



Variation of the quality parameters in bergamot fruits according to the area of cultivation

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Summary

Background of the study – The main bergamot cultivated area in the world for the essential oil extraction is located along the coastal strip of Reggio Calabria, Italy. In recent years, the fruit is appreciated for its health properties of the pulp and juice. **Objectives** – The work aimed to verify how the organoleptic, nutraceutical, and essential oil characteristics of the fruit change between traditional bergamot cultivation area and one of newly introduced. **Methods** – The experiment was carried out on the ‘Fantastico’ bergamot cultivar during two years along the Ionian coast (traditional bergamot cultivation area: Bianco, Bova Marina, Brancaleone, Caulonia, Casignana, Gerace, Melito P.S., Palizzi, Pellaro, Portigliola, San Carlo, Siderno) and one of newly introduced on Tyrrhenian coast (Rizziconi). **Results** – The environment has influenced the production of bergamot, both for fresh consumption and for essential oil. The bergamots coming from Rizziconi have the best biometric parameters (fresh weight 281.24 g), but the lower peak of component of essential oil (linalool 2.17E+04 pA, linalyl-acetate 2.52E+07 pA, and limonene 6.86E+07 pA); the fruit of Portigliola, Siderno, and Gerace on Ionian coast have pulp with high health parameters (total polyphenols content 0.875 mg GAE g⁻¹ FW, total antioxidant capacity 4.367 μmol trolox g⁻¹ FW), and ascorbic acid 57.84 mg 100 mL⁻¹, respectively); the fruit of Portigliola has also a high peak of an important component of essential oil (linalool 2.49E+06 pA). The fruit of Bova Marina and San Carlo shows good biometric parameters such as fresh weight (280.82 g and 278.66 g, respectively), juice with high titratable acidity (5.88% and 5.60%, respectively), optimal content of linalool (3.08E+04 and 5.13E+04, respectively), and limonene (2.43E+08 and 1.19E+08, respectively). The bergamot of Bianco has the highest content of dry weight (16%) and Total soluble solids (9.70° Brix), but also a good content of limonene (2.25E+08 pA). The fruit of Pellaro has high ascorbic acid content (38.13 mg 100 mL⁻¹) and high linalyl-acetate in flavedo (4.04E+07 pA). **Conclusion** – The work has shown that the components that define the essence remains the prerogative of the traditional area located on the Ionian coast, whereas on the Tyrrhenian coast, the fruits have shown better biometric performances.

Keywords

citrus, essential oil, fresh fruit, organoleptic, polyphenols, total antioxidant capacity

Significance of this study

What is already known on this subject?

- The coastal strip of Reggio Calabria at the Ionian coast is the main area productive in the world of bergamot. It is able to provide a high quality of essential oil extracted.

What are the new findings?

- It has been found that some bergamot fruit parameters are strongly dependent by environment both for fresh consumption and for essential oil extraction.

What is the expected impact on horticulture?

- Some areas are more specialised for the fresh fruit size, other for the organoleptic and nutraceutical characteristics of juice, and others for essential oil.

Introduction

The cultivation of bergamot (*Citrus bergamia* Risso) began in Italy around the 17th century. In Tuscany, bergamot was introduced as the ornamental tree at the end of the 17th century (Dugo and Bonaccorsi, 2013). This citrus fruit spread starting from the middle of 1700 in the province of Reggio Calabria. The first bergamot plantation was done in 1750 in a fund called “Giunchi”, and it has given rise to the cultivation of bergamot. It is not easy to establish precisely how this citrus came to Calabria and, above all, what the origin of the name is.

It is successfully cultivated in the province of Reggio Calabria, along the coastal strip that extends from Scilla to Monasterace. This stretch of coast is the exclusive productive area of the world bergamot production (Dugo and Bonaccorsi, 2013). Indeed, it is able to provide more than 90% of world bergamot production with a high quality of essential oil extracted from fruit flavedo (Lauro et al., 2016). However, in recent years, the bergamot is appreciated by consumers for its nutraceutical components, such as the polyphenolic fraction of juice and albedo, and then for its health properties (Lamiquiz-Moneo et al., 2019; Navarra et al., 2014; Visalli et al., 2014; Toth et al., 2015; Capomolla et al., 2019). These properties can contrast many metabolic diseases (Celep and Rastmanesh, 2013).

Furthermore, in the last years, the bergamot has found collocation in new cultivation zones of the Calabrian region, which had already hosted this species in the past years, or in newly introduced zones.

Therefore, the work aimed to verify how the organoleptic and nutraceutical characteristics of the fruit change, both

among traditional bergamot cultivation areas and newly introduced, without neglecting the main essential oil parameters.

Materials and methods

Orchards

The experiment was carried out over two years (2018–2019) on the 'Fantastico' bergamot cultivar grafted on the same sour orange.

It involved 12 sites distributed in the traditional bergamot cultivation area, along the Ionian coast of Calabria Region, Southern Italy and one in a newly introduced area on the Tyrrhenian coast of this region (Supplemental Figure S1).

In particular, the sites involved in the research activity were: Bianco (IC), Bova Marina (IC), Brancaleone (IC), Casignana (IC), Caulonia (IC), Gerace (IC), Melito P.S. (IC), Palizzi (IC), Pellaro (IC), Portigliola (IC), San Carlo (IC), and Sidero on the Ionic Coast (IC) and Rizziconi (the new cultivation area) on the Tyrrhenian Coast (TC) (Supplemental Figure S1).

The mean annual precipitation, annual temperature and autumn temperature of the different sites are shown in Supplemental Table S1.

For each site, soil data were taken with reference to the soil map of Calabrian Region (ARSAC); the physical parameters (% clay, sand, silt) and chemical parameters: pH (in water), Ec (mS cm⁻¹), nitrogen (g kg⁻¹), P₂O₅ (ppm), K₂O (ppm), organic matter (%), MgO (ppm), Na (ppm) were taken into account (Supplemental Table S2).

