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Quality Evaluation of Bergamot Fruits Produced in Different Areas of Calabria Region

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Abstract: Citrus fruits are extensively cultivated worldwide, with Italy and Spain being major producers. Southern Italy, particularly in Reggio Calabria, a typical citrus fruit is produced, namely, bergamot (*Citrus bergamia Risso* et Poiteau), known for its mysterious origins and exceptional quality essential oil protected by the EU's PDO designation. Despite historical challenges, bergamot has regained prominence for its nutraceutical potential, especially its flavonoid-rich juice, offering significant health benefits. However, little attention has been paid to understanding the qualitative and quantitative differences of bergamot juice in Calabrian production areas. For this reason, this work aims to investigate the quality characteristics of bergamot juice produced in different areas of Calabria sites. The results showed the best quality attributes of bergamot fruits harvested in the DPO area. In particular, higher levels of total soluble solids, stable acidity and higher juice were found. In addition, higher contents of ascorbic and citric acids, which are nutritionally valuable and tasteful, were found. The phenolic profile, characterized by the key compounds of bergamot, highlighted the better nutraceutical potential of the fruit grown in the DPO area.

Keywords: bergamot; citrus fruit; fantastico cultivar; flavonoids; juice; nutraceutical potential; polyphenols

1. Introduction

One of the most widespread fruit crops in the world is citrus fruit. In Europe (EU), the latest available data referring to the year 2022 shows a total production of 10325t, strongly concentrated in mediterranean countries, including Italy second only to Spain with a production of 3061t [1]. Southern Italy is well known for its citrus fruit production especially in Calabria and Sicily where are produced particular citrus fruits such as pigmented oranges or orange blood, lemons, mandarins, bergamots [2,3].

Bergamot (*Citrus bergamia* Risso et Poiteau), cultivated in the province of Reggio Calabria, is a citrus fruit cloaked in mystery regarding its origins, adding to its enigmatic allure. Some researchers indicate its origin derived from bitter orange (*Citrus aurantium*) and lemon (*Citrus limon*) [4], others from bitter orange and lime (*Citrus aurantifolia* (Christm.) [5] or hybrid of citron (*Citrus medica*) and bitter orange [6–9]. During the last century, this crop experienced a series of events that led to a collapse in the price of bergamot, significantly reducing the presence of this citrus fruit [10,11], although today it enjoys an excellent diffusion. Its success is owing to the fact that the quality of its essential oil produced in a small area of Calabria, obtained by screwing and cold-pressing the peel, is not comparable to that obtained elsewhere in the world. In fact, it is protected by the European Union with the PDO quality mark. The compositional differences of the essential oil of bergamot

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(BEO) produced in the area and outside of this area have been extensively studied. Several research reported by Dugo et al. [12], highlighted the differences among the different production areas.

It is acclaimed that bergamot essential oil produce in other place are not comparable to that of the designed area [13]. The reason of bergamot's essential oil quality in the area of Reggio Calabria is highly suitable thanks to the microclimate and soil properties [14,15] that determine the qualitative characteristics of BEO [13].

Until a few years ago, bergamot was only harvest for the production of essential oil, so that everything else (juice, pulp and seeds) were considered industrial waste [16,17]. Over the years, studies have uncovered interesting nutraceutical potential and human health benefits for which fresh consumption and juice have been of interest. Compared to other fruits belonging to the same genus, bergamot is abundant in many phytochemicals and nutraceuticals such as organic acids, limonoids, phenolic acids and flavonoids [18,19]. Recently were discovered new flavonoids known as 3-hydroxy-3-methylglutaric acid conjugates of naringin and neohesperidin, called melitidin and brutieridin. These molecules with statin-like action are very compelling for their remarkable cholesterol-lowering effect [20,21]. Bergamot juice (BJ) has a flavonoid-rich profile including naringin, neohesperidin, neoeriocitrin (glycosylated flavanones) constitute the primary compounds of the polyphenol fraction in addition to C-glucosides, flavone O-glycosides and flavanone O-glycosides [22,23]. For this reason, the consumption of bergamot as fruits was greatly increase and their nutritional characteristics joined to the juice's yield are becoming very important for the evaluation.

