4
2021	Feb	12.3	41.6
2021	Mar	11.7	54.2
2021	Apr	13.8	17.8
2021	May	19.0	15.4
2021	Jun	24.7	12.0
2021	Jul	27.1	4.4
2021	Aug	28.2	10.4
2021	Sep	24.0	52.6
2021	Oct	18.6	97.1
2021	Nov	16.8	104.6
2021	Dec	12.5	87.0
2022	Jan	10.9	49.4
2022	Feb	11.9	50
2022	Mar	10.9	44
2022	Apr	14.6	14.2
2022	May	19.6	15.2
2022	Jun	26.5	5.4

Determination of individual Phenolic Compounds (PC) on BJ

The method reported by De Bruno et al. 2023 was followed for the chromatographic analysis of PC. BJ was centrifuged (8.000 rpm, 5 min, 4°C) with a centrifuge apparatus (NF-1200R, Nüve, Ankara, Turkey), and 2 µL of supernatant (filtered with RC, 0.45 µm, diameter 15 mm) was injected into a UHPLC PLATINblue instrument (Knauer, Berlin, Germany). A Knauer blue orchid C18 column (1.8 mm, 100 × 2 mm) was used for the compound's separation. Chromatographic separation followed the gradient reported in Table 2. Phenolic compounds were detected at their specific absorption maxima: Neoeriocitrin, Naringin, Neohesperidin, Eriocitrin, Narirutin, Melitidin, and Brutieridin were monitored at 280 nm, while p-coumaric acid and ferulic acid were detected at 305 nm.

Quantification was performed using external calibration curves prepared in the range 1-100 mg/L (R^2>0.999). The method was validated by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ), calculated as 3.3 × SD and 10 × SD, respectively, where SD corresponds to the standard deviation of the analytical response. Only calibration points showing RSD ≤10% were accepted. Results were expressed as mg L^{-1} of juice. All samples were analyzed using three biological replicates per sampling date, each measured in duplicate.

In addition, the dilution effect of juice polyphenols during ripening was assessed to understand variations in polyphenol content as the juice yield changes. It was obtained by multiplying the average juice yield by the average fruit weight by the total polyphenol content as follows:

JW (kg) = (FW x JY)/1000
JP (mg/kg) = JW x TP
Where:
FW: Fruit Weight
Jy: Juice Yield
Jw: Juice Weight
Tp: Total Polyphenols
Jp: Juice Polyphenols

Determination of the volatile fraction of BEO

The methodology described by Gionfriddo et al. 2003 was used to carry out the characterization of BEO. A GC2010 gas chromatograph equipped with a Flame Ionization Detector (FID) and a capillary column made of fused silica (DB-5MS: Inner Diameter: 0.25mm, Length: 30 m, Film: 0.25µm) was used. The temperature program consisted of heating at 70°C for 10 minutes, followed by heating at 3°C min^{-1} to 120°C, heating from 130°C to 220°C at 4°C min^{-1}, maintaining at 220°C for 5 minutes, heating from 220°C to 280°C at 15°C min^{-1}, and maintaining at 280 °C for 10 minutes. The operative conditions involved using helium as carrier gas with a flow rate of 1.5 mL min^{-1}, a split ratio of 1:100, and the injector was set at 230°C. FID was set at 250°C, and the injection volume was 0.2 µL, manually injected in split mode. Three injections were performed for each sample. Compounds identification was performed considering their retention times and the retention times of the external standards used. Peak area percentages were handled using GCsolution software (Shimadzu, Japan), and relative concentrations of components were obtained by peak area normalization (%).

The performance method was verified by monitoring retention-time stability across the analytical sequence and confirming consistent peak-shape resolution in the three technical injections per sample.