For each planting water was administered to raise the soil water potential from -40 cb to 0 cb. Generally, one or two interventions every seven/ten days, distributing 80 L of water from June to September. In addition, plants were fertilized in the fall with a NKP fertilizer; however, other nitrogen fertilization was performed by irrigation during the growing cycle. Moreover, the plants have been subjected to the normal pest treatments.

Experimental design

In each of 13 municipalities (Treatment = T), a representative commercial planting was selected. The compared sites had the planting with the same rootstock (sour orange; *Citrus aurantium* L.), similar age (ranged for 20 to 25 years), and same tree shape (globe training system). Furthermore, all plantings had the drip irrigation system with 4 drippers per plant (4 L h⁻¹). In each planting, ten plants were randomly selected, and on these, the fruit samplings were done.

Fruit colour measurements

Eighty fruits (8 fruit per tree on ten trees) were randomly sampled for each orchard at harvest, at the end of autumn, from 230 to 240 days after flower bloom (DAFB) in both years.

The fruits sampled were immediately used to determine: peel colour in terms of CIELAB space colour [*L**, *a**, *b**, Chroma and Hue angle (tint)] using a Minolta CM-700d spectrophotometer (Minolta, Osaka, Japan).

Fruit biometric measurements

The height (H), diameter (D) and peel thickness (PT) of the fruit was measured using a digital calibre mod. CDJB15 (Borletti, Montichiari, Bs, Italy). Relative length (H/D, diametric ratio) was calculated. Successively, the fresh weight (FW) of the fruit was measured using an electronic balance

(Mettler-Toledo, Greifensee, Switzerland). Then, the fruit volume (FV) was found by water displacement of fruit into a graduated cylinder and it was expressed in cm³. Finally, the flavedo was removed using a grater. The remaining grated flavedo on the grater was removed with water.

The resulting mixture was pressed and filtered, and the resulting product was placed in Eppendorf and centrifuged for 20 min at 15,000 rpm at a temperature of 4°C. The essential oil (supernatant) was separated by a micropipette and it was used to gas chromatography.

Maturation index

A half pulp portion of the fruit was used to evaluate nutraceuticals parameters and dry weight (DW/FW) * 100. The remaining pulp portion was used to measure the Juice Yield (JY) using a juice extractor; it was expressed as a percentage on fresh weight [(pulp weight - juice pellet after centrifugation)/pulp weight] * 100.

The total soluble solids (TSS) of juice was measured using a digital refractometer (PR-1; Atago, Tokyo, Japan). Titratable acidity (TA) was measured using 10 mL juice diluted with distilled water (1:1) and titrated to pH 8.2 with 0.1 N NaOH. The titratable acidity was expressed as the percentage of citric acid monohydrate (C₆H₈O₇·H₂O). The TSS/TA ratio was calculated; it is an important index, able to define the internal and, therefore, the sensory quality of the fruits (Niu et al., 2008; Lado et al., 2014).

Nutraceutical parameters

Ascorbic acid (AA) content was determined using the procedure based on the reduction of the dye 2,6-dichlorophenolindophenol (DIP) by ascorbic acid (mg ascorbic acid 100 mL⁻¹) (da Silva et al., 2017).

Total polyphenols content (TPC) and total antioxidant capacity (TAC) were performed; a pulp portion (20 g) was homogenised using an Ultraturrax blender (20,000 rpm, T25 Basic; IKA Werke, Germany). The TPC and TAC were separately analysed using a Lambda 35 spectrophotometer (PerkinElmer Corporation, Waltham, Mass., U.S.A.). Before measuring the TPC and TAC, standard curves were prepared for each test. The TPC (mg gallic acid equivalents g⁻¹ FW) was determined using the Folin-Ciocalteu method (Slinkard and Singleton, 1997). The TAC was determined using the modified TEAC assay and expressed as mmol Trolox equivalents g⁻¹ FW (Pellegrini et al., 1999; Re et al., 1999). The TEAC assay included both the hydrophilic and the lipophilic contributions (Scalzo et al., 2005) of the orange samples.

Total flavonoid content (FDT) of the samples was measured using a colorimetric method (Zhishen et al., 1999; Dewanto et al., 2002). The methanolic extract (250 µL) was mixed respectively with 1.25 mL of water and 75 µL of 5% NaNO₂ solution, then allowed to mix for 6 min. After addition of 150 µL of 10% AlCl₃ solution and mixing for 5 min, the reaction was initiated by adding 0.5 mL of 1 M NaOH, and the total volume was made up to 2.5 mL with water. Sample absorbance was read at 510 nm using a UV/Vis spectrophotometer (Lambda 35, PerkinElmer Corp., U.S.A.). Total flavonoid content was expressed as mg (+)-catechin equivalents (CA) g⁻¹ FW.

The preparation of samples for total flavonols content (FLT) determination was done according to the aluminium chloride colorimetric technique: 0.5 mL of each extract was mixed with 0.5 mL aluminium chloride (2%), and then 1.5 mL potassium acetate (5%) was added. After 150 min, the absorbance was determined at 440 nm. Total flavonols content was expressed as mg (+)-catechin equivalents (CA) g⁻¹ FW.

The calibration curve was plotted by different concentration of quercetin equivalents (Miliauskas et al., 2004).

Total Chl ($a+b$) (TChl) and carotenoid (TCar) were calculated according to Porra et al. (1989).

