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RESEARCH ARTICLE

# *Citrus bergamia*, Risso: the peel, the juice and the seed oil of the bergamot fruit of Reggio Calabria (South Italy)

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ABSTRACT

Bergamot is a characteristic citrus fruit of the province of Reggio Calabria (South Italy) and until today it was mainly known for the production of its peel essential oil. Since some year, due to the positive feedback from consumers, there has been a growing interest in the bergamot juice which in the past was not used as a beverage because it was considered very sour. The recent studies on the functional aspects of citrus fruits and therefore also on bergamot have made this fruit known to a new youth. They exist three cultivars: *Castagnaro*, *Fantastico* and *Femminello*. In this study the biometrics of the fruits and the physico-chemical characteristics of the juice have been taken into consideration, such as: Brix degrees, pH, pulp content in the squeezed juice, turbidity, acidity, vitamin C, Formol number, single and total flavonoids. Total flavonoids in the bergamot juice ranged between 362 and 520 mg/L whereas in the *Cloudy* juice they ranged between 4660 and 8468 mg/L. The composition in fatty acids and in sterols of the seed oil of this fruit was also analysed to evaluate its potential. Oleic acid (30.15-34.36%) and linoleic acid (27.01-29.84%) were found in similar quantity, while the most present sterol was $\beta$-sitosterol (76.9-79.2%).

**Keywords:** Castagnaro; Essential oil; Fatty acids; Fantastico; Femminello; Flavonoids; Limonene; Sterols

## INTRODUCTION

Calabria (South Italy) is a very active region from the point of view of quality and many agricultural products have obtained the certification of their quality. Among these, very important is the citrus production and mainly the bergamot. Many studies have been conducted on citrus fruits (Giuffrè et al., 2017a; Proto and Zimbalatti, 2010; Giuffrè, 2019). Much more can be said about bergamot especially because in the last decade there has been a renewed and increased demand for this fruit to be used both for the extraction of the peel essential oil (Gioffrè et al., in press) and for its juice due to the presence of functional compounds. Also the bergamot by-products were studied (Di Donna et al., 2020). Very important is therefore the possibility to extract bioactive compounds from the various parts of the fruit, in addition, the seed oil could be used as a food or for industrial uses (bio-diesel, pharmaceutical products). The use of the peel for the preparation of sweets or candied fruit has also to be considered. The preparation of biscuits, sweets, creams, pastries, chocolates, nougat and other types of bergamot flavored desserts is quite widespread. The cultivation area of the bergamot is included between Scilla and Monasterace, as indicated by the article No. 3 of the Regulations for the protected designation of origin 'Bergamot of Reggio Calabria-Essential oil' (Politiche agricole, 2020) in a coastal strip that, measured by coast towards the hinterland, it is deep up to 12 km. Bergamot juice is rich in flavonoids for which an antioxidant action has been proven with the ability to scavenge free radicals and which is manifested in protecting the cell wall of the stomach (Bigoniya and Singh, 2014), improving vascular integrity and decreasing the vascular permeability (Pizzorno and Murray, 2019). Flavonoids exert pharmacological properties with anti-inflammatory and analgesic effects (Galati et al., 1994) and protect against an increase in blood pressure and cholesterol in the blood (Benavente-Garcia et al., 1997; Pszczola, 1998). Bergamot peel essential oil was found to have antimicrobial, anti-inflammatory, analgesic and anti-

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proliferative effects (Navarra et al., 2015) and a mixture of essential oils inhaled before sleeping was found to have a beneficial effect for patients in cardiac rehabilitation (McDonnell and Newcomb, 2019).

The purpose of this scientific work is to study and obtain recent information on the biometric characteristics of fruits, the physico-chemical properties of the juice, the seed oil composition and the peel essential oil profile of the three bergamot cultivars grown in the province of Reggio Calabria.

## MATERIALS AND METHODS

### Vegetable material

The experiment was conducted in the geographical area of Bianco, a small town in the Ionian side of the province of Reggio Calabria, South Italy, in the 2018-2019 harvest year, when samples were collected in different maturation stages. All the three known cultivars have been considered, i.e.: *Castagnaro*, *Fantastico* and *Femminello* (Figs. 1-3). For each cultivar, a total of 30 kg of fruit was collected at random from 20 bergamot trees, each month from October to March. The fruits were immediately taken to the laboratory to be subjected to biometric and physico-chemical analyses. The analytical values reported in the tables of this work and here are reported the means between all collection periods.

Fig 1. The bergamot fruit (*Castagnaro* cv).

Fig 2. The bergamot fruit (*Fantastico* cv).

Fig 3. The bergamot fruit (*Femminello* cv).

First, the fruits were subjected to the peel essential oil extraction. After this, the remaining part, consisting of the albedo and the remaining part of the pulp which is called 'pastazzo', was homogenized and ground by adding water in a water/pastazzo ratio 2:1, to facilitate the flavonoids extraction. The mixture was pressed to separate the pulp (from which pectins are extracted in a specific industrial plant) from the liquid phase. Pulp contained pectins non-soluble and soluble in water. The non-soluble in water pectins represent a problem when the liquid phase has to be separated from the pulp. To facilitate pectin degradation, it was used pectinase at 50-60 °C that breaks the wall of cell and the obtained juice is called 'cloudy juice' (Li et al., 2006; Laroze et al., 2020; Roggia Ruviaro et al., 2019). This method is applied in the edible juice extraction industry.