Statistical analysis

The experimental design consisted of a fully crossed factorial arrangement with two cultivars (FA, FE) and eight harvest dates. For each cultivar × harvest combination, three biological replicates were used as experimental units. All measurements were performed with technical replicates (triplicates for UHPLC and spectrophotometric assays; duplicates for GC), which were averaged prior to analysis. For each response variable, the effects of cultivar, harvest date, and their interaction were evaluated using two-way ANOVA. When significant main or interaction effects were detected, multiple comparisons were performed using Tukey's HSD test at a significance level of $p < 0.05$. Statistical analyses were carried out using SPSS (Version 15.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Polyphenol composition of bergamot juice

Dynamics of hydroxycinnamic acids

Hydroxycinnamic acids represented a minor but consistent fraction of the bergamot phenolic profile, with p-coumaric acid and ferulic acid being the only compounds detected, in agreement with previous findings in *Citrus* spp. juices (Jabri and Marzouk 2013). These metabolites, which contribute to lignin formation and stress-response pathways (Ismail and Brown 1975; Ledesma-Escobar et al. 2018), exhibited limited seasonal variation compared with the other phenolic classes.

Table 2. Elution condition of the UHPLC analysis

Time (min)	Solvent A (%)	Solvent B (%)	Flow (mL min^{-1})	Column temperature
Initial	95	5	0.4	25°C
3	95	5	0.4	25°C
15	60	40	0.4	25°C
16	0	100	0.4	25°C
18	95	5	0.4	25°C
20	95	5	0.4	25°C

Note: Solvent A: Water acidified with formic acid until pH 3.10 and Solvent B: Acetonitrile

Across the eight-month harvest period, p-coumaric acid showed relatively stable concentrations in both cultivars, with only minor fluctuations and no marked month-to-month variation, except for a modest increase in Femminello (FE) at the end of the season (Table 3). Ferulic acid displayed slightly greater temporal variability, with a significant effect of harvest time ($p<0.01$): in Fantastico (FA), the highest concentrations were observed at the beginning of the season, followed by a gradual decline, whereas FE reached maximum values during late spring (May-June).

Overall, the narrow concentration ranges observed for both acids indicate that hydroxycinnamic acids are only marginally influenced by ripening processes. Cultivar-related differences became more evident in the final harvest months, when FE accumulated higher levels of both compounds compared with FA, suggesting a limited but genotype-dependent modulation at advanced ripening stages.

Evolution of major flavanones

Flavanones constituted the dominant phenolic class in bergamot juice, with neoeriocitrin, naringin, and neohesperidin representing the most abundant compounds (Table 4 and Figure 1). These neohesperidosides are known to strongly contribute to bergamot bitterness and bioactivity, in agreement with previous findings in bergamot and other citrus species (Gionfriddo et al. 1996; Giuffrè 2019; Li et al. 2023; Gattuso et al. 2024). In contrast, the rutinosides eriocitrin and narirutin were present at lower concentrations and showed more irregular seasonal patterns, consistent with their more limited sensory contribution.

Neoeriocitrin exhibited a clear late-season accumulation pattern in both cultivars, with relatively stable concentrations from November to February, followed by a marked increase from March onward. Significant cultivar differences emerged mainly in late spring (May-June), when FE accumulated higher levels than FA, in line with cultivar-dependent regulation of flavanone biosynthesis reported for citrus fruits (Li et al. 2023).

Naringin showed the most pronounced and consistent increase throughout ripening, confirming its key role in determining bergamot juice bitterness. Although both cultivars displayed progressive accumulation, FA showed an earlier increase, resulting in significant differences at mid-season (March-April). By the end of the harvest period, however, concentrations converged toward similarly high levels in both cultivars.

Neohesperidin followed a clearly cultivar-dependent trajectory: FA maintained relatively stable concentrations throughout the season, whereas FE showed a progressive and significant increase, particularly from March onward. This behavior is noteworthy considering the broad spectrum of biological activities attributed to neohesperidin, including antioxidant, anti-inflammatory, neuroprotective, and antidiabetic effects, which have stimulated increasing interest in its nutraceutical and pharmaceutical potential (Ortiz et al. 2022).