Total chlorophyll ($a+b$) and carotenoid content of pulp from each treatment were extracted with 80% acetone and centrifuged at 20,000 rpm for 20 min at 4°C. All pigment extraction was done in the dark with the samples kept on ice. The absorbance of the supernatant was measured using a dual-wavelength/double beam spectrophotometer (Lambda 35, PerkinElmer Corp., U.S.A.). The equation used for the quantification of Chlorophyll a , Chlorophyll b , and carotenoids are given below:

$$\text{Chl } a \text{ (}\mu\text{g mL}^{-1}\text{)} = (12.25 \times A_{663.2}) - (2.79 \times A_{648.8});$$

$$\text{Chl } b \text{ (}\mu\text{g mL}^{-1}\text{)} = (21.50 \times A_{648.8}) - (5.10 \times A_{663.2});$$

$$\text{Total Chl (}\mu\text{g mL}^{-1}\text{)} = (17.67 \times A_{648.8}) + (7.12 \times A_{663.2});$$

$$\text{Carotenoids (}\mu\text{g mL}^{-1}\text{)} =$$

$$[(1,000 \times A_{470}) - (1.82 \times \text{Chl } a) - (85.02 \times \text{Chl } b)] / 198.$$

All these measurements were carried in the Laboratory of Tree Cultivation of the Department of Agraria (Mediterranean University of Reggio Calabria).

Gas chromatography

The essential oils (1 μL) were injected into a gas chromatograph (GTrace1310, Thermo Fisher), coupled with a single quadrupole mass spectrometer (ISQ Lt. Thermo Fisher), equipped with a capillary column (TG-5MS - 30 m \times 0.25 μm \times 0.25 μm). Transfer line, ion source and injector were set at temperatures of 250°C, 260°C and 200°C, respectively, with a helium flow in the column of 1 mL min^{-1} and a split ratio of 60. The analysis was conducted in a full scan (40–400 amu), and the programmed temperature, used for sample separation, was set as follows: isothermal for 3 min at 60°C followed by an increase of 6°C min^{-1} until reaching 240°C and isothermal again at 240°C for 4 min.

The metabolites were subsequently identified according to the retention index [calculated based on a mixture of alkanes (C8–C40) injected separately] and comparing the mass

spectra with those reported in the spectral libraries (NIST 2005, PerkinElmer Inc., Waltham, MS; Wiley 7.0, John Wiley & Sons Ltd., Hoboken, NJ). After the analysis, the quantification of metabolites was performed based on the intensities of the individual normalised peaks (pA).

Statistical analysis

All data were analysed using two-way ANOVA tests for means comparisons with standard errors and the means were separated by Tukey's test when the ANOVAs indicated significant ($p < 0.05$) variable effects. The data of the fruit quality and the nutritional analysis are reported as the means from both years. The Principal component analysis (PCA) was performed; for the PCA, the square cosines values near or over 0.50 were accepted. All the analysis were performed using the IBM® SPSS® Statistic, v. 22 (SPSS Inc., IBM Company, Armonk, New York, U.S.A.).

Results and discussion

Pedoclimatic aspects of environments

The Mediterranean climate of these areas is a subtype of the temperate climate, according to the classification provided by Koppen-Geiger (Csa). It is characterised by concentrated rainfall in winter and dry and very hot summers. The average annual rainfall, altimetry, temperature showed by different changed among sites (Supplemental Table S1). The altimeters ranged from 5 m a.s.l. of Palizzi to 72 m a.s.l. of San Carlo; annual temperature ranged from 14.7°C of Melito P.S. and San Carlo to 19.9°C of Pellaro; finally the annual rainfall was highest in Casignana, 1,174.6 mm, and lowest in Bova Marina, Melito P.S., and San Carlo (527 mm) (Supplemental Table S1). The physical and chemical characteristics of the soil also differed between sites (Supplemental Table S2). Nine sites had sandy-loam soil (Bova Marina, Caulonia, Gerace, Palizzi, Pellaro, Portigliola, Rizziconi, San Carlo, Siderno), whereas four sites had clay-loam soil (Bianco, Brancaleone, Casignana, Melito P.S.) (Supplemental Table S2). The

TABLE 1. Biometric indices (fresh weight, relative length, fruit volume, peel thickness, and juice yield) in *Citrus bergamia* fruit, cv. 'Fantastico' in the bergamot cultivation sites in observation on the Ionian coast (IC) and the Tyrrhenian coast (TC) of Calabrian Region, Italy.

Areas	Fresh weight (FW) (g)	Relative length (H/D)	Fruit volume (FV) (cm^3)	Peel thickness (PT) (mm)	Juice yield (JY) (%)
Bianco (IC)	181.54 \pm 7.11d	0.91 \pm 0.01de	191.73 \pm 6.03c	2.94 \pm 0.20f	32.59 \pm 4.07n.s.
Bova Marina (IC)	280.82 \pm 10.89a	0.92 \pm 0.02de	283.15 \pm 9.05a	5.18 \pm 0.20a	44.05 \pm 1.31
Brancaleone (IC)	265.23 \pm 12.07ab	0.96 \pm 0.01abc	270.97 \pm 10.4ab	4.99 \pm 0.21ab	42.24 \pm 0.77
Casignana (IC)	210.53 \pm 8.09c	0.91 \pm 0.01de	194.97 \pm 8.15c	3.23 \pm 0.14ef	43.32 \pm 1.26
Caulonia (IC)	209.53 \pm 7.11c	0.92 \pm 0.01de	193.39 \pm 8.08c	3.99 \pm 0.24def	41.19 \pm 2.30
Gerace (IC)	232.90 \pm 11.38bc	0.96 \pm 0.01abc	268.45 \pm 10.21ab	4.11 \pm 0.21bcde	38.76 \pm 6.44
Melito P.S. (IC)	261.05 \pm 9.86ab	0.99 \pm 0.01a	296.81 \pm 10.02a	5.55 \pm 0.22a	38.91 \pm 2.35
Palizzi (IC)	209.40 \pm 7.59c	0.86 \pm 0.01e	200.22 \pm 9.04c	3.92 \pm 0.13de	39.20 \pm 2.14
Pellaro (IC)	260.80 \pm 9.18ab	0.96 \pm 0.01abc	252.96 \pm 9.56ab	4.16 \pm 0.11bcd	41.54 \pm 5.34
Portigliola (IC)	177.85 \pm 5.49d	0.93 \pm 0.01de	202.33 \pm 9.02c	3.23 \pm 0.11ef	42.86 \pm 2.55
Rizziconi (TC)	281.24 \pm 10.72a	0.97 \pm 0.01ab	275.14 \pm 10.10a	4.95 \pm 0.23ab	41.96 \pm 1.80
San Carlo (IC)	278.66 \pm 11.61a	0.90 \pm 0.01de	273.15 \pm 9.89a	4.92 \pm 0.21abc	44.18 \pm 1.26
Siderno (IC)	266.03 \pm 9.27ab	0.91 \pm 0.01de	252.87 \pm 9.58ab	5.63 \pm 0.22a	33.93 \pm 2.98
Year	*	*	*	*	*
T \times Years	n.s.	n.s.	n.s.	n.s.	n.s.