Despite the general and distinguishing characteristics of bergamot have been studied mainly with regard to the essence, insufficient attention has been paid to the qualitative and quantitative differences in Bergamot juice obtained in different Calabrian production areas. Recently some studies were appeared, these reported a characterisation of fruit quality harvested on the typical area located on the Ionian coast of Reggio Calabria [24,25].

The aim of this study was to evaluate the qualitative parameters of bergamot fruit (Fantastico cultivar) focusing mainly on the juice. Two different growing areas and two harvesting years were considered. Specifically, the fruits were harvested in province of Reggio Calabria on the Ionian (Melito di Porto Salvo) in the area designed as typical for bergamot essence production and defined inside that of DPO, and an area comprised on Tyrrhenium side (Rizziconi), this shows completely different characteristics in term of soil and climate.

2. Materials and Methods

2.1. Fruits Sampling

Bergamot fruits were harvested from "Fantastico" (F) plants grafted onto sour orange trees in two experimental field located in Melito di Porto Salvo (Ionian coast, DPO area) and Rizziconi (Reggio Calabria, Thyrrenium site).

The site of Melito di Porto Salvo, so the Ionian coast of Reggio Calabria district, is characterised by an arid climate with an average monthly temperature greater all year respect to the Tyrrhenian side. It shows less rainfalls respect the Rizziconi site (Thyrrenian side), and are concentrated during the autumn-winter season. The Rizziconi's rainfalls are better distributed along the year.

Harvesting took place in two harvest seasons (2022/23 and 2023/24) in Dicember and January by sampling three replicates (twelve fruits each) for both cultivars. The fruits were promptly transported to the Food Technology laboratory at the Mediterranea University of Reggio Calabria for analysis on the same day of harvesting.

Bergamot samples were hand squeezed with a commercial juicer and the obtained juice was immediately analysed.

Samples were named "F-M" (Fantastico bergamot fruit harvested in Melito) and "F-R" (Fantastico bergamot fruit harvested in Rizziconi).

2.2. Chemical Characterisation of Bergamot Juice (BJ)

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2.2.1. Chemical Determinations

Bergamot Juice (BJ) was manually extracted using a commercial juicer (Metro Professional GJU2001, METRO Markets GmbH, Germany). The juice was analysed for total soluble solids (TSS) expressed in °Brix using a digital refractometer (DBR 047 SALT); pH was determined with a pH meter (Crison Basic 20, Spain); titratable acidity (TA) was acquired by titrating juice with 0.25N NaOH until pH of 8.1, following the International Federation of Fruit Juice Producers (IFU) method [26]. Juice yield (JY) was calculated dividing the juice weight by the fruit weight and the multiplied by 100 (JY %).

2.2.2. Organic Acid Determination

Analysis of organic acids in BJ samples was conducted according to Boninsegna et al. method [27]. Briefly, BJ was centrifuged in a refrigerated centrifuge (NF 1200R, Nüve, Ankara, Turkey) at 7000 rpm for 8 min (4°C), filtered with RC 0.45µm syringe filter and diluted with HPLC grade water (dilution 1:5). The analysis of organic acids was carried out in a HPLC-DAD system (Knauer HPLC Smartline Pump 1000; Knauer Smartline UV Detector 2600) using a SYNERGI HYDRO-RP coloumn (250 mm × 4.6 mm i.d., 4 µm) thermostatically controlled at 22 °C injecting 20 µL of sample. Setting conditions were isocratic elution with a mobile phase solution of potassium phosphate 20 mM acidified (pH 2.9), flow rate of 0.7 mL min^{-1}. Ascorbic acid was detected at 254 nm, citric acid at 210 nm and results were reported as mg of acid per L^{-1} of BJ.