### Quantification and determination of the essential oil

The peel essential oil extraction was made from bergamot fruits by breaking the oil glands existing in the peel and by collecting the essential oil on a laboratory glass. The essential oil was sampled by a specific syringe and was injected into a GC-MS system (Gionfriddo et al., 2003). The identification of the volatiles was based on the comparison of their mass spectra with those of NIST/EPA/NIH Mass spectral library Version 2.0. In many cases, the identification was further confirmed by comparing the retention times of pure standards separately injected, in addition, the retention times of the analytes were confirmed with those from the literature.

### Determination of the brix degree of juice

It was conducted using a refractometer from the Mettler-Toledo company, placing a drop of juice, after the instrument calibration with bi-deionised water (IFUMA, 2006).

### Determination of pH of juice

It was performed by a measurement with an instrument provided by the Mettler-Toledo company (IFUMA, 2006).

### Determination of pulp content in the juice

This parameter indicates the solid fraction (as a percentage) existing in the juice after bergamot fruit pressure and it was quantified after centrifugation for 10 minutes at 3500 rpm. Pulp was separated from juice because to the specific weight differences.

### Determination of turbidity of the juice

A solution containing 12% pressed bergamot in deionised water was prepared, and the transmittance was measured at 578 nm and it was expressed as a percentage ratio between the intensity of the incident light (IFUMA, 2006).

### Determination of titratable acidity of the juice

The sample was prepared by weighing 10 g of the bergamot juice in a beaker and by adding 150 mL of deionised water with a subsequent 10 min boiling. At this point the acidity was determined by titration with an aqueous solution of 0.1N NaOH, up to pH 8.1. Acidity was expressed as grams of citric acid monohydrate per liter of juice (IFUMA, 2006).

### Determination of vitamin C in the juice

Quantification of vitamin C was performed by iodometric titration. Ten mL of juice and 5 mL of deionised water were placed in a beaker. The solution was titrated with a 0.01 N iodine solution using 2% starch as an indicator. The result was expressed as mg of ascorbic acid / L of juice (IFUMA, 2006).

### Determination of the formol number in the juice

It was carried out by titration with an aqueous solution of 0.1N NaOH and by using phenolphthalein as an indicator; the method EN 1133, IFUMA 30 (IFUMA, 2006) was applied.

### Determination of flavonoids in the juice

It was conducted using an HPLC method with a Knauer (Berlin, Germany) instrument equipped with a diode-array detector (PDA), a thermal conditioning furnace, a separation chromatographic column (Kinetex 5μ C18 100 Å, 150 mm length, 4.6 mm internal diameter). The mobile phase used in this experiment was 0.1% formic acid in deionised water (A) and methanol (B) with the following conditions: 80% A in isocratic (5 min); from 80% A to 45% A in gradient (42 min); 45% A in isocratic (5 min); from 45 to 20% A (7 min); 20% A in isocratic (5 min); from 20 to 80% A (5 min). The injection volume was 20 μL, the flow rate was set at 1 mL/min and the UV detector was set at 280 nm. The system was supported by Chromera software version 3.4.0.5712 (Giuffrè et al., 2019).

### Determination of fatty acids in the seed oil

The oil was extracted from the dried seeds using a Soxhlet apparatus with *n*-hexane as the extracting solvent. The fatty acids were transformed into their methyl esters and determined by gas-chromatography. The samples were prepared following the indications given by the cold method proposed by the EEC Reg., Annex XA (EEC, 2015), the gas-chromatographic conditions used are those described in another scientific work (Giuffrè and Capocasale, 2016a).

### Determination of sterols in the seed oil

The seed oil, extracted as described in 2.11, was analysed also for sterol quantification. The samples were prepared according to the method proposed by the EEC Reg., Annex V (EEC, 2015), the gas-chromatographic conditions were those described in previous papers (Giuffrè and Capocasale, 2016b). In short, the oil was subjected to saponification and the unsaponifiable fraction was separated by thin layer chromatography. At this point the sterol fraction was extracted and subjected to silanisation before the gas-chromatographic analysis.

### Statistical Analysis

Means were calculated on 3 replicates and the Excel 2010 software was used. Statistical differences were calculated by one-way ANOVA and Tukey test for post hoc analysis at $p<0.05$ and the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used.

**Table 1: Biometrics of bergamot fruit. *** significance at p<0.001; * significance at p<0.05. Means in the same column are distinguished by different letters**

| Cultivar | Fruit weight (g) | Vertical diameter (cm) | Horizontal diameter (cm) | Peel weight (g) | Juice weight / fruit (g) |
|---|---|---|---|---|---|
| Castagnaro | 343$^{a}$ | 8.91$^{a}$ | 9.34$^{a}$ | 53.30$^{a}$ | 30.19$^{b}$ |
| Fantastico | 222$^{b}$ | 7.61$^{b}$ | 7.83$^{b}$ | 37.95$^{b}$ | 32.57$^{a}$ |
| Femminello | 159$^{c}$ | 6.68$^{c}$ | 6.86$^{c}$ | 22.82$^{c}$ | 32.49$^{a}$ |
| *Significance* | *** | *** | *** | *** | * |