Asterisk (*) and different letters indicate significant differences per $p \leq 0.05$; n.s. = no significant differences.

pH soil ranged from slightly acid (Rizziconi, pH 6.1) to middle alkaline (Pellaro, pH 8.1); the total nitrogen content in the soil was “low” in five sites (Caulonia, Gerace, Pellaro, Rizziconi, Siderno), “high” in one site (Bova Marina), whereas in the other six sites (Bianco, Brancaleone, Casignana, Melito P.S., Palizzi, and San Carlo) it was medium (Supplemental Table S2). In all sites the organic matter (O.M.) was high (> 1.29%) and the Ec was very low (Supplemental Table S2). The available potassium (K_2O) soil content was good in four sites (Bianco, Casignana, Caulonia, and Palizzi), in three sites high (Gerace, Pellaro and Portigliola), whereas in six sites very high (Bova Marina, Brancaleone, Melito P.S., Rizziconi, San Carlo, and Siderno) (Supplemental Table S2). The available phosphorus (P_2O_5) was very low in eight sites (Bianco, Brancaleone, Casignana, Gerace, Palizzi, Portigliola, Rizziconi and Siderno), whereas it was high in the others sites [ranged from 32 ppm (Melito P.S.) to 58 ppm (Bova Marina)] (Supplemental Table S2). However, the soil characteristics reported of the 13 sites (Supplemental Table S2) have been suitable for citrus cultivation (Srivastava and Kohli, 1997). The trees selected has been grafted onto the same rootstock, sour orange. Therefore, there was no risk of finding physiological changes induced by the rootstock (Gullo et al., 2018).

Fruit biometric measurements

The experiment has shown substantial differences among the quality of bergamot fruit sampled from the 13 sites distributed along the Calabria coast. Indeed, the fresh fruit weight ranged from 177.85 g to 281.24 g.

The PT ranged from 2.94 to 5.63 mm. In the sites of Bova Marina, Brancaleone, Melito P.S., Rizziconi, San Carlo, and Siderno the average peel thickness was 66% higher than in the sites of Bianco, Casignana, Caulonia, and Portigliola where the lower average value was recorded (Table 1). The juice yield (JY) ranged from around 33% to 40%, and it did not show significant differences among the 13 compared zones (Table 1). Although peel color has been widely used as a maturity index, important variation exists between cultivation sites, because color development is affected by climatic conditions such as light and temperature (Table 2).

The TSS/TA ratio showed significant differences among compared sites. The highest ratio was found in fruit collected from Portigliola (3.00, more sweetness and better suited for fresh consumption), whereas the lowest value was found to Bova Marina (1.49, less sweetness).

The dry matter content showed that the maximum value in fruit was detached in Gerace, whereas the lowest in Rizziconi (Table 3).

In our trial, differences in the content of different health-related bioactive compounds considered important attributes for marketability and differential quality in fruits were sought. Therefore, the TAC showed the high correlation with TPC, FLT, AA, and TCar, whereas TAC vs. FDT correlation was low (Table 4).

The composition of bergamot essential oil can change in terms of quality and quantity, depending on the pedoclimatic environment.

The bergamot essential oil is composed of a volatile fraction (93–96% of the total) and a non-volatile fraction (4–7% of the total) (Costa et al., 2010; Dugo and Bonaccorsi, 2013; Navarra et al., 2015). The non-volatile fraction contains pigments, psoralins, coumarins and waxes (Dugo et al., 2000); instead, the volatile fractions composed of more than 100 molecules (Dugo et al., 1987). However, the ones that contribute most to characterise the bergamot essence are linalyl acetate, linalool and limonene.

Bergamot oil is characterised by a high content of oxygenated compounds, especially linalool (LL) and linalyl-acetate (LA), which can represent more than 50% of the entire volatile fraction. In the other citrus essential oils extracted from the peel, the total content of oxygenated compounds hardly reaches 5%.

The LL presents values with higher peaks in the fruits coming from the sites of Portigliola, Siderno and Brancaleone (Supplemental Figure S2). The bergamot coming from the zones of Portigliola, was also characterised to have higher peaks of LA in the essential oil in comparison to those coming from the other sites; in particular, the minor peaks, have been recorded for the fruits coming from the zones of Gerace and Rizziconi (Supplemental Figure S3).

TABLE 2. Main colourimetric indices in *Citrus bergamia* fruit, cv. ‘Fantastico’, in the bergamot cultivation sites in observation on the Ionian coast (IC) and the Tyrrhenian coast (TC) of Calabrian Region, Italy.