2.2.3. Individual Polyphenols Determination

The quali-quantitative analysis of polyphenols in BJ was conducted using an ultra-high performance liquid chromatography system (UHPLC), as reported by De Bruno et al. [28]. The UPLC PLATINblue system (Knauer, Berlin, Germany) was equipped with a binary pump, Knauer blue orchid column C18 (1.8 µm, 100 × 2 mm) thermostat at 30 °C, and a Photo Diode Array Detector (PDA–1) PLATINblue. 2 µL of filtered BJ sample were injected into the system and the flow rate was set at 0.4 mL min^{-1}. The elution rate was performed using acidified water (formic acid-pH 3.10) in pump A and acetonitrile in pump B with the following gradient: 1)95% A (0-3 min); 95-60% A (3-15 min); and 60-0% A (15-15.5). The quantification of phenol compounds was achieved using external standards and results were expressed as mg 100 mL^{-1} of BJ.

2.3. Statistical Analyses

Mean and standard deviation of five measurements were calculated and analysis of variance (one-way ANOVA) was carried out applying the Tukey post hoc test at $p<0.05$ by SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Qualitative Characteristic of Bergamot Fruits

The sugar content (TSS), pH and acidity are crucial aspects that allow for defining the fruit's ripening characteristics of bergamot fruits.

Total soluble solids (TSS) are predominantly sugars (glucose, fructose and sucrose) and are indicated as an indicator of fruit internal quality [29]. TSS content showed statistical differences between the two samples of BJ with higher values in both monitoring times in F-M (Table 1). The same trend was observed in all two years considered. Moreover, it is worth to note that in F-M the TSS remained constant in F-M while tending to decrease in F-R during ripening. The annual data showed higher values in F-M and during the second year reaching 11.28 °Brix in the December. Data measured on the juices agreed with those reported by Cautela et al. [30] the TSS observed in fruits from Ionian area were higher to that reported by Gullo et al. for the same location [24] but were comparable to other reported by the same authors in the same area. The fruits collected in the Tyrrhenian site gave similar TTS value to that of juice obtained in the same area by Gullo et al. [24].

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Differences could be due to the different season, as it is possible observe in this work the two years of observations release different results.

As reported in Table 1, pH values ranged between 2.35 and 2.62. During ripening process no statistical differences in F-M and F-R samples were found during the first year, but in the second year a different trend was observed showing statistical increment in F-M and a decrease in F-R.

TA showed high statistical differences in all ripening stages and in the two considered years except for F-M in the first were, despite a low decrease, no statistical differences were found. In general, the trend followed by acidity is decreasing as reported by Di Matteo et al. [31] who found the same pattern in lemon fruits. Also, Giuffrè [25] observed the same trend and similar contents for juice obtained from bergamot fruits harvested in the Ionian area. Otherwise Gullo et al. [24] did not observe pronounced differences between the fruits of the sample collected on Tyrrhenian site respect to those of Ionian, these were in a greater number and with a wide range of variability. Respect to industrial produced juice the values observed in this work agree with those of Cautela [30].

Table 1. TSS, pH and TA in bergamot samples.

I YEAR	**TSS (°Brix)**			**pH**			**TA (g L^{-1} Citric ac. m)**		
	Dic	Jan	Sign	Dic	Jan	Sign	Dic	Jan	Sign
F-M	10.36±0.13	10.24±0.19	n.s.	2.52±0.09	2.57±0.07	n.s.	49.28±1.07	48.58±1.21	n.s.
F-R	8.84±0.54	7.7±0.21	**	2.59±0.06	2.61±0.1	n.s.	43.65±1.78	41.23±1.11	**
Sign	**	**		*	**		**	**	
II YEAR	**TSS (°Brix)**			**pH**			**TA (g L^{-1} Citric ac. m)**		
	Dic	Jan	Sign	Dic	Jan	Sign	Dic	Jan	Sign
F-M	11.28±0.13	11.04±0.53	n.s.	2.35±0.02	2.62±0.08	**	55.37±1.86	49.04±0.55	**
F-R	8.74±0.25	7.58±0.18	**	2.57±0.06	2.48±0.02	**	50.09±1.48	42.56±3.06	**
Sign	**	**		**	**		**	**	

Statistical analysis significance based on Tukey's post hoc test: ** for significance at p>0.01; * for significance at p>0.05 and n.s. for not significant.