## RESULTS AND DISCUSSION

### Biometrics of fruit

Fruits of the three cultivars differed with regard to the biometrics (Table 1). The fruit of the *Castagnaro cv.* was the heaviest (343 g), i.e about twice that of the *Femminello cv.* (159 g), ($p<0.001$). The vertical diameter showed very high significant differences between cultivars ($p<0.001$) and measured 8.91, 7.61 and 6.68 cm for *Castagnaro*, *Fantastico* and *Femminello cvs.* respectively. The weight of the peel was one of the most interesting parameters. The *Castagnaro cv.* was characterised by the highest peel content (53.30 g on average), i.e.15-16% of the fruit weight, this is important in the production of the essential oil or in the production of candied fruit. If the quantity of juice per fruit is examined, it can be seen that the fruit of *Castagnaro cv.* showed a quantity of juice lower than the other two cultivars ($p<0.05$), namely 30.19 g, compared to 32.57 g of *Fantastico cv.* and 32.49 g of the *Femminello cv*.

### Volatiles in the essential oil fraction

The color of the essential oil is green at the beginning of the ripening stage of the fruit and it changes to yellow at the end of ripening. At the same time this variation happens in the peel of fruit from October to March. The peel color of fruits picked at the green stage turn to yellow after a prolonged storage.

Volatiles are the components of a fragrance with a low molecular weight which can be described in terms of notes (top, middle and bottom). The top notes (the most volatile) are immediately perceived when the container of an essential oil is opened, the middle notes are the constituents of the body of a fragrance and the bottom notes are the longest lasting volatiles. The characteristics of each fragrance are due to the balance of the three notes. The very high value of an essential oil as the one of bergamot is related to the presence of a large number of volatiles which compose the fragrance instead of a synthetic fragrance containing a very low number of molecules (Draelos, 2013). In this work 66 volatiles were revealed. For all the three cultivars the prevailing compound was limonene 39.40% (*Castagnaro*), 35.03% (*Fantastico*), 31.10% (*Femminello*) showing very high significant differences between the three cultivars ($p<0.001$), this was confirmed by other studies (Poiana et al., 2003; Costa et al., 2010). Linalyl acetate was the second most notably component: 33.40% (Castagnaro), 37.73% (*Fantastico*), 38.61% (*Femminello*), ($p<0.001$). Limonene and linalyl acetate are the most potent odorants in the volatiles based on their lowest threshold odor values. The higher their quantity the higher the perception of bergamot by sensory.

A considerable detected quantity was that of γ-terpinene between 5.00% (in *Fantastico cv*) and 8.14% (in *Femminello cv*), ($p<0.001$). Linalool, which is a terpenoid widely used in cosmetics (Draelos, 2013) was one of the predominant compounds and was found between 6.00% (*Castagnaro*) and 9.00% (*Femminello*), ($p<0.001$). β-Pinene was the fifth compound in order of quantity detected in the bergamot essential oil ranging between 4,12% (*Castagnaro*) and 5,78% (*Femminello*), ($p<0.001$). All other volatiles were quantified for less than 1% (Table 2).

### Brix degree of juice

The Brix degree can be defined as the measurement of the solid state of substances dissolved in a liquid. For this measure the sucrose expressed as g/100 of the analysed juice is taken as the reference substance. For all three bergamot cultivars, the °Brix found was between 8.63 (*Femminello*) and 8.83 (*Fantastico*) ($p<0.05$), (Table 3) reflecting the low sugar content present in this juice, that is in a comparable quantity to that found in juices of Spanish lemon (Marín et al., 2003), and less than that found in tangerine juice (10.42-14.17) or sweet orange (11.3-12.58) detected on fruits obtained from plants grown in China (Xu et al., 2008).

### pH of juice

The bergamot juice is very acidic and contains mainly ascorbic acid and citric acid which determine the final pH value. In the samples analysed in this study the pH value was between 2.43 in the *Femminello* cv and 2.58 in *Castagnaro* ($p<0.05$) (Table. 3). These values are lower than those recorded in other citrus juices 3.05 in grapefruit, 3.63 in orange, and similar to that found in lemon juice (2.43), in fruits from a local Hungarian vegetable market (Cserhalmi et al., 2006).

### Pulp content

Pulp is the solid fraction present in the juice obtained by pressing. After the fruit is mechanically pressed, the obtained mixture is sent to the subsequent production phases. Pulp is a problem from a technological point of view when the juice has to be subjected to industrial treatments (mainly for the concentration or conservation), and for this reason it must necessarily be eliminated. Separation is conducted by centrifugation. In the juice of the three cultivars the pulp content was closed between 9.37% (*Femminello*) and 9.83% (*Castagnaro*), ($p<0.05$) (Table 3).

### Turbidity

It defines the greater or lesser clarity of the juice and is a parameter that is also required for other types of products derived from other vegetable juices. Rather similar values have been found among the three cultivars varying between 37.71% (*Fantastico*) and 39.81% (*Femminello*), ($p<0.001$) (Table. 3).