Areas	Lightness (L^*)	a^*	b^*	Chroma	°Hue
Bianco (IC)	61.89±0.93ab	-5.23±0.39ab	27.06±1.05de	27.76±0.99ed	102.58±1.03cde
Bova Marina (IC)	56.10±0.69c	-7.28±0.12cf	21.32±0.84f	22.59±0.80f	109.72±0.69a
Brancaleone (IC)	62.70±0.93ab	-4.87±0.49ab	29.35±1.09cde	29.98±1.04edc	101.33±1.02cdef
Casignana (IC)	62.26±0.83ab	-5.02±0.27ab	28.46±1.01de	29.01±0.96ed	101.00±0.86cdef
Caulonia (IC)	65.15±0.89a	-5.12±0.29ab	30.96±0.88ab	31.48±0.84bcd	100.03±0.72ef
Gerace (IC)	59.77±0.69b	-6.46±0.18bc	25.73±0.76ef	26.60±0.72ef	104.75±0.69bc
Melito P.S. (IC)	61.39±0.97ab	-5.64±0.28abc	26.85±0.96de	27.56±0.91de	103.04±0.88cde
Palizzi (IC)	53.83±0.65d	-8.36±0.20d	28.29±0.81de	29.56±0.77de	107.00±0.64ab
Pellaro (IC)	63.06±1.19ab	-4.86±0.54ab	30.04±0.89cde	30.68±0.82cde	100.51±1.11def
Portigliola (IC)	61.56±0.82ab	-7.15±0.34cd	36.55±0.84a	37.36±0.78a	101.60±0.73cdef
Rizziconi (TC)	60.53±1.12b	-3.97±0.52b	33.75±1.06abc	34.20±1.00abc	98.31±0.92fg
San Carlo (IC)	60.34±0.85b	-5.72±0.3abc	25.67±1.04ef	26.45±0.99ef	104.28±0.84bcd
Siderno (IC)	64.90±0.89a	-0.85±0.62a	34.80±1.34ab	35.03±1.23ab	95.87±0.61g
Year	*	*	*	*	*
Year x Treatment	n.s.	n.s.	n.s.	n.s.	n.s.

Asterisk (*) and different letters indicate significant differences per $p \leq 0.05$; n.s. = no significant differences.

TABLE 3. Main maturation indices (total soluble solids, titratable acidity, their ratio, dry weight) in *Citrus bergamia* fruit, cv. 'Fantastico' in the bergamot cultivation sites in observation on the Ionian coast (IC) and the Tyrrhenian coast (TC) of Calabrian Region, Italy.

Areas	Total soluble solids (TSS) (°Brix)	Titratable acidity (TA) (%)	TSS/TA	Dry weight (%)
Bianco (IC)	9.70±0.29abc	5.30±0.04ab	1.83±0.06def	16.0±0.40ab
Bova Marina (IC)	8.75±0.03bc	5.88±0.15a	1.49±0.03f	14.43±0.27cdef
Brancaleone (IC)	9.53±0.19bc	3.93±0.07de	2.42±0.05bc	14.86±0.43bcde
Casignana (IC)	10.05±0.60ab	4.62±0.10cd	2.18±0.15bcd	15.84±0.18abc
Caulonia (IC)	8.43±0.35bc	4.35±0.17cde	1.94±0.04def	15.98±0.29abc
Gerace (IC)	9.40±0.32bc	3.62±0.22e	2.63±0.20ab	16.88±0.29a
Melito P.S. (IC)	8.55±0.16bc	5.02±0.07bc	1.70±0.04ef	12.49±0.72g
Palizzi (IC)	8.45±0.03bc	4.73±0.13cd	1.79±0.06def	13.88±0.23efg
Pellaro (IC)	9.43±0.17bc	4.98±0.03bc	1.89±0.04def	14.09±0.16defg
Portigliola (IC)	11.33±0.89a	3.76±0.19e	3.00±0.10a	15.65±0.25abcd
Rizziconi (TC)	8.25±0.05c	4.63±0.18bcd	1.79±0.07def	12.86±0.19fg
San Carlo (IC)	8.53±0.13bc	5.60±0.34bc	1.70±0.08ef	13.42±0.22efg
Siderno (IC)	8.70±0.21bc	4.07±0.14de	2.15±0.07cde	14.42±0.20cdef
Year	*	*	n.s.	*
Year × Treatment	n.s.	n.s.	n.s.	n.s.

Asterisk (*) and different letters indicate significant differences per $p \leq 0.05$; n.s. = no significant differences.

TABLE 4. Main nutraceutical parameters (total antioxidant capacity, total polyphenols content, ascorbic acid, flavonoids, flavonols, chlorophyll, and carotenoids) in *Citrus bergamia* fruit, cv. 'Fantastico' in the bergamot cultivation areas in observation on the Ionian coast (IC) and the Tyrrhenian coast (TC) of Calabrian Region, Italy

Areas	TAC (μmol trolox g ⁻¹ FW)	TPC (mg GAE g ⁻¹ FW)	AA (mg 100 mL ⁻¹)	FDT (mg querc. g ⁻¹ FW)	FLT (mg querc. g ⁻¹ FW)	TChl (μg g ⁻¹ FW)	TCar (μg g ⁻¹ FW)
Bianco (IC)	5.066b	0.575de	45.40bc	0.441cd	0.177def	2.056bc	0.411ab
Bova Marina (IC)	3.780c	0.453e	41.19abc	0.504cd	0.172ef	0.193e	0.226bcd
Brancaleone (IC)	5.172b	0.662cd	45.02abc	0.510cd	0.199abcde	0.725de	0.322abcd
Casignana (IC)	4.709bc	0.792bc	49.23cd	0.627bcd	0.188bcdef	0.444de	0.226bcd
Caulonia (IC)	1.853d	0.501de	35.67a	0.531cd	0.170f	0.462de	0.166d
Gerace (IC)	5.542ab	0.843abc	57.84d	0.894ab	0.204abcd	4.070a	0.401ab
Melito P.S. (IC)	4.237bc	0.556de	39.33ab	0.592cd	0.178cdef	0.348de	0.203cd
Palizzi (IC)	4.710bc	0.774bc	47.32bc	0.561cd	0.173ef	1.293cd	0.419ab
Pellaro (IC)	4.971bc	0.848abc	38.13ab	0.706abcd	0.206abc	1.352bcd	0.398abc
Portigliola (IC)	6.760a	0.875a	44.42abc	0.696abcd	0.220a	1.800bc	0.465a
Rizziconi (TC)	5.339b	1.025a	45.02abc	0.937a	0.209ab	0.240e	0.269abcd
San Carlo (IC)	4.969bc	0.567de	42.01abc	0.433d	0.171ef	2.332b	0.4016ab
Siderno (IC)	4.367bc	0.871ab	47.32bc	0.714abc	0.221a	0.754de	0.433a
Year	*	*	n.s.	*	n.s.	*	*
Year × Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Asterisk (*) and different letters indicate significant differences per $p \leq 0.05$; n.s. = no significant differences.