Generally, juice yield in citrus fruits is considered one of the most important parameters for fruit harvesting and processing, for this reason many techniques have been conducted in order to enhance the juice content [32,33].

The results reported in Figure 1 suggested that during ripening, both samples showed a general improvement in juice yield, although with different significance. In F-M fruits was obtained a higher JY compared to F-R samples with the exception of second-year sampling probably due to the extremely hot and dry weather conditions in the production area in the summer and autumn of 2023. In F-M samples JY was always above 40%, reaching 54.51% in the January sampling Year 2022. On the other hand, in F-R sample, it was always below 40% except in the second year's sampling, which despite being a great juice content for citrus fruit is lower than that found in F-M. Nevertheless, these findings suggested delayed harvesting to optimize juice extraction and have riper fruit.

The yield observed in the Ionian site were higher respect those reported by Giuffrè [25] and Gullo et al. [24]. This could be due for different reasons such as different season.

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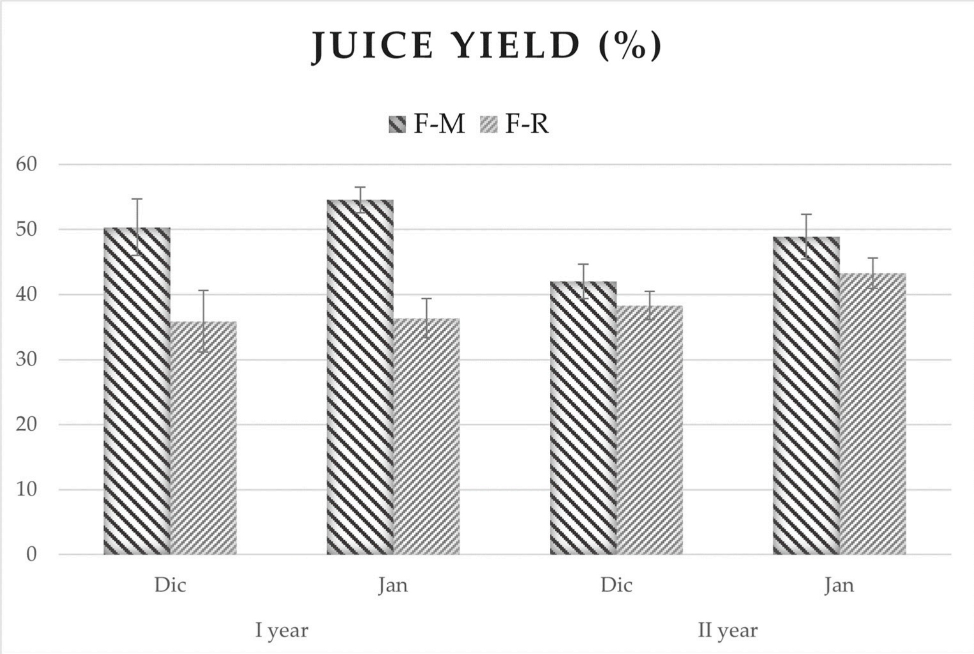

Figure 1. Juice content in Bergamot fruits.

3.2. Qualitative Characteristic of Bergamot Fruits

The main organic acids detected in BJ through liquid chromatographic analysis were reported in Table 2. They are strongly associated to metabolic plant pathways and responsible of flavor [34,35]. Ascorbic acid (AA), or vitamin C, is among the vitamins of fundamental importance for humans, as our bodies are unable to synthesize them on their own. AA is widely recognized for its numerous biological functions [36,37].

During monitoring time, AA showed lower values in F-R sample. The highest concentration was detected in F-M in January 2023 (0.61 g L^{-1}). In this year were not observed statistical differences over time between the monitoring times. Conversely, in the second year, the AA content was reduced in the second monitoring time with high statistical differences due to degradation of organic acids during ripening.