**Table 2: Percentage content of the volatile compounds of the peel essential oil of the three Bergamot cultivars: *Castagnaro*, *Fantastico* and *Femminello*. Values are expressed as mean of three replicates±SD. *** significance at p<0.001; ** significance at p<0.01; * significance at p<0.05; n.s., not significant. Means in the same line are distinguished by different letters**

| | VOLATILES (%) | *Castagnaro* | *Fantastico* | *Femminello* | *Signif.* |
|---|---|---|---|---|---|
| 1 | Tricyclene | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 2 | α-Tujene | 0.22[b] | 0.32[a] | 0.11[c] | *** |
| 3 | α-Pinene | 0.53[a] | 0.21[c] | 0.33[b] | *** |
| 4 | Camphene | 0.04[b] | 0.07[a] | 0.02[c] | *** |
| 5 | Sabinene | 0.66[a] | 0.48[b] | 0.47[b] | ** |
| 6 | β-Pinene | 4.12[c] | 5.22[b] | 5.78[a] | *** |
| 7 | 6-Methyl-5-hepten-2-one | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 8 | Myrcene | 0.73[a] | 0.57[b] | 0.54[bc] | *** |
| 9 | Octanal | 0.04[a] | 0.04[a] | 0.03[a] | n.s. |
| 10 | α-Phellandrene | 0.07[b] | 0.09[a] | 0.01[c] | *** |
| 11 | Δ3-Carene | 0.01[a] | 0.01[a] | tr | n.s. |
| 12 | α-Terpinene | 0.23[b] | 0.35[a] | 0.11[c] | *** |
| 13 | p-Cymene | 0.17[c] | 0.30[a] | 0.28[ab] | *** |
| 14 | Limonene | 39.40[a] | 35.03[b] | 31.10[c] | *** |
| 15 | (Z)-β-Ocimene | 0.06[c] | 0.11[a] | 0.09[b] | *** |
| 16 | (E)-β-Ocimene | 0.25[a] | 0.24[a] | 0.16[b] | ** |
| 17 | γ-Terpinene | 7.42[b] | 5.00[c] | 8.13[a] | *** |
| 18 | cis-Sabinene hydrate | 0.09[a] | 0.07[b] | 0.05[c] | *** |
| 19 | Terpinolene | 0.42[a] | 0.41[a] | 0.31[b] | ** |
| 20 | Linalool | 6.00[c] | 8.67[b] | 9.00[a] | *** |
| 21 | Nonanal | 0.03[a] | 0.03[a] | 0.01[b] | ** |
| 22 | Hepthyl acetate | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 23 | cis-Limonene oxide | 0.01[bc] | 0.02[b] | 0.04[a] | ** |
| 24 | trans-Limonene oxide | 0.02[a] | 0.01[b] | 0.02[a] | * |
| 25 | Camphor | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 26 | Citronellal | 0.10[a] | 0.05[b] | 0.06[b] | ** |
| 27 | Terpinen-4-ol | 0.04[b] | 0.04[b] | 0.09[a] | ** |
| 28 | α-Terpineol | 0.08[a] | 0.07[ab] | 0.07[ab] | ** |
| 29 | Decanal | 0.06[b] | 0.01[c] | 0.11[a] | *** |
| 30 | Octyl acetate | 0.11[a] | 0.11[a] | 0.10[a] | n.s. |
| 31 | Nerol | 0.01[c] | 0.05[b] | 0.08[a] | *** |
| 32 | Citronellol | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 33 | Neral | 0.12[c] | 0.31[b] | 0.44[a] | *** |
| 34 | Carvone | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 35 | Linalyl acetate | 33.40[c] | 37.73[b] | 38.61[a] | *** |
| 36 | Geranial | 0.68[a] | 0.26[c] | 0.39[b] | *** |
| 37 | Isobornyl acetate | 0.02[ab] | 0.02[ab] | 0.03[a] | ** |
| 38 | Undecanal | 0.03[c] | 0.06[b] | 0.11[a] | *** |
| 39 | Nonyl acetate | 0.11[a] | 0.05[bc] | 0.07[b] | *** |
| 40 | Methyl geranoate | 0.02[a] | 0.01[ab] | 0.01[ab] | ** |
| 41 | Linalyl propionate | 0.05[a] | 0.04[ab] | 0.03[c] | ** |
| 42 | δ-Elemene | 0.02[ab] | 0.03[a] | 0.02[ab] | ** |
| 43 | α-Terpinyl acetate | 0.31[ab] | 0.34[a] | 0.15[c] | ** |
| 44 | Citronellyl acetate | 0.11[a] | 0.03[c] | 0.09[b] | *** |
| 45 | Neryl acetate | 0.67[a] | 0.26[c] | 0.50[b] | *** |
| 46 | Geranyl acetate | 0.74[a] | 0.52[b] | 0.32[c] | *** |
| 47 | Dodecanale | 0.09[a] | 0.09[a] | 0.03[b] | * |
| 48 | Decyl acetate | 0.06[bc] | 0.07[ab] | 0.08[a] | ** |
| 49 | cis-α-Bergamotene | 0.03[b] | 0.03[b] | 0.04[a] | * |
| 50 | trans-α-Bergamotene | 0.51[a] | 0.44[b] | 0.40[b] | ** |
| 51 | β-Caryophyllene | 0.46[b] | 0.54[a] | 0.55[a] | * |
| 52 | (Z)-β-Farnesene | 0.02[a] | 0.01[ab] | 0.01[ab] | * |
| 53 | (E)-β-Farnesene | 0.06[a] | 0.05[ab] | 0.04[b] | * |

(*Contd...*)