The ratio between LA and LL is considered one of the most important quality indexes of bergamot essential oil. In the province of Reggio Calabria, the ratio generally reaches values close to 0.3 (Di Giacomo and Mincione, 1995; Bouzouita et al., 2010). As far as α -pinene (α -P) and limonene (LM), the higher values were recorded in the sites of Bova Marina, Gerace, and Bianco, whereas the lower values were recorded in the sites of Rizziconi, Siderno and Portigliola, (Supplemental Figures S4 and S5). The γ -terpinene (γ -T) was higher to Bova Marina, Gerace, Melito P.S., Pellaro, and Bianco and lower values were detected to Rizziconi, Portigliola, and Siderno (Supplemental Figure S6).

The higher values of β -pinene (β -P) were found in the sites of Gerace, Bova Marina, whereas the lower values were found in the sites of Brancaleone, Siderno and Rizziconi (Supplemental Figure S7).

PCA

Factors F1, F2, F3, and F4 of the PCA accumulated 27.57%, 19.53%, 11.90% and 8.64% of the variance, respectively, and totaled 67.63% of the initial variability (Figure 1)

In particular, TSS/TA, FLT, TPC, L^* , a^* , b^* , TChr, and LL, Plu, were placed on the right side of F1-F2 plane, whereas TA, α -P, β -P, γ -T, LM, °Hue, N, and T (°C) were located on the left

TABLE 5. Correlation matrix (Pearson coefficient) between the parameters observed in bergamot fruit, cv. 'Fantastico', and the climatic and soil parameters.

