

Bergamot (*Citrus bergamia*, Risso): the effects of cultivar and harvest date on functional properties of juice and cloudy.

Angelo Maria Giuffrè

Università degli Studi Mediterranea di Reggio Calabria, AGRARIA - Dipartimento di Agricoltura, Risorse forestali, Ambiente Risorse zootecniche, Ingegneria agraria, Alimenti – Contrada Melissari, 89124 - Reggio Calabria (Italia).

Correspondence: Dr. Angelo M. Giuffrè, Dipartimento di Agraria, Università degli Studi 'Mediterranea' di Reggio Calabria, Contrada Melissari, (89124, Reggio Calabria, Italy). E.mail: amgiuffre@unirc.it.

Phone +39 (0) 965.1694362.

Keywords Antioxidants, Bergamot, Bioactive compound, Biometrics, Biomolecules, *Citrus bergamia* Risso, Cloudy.

Abstract

Reggio Calabria province (South Italy) is known for being almost the only area of cultivation of the bergamot fruit, grown principally for its essential oil, but today much studied for the health benefits of its juice. The biometrics and physico-chemical properties of the three (*Citrus bergamia* Risso) existing genotypes namely Castagnaro, Fantastico and Femminello were studied during fruit ripening from October to March. Castagnaro cv had the biggest and heaviest fruit during this harvest period. °Brix (7.9-10.0), pH (2.2-2.8) and Formol number (1.47-2.37 mL NaOH 0.1N/100mL) were shown to be influenced by both the genotype and by the harvest date. Titratable acidity (34.98-59.50 g/L) and Vitamin C (Ascorbic acid) (341-867 g/L) decreased during fruit

ripening. The evolution of flavonoids such as neoeriocitrin, naringin, neohesperidin brutieridin and melitidin was studied both in bergamot juice and in the bergamot cloudy which is the aqueous extract of bergamot during fruit processing. Bergamot cloudy contained a higher quantity of flavonoids compared to the juice. This study gives important information regarding the cultivar and the harvest date for producers who want to obtain the highest juice quantity or the highest juice quality from the bergamot fruit.

1. Introduction

Bergamot (*Citrus bergamia*, Risso) is an evergreen tree almost exclusively growing on the Ionian and Tyrrhenian Coast of Reggio Calabria Province (South Italy), in a strip of land between 1 to 12 km wide. It is very important for the region where it is cultivated given its economic benefits [1]. Three varieties (genotypes) are known: Castagnaro, Fantastico and Femminello. In 2017, 1,500 hectares of bergamot trees were cultivated in Reggio Calabria, producing 18,750 tons of fruits [2]. Bergamot is a non-climacteric fruit and in the past was picked when the highest essential oil content in the peel was reached. Today the juice has also to be considered. The fruit was commonly cultivated for its essential oil extracted from the peel and which is used in cosmetic, perfumery and in food industry. Very recently the bioconversion of juice and peel into wines and vinegars was positively conducted [3]. More recently there has been an increasing interest in the use of its juice as a beverage also in a blend with other fruit juices. This interest is related to the demand for minimally processed foods and functional foods containing antioxidants and biomolecules whose beneficial effect on human health have been widely studied regarding diabetes, cancers, Alzheimer's disease, insulin resistance and neuro-disease [4]. There is no universally accepted definition of functional food. The following definition could be applied: 'Natural or processed foods that contain known or unknown biologically-active compounds; which in defined amounts provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease [5]. This merges and updates the definitions stated by the National Academy of Sciences Food and Nutrition Board in the US [6], by the Institute of Food Technologists [7], the American Dietetic Association [8] and what De Felice stated for Nutraceuticals [9]. The aim of this research was to investigate the evolution of biometrics and the anti-oxidative properties during the fruit ripening, the effect of cultivar was also studied.

2. Materials and methods

2.1. Plant Material

All three cultivars were grown in the same area, in mono-cultivar plots. The bergamot trees were cultivated on level ground and were planted with a distance of 6 m between each row and the trees 5 m apart within each row. All the trees were irrigated and fertilized in the same way. Fruits were collected early in the morning and carefully placed in plastic containers commonly used for citrus fruit picking. Thereafter, fruits were immediately transferred to the laboratory for biometric analyses.