**Table 2: (*Continued*)**

| | VOLATILES (%) | *Castagnaro* | *Fantastico* | *Femminello* | *Signif.* |
|---|---|---|---|---|---|
| 54 | α-Humulene | 0.03[a] | 0.03[a] | 0.02[ab] | * |
| 55 | β-Santalene | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 56 | Germacrene D | 0.11[b] | 0.21[a] | 0.08[c] | *** |
| 57 | α-Farnesene | 0.25[c] | 0.32[a] | 0.30[ab] | ** |
| 58 | trans-β-Bergamotene | 0.04[a] | 0.02[b] | 0.01[b] | * |
| 59 | Bicyclogermacrene | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 60 | (Z)-α-Bisabolene | 0.06[b] | 0.08[a] | 0.07[ab] | * |
| 61 | β-Bisabolene | 0.83[a] | 0.73[b] | 0.31[c] | *** |
| 62 | (E)-α-Bisabolene | 0.01[a] | 0.01[a] | 0.01[a] | n.s. |
| 63 | (E)-Nerolidol | 0.04[a] | 0.03[ab] | 0.03[ab] | * |
| 64 | Campherenol | 0.02[a] | 0.01[ab] | 0.01[ab] | * |
| 65 | α-Bisabolol | 0.02[a] | 0.01[ab] | 0.01[ab] | * |
| 66 | Nootkatone | 0.09[a] | 0.02[c] | 0.06[b] | *** |
| | Total | 100.00 | 100.00 | 100.00 | |

**Table 3: Physicochemical properties of the bergamot juice. *** significance at p<0.001; ** significance at p<0.01; * significance at p<0.05. Means in the same column are distinguished by different letters**

| Cultivar | °Brix | pH | Pulp (%) | Turbidity (%) | Acidity (g Citric acid monohydrate/L) | Vitamin C (mg/L) | Formol number (mL NaOH 0.1N/ 100 mL) |
|---|---|---|---|---|---|---|---|
| Castagnaro | 8.79[ab] | 2.58[a] | 9.83[a] | 38.22[b] | 45.54[b] | 568[b] | 19.4[a] |
| Fantastico | 8.83[a] | 2.51[ab] | 9.78[ab] | 37.71[c] | 49.69[a] | 591[a] | 18.6[b] |
| Femminello | 8.63[b] | 2.43[b] | 9.37[c] | 39.81[a] | 49.51[a] | 541[c] | 17.4[c] |
| *Significance* | * | * | * | *** | ** | *** | *** |

### Titratable acidity

Acidity was quantified as 45.54 g/L of citric acid monohydrate in *Castagnaro* cv, while *Fantastico* and *Femminello* showed a similar and higher content: 49.69 and 49.51 g/L, respectively, ($p<0.01$) (Table. 3). Similar values (40.9 g/L) or higher values (77.0 g/L) were quantified by other authors in lemon juice of *Verna* cv grown in Spain (Marín et al., 2002), whereas much lower values were observed in mandarin juice (8.6-18.7 g/L) and sweet orange (8.6-13.8 g/L) cultivated in China (Xu et al., 2008). In a previous study was found that titratable acidity decreased with fruit ripening from October to March: from 53.86 to 34.98 g/L in *Castagnaro*, from 58.67 to 39.83 g/L in *Fantastico* and from 54.28 to 41.90 g/L in *Femminello* (Giuffrè, 2019).

### Vitamin C

Vitamin C is found almost exclusively in foods of vegetable origin. Its absorption into the human body occurs in the buccal mucosa, in the stomach and in the small intestine. Buccal absorption occurs by passive diffusion through the mucosal cell membrane. The gastrointestinal absorption of vitamin C is rapid and efficient (Basu and Donaldson, 2003). The maximum amount of Vitamin C (ascorbic acid) was observed in *Fantastico* juice (591 mg/L) while in the *Femminello* juice the minimum content (541 mg/L) was observed, ($p<0.001$) (Table 3). These quantities are slightly lower than those present in the Italian red orange (603-680 mg/L) (Giuffrè et al., 2017a), and similar or higher than those found in juice of lemons cultivated in China (252-532 mg/L) (Xu et al., 2008). In a study previously published and conducted from October to March was found a decreasing trend in the Vitamin C content in the juice during bergamot fruit ripening from 831 to 341 g/L in *Castagnaro*, from 867 to 457 g/L in *Fantastico* and from 669 to 349 g/L in *Femminello* (Giuffrè, 2019).

### Formol number

The Formol number is a useful parameter to determine the content of total amino acids in a fruit juice (Berk, 2016). The highest value (19.4) was observed in *Castagnaro* cv, followed by 18.6 in *Fantastico* cv and 17.4 in *Femminello* cv, ($p<0.001$), (Table. 3). If these values are compared with those found in other Italian and foreign citrus fruit juices, it can be seen that the samples considered in this study show a Formol number lower than that found in common orange juice from fruits of the Czech Republic market (21.5-25.5) (Šnurkovič, 2013), but greater than that found in red orange juice from Sicily in Southern Italy (17.3) (Giuffrè et al., 2017a).

### Flavonoids

Flavonoids are compounds present and studied in different plant matrices (Giuffrè, 2013; Sidari et al., 2018; Panuccio et al., 2019; Di Donna et al., 2011; Di Donna et al., 2013; Di Donna et al., 2014; Fiorillo et al., 2019). Flavonoids in bergamot albedo and juice are recognised to have a beneficial effect in the contrast of atheroslerosis and in reduction of serum level of lipids (Lamiquiz-Moneo, in press), an anticancer activity (Navarra et al., 2014; Visalli et al., 2014), a cardio protective effect with a 150 mg daily dose (Toth et al. 2015), a robust improvement of dyslipidemia (reduction of triglycerides, total cholesterol and low-density lipoprotein cholesterol) (Capomolla et al., 2019) and a anti-inflammatory and antiproliferative potential (Russo et al. 2015).