Results of AA are in accordance with Cuzzocrea [38] and with other authors who found similar values in orange varieties [39,40]. Juice obtained in the Ionian area by Giuffrè [25] showed a different trend with a stable or slight decrease between December and January-February collected fruits with values similar,

Citric acid (CA) is the primary organic acid in BJ which accumulates inside the cells of the citrus fruit juice sac, contributing significantly to the taste and quality of fruits. It is an organic triprotic acid found in abundance in citrus fruits [41]. CA content in BJ varied significantly between the two areas ranging from 36.87 g L^{-1} in F-R to 49.13 g L^{-1} in F-M.

The comparison between the two juices revealed that the citric acid content during the two different periods of the year remained relatively stable in both samples. The main differences were noted between F-M and F-R in which, as with AA, the highest concentration was detected in F-M.

The differences in organic acids concentration could be attributable to temperature effect which impact fruit acidity by altering metabolism and storage of organic acids within vacuoles. Lin et al., 2016 [34] observed the primary factors affecting open field fruit quality in winter are the cold temperatures and physiological drought.

Table 2. Organic acids in Bergamot juice.

I YEAR	AA (g L^{-1})			CA (g L^{-1})		
	Dic	Jan	Sign	Dic	Jan	Sign

F-M	0.57±0.04	0.61±0.07	n.s.	46.44±2.64	49.13±3.14	n.s.
F-R	0.43±0.07	0.48±0.02	n.s.	38.87±3.24	40.35±2.04	n.s.
Sign	**	**		**	**	
II YEAR	**AA (g L-1)**			**CA (g L-1)**		
	Dic	Jan	Sign	Dic	Jan	Sign
F-M	0.59±0.02	0.48±0.06	**	47.96±1.08	47.46±2.2	n.s.
F-R	0.42±0.02	0.32±0.04	**	47.25±2.98	44.8±2.54	n.s.
Sign	**	**		n.s.	n.s.	

Statistical analysis significance based on Tukey's post hoc test: ** for significance at p>0.01; * for significance at p>0.05 and n.s. for not significant.

A low concentration of acids in the fruit may be linked to higher levels of secondary metabolites such as alcohols and aldehydes, which may adversely affect the flavor of the fruit pre- and post-harvest [42]. The content and concentration variations of organic acids influence the taste of fruit and play a role in controlling the ripening and storage quality of fruit. The inverse relationship between organic acid levels and fruit weight loss during post-harvest storage emphasizes the importance of organic acids in preserving fruit quality and storability, as they can lead to increased post-harvest losses, resulting in significant economic damage to the citrus industry [43].

These chemical-physical findings from bergamot fruits grown in various locations in Calabria, highlighted the effect reported in the literature on other citrus fruits. Quality characteristics such as color, acidity, size, juice and total soluble solids content are strongly influenced by environmental factors such as radiation, relative humidity and temperature [44].

3.3. Comparison of Phenolic Compounds in Bergamot Samples

Bergamot juice (BJ) has gained increased societal attention in recent years for its nutraceutical and medical effects in the prevention and treatment of many diseases due to the presence of high level of polyphenols [45].

The main phenols detected in BJ (Table 3) showed a similar chromatographic profile of bergamot pomace [46]. p-coumaric acid and ferulic acid (hydroxycinnamic acids) were recorded in great quantity with values renging from 1.42 mg 100 mL^{-1} in F-M to 0.61 mg 100 mL^{-1} in F-R for p-coumaric acid while ferulic acid from 1.29 mg 100 mL^{-1} in F-M to 0.61 mg 100 mL^{-1} in F-R. Concentrations of p-coumaric acid followed different trends in both samples for both years, exhibiting an ever higher content in BJ F-M.

The higher content of ferulic acid was also detectet in F-M between the analysed samples in the two harvest years. Values were stable (no statistical differences) between the first and the second monitoring time, except for F-R juice in which during the second year was observed a statistical (p<0.01) reduction.