2.2. *Juice extraction*

The bergamot fruit is cultivated for essential oil extraction (from peel) and juice extraction. In the present study both the 'albedo' and the remaining pulp which are commonly known as 'pastazzo' were processed into a hammer mills where they were grinded and homogenised with water to solubilise polyphenols. The obtained mixture undergoes various steps in steel tanks to allow the flavonoids to be extracted as much as possible from the 'pastazzo' and transferred to the liquid phase. A first rough separation is conducted by a press, which divides the pulp destined for the subsequent recovery of the pectins in another production plant, from the liquid phase which is stored in steel tanks for the subsequent processing. The pressed pulp contains a big quantity of pectins, both in the water soluble and non soluble, which makes difficult the separation of pulp from the liquid fraction. For this reason pectinase was used as a pectolytic enzyme at 50-60 °C to facilitate pectins degradation. The juice obtained by this procedure is called 'cloudy'.

2.3. *Chemicals*

Chemicals of both analytical grade and chromatographic grade were purchased from Carlo Erba, Milan, Italy. Pure standards of fatty acid methyl esters and sterols were from Sigma Chemical Co., St Louis, MO, USA.

2.4. *Pulp content*

Pulp content is the solid fraction quantified as a percentage in a bergamot juice after centrifugation for 10 mins at 3500 rpm. Pulp and juice are separated by difference of gravity.

2.5. *Turbidity*

A 12% Bergamot juice in bi-deionised water was prepared and transmittance was read at 578 nm. Turbidity was expressed as percentage ratio between the intensity of the incident light and intensity of light emission from the cuvette.

2.6. *pH*

A Mettler-Toledo instrument was used after calibration pH 7.0 and pH 4.0.

2.7. *°Brix*

The degree Brix was determined by a Mettler-Toledo refractometer on a drop of bergamot juice sample after zero-set of the instrument by a drop of bi-deionised water.

2.8. *Titratable Acidity*

A 10 g aliquot of bergamot juice and 150 mL of bi-deionised water were placed in a glass beaker. The mixture was boiled for 10 mins. Thereafter acidity was determined by titration with a 0.1 NaOH aqueous solution up to pH 8.1. Acidity was expressed as g of citric acid monohydrate per litre of juice [10].

2.9. *Vitamin C*

Vitamin C was quantified by an iodometric titration. In a glass beaker, 1 mL of bergamot juice and 5 mL of bi-deionised water were mixed and titrated by a 0.01 N iodine solution using a 2% starch solution as an indicator. The result was expressed as mg ascorbic acid / L of juice [10].

2.10. *Formol number*

In a glass beaker, 10 mL of bergamot juice, 10 mL of 40% by volume formaldehyde solution (pH 2.8) and 7 drops of phenolphthalein (1% in ethanol) were measured. The mixture was stirred and titrated by a 0.1 NaOH solution (IFUMA 30, method EN 1133) [11].

2.11. *Flavonoids*

The analysis was carried out using the method suggested by Giuffrè et al.[10] and modified using a HPLC-PDA system coupled with a column conditioning system at 25°C. The separation column was a Kinetex 5 μ C18 100 Å, 150 mm length, 4.6 mm internal diameter. The system was supported by Chromera software version 3.4.0.5712.

2.12. *DPPH and FRAP assays*

The analyses were conducted spectrophotometrically as suggested by Panuccio et al and Sicari et al [12-13].

2.13. *Statistical analysis*

Thirty kilogrammes of bergamot fruits were randomly collected from 20 trees at each harvest date for each cultivar (Castagnaro, Fantastico and Femminello) in the mid of each month from October 2016 to March 2017. For each cultivar, two batches (15 kg each) were prepared at each harvest date

and two replicates were obtained from each batch. The same experimental design was replicated in the harvest year 2017-2018. Means and standard deviations were calculated on 8 replicates [4 replicates x two harvest years) by Excel 2010 software. Statistical differences were calculated by one-way ANOVA and Tukey test for post hoc analysis at $p < 0.05$ using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA); the variables were: the cultivar and the harvest date of bergamots. Principal component analysis (PCA) was carried out using the software XLSTAT version 2009.1.01.