In our work the most representative flavonoids found in bergamot juice and in cloudy juice are reported. Regarding bergamot juice, the greatest total amount of flavonoids was detected in the *Femminello* cv (520 mg/L), followed by the quantity detected in *Fantastico* (460 mg/L and in *Castagnaro* (362 mg/L), ($p<0.001$) (Table 4). If each single compound is considered, it can be observed that naringin was the most represented in the juice of all three cultivars, 33.69%, 31.90% and 26.82% respectively for *Castagnaro*, *Femminello* and *Fantastico*, ($p<0.001$). The other predominant flavonoids were neohesperidin (19.53-22.10%) and neoeriocitrin (14.20-14.88%). In recent years, two compounds have been isolated and identified in bergamot juice, namely brutieridin and melitidin (Di Donna et al., 2009; Di Donna et al., 2011; Di Donna et al., 2013; Di Donna et al., 2014; Fiorillo et al., 2018; Giuffrè, 2019). In the samples studied in our work, brutieridin was highest in the *Fantastico* cv (27.33%) whereas accounted for 22.12% and 22.56% respectively for *Castagnaro* and *Femminello*, ($p<0.01$). Melitidin was higher in the *Castagnaro* juice (10.86%) than in *Femminello* (8.98%) and *Fantastico* (7.41%), ($p<0.001$). In the cloudy juice, the quantity of total flavonoids was much greater than that of the juice. It was 4660 mg/L in Castagnaro, but it was about 1.46 times higher in *Fantastico* (6787 mg/L), and 1.81 times higher in *Femminello* (8438 mg/L), ($p<0.001$) (Table. 5). In the cloudy juice, naringin was the most represented flavonoid and varied from a high 37.64% in *Castagnaro* and a low 32.80% in *Fantastico*, ($p<0.001$). Smaller amounts of brutieridin and melitidin were found in comparison to those of the respective juices, in fact brutieridin ranged between 12.07% in *Fantastico* cloudy juice and 9.65% in the *Femminello* cloudy juice, ($p<0.01$).

Melitidin content was greatest in the *Castagnaro* cloudy juice (5.81%), followed by *Fantastico* (4.85%) and *Femminello* (3.85%), ($p<0.001$), (Table. 5).

### Fatty acids of the bergamot seed oil

The fatty acid composition of a vegetable edible oil is very important in the human diet, in fact the omega-3 have beneficial effects against the risks of coronary diseases (Brouwer, 2008), moreover the poly-unsaturated fatty acids (n-3) have a role in the prevention of certain types of cancer (Arab-Tehrany et al., 2012).

The analysis of the fatty acid profile of the bergamot seed oil showed that oleic acid was found between 30.15% (*Castagnaro*) and 34.36% (*Fantastico*), ($p<0.001$) (Table. 6), i.e. in a similar content to edible vegetable oils such as: sesame (36.82-41.75%) (Anastasi et al., 2017), low oleic sunflower (32.47%) (Giuffrè et al., 2017b), or in lower quantity than other edible vegetable oils such as peanut seed oil 44.61- 50.94% (Giuffrè et al., 2016), or olive oil 61.41-75.77% (Louadj, 2010). Oleic acid of other citrus seed oils was found to be: 20.78% in cold pressed grapefruit seeds and 20.74% in enzyme treated grapefruit seeds (Yilmaz et al., 2019), 30.86% and 30.27% in cold pressed and in solvent extracted lemon seeds respectively (Yilmaz and Aydeniz, 2017). Linoleic acid was found to be between 27.01% (*Fantastico*) and 29.82% (*Femminello*) ($p<0.01$) while the ratio linoleic/linolenic acid was 2.60, 2.15 and 2.97 for *Castagnaro*, *Fantastico* and *Femminello*, respectively, ($p<0.001$) (Table. 6). Findings of other authors on linoleic acid were: 39.9% (*Citrus sinensis*), 42.1% (*Citrus nobilis*), 26.8% (*Citrus limon, var. Interdonato*), 44.5% (*Citrus limon*, var kütdiken) and 19.5% (*Citrus paradisi*), (Matthaus and Özcan, 2012). As a whole it can be seen that bergamot seed oil has a low total saturated fatty acid content 25.85-28.78%, ($p<0.01$) (Table. 6) and conversely the total unsaturated ones varied between 74.15% (*Fantastico*) and 71.22% (*Castagnaro*), ($p<0.01$), in very similar quantity to what found in tomato seed oil, that is another vegetable whose seeds could be considered a by-product and not one waste (Giuffrè et al., 2015). The highest oleic/linoleic acid and the oleic/palmitic ratios were found in *Fantastico*: 1.27 and 1.63 respectively. The sum of 18 carbon-chain fatty acids accounted for 72.60, 76.13 and 78.13% in *Castagnaro*, *Fantastico* and *Femminello* respectively ($p<0.001$); the polyunsaturated fatty acids (EFAs) accounted for about 40% in all the three cultivars.