Among the rutinosides, eriocitrin and narirutin remained minor constituents and exhibited non-linear and less structured seasonal trends, as commonly reported for these flavanones in bergamot and other citrus juices (Sicari and Pellicanò 2016). Despite their lower abundance, narirutin is recognized as a biologically active dietary flavonoid with documented anti-allergic, neuroprotective, antidiabetic, and hepatoprotective properties (Mitra et al. 2022). The irregular accumulation patterns of these compounds suggest a stronger sensitivity to environmental factors and a limited involvement in the main ripening-related metabolic transitions.

Overall, major flavanones displayed pronounced seasonal dynamics, with marked late-season increases in bitter neohesperidosides and clear cultivar × harvest time interactions for naringin and neohesperidin. These results confirm that flavanone accumulation in bergamot juice is closely linked to ripening progression and modulated by cultivar-specific metabolic regulation.

Table 3. Phenolic acid (mg L^{-1}) content in BJs during ripening (mean values ± standard deviation)

Month	*p*-Coumaric acid			Ferulic acid		
	FA	FE	Sign	FA	FE	Sign.
NOV	14.0±0.1^{a}	14.7±1.56^{abc}	ns	13.9±0.3^{a}	14.0±1.3^{ab}	ns
DEC	13.0±1.9^{ab}	12.3±1.0^{c}	ns	10.6±1.6^{bc}	12.9±1.6^{abc}	ns
JAN	11.3±0.8^{b}	12.0±0.7^{c}	ns	9.3±0.7^{c}	10.0±0.8^{c}	ns
FEB	12.4±1.3^{ab}	12.1±0.4^{c}	ns	11.1±1.0^{bc}	11.0±0.3^{bc}	ns
MAR	13.5±0.5^{ab}	13.3±0.1^{bc}	ns	10.4±0.6^{c}	11.0±0.5^{bc}	ns
APR	13.7±0.6^{ab}	13.1±0.7^{bc}	ns	10.4±0.5^{c}	10.8±1.5^{c}	ns
MAY	14.8±0.9^{a}	16.5±0.9^{abc}	ns	12.8±0.6^{ab}	14.3±0.3^{a}	*
JUN	11.2±0.4^{b}	15.4±1.6^{b}	*	10.8±0.3^{bc}	14.0±1.3^{ab}	*
Sign.	**	**		**	**	

Note: Data were reported as mean ± standard deviation of 5 replications. Lowercase letters within a column indicate significant differences as determined by Tukey's post hoc analysis. Sign.: Significance, **: Significance at $p < 0.01$, *: Significance at $p < 0.05$, ns: Not significant