3. Results and discussion

3.1. *Biometrics*

The vertical diameter length was longest in Castagnaro fruit which showed the most constant increase in length, from 6.63 cm in October to more than 9 cm after December. The vertical diameter length of Fantastico and Femminello fruits showed a slight decrease in March at the end of the ripening period (7.15 and 6.50 cm respectively), (Table 1). The horizontal diameter was greatest in Castagnaro at each monthly sampling and showed a tendency to increase during the ripening of Castagnaro and Fantastico, whereas in Femminello a slightly fluctuating rate was found (Table 1). The pulp in juice content showed very high significant differences ($P < 0.001$) for each cultivar during fruit ripening but no significant differences were found between cultivars on October, December and January (Table 1). Vertical diameter increased with horizontal diameter ($r = 0.958$), with fruit weight ($r = 0.87$) and with peel weight ($r = 0.880$), (Table 7). Fruit weight increased very highly significantly ($P < 0.001$) during fruit ripening of all cultivars. From October to March, Castagnaro fruit showed both the highest increase in weight during ripening (72%) and the highest weight in each month (245 g on October and 421 g on March). Fantastico fruit increased by 49% during ripening (173 g on October and 258 g on March). Femminello fruits showed both the lowest weight on each harvest date and the lowest increase in weight (23%) from October to February (Table 2). The peel weight was always greatest in Castagnaro, 44.1 g on October, 55.01 g on December and 74.02 g on February but a substantial fall in weight was measured in March in all the three cultivars (Table 2). The percentage of juice was highest in Fantastico at the first stage of ripening (29.33-30.50%) and in Fantastico and Femminello at the end of the ripening period (39.97% and 40.01% respectively), (Table 1). Pulp in juice is a negative parameter because it has to be removed during the industrial process before using or storing the fruit juice. The juice turbidity was very highly significantly different ($P < 0.001$) during ripening and the same significance of differences was found between cultivars at each monthly sampling (Table 2). In the correlation matrix, fruit weight had a strong positive correlation with the vertical diameter ($r = 0.875$; $P <$

0.001; $r^2 = 0.766$; $t = 19.33$) and stronger with the horizontal diameter ($r = 0.920$; $P < 0.001$; $r^2 = 0.846$; $t = 19.25$), (Table 7). The increase in peel weight in the three cultivars was strongly correlated with the vertical diameter ($r = 0.880$; $P < 0.001$; $r^2 = 0.774$; $t = 14.76$) and with the horizontal diameter ($r = 0.830$; $P < 0.001$; $r^2 = 0.689$; $t = 14.62$), (Table 7). Fruit weight showed a weak correlation with juice content ($r = 0.112$; $P < 0.001$; $r^2 = 0.013$; $t = 17.23$) but it was strongly correlated with peel weight ($r = 0.815$; $P < 0.001$; $r^2 = 0.664$; $t = 16.56$) this means that the increase in weight during fruit ripening is mainly due to the increase in peel and not that of juice.

3.2. pH

Bergamot juice is very acidic and mainly contains ascorbic and citric acid which contribute significantly to the composition of this parameter. Between cultivars, no significant differences were found in November, February and March. A very high significant pH increase ($P < 0.001$) was found in Castagnaro and Femminello fruit juices and high significant differences were found in Fantastico juice ($P < 0.01$) (Table 3). pH of juice was negatively and moderately correlated with Vitamin C ($r = 0.643$; $P < 0.001$; $r^2 = 0.413$; $t = 31.32$) but strongly and negatively correlated with titratable acidity ($r = 0.740$; $P < 0.001$; $r^2 = 0.548$; $t = 50.76$), (Table 8). The pH of the bergamot juice was lower than Grapefruit juice (3.05), Orange juice (3.63) and Tangerine juice (3.41) but similar or higher than Lemon juice (2.43) [14].