Variables	TC	Clay	Sand	pH	Ec	O.M.	N	P ₂ O ₅	K ₂ O	MgO	Na	PLUVIOMETRY	TAC	TPC	FDT	FLT	TCH	TcR	AA	TSS	TA	TSS/TA	DW	FW	H	D	H/D	FV	IV	PT	1°	a*	b*	Chr	hue	α-P	β-P	IV	Y-T	LL	LA		
TC	1.00	0.40	-0.34	-0.29	-0.32	-0.47	0.75	0.37	0.20	0.24	0.24	0.24	-0.23	-0.02	-0.57	-0.72	0.06	0.10	-0.14	0.12	-0.02	0.51	-0.32	-0.01	-0.13	-0.14	0.07	-0.36	-0.04	0.15	-0.15	-0.71	-0.71	-0.76	-0.76	0.86	0.32	0.44	0.18	0.64	0.32	0.14	
Clay	0.40	1.00	-0.99	0.68	0.30	0.16	-0.07	0.27	-0.20	0.32	0.38	-0.14	-0.12	-0.21	-0.39	-0.34	-0.09	-0.07	-0.04	-0.05	0.23	-0.25	-0.06	-0.11	-0.18	-0.18	-0.14	-0.18	-0.03	-0.14	0.01	-0.02	-0.31	-0.33	0.07	0.06	0.01	0.18	0.29	-0.24	-0.10		
Sand	-0.34	-0.99	1.00	-0.68	-0.30	-0.24	0.08	-0.28	0.15	-0.28	-0.31	0.22	0.10	-0.27	0.37	-0.44	-0.22	0.14	0.09	0.03	0.22	0.18	0.04	0.40	-0.28	-0.26	-0.21	-0.28	-0.27	0.24	0.11	-0.09	-0.33	-0.33	-0.19	0.30	0.24	0.40	0.31	-0.01	0.15		
pH	0.29	0.68	-0.68	1.00	-0.37	-0.24	0.05	-0.29	0.23	0.21	-0.27	-0.25	0.37	-0.44	-0.22	0.14	0.09	-0.08	-0.07	0.52	0.62	-0.32	0.56	0.02	-0.14	0.11	0.04	0.15	0.07	0.03	-0.25	-0.18	-0.15	0.27	0.29	-0.04	-0.30	-0.35	-0.26	-0.34	0.20	0.07	
Ec	-0.32	0.30	-0.38	0.37	1.00	0.79	-0.28	0.13	-0.05	-0.10	-0.12	-0.20	0.06	0.22	0.15	0.23	0.03	0.19	-0.34	0.09	0.19	-0.10	-0.13	0.17	0.15	0.00	0.25	0.08	0.11	0.07	0.12	0.22	0.06	0.06	0.05	-0.14	-0.12	-0.10	0.20	-0.20	-0.19		
O.M.	-0.47	0.15	-0.24	-0.14	0.79	1.00	-0.40	-0.08	0.04	-0.19	-0.13	0.00	0.15	0.51	0.44	0.33	0.13	0.08	-0.31	0.44	-0.20	0.44	-0.20	0.41	-0.16	0.12	0.22	0.43	-0.14	0.33	0.18	0.13	-0.78	-0.72	-0.73	0.90	0.67	0.71	0.58	0.64	-0.20	-0.44	
N	0.75	-0.07	0.08	-0.28	0.48	1.00	0.43	0.24	-0.16	-0.29	-0.40	0.06	-0.46	-0.51	-0.27	0.34	0.00	0.41	-0.19	0.65	-0.47	-0.12	0.37	0.25	0.39	0.39	0.39	0.34	0.33	0.18	0.33	-0.78	-0.74	-0.75	0.90	0.67	0.71	0.58	0.64	-0.20	0.05		
P ₂ O ₅	-0.08	-0.20	0.15	-0.29	-0.06	0.04	0.24	1.00	0.06	-0.34	-0.56	-0.66	-0.13	0.07	0.10	-0.28	-0.19	-0.22	-0.46	0.15	-0.29	-0.68	0.86	0.87	0.87	0.91	0.47	0.92	0.17	0.95	-0.04	0.36	-0.16	-0.16	0.02	-0.08	0.05	-0.10	-0.10	0.08	-0.05		
K ₂ O	0.32	0.32	-0.28	0.23	-0.10	-0.19	-0.16	0.34	1.00	-0.06	-0.34	0.28	0.25	0.08	-0.33	0.08	-0.19	-0.16	0.08	0.54	-0.05	0.29	0.24	-0.09	0.24	-0.09	-0.09	-0.14	-0.03	0.33	0.15	0.07	-0.18	0.15	-0.14	0.04	-0.02	-0.14	0.16	0.13	0.36	0.29	
Na	0.24	0.38	-0.31	0.21	-0.12	-0.13	-0.29	-0.23	-0.34	0.73	1.00	0.65	0.05	0.08	-0.10	-0.26	-0.31	0.24	0.37	0.34	0.34	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	
PLUVIOMETRY	-0.23	-0.14	0.22	-0.27	-0.20	0.00	-0.48	-0.40	-0.56	0.28	0.65	1.00	0.28	0.53	0.40	0.36	-0.09	-0.07	0.38	0.48	-0.31	0.40	0.39	-0.46	-0.45	-0.58	-0.06	-0.52	0.03	-0.56	0.25	0.20	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
TAC	-0.02	-0.12	0.10	-0.25	0.06	0.15	0.06	-0.36	-0.06	0.25	0.05	0.28	1.00	0.60	0.37	0.59	0.47	0.69	0.52	0.62	-0.32	0.56	0.02	-0.14	0.11	0.04	0.15	0.07	0.03	-0.25	-0.18	-0.15	0.27	0.29	-0.02	-0.04	-0.13	-0.03	-0.10	0.32	0.16		
TPC	-0.57	-0.22	0.17	-0.37	0.22	0.51	0.44	-0.45	-0.51	-0.13	-0.16	0.08	0.53	1.00	0.84	0.82	0.15	0.44	0.46	0.54	0.47	-0.02	0.02	0.02	0.02	0.10	0.16	-0.05	-0.06	-0.11	0.08	0.29	0.67	0.67	-0.52	-0.49	-0.49	-0.44	-0.54	0.20	0.12		
FDT	-0.72	-0.34	0.29	-0.22	0.23	0.33	-0.55	-0.34	0.10	0.03	-0.02	0.36	0.59	0.82	0.72	1.00	0.13	0.46	0.36	0.44	-0.65	0.67	0.11	0.05	0.20	0.01	0.37	0.11	-0.12	0.05	0.38	0.47	0.71	0.72	-0.62	-0.40	-0.41	0.23	0.35	0.52	0.45	0.26	0.14
FLT	0.06	-0.09	0.08	0.14	0.03	-0.13	0.34	-0.30	-0.28	-0.19	-0.26	-0.09	0.47	1.00	0.44	0.12	0.45	0.44	0.48	0.29	0.29	0.43	0.15	-0.24	-0.20	-0.05	-0.31	-0.16	-0.35	-0.24	-0.11	-0.01	0.30	0.31	-0.05	-0.01	-0.13	-0.01	-0.25	0.72	0.64		
TCH	-0.14	-0.07	0.03	0.09	0.19	0.08	0.00	-0.36	-0.19	-0.16	-0.31	-0.07	0.69	0.44	1.00	0.41	0.39	-0.32	0.43	1.00	0.25	-0.49	0.49	0.44	-0.18	-0.05	0.00	-0.10	-0.04	-0.26	-0.21	-0.24	-0.03	-0.03	0.07	0.28	0.20	0.37	-0.09	-0.02	-0.22		
AA	0.12	-0.04	0.08	-0.03	-0.34	-0.23	0.14	-0.41	-0.22	0.08	0.24	0.38	0.52	0.48	0.46	0.36	0.59	0.41	1.00	0.25	-0.49	0.49	0.44	-0.18	-0.05	0.00	-0.10	-0.04	-0.26	-0.21	-0.24	-0.03	-0.03	0.07	0.28	0.20	0.37	-0.09	-0.02	-0.22			
AA	-0.02	-0.05	0.06	0.22	0.09	-0.16	-0.20	-0.19	-0.46	0.54	0.37	0.48	0.62	0.24	0.05	0.44	0.29	0.39	0.25	1.00	-0.38	0.71	0.57	-0.61	-0.33	-0.50	0.04	-0.42	0.08	-0.64	0.20	-0.19	0.29	0.31	-0.07	0.04	-0.07	0.06	-0.01	0.60	0.56		
TA	0.51	0.23	-0.18	0.07	0.17	0.09	0.44	0.65	0.15	-0.05	-0.10	-0.31	-0.32	-0.54	-0.50	-0.65	-0.55	-0.32	-0.49	0.38	1.00	-0.87	-0.43	0.26	0.06	0.23	-0.17	0.15	0.08	0.12	-0.38	-0.21	-0.62	-0.62	0.48	0.27	0.25	0.77	0.53	-0.60	-0.18		
TSS/TA	-0.32	-0.25	0.22	0.04	-0.10	-0.19	-0.31	-0.47	-0.29	0.29	0.20	0.40	0.56	0.47	0.38	0.67	0.44	0.43	0.49	0.77	-0.87	1.00	0.60	-0.46	-0.52	-0.59	-0.20	-0.57	-0.17	-0.67	0.23	-0.11	0.00	0.01	0.42	-0.39	-0.08	-0.12	-0.09	-0.32	0.71	0.42	
DW	-0.01	-0.06	0.12	0.40	-0.13	-0.38	-0.16	-0.12	-0.68	0.24	0.27	0.39	0.02	-0.03	0.01	0.11	0.48	0.15	0.44	0.57	0.43	0.60	1.00	-0.46	-0.66	-0.66	1.00	0.85	0.71	0.89	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	
FW	-0.13	-0.11	0.05	-0.26	0.15	0.27	0.23	0.25	0.87	0.00	-0.38	-0.45	0.11	0.02	0.32	0.30	-0.05	-0.20	-0.05	-0.16	-0.52	0.82	1.00	0.82	0.82	0.82	1.00	0.85	0.77	0.89	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	
H	0.07	-0.11	0.05	-0.26	0.15	0.27	0.23	0.25	0.87	0.00	-0.38	-0.45	0.11	0.02	0.32	0.30	-0.05	-0.20	-0.05	-0.16	-0.52	0.82	1.00	0.82	0.82	0.82	1.00	0.85	0.77	0.89	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	
D	0.07	-0.11	0.05	-0.26	0.15	0.27	0.23	0.25	0.87	0.00	-0.38	-0.45	0.11	0.02	0.32	0.30	-0.05	-0.20	-0.05	-0.16	-0.52	0.82	1.00	0.82	0.82	0.82	1.00	0.85	0.77	0.89	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91		
FV	0.07	-0.11	0.05	-0.26	0.15	0.27	0.23	0.25	0.87	0.00	-0.38	-0.45	0.11	0.02	0.32	0.30	-0.05	-0.20	-0.05	-0.16	-0.52	0.82	1.00	0.82	0.82	0.82	1.00	0.85	0.77	0.89	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91		
H/D	0.07	-0.11	0.05	-0.26	0.15	0.27	0.23	0.25	0.87	0.00	-0.38	-0.45	0.11	0.02	0.32	0.30	-0.05	-0.20	-0.05	-0.16	-0.52	0.82	1.00	0.82	0.82	0.82	1.00	0.85	0.77	0.89	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91		
IV	0.07	-0.11	0.05	-0.26	0.15	0.27	0.23	0.25	0.87	0.00	-0.38	-0.45	0.11	0.02	0.32	0.30	-0.05	-0.20	-0.05	-0.16	-0.52	0.82	1.00	0.82	0.82	0.82	1.00	0.85	0.77	0.89	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91		
PT	0.07	-0.11	0.05	-0.26	0.15	0.27	0.23	0.25	0.87	0.00	-0.38	-0.45	0.11	0.02	0.32	0.30	-0.05	-0.20	-0.05	-0.16	-0.52	0.82	1.00	0.82	0.82	0.82	1.00	0.85															