Table 4. Flavonoid composition and total polyphenol content in BJs

Month	Neoeriocitrin			Naringin			Neohesperidin			Eriocitrin		
	FA	**FE**	**Sign.**	**FA**	**FE**	**Sign.**	**FA**	**FE**	**Sign.**	**FA**	**FE**	**Sign.**
NOV	120.0±1.9^{d}	110.3±15.5^{c}	ns	131.3±30.5^{b}	68.7±4.7^{c}	*	105.6±23.0	69.0±28.9^{bc}	ns	5.5±0.9^{b}	4.2±0.3^{b}	ns
DEC	107.9±13.0^{d}	115.4±18.3^{c}	ns	120.4±23.3^{b}	90.6±9.8^{c}	ns	103.1±6.1	69.7±11.5^{bc}	*	6.9±1.3^{ab}	3.8±0.2^{b}	*
JAN	115.3±20.7^{d}	95.0±7.1^{c}	ns	123.6±24.9^{b}	127.1±27.5^{b}	ns	104.9±18.8	61.8±6.2^{c}	*	6.2±0.6^{ab}	3.±0.2^{b}	**
FEB	173.0±17.6^{c}	194.2±8.3^{b}	ns	165.8±24.9^{ab}	134.9±9.4^{b}	ns	107.1±9.0	88.6±0.9^{abc}	*	6.6±0.9^{ab}	5.9±0.5^{a}	ns
MAR	169.0±10.3^{c}	201.3±7.2^{b}	*	204.7±17.0^{a}	144.2±3.6^{b}	**	102.8±8.5	79.3±5.8^{abc}	*	5.9±0.5^{b}	4.7±0.3^{ab}	*
APR	214.5±15.9^{b}	209.4±10.2^{b}	ns	201.7±32.5^{a}	143.2±5.^{b}	*	122.0±21.5	70.2±13.1^{bc}	*	7.2±0.4^{ab}	4.4±1.3^{ab}	*
MAY	256.7±15.1^{a}	360.4±6.7^{a}	**	216.9±24.9^{a}	245.0±3.60^{a}	ns	110.1±18.4	114.4±7.9^{a}	ns	8.2±0.5^{a}	4.9±0.8^{ab}	**
JUN	258.1±13.1^{a}	381.0±15.8^{a}	**	233.2±1.6^{a}	243.6±17.3^{a}	ns	121.7±12.5	99.2±1.0^{ab}	*	5.7±0.3^{b}	3.4±0.2^{b}	**
Sign.	**	**		**	**		n.s.	**		**	**	
	Narirutin			**Melitidin**			**Brutieridin**			**Total polyphenols**		
	FA	**FE**	**Sign.**	**FA**	**FE**	**Sign.**	**FA**	**FE**	**Sign.**	**FA**	**FE**	**Sign.**
NOV	3.5±0.3^{b}	6.2±0.2^{a}	**	76.4±18.5	58.7±15.0^{b}	ns	147.0±17.8	155.2±12.1^{b}	ns	617.3±71.8^{c}	500.9±17.4^{c}	ns
DEC	5.3±0.8^{a}	2.0±0.1^{cd}	**	57.5±10.5	43.9±4.9^{bc}	ns	147.7±21.1	143.6±21.2^{b}	ns	572.3±68.9^{c}	494.0±65.5^{c}	ns
GEN	3.8±0.3^{ab}	1.7±0.3^{d}	**	59.8±7.9	37.4±1.8^{c}	**	160.5±23.0	153.5±24.0^{b}	ns	594.6±90.4^{c}	502.0±1.3^{c}	ns
FEB	5.3±1.2^{a}	4.1±0.2^{b}	ns	66.7±17.1	61.3±6.7^{b}	ns	150.0±34.3	179.7±4.6^{ab}	ns	698.0±79.5^{bc}	691.9±26.5^{b}	ns
MAR	4.7±0.3^{ab}	2.1±0.1^{cd}	**	58.9±6.4	49.8±3.3^{bc}	ns	165.4±8.9	167.2±5.8^{b}	ns	735.2±43.2^{abc}	672.8±16.7^{b}	ns
APR	5.0±0.5^{ab}	2.6±0.1^{c}	**	75.6±6.5	57.5±4.5^{b}	*	174.9±3.6	155.6±17.1^{b}	ns	824.9±79.0^{ab}	666.8±43.8^{b}	*
MAG	5.4±0.2^{a}	2.4±0.4^{cd}	**	67.7±6.2	82.5±6.0^{a}	*	190.3±13.2	217.7±8.5^{a}	*	882.9±77.3^{ab}	1058.0±7.9^{a}	*
JUN	4.4±0.6^{ab}	2.4±0.3^{cd}	**	72.7±2.4	86.9±1.3^{a}	**	192.0±11.7	215.6±9.9^{a}	ns	909.6±32.2^{a}	1061.4±44.0^{a}	**
Sign.	**	**		n.s.	**		*	**		**	**	

Note: Lowercase letters within a column indicate significant differences as determined by Tukey's post hoc analysis. Sign.: Significance, **: Significance at $p < 0.01$, *: Significance at $p < 0.05$; ns: Not significant