3.3. °Brix

The degree Brix is the sugar content expressed as g/100 g juice. It is directly proportional to the sweetness of the fruit and therefore to its organoleptic pleasantness. This value did not exceed 10, which was reached by Fantastico cv in November (Table 3). The analysis of variance showed very highly significant differences during ripening ($P < 0.001$) in all the cultivars. If the cultivar effect is considered, very high significant differences in November, January and March ($P < 0.001$) were found, high significant differences in October and December ($P < 0.01$) and significant differences ($P < 0.05$) in February (Table 3). The Brix (%)/Titratable acidity (%) is a maturity index and the highest value for all the three cultivars was found in the last month of sampling, with a tendency to increase during ripening (Table 3). The °Brix (%)/Titratable Acidity (%) ratio had a strong negative correlation with total flavonoids in juice ($r = 0.920$; $P < 0.001$; $r^2 = 0.846$; $t = 16.61$), (Table 8). Other Authors, in other citrus juices found a different °Brix: 5.10 (grapefruit), 1.16 (lemon), 4.53 (orange), 6.50 Tangerine^[14], and 11.0 °Brix in squeezed blood orange juice cultivated in Calabria [15].

3.4. *Titratable Acidity*

The titratable acidity is an important parameter to determine the maturity of the fruit and the acidic taste in citrus fruit. The degree of maturity of a fruit is one of the most important factors to determine conservation methods and control quality parameters such as taste and flavour. An immature fruit usually has a low sugar content in relation to acidity, compared to the ripe fruit that has a high level of sugar in relation to acidity. In bergamot juice a very high significant difference in titratable acidity between cultivars ($P < 0.001$) was observed, from a low 53.86 g/L in Castagnaro to a high 58.67 g/L in Fantastico measured at the earliest sampling event (Table 3). During fruit ripening a decreasing trend in titratable acidity in all cultivars was observed. At the last sampling event in Castagnaro the lowest content (34.98 g/L) was seen, namely a decrease of 35.05% from October to March, whereas the highest value was not found in Fantastico (as at the earliest sampling event) but in Femminello (41.90 g/L) with a decrease rate of 22.81%.

3.5. *Vitamin C*

The human body cannot synthesize vitamins, therefore they have to be part of our diet. Vitamin C (ascorbic acid) is water soluble, has an antioxidant potential [16], prevents scurvy [17] and degenerative diseases, particularly those that are ageing-related [18] and has possible protective effect on the bones of older adults. Vitamin C can be oxidised by storage at room temperature, the addition of baking soda, overcooking and contact with copper contact and the over intakes of zinc (cooking tools), alcohol and pectin [19]. In the studied samples, vitamin C decreased dramatically during fruit ripening: Castagnaro (59%), Fantastico (47%) and Femminello (48%) from October to March. In October a very high significant difference in vitamin C between cultivars ($P < 0.001$) was found: 831 mg/L in Castagnaro, 867 mg/L in Fantastico and 669 mg/L in Femminello (Table 3). Vitamin C content was not influenced by cultivars in February but very highly affected by this variable ($P < 0.001$) in all other months (Table 3). Findings of other Authors on vitamin C content in citrus fruit juices have revealed 680 mg/L and 455 mg/L respectively in Marsh and Star Ruby i.e. two grapefruit cultivars [20], 680 mg/L in blood orange [10], 220 mg/L in pomelo [21] and 355 mg/L in lemon analysed by HPLC [14].

3.6. *Formol Number*

The Formol number can represent, in a normal chemical industrial control, a useful index for the global quantitative evaluation of amino acids present in fruit juices. The Formol number is not influenced by the presence of many natural constituents of fruit juice (sugars, vitamins, flavourings, colourings) and it is applied in the quality determination of fruit juice and beverages because it

expresses the total number of amino acids found. In bergamot juice the cultivar did not influence the Formol number in October but very high significant differences ($P<0.001$) were found between cultivars from November to March. The harvest date had significant influence ($P<0.001$) on the Formol Number for the three cultivars (Table 3). The Formol number varied from 23.7 mL NaOH 0.1N/100 mL (Femminello in March) to 14.3 mL NaOH 0.1N/100 mL (Castagnaro in December and Femminello in February) and exceeded 20 mL NaOH 0.1N/100 mL at the same time in all the three cultivars only in October. No correlation was found between Formol number and melitidin in juice ($r = 0$).