### Sterols of the bergamot seed oil

Sterols are ascribed to the so-called minor compounds that play a very important role in the study of a vegetable oil and which are considered together with other compounds such as alkanes (Giuffrè and Capocasale, 2016c) and policosanols (Giuffrè and Capocasale, 2015), to evaluate the physico-chemical properties of a vegetable oil.

**Table 4: Flavonoids of the Bergamot juice. *** significance at $p<0.001$; ** significance at $p<0.01$. Means in the same column are distinguished by different letters**

| Cultivar | Neoeriocitrin (%) | Naringin (%) | Neohesperidin (%) | Melitidin (%) | Brutieridin (%) | Total Flavonoids (mg/L) |
|---|---|---|---|---|---|---|
| Castagnaro | $14.20^{b}$ | $33.69^{a}$ | $19.53^{c}$ | $10.86^{a}$ | $22.12^{b}$ | $362^{c}$ |
| Fantastico | $14.53^{ab}$ | $26.82^{c}$ | $22.10^{a}$ | $7.41^{c}$ | $27.33^{a}$ | $460^{b}$ |
| Femminello | $14.88^{a}$ | $31.90^{b}$ | $21.69^{b}$ | $8.98^{b}$ | $22.56^{b}$ | $520^{a}$ |
| *Significance* | ** | *** | *** | *** | ** | *** |

Studies conducted in humans have evidenced that sterols are bioactive, in fact if they are assumed with the diet and reduce the absorption of the intestinal cholesterol; in addition, phytosterol intake was found to be inversely related to cholesterol in serum (Ellegard et al., 2000; Ostlund et al., 2002; Ostlund et al., 2003; Kliberg et al., 2008).

**Table 5: Flavonoids of the Bergamot *Cloudy* juice. *** significance at p<0.001; ** significance at p<0.01; * significance at p<0.05. Means in the same column are distinguished by different letters**

| Cultivar | Neoeriocitrin (%) | Naringin (%) | Neohesperidin (%) | Melitidin (%) | Brutieridin (%) | Total Flavonoids (mg/L) |
|---|---|---|---|---|---|---|
| Castagnaro | 22.32a | 37.64$^{a}$ | 23.36$^{c}$ | 5.81$^{a}$ | 10.94$^{b}$ | 4660$^{c}$ |
| Fantastico | 21.49$^{b}$ | 32.80$^{c}$ | 28.84$^{a}$ | 4.85$^{b}$ | 12.07$^{a}$ | 6787$^{b}$ |
| Femminello | 21.45$^{b}$ | 36.66$^{b}$ | 28.50$^{ab}$ | 3.85$^{c}$ | 9.65$^{c}$ | 8438$^{a}$ |
| *Significance* | * | *** | ** | *** | *** | *** |

**Table 6: Fatty acid composition of the bergamot seed oil. *** significance at p<0.001; ** significance at p<0.01; * significance at p<0.05. Means in the same line are distinguished by different letters**

| | Castagnaro | Fantastico | Femminello | *Significance* |
|---|---|---|---|---|
| Fatty acid | | | | |
| Myristic (%) | 0.01$^{b}$ | 0.02$^{a}$ | 0.01$^{b}$ | * |
| Palmitic (%) | 23.03$^{a}$ | 21.02$^{c}$ | 22.10$^{b}$ | *** |
| Palmitoleic (%) | 0.11$^{a}$ | 0.06$^{c}$ | 0.09$^{b}$ | *** |
| Margaric (%) | 0.14$^{a}$ | 0.10$^{c}$ | 0.13$^{ab}$ | * |
| Margaroleic (%) | 0.08$^{a}$ | 0.05$^{c}$ | 0.07$^{ab}$ | ** |
| Sterаric (%) | 5.20$^{b}$ | 4.21$^{c}$ | 6.11$^{a}$ | *** |
| Oleic (%) | 30.15$^{c}$ | 34.36$^{a}$ | 31.11$^{b}$ | *** |
| Linoleic (%) | 29.44$^{ab}$ | 27.01$^{b}$ | 29.82$^{a}$ | ** |
| Linolenic (%) | 11.34$^{b}$ | 12.55$^{a}$ | 10.03$^{c}$ | *** |
| Arachidic (%) | 0.25$^{b}$ | 0.33$^{a}$ | 0.20$^{c}$ | *** |
| Eicosenoic (%) | 0.11$^{b}$ | 0.12$^{b}$ | 0.16$^{a}$ | *** |
| Behenic (%) | 0.05$^{bc}$ | 0.06$^{b}$ | 0.09$^{a}$ | ** |
| Lignoceric (%) | 0.10$^{a}$ | 0.11$^{a}$ | 0.08$^{b}$ | * |
| Monounsaturated (%) | 30.44$^{c}$ | 34.59$^{a}$ | 31.43$^{b}$ | *** |
| Polyunsaturated (%) | 40.78$^{a}$ | 39.56$^{bc}$ | 39.85$^{b}$ | ** |
| Unsaturated (%) | 71.22$^{b}$ | 74.15$^{a}$ | 71.28$^{b}$ | ** |
| Saturated (%) | 28.78$^{a}$ | 25.85$^{b}$ | 28.72$^{a}$ | ** |
| Oleic + Palmitic (%) | 0.11$^{a}$ | 0.06$^{c}$ | 0.09$^{b}$ | *** |
| Σ 16 Carbon-Chain (%) | 23.14$^{a}$ | 21.08$^{c}$ | 22.19$^{b}$ | *** |
| Σ 17 Carbon-Chain (%) | 0.21$^{a}$ | 0.21$^{a}$ | 0.15$^{b}$ | * |
| Σ 18 Carbon-Chain (%) | 72.60$^{c}$ | 76.13$^{b}$ | 78.13$^{a}$ | *** |
| Unsaturated/Saturated | 2.48$^{b}$ | 2.87$^{a}$ | 2.48$^{b}$ | * |
| Linoleic/Linolenic | 2.60$^{b}$ | 2.15$^{c}$ | 2.97$^{a}$ | *** |
| Oleic/Linoleic | 1.02$^{b}$ | 1.27$^{a}$ | 1.04$^{b}$ | ** |
| Oleic/Palmitic | 1.31$^{c}$ | 1.63$^{a}$ | 1.41$^{b}$ | *** |
| Palmitic/Linoleic | 0.78$^{a}$ | 0.78$^{a}$ | 0.74$^{b}$ | * |