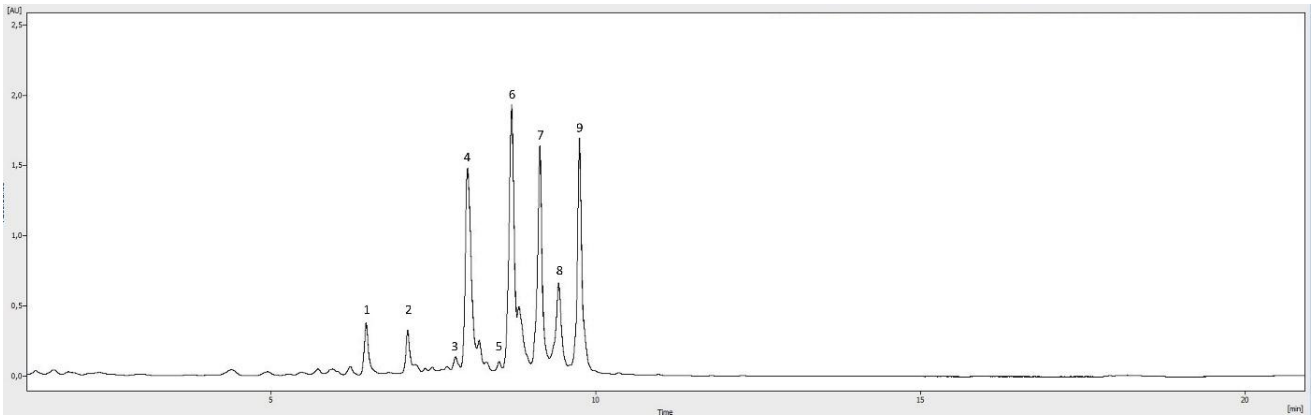

Figure 1. Example of chromatogram of Bergamot Juice (UHPLC). 1. *p*-coumaric acid, 2. Ferulic acid, 3. Eriocitrin, 4. Neoeriocitrin, 5. Narirutin, 6. Naringin, 7. Neohesperidin, 8. Melitidin, and 9. Brutieridin

HMG-flavanone derivatives: Melitidin and brutieridin

Melitidin and brutieridin, two highly bioactive HMG-flavanone derivatives with recognized statin-like properties, represented a substantial portion of the bergamot phenolic profile throughout the season (Table 4). Their accumulation patterns highlighted a strong cultivar-dependent response. In FA, both compounds remained relatively stable across the entire harvest period, showing no significant seasonal fluctuations. In contrast, FE exhibited a progressive and marked increase from March onward, reaching maximum concentrations in May-June. This divergence indicates a clear cultivar × harvest time interaction, reflecting a higher metabolic responsiveness of FE during late ripening stages. Correlation analyses further indicated that total phenolic content was positively associated with temperature and negatively correlated with rainfall in both cultivars, suggesting that warmer and drier conditions favor polyphenol accumulation in bergamot juice.

Polyphenol dynamics and the dilution effect

To correctly interpret polyphenol dynamics during ripening, compositional data must be considered alongside changes in juice yield, as fruit growth can induce a dilution effect on metabolite concentrations. As reported for other citrus species, flavonoid accumulation tends to slow during cell expansion phases, resulting in lower concentrations per unit of juice volume (Berhow 2002). In the present study, juice yield increased until February and subsequently declined, whereas polyphenol concentrations continued to rise, generating the inverse trend illustrated in Figure 2. This behavior reflects a typical dilution-concentration mechanism: during early ripening, rapid pulp growth leads to an apparent dilution of phenolics, while during later stages, reduced water content and enhanced secondary metabolism promote net polyphenol concentration. Polynomial regression analysis described these trends effectively, with coefficients of determination of 0.919 for FA and 0.813 for FE. The steeper curvature observed in FA indicates a more pronounced late-season concentration phase, whereas FE displayed a smoother and more gradual increase. Overall, these results demonstrate that polyphenol levels in bergamot juice are governed not only by biosynthetic activity but also by cultivar-specific differences in juice accumulation and water dynamics during ripening.

Changes in Bergamot Essential Oil (BEO) composition

The volatile profile of Bergamot Essential Oil (BEO) displayed the characteristic composition previously described for this species (Dugo et al. 1987; Dugo et al. 2000), dominated by monoterpene hydrocarbons (e.g., limonene, γ-terpinene, β-pinene), followed by oxygenated monoterpenes (such as linalool, neral, and geranial) and by acetate esters, including linalyl and neryl acetate. A total of 50 components were identified (Figure 3), in line with recent high-resolution chromatographic studies reporting an even broader array of minor volatiles (Marzocchi et al. 2019).