Hue and then produced bergamot fruit with high values for these parameters most suitable for essence extraction (Supplemental Table S4; Figure 2).

In contrast to Bova Marina, Portigliola and Siderno were correlated positively with F1 variables as TPC, TSS/TA, FL, b^* , TChr, Hue, and LL.

Bianco and Rizziconi showed high square cosine with F2. Rizziconi showed high values of D, H, V, FW, and PT, whereas Bianco showed high values of DW, TSS, but for its position also good values of LM, α -P, β -P e γ -T (Supplemental Table S4).

Gerace and Pellaro were placed in the lower right and lower left side of the F3-F4 plane, respectively (Figure 2); Gerace participated to F3 formation and the fruit showed higher AA and TAC, whereas Pellaro centroid participated to F4 construction and showed high square cosines with exception LA (Figure 2).

The other sites or showed lower square cosines or participated to other factors (Table 5, Supplemental Table S4).

Conclusion

The environment has influenced the production of bergamot, both for fresh consumption and for essential oil. However, the PCA has found that some fruit parameters are strongly dependent by environment only in seven sites. Indeed, the bergamots coming from Rizziconi on the Tyrrhenian coast have the best biometric parameters [FW (281.24 g \pm 0.72)], but the lower peak of component of essential oil [LL (2.17E+04 pA), LA (2.52E+07 pA), and LM (6.86E+07 pA)]; the fruit of Portigliola, Siderno, and Gerace on the Ionian coast have pulp with high health parameters [TPC (0.875 mg GAE g⁻¹ FW), TAC (4.367 μ mol trolox g⁻¹ FW), and AA (57.84 mg 100 mL⁻¹), respectively]; furthermore, the fruit of Portigliola has a pulp more sweetness (TSS/TA = 3 \pm 0.10), flavado more yellow, but also a high peak of an important component of essential oil [LL (2.49E+06 pA)]. The fruit of Bova Marina and San Carlo shows the good biometric parameters such as FW (280.82 g \pm 10.89; 278.66 g \pm 11.61, respectively), flavado greenish, juice with high titratable acidity (5.88% \pm 0.15 pA; 5.60% \pm 0.34 pA, respectively), optimal content of LL (3.08E+04 pA; 5.13E+04 pA), LM (2.43E+08 pA, 1.19E+08 pA) α -P (2.43E+08 pA; 3.18E+06 pA), β -P (3.38E+07 pA, 2.29E+07 pA), and γ -T (7.82E+04 pA, 6.10E+04 pA, respectively). The bergamot of Bianco has the highest content of DW (16% \pm 0.40) and TSS (9.70 °Brix \pm 0.29), but also fruit greenish with a good content of LM (2.25E+08 pA). Finally, the fruit of Pellaro has high AA content (38.13 mg 100 mL⁻¹) and high LA in flavado (4.04E+07 pA). Therefore this work has shown that the components that define the essence remain the prerogative of the traditional area located on the Ionian, whereas on the Tyrrhenian coast, the fruits have shown better biometric performances.

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SUPPLEMENTAL INFORMATION – TABLES S1–S4 AND FIGURES S1–S7For Supplemental Information see www.ishs.org/eJHS.