The other compounds detected belong to the class of flavonoids. Small quantities of eriocitrin and narirutin were found, and both flavonones showed similar behaviour with a statistical increase in F-M in the first year only, confirming a stable level (no statistical differences) in F-M in the second year and in F-R in both years.

Neoeriocitrin, naringin and neohesperidin were detected in high concentrations in BJ with values comparable to those reported by Da Pozzo et al., 2018 [47].

Neoeriocitrin was lower in F-R only in the first monitoring time of the second year (9.87 mg 100 mL^{-1} in F-M, 8.59 mg 100 mL^{-1} in F-R). For the other two major flavonoids naringin and neohesperidin, the differences were considerable between the sample juices, reaching up to twice the F-R content in F-M. The concentration levels of naringin (values comprised between 6.45 and 13.88 mg 100 mL^{-1}) showed a statistical increment in F-M in the first year and was stable in the second year. BJ obtained in Rizziconi did not exhibited changes from the first to the second measurement. A similar trend was observed for neohesperidin without any variation between the different times analysed and over the different years. Statistical analysis showed relevant differences (p<0.01) between F-M and F-R, where the content is almost always reduced by half. For istance, during January 2024, 12.11 mg 100 mL^{-1} were found in F-M and 4.07 mg 100 mL^{-1} in F-R.

Melitidin and brutieridin, C-Glycosyl flavones, are characteristic of bergamot fruits and well known statins for their anticolesterolemic activity [48]. The statistical differences showed a clearly higher concentration in F-M sample than in F-R in both years and in both harvesting times. In particular, brutieridin was at least twice the concentration in F-M, and during the second half of the second year it tripled.

The compositional differences in BJ samples obtained from the fruits of the two different areas are highlighted by the total polyphenols content of the main quantified phenols, which showed that in all periods in which the BJ were analysed, the polyphenol content was significantly ($p<0.01$) lower in F-R as clearly visible in Figure 2.

Table 3. Polyphenols composition of BJ samples.

mg 100 mL^{-1}		I YEAR			II YEAR		
		F-M	**F-R**	**Sign**	**F-M**	**F-R**	**Sign**
p-coumaric acid	Dic	1.02±0.02	0.83±0.03	**	1.42±0.08	1.14±0.08	**
	Jan	1.09±0.01	0.73±0.13	**	1.22±0.1	0.75±0.09	**
Sign		**	n.s.		n.s.	**	
Ferulic acid	Dic	0.98±0.18	0.78±0.09	n.s.	1.29±0.14	0.88±0.06	**
	Jan	0.98±0.14	0.66±0.1	**	1.06±0.13	0.61±0.09	**
Sign		n.s.	n.s.		n.s.	**	
Eriocitrin	Dic	0.57±0.05	0.6±0.15	n.s.	1.27±0.08	0.74±0.04	**
	Jan	0.68±0.06	0.56±0.09	n.s.	1.24±0.02	0.62±0.09	**
Sign		*	n.s.		n.s.	n.s.	
Neoeriocitrin	Dic	9.62±0.83	9.13±0.38	n.s.	9.87±0.34	8.59±0.55	*
	Jan	10.89±0.68	12.15±1.88	n.s.	11.07±1.61	9.37±1.25	n.s.
Sign		n.s.	*		n.s.	n.s.	
Narirutin	Dic	0.22±0.05	0.22±0.05	n.s.	1.21±0.02	0.85±0.12	**
	Jan	0.37±0.01	0.15±0.03	**	1.26±0.45	0.76±0.07	n.s.
Sign		**	n.s.		n.s.	n.s.	
Naringin	Dic	9.69±1.55	6.66±0.85	*	13.44±0.6	7.35±0.26	**
	Jan	12.49±1.25	8.49±1.83	*	13.88±0.99	6.45±1	**
Sign		*	n.s.		n.s.	n.s.	
Neohesperidin	Dic	9.12±2	4.1±1	**	11.93±0.6	5.04±0.41	**
	Jan	8.92±1.2	5.15±1.22	**	12.11±1.92	4.07±0.64	**
Sign		n.s.	n.s.		n.s.	n.s.	
Melitidin	Dic	3.99±0.72	2.05±0.77	**	8.26±0.47	4.67±0.35	**
	Jan	5.09±0.83	2.14±0.39	**	8.55±1.06	3.48±0.25	**
Sign		n.s.	n.s.		n.s.	**	
Brutieridin	Dic	21.74±2.2	9.56±2.85	**	23.05±0.66	10.97±0.72	**
	Jan	24.53±0.69	8.56±1.28	**	22.19±2.38	7.21±0.43	**
Sign		n.s.	n.s.		n.s.	**	
Total Polyphenols	Dic	56.95±6.01	33.93±4.93	**	71.75±2.58	40.23±1.98	**
	Jan	65.04±3.53	38.6±6.5	**	72.59±8.47	33.31±3.05	**
Sign		n.s.	n.s.		n.s.	*	