In contrast to most previous investigations, which typically restrict sampling to early or mid-season harvests, the present study covers the entire November-June window. This extended sampling allowed the identification of ripening-related metabolic shifts occurring at advanced maturity stages, providing a more comprehensive description of seasonal changes in aroma-active compounds relevant for industrial applications.

Figure 2. Juice polyphenols dilution effect during ripening

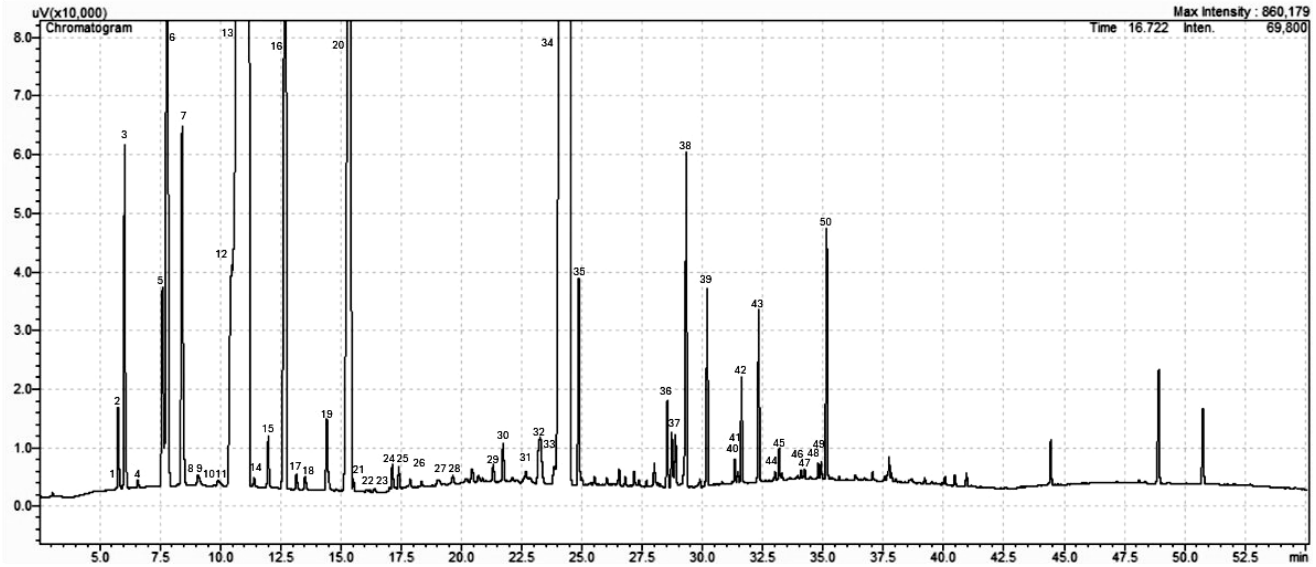

Figure 3. BEO sample gas chromatogram. 1. Tricyclene, 2. α-Thujene, 3. α-Pinene, 4. Camphene, 5. Sabinene, 6. β-Pinene, 7. Myrcene, 8. Octanal, 9. α-Phellandrene, 10. δ-3-Carene, 11. α-Terpinene, 12. *p*-Cymene, 13. Limonene, 14. (Z)-β-Ocimene, 15. (E)-β-Ocimene, 16. γ -Terpinene, 17. Trans-Sabinene hydrate, 18. Octanol, 19. Terpinolene, 20. Linalool, 21. Nonanal, 22. Cis-Limonene oxide, 23. Trans-Limonene-oxide, 24. Isopulegol, 25. Camphor, 26. Citronellal, 27. Terpinen-4-ol, 28. α-Terpineol, 29. Decanal, 30. Octyl-acetate, 31. Nerol, 32. Neral, 33. Cis-Sabinene-hydrate-acetate, 34. Linalyl-acetate, 35. Geranial, 36. α-Terpinyl-acetate, 37. Citronellyl-acetate, 38. Neryl-acetate, 39. Geranyl-acetate, 40. Dodecanal, 41. Decyl-acetate, 42. (β)-Caryophyllene, 43. trans-α-Bergamotene, 44. α-Humulene, 45. Cis-β-Farnesene, 46. Germacrene D, 47. Sesquiterpene, 48. Sesquiterpene, 49. α-Farnesene, 50. β-Bisabolene