**Table 7: Sterol composition of the bergamot seed oil. *** significance at p<0.001; ** significance at p<0.01; * significance at p<0.05 ; n.s., not significant. Means in the same column are distinguished by different letters**

| | *Castagnaro* | *Fantastico* | *Femminello* | *Significance* |
|---|---|---|---|---|
| Cholesterol (%) | 1.0$^{a}$ | 0.8$^{bc}$ | 0.9$^{b}$ | * |
| 24-Methylen cholesterol (%) | 0.2$^{a}$ | 0.1$^{b}$ | 0.2$^{a}$ | * |
| Campesterol (%) | 10.2$^{c}$ | 12.6$^{a}$ | 11.9$^{b}$ | ** |
| Campestanol (%) | 0.1$^{a}$ | 0.1$^{a}$ | 0.1$^{a}$ | n.s. |
| Stigmasterol (%) | 3.1$^{b}$ | 2.9$^{c}$ | 3.8$^{a}$ | *** |
| Clerosterol (%) | 1.3$^{a}$ | 1.2$^{ab}$ | 1.0$^{c}$ | ** |
| β-Sitosterol (%) | 79.2$^{a}$ | 77.8$^{b}$ | 76.9$^{c}$ | *** |
| Sitostanol %) | 0.4$^{b}$ | 0.5$^{a}$ | 0.4$^{b}$ | * |
| Δ5-Avenasterol (%) | 2.3$^{c}$ | 2.7$^{b}$ | 3.1$^{a}$ | *** |
| Others (%) | 2.2$^{a}$ | 1.3$^{c}$ | 1.7$^{b}$ | *** |
| Total sterols (mg/kg) | 3192$^{c}$ | 3286$^{b}$ | 3371$^{a}$ | *** |

The intake of 1.8 g/d of plant sterols during the day for 5 days lower the LDL-C by 0.21 mmol/L, but the ingestion of the same quantity once a day with breakfast did not lower LDL-C levels (AbuMweis, 2009).

The most prominent sterol of bergamot seed oil was β-sitosterol: 76.5% in *Femminello* and 79.8% in *Castagnaro*, ($p<0.001$), (Table. 7), and in greater quantity than that found in an edible vegetable seed oil such as the high oleic sunflower oil (49.8-57.2%) (Anastasi et al., 2010). The second predominat sterol was campesterol with a low 10.2% in *Castagnaro* and a high 12.6% in *Fantastico*.

The Δ5-Avenasterol was always less than 3.5% of the total sterol content, in a slightly lower quantity than that found in Turkish orange (*Citrus sinensis*) seed oil (4.59%) (Aydeniz Güneşer and Yilmaz, 2017) and with a similar content of Turkish lemon (*Citrus limon*, cv Interdonato*)* seed oil (3.6%) (Matthaus and Özcan, 2012). Cholesterol ranged between 0.8 % in *Fantastico* cv and 1.0% in *Castagnaro* cv ($p<0.05$), similarly to grape-fruit (*Citrus paradisi*) seed oil (0.8%) and in lower amount with respect to other Citrus seed oil (Matthaus and Özcan, 2012). The total sterol content found in the bergamot seed oil was: 3192 mg/kg in *Castagnaro*, 3286 mg/kg in *Fantastico* and 3371 mg/kg in *Femminello*, showing very high significant differences between the three cultivars ($p<0.001$), (Table.7), in higher amount than in bitter orange (*Citrus sinensis*) seed oil (2038 mg/kg) and similarly to sweet orange (*Citrus aurantium*) seed oil (3199 mg/kg), (Matthaus and Özcan, 2012).

## CONCLUSIONS

Bergamot is a precious citrus fruit cultivated almost exclusively in the province of Reggio Calabria and more precisely in the coastal strip. The fruits of the three cultivars sampled in the geographical area of Bianco town (2018-2019 harvest season) have produced fruits with different biometric characteristics and also different was the juice and the cloudy juice composition. The total flavonoid content was higher in the *Femminello* than in the other two cultivars. The peel essential oil of the three cultivars was characterised by the same volatile compounds but amounting in different quantities, mainly: limonene, linalyl acetate, γ-terpinene, linalool and β-pinene. The bergamot seed oil showed a fatty acid composition with more than 40% essential fatty acids, and a sterol profile with β-sitosterol as the predominant and cholesterol accounting for a maximum of 1%, similarly to other vegetable oils used in human nutrition.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest

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