Statistical analysis significance based on Tukey's post hoc test: ** for significance at $p>0.01$; * for significance at $p>0.05$ and n.s. for not significant.

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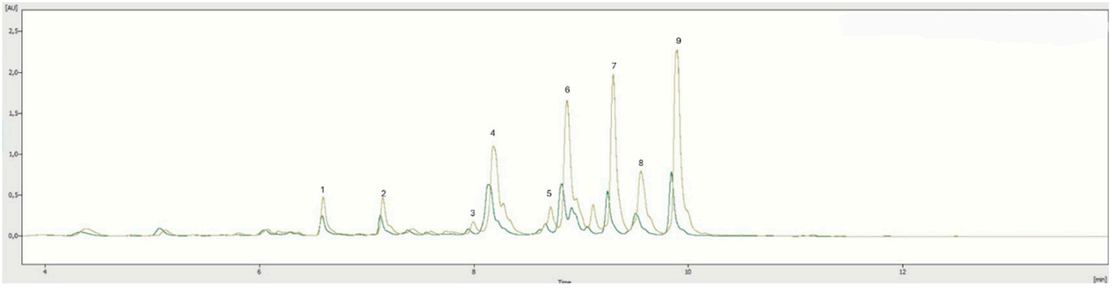

Figure 2. Chromatographic profiles comparison of F-M and F-R BJ samples (UHPLC). (1) p-coumaric acid; (2) ferulic acid (3); eriocitrin; (4) neoeriocitrin; (5) narirutin; (6) naringin; (7) neohesperidin; (8) melitidin and (9) brutieridin. Mustard colour: F-M sample; green: F-R sample.

4. Conclusions

The results of this work, which lasted two years and was conducted at two different harvest times (December and January), showed how the production area highly suited to the cultivation of bergamot strongly influences the characteristics of the fruit. Although it was already known that the characteristics of the essence find their maximum qualitative expression in the designated Ionian coastal strip of the province of Reggio Calabria, it has been shown that the chemical-physical characteristics of the fruit are also strongly influenced by the area and thus by the microclimatic conditions. Even if the seasonal variability could be influencing the characteristics, juice yields, the content of organic acids, flavonoids for which there is growing nutraceutical and health interest, showed a higher quality in the Melito Porto Salvo area than in Rizziconi, even though both areas are in the Calabria region (Italy).

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Data comparison of physicochemical changes in the two different years.; Figure S2: Main polyphenols in BJ: comparison over the two years.

Author Contributions: Conceptualization, A.G., R.M, S.C. and M.P.; methodology, A.G. and A.D.B.; software, A.G. and A.D.B.; validation, R.M. and M.P.; formal analysis, A.G. and D.M.; investigation, A.G. and D.M.; resources, R.M. and M.P..; data curation, A.G. and A.D.B..; writing—original draft preparation, A.G. and A.D.B.; writing—review and editing, M.P., S.C. and R.M.; visualization, R.M. and A.D.B.; supervision, M.P. All authors have read and agreed to the published version of the manuscript.

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