Seasonal dynamics of major monoterpenes

Limonene, γ-terpinene, and β-pinene were the dominant monoterpene hydrocarbons in both cultivars, confirming their role as key markers of bergamot authenticity and quality (Mondello et al. 1995; Marzocchi et al. 2019). Although their relative abundances varied throughout the season, these compounds consistently represented the structural backbone of the volatile fraction. During early ripening stages, monoterpene hydrocarbons were slightly less abundant, followed by an increase toward mid-season, likely associated with active terpene biosynthesis in developing oil glands. In later harvests, their levels tended to stabilize or moderately decline, suggesting a shift in metabolic allocation as fruit maturity progressed. Beyond their sensory contribution, these monoterpenes have been associated with several biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Salehi et al. 2019; Anandakumar et al. 2021), reinforcing the multifunctional value of bergamot essential oil.

Linalool-linalyl acetate conversion

The most relevant compositional change observed during ripening was the progressive conversion of linalool into linalyl acetate, a reaction widely described in bergamot metabolism and associated with terpene alcohol acetyl transferase activity (Di Giacomo and Mincione 1995; Marzocchi et al. 2019). This transformation has important implications for aromatic quality, as linalool contributes fresh and green notes, whereas linalyl acetate imparts sweeter and more persistent floral nuances. Consistent with this process, the ratio between linalool and linalyl acetate (essence degree) was highest at the beginning of the harvest season and declined steadily as ripening advanced (Figure 4). This trend was mirrored by the individual compound dynamics, with a marked decrease in linalool and a concomitant increase in linalyl acetate (Figures 5 and 6).

Polynomial regression analysis confirmed the temporal evolution of both compounds, indicating a continuous esterification process during ripening. The sharper decline in linalool relative to the increase in linalyl acetate suggests that ester formation does not fully compensate for the reduction in precursor availability, particularly at advanced maturity stages. These results are consistent with previous reports showing enhanced ester accumulation in mid- to late-season bergamot essential oils (Russo et al. 2013; Marzocchi et al. 2019).

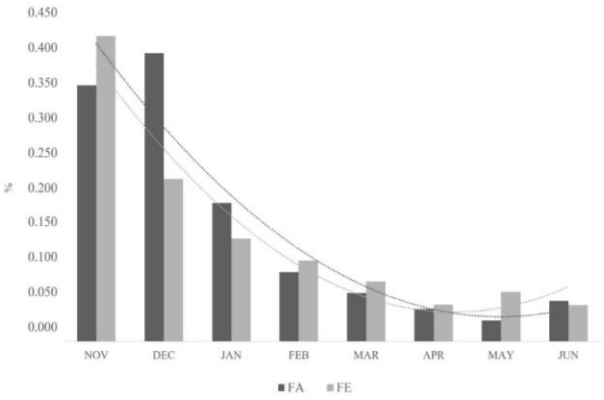

Figure 4. Ratio between Linalool and Linalyl acetate in BEO samples during fruit growth

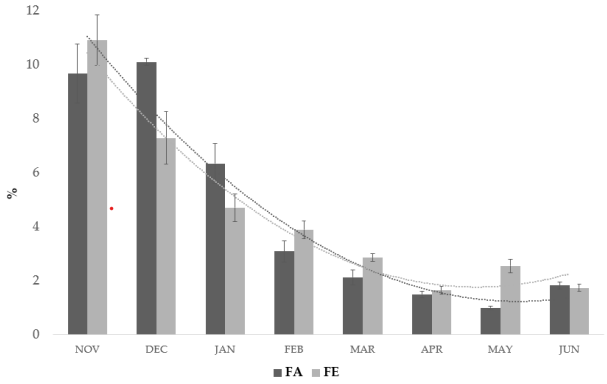

Figure 5. Linalool trend during ripening in FA and FE

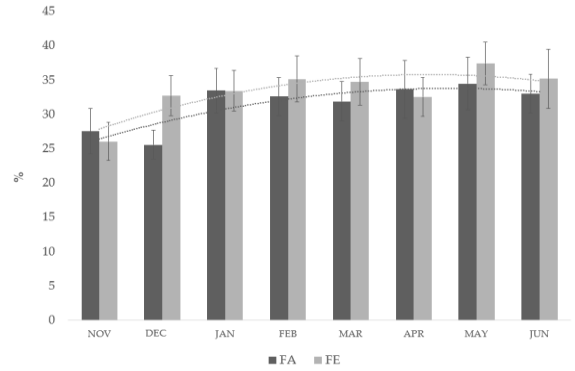

Figure 6. Changes in Linalyl acetate in FA and FE essential oil

Cultivar-specific traits and industrial relevance

Both cultivars followed similar overall metabolic trajectories, although cultivar-specific differences were evident. Fantastico tended to retain slightly higher linalool levels during early harvests, favoring fresher and more vibrant aromatic profiles. In contrast, Femminello showed a more pronounced accumulation of linalyl acetate at full maturity, consistent with smoother and more persistent fragrance characteristics. These differences are particularly relevant from an industrial perspective, as the major volatile constituents of bergamot essential oil, especially limonene, β-pinene, γ-terpinene, linalool, and linalyl acetate, have been linked to biological activities of interest for nutraceutical, pharmaceutical, and cosmetic applications (Russo et al. 2013; Anandakumar et al. 2021).

Implications for optimal harvest timing

The seasonal evolution of key volatile compounds provides practical indications for defining harvest strategies tailored to specific industrial uses. Early-season fruits, characterized by higher linalool proportions, yield essential oils with fresher and greener aromatic notes, suitable for applications emphasizing top-note intensity, such as fine fragrances and selected cosmetic products. As ripening progresses, the increased accumulation of linalyl acetate results in oils with smoother, sweeter, and more persistent aromatic profiles, making mid- to late-season harvests particularly appropriate for high-end perfumery and formulations requiring longer-lasting fragrance characteristics. Overall, the extended November-June dataset demonstrates that both cultivars consistently produce high-quality essential oil throughout the harvest window, while expressing distinct cultivar-specific aromatic signatures. This variability offers valuable flexibility for growers and industry stakeholders to optimize harvest timing according to the desired sensory profile and final product destination.

In conclusion, this study presents a season-long evaluation of the two main bergamot cultivars cultivated in the Calabrian PDO area, Fantastico and Femminello, by integrating analyses of juice phenolic compounds and essential oil volatiles from November to June. Phenolic concentrations in juice increased progressively during ripening, rising from about 500-620 mg L^{-1} in early harvests to more than 900 mg L^{-1} in late-season fruits, with the highest levels recorded in Femminello (>1,050 mg L^{-1} in June). These patterns reflect combined effects of biosynthesis and fruit growth, indicating that delayed harvests are advantageous for maximizing polyphenol content in nutraceutical and functional food applications. In contrast, essential oil composition shifted seasonally from monoterpene alcohols to acetate esters, driven mainly by the conversion of linalool to linalyl acetate. Early harvests produced linalool-rich oils with fresher aromatic profiles, while mid- to late-season harvests yielded ester-rich oils with smoother and more persistent fragrances. Although both cultivars showed similar ripening trends, Fantastico retained higher linalool levels early in the season, whereas Femminello accumulated more linalyl acetate at full maturity. Overall, optimal harvest timing depends on the intended end use, and this integrated approach supports cultivar-specific harvest management and targeted valorization of bergamot within the PDO system.

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