3.7. *Flavonoids*

Flavonoids are polyphenols with an antioxidant and radical scavenging role, and are described by the scientific literature to have many beneficial effects on human health. They are biomolecules that prevent from the risk of primary open-angle glaucoma [21], have an antiplatelet effect [22], maintain the anti-inflammatory action of cortisol under pro-oxidant conditions [23], protect vascular endothelial function [24], have an anti-obesity activity [25], reduce the risk of cardiovascular disease [26] and have antimicrobial [27] antiviral [28] and anti-inflammatory effects [29]. Neoerocitrin was significantly high in Femminello and low in Castagnaro and Fantastico ($P<0.001$) at the earliest sampling event. This compound showed a tendency increase in bergamot juice of Castagnaro and Fantastico as fruits ripened from October to February, with a fall in March (Table 4). Naringin, neohesperidin and brutieridin were the major flavonoids in the bergamot juice (Table 4) whereas neoeriocitrin, naringin and neohesperidin prevailed in bergamot cloudy (Table 5) this was probably due to a higher water solubility. Naringin was very highly significantly influenced ($P<0.001$) by both cultivar and harvest date variables (Tables 4-5). Neoeriocitrin in both bergamot juice and bergamot cloudy was highest in the last fruit sample dates (February and March) for all the three cultivars (Tables 4-5). In the bergamot juice, naringin content was highest on the last sample date (42.61%, 28.63% and 42.30% of the total flavonoids, respectively for Castagnaro, Fantastico and Femminello). Neohesperidin in bergamot juice was significantly different at each sample date ($P<0.001$) with January being the month in which the highest quantity was measured. Almost always and in both the juice and the cloudy of the three cvs of bergamot, the neohesperidin content was highest in Fantastico (Tables 4-5). Brutieridin and melitidin are two molecules identified and described in the bergamot juice by Di Donna et al. [30-33] and Fiorillo et al [34]. The name brutieridin comes from the ancient name of one of the Calabrian cities (Brutium, today Cosenza) where brutieridin and melitidin were studied, whereas melitidin derives from the name of one of the most important towns (Melito Porto Salvo) where the bergamot tree is cultivated. In the samples studied in our work, brutieridin was always greater than melitidin both in bergamot juice

and in bergamot cloudy (Tables 4-5). On the first sample dates (October-December), melitidin was higher in Castagnaro and Femminello juice than in Fantastico, the same situation was found on the last sample date (Table 4). Melitidin in cloudy was significantly lower in March when its content was lower than 3% in all the three cultivars, whereas it was double or almost double in the early period of ripening from October to December (Table 5). A significant decreasing tendency in the brutieridin content of bergamot juice was recorded in Castagnaro and Fantastico ($P<0.001$) whereas a fluctuating rate was found, during fruit ripening, in Femminello juice and in the cloudy of the three cultivars. Brutieridin detected in both bergamot juice and bergamot cloudy was almost always greatest in Fantastico cv from October to March, except in February when the highest Brutieridin content was found in Femminello (26.94%) for Bergamot juice and in Castagnaro (14.33%) for bergamot cloudy. Harvest date and cultivar variables showed very high significant differences ($P<0.001$) between means (Tables 4-5). The correlation between each single flavonoid in juice and its homologous in cloudy was between $r = 0.217$ of brutieridin and $r = 0.582$ of melitidin (Table 8). In the bergamot juice the total flavonoid content constantly increased with fruit ripening in Fantastico from 361 mg/L in October to 678 mg/L in March (namely an increase rate of 87.81%) and in Femminello from 287 mg/L in October to 824 mg/L in March (namely an increase of 187.11%). Also in Castagnaro juice the flavonoid content was higher in the last period of fruit ripening compared to October and November (Table 4). Studies on flavonoid content in other citrus juice during storage at 4°C showed a decreasing trend^[35], which indicates fruit should be picked later, and juice should be consumed as soon possible after picking.

3.8. *DPPH assay and FRAP assay*

A citrus juice contains more than one class of antioxidants which have different behaviours. For this reason many authors suggest applying more than one assay to evaluate antioxidant activity. In the present study we applied DPPH assay and FRAP assay which are two of the most common tests used on many matrices such as common orange [13] blood orange juice [10], edible vegetable oils and potential industrial vegetable oils [36-38], apples, bananas, strawberries, kiwifruit, cauliflower [39]. Vitamin C and flavonoids are the most important antioxidants in bergamot juice and show an inverse ratio during fruit ripening: the vitamin C showed a decreasing trend (Table 3) in opposition to total flavonoid content which increased with harvest date (Table 4). In all the three cultivars DPPH value showed a very high significant difference at each month of sampling (Table 6). The correlation between antioxidant activity of the bergamot juice measured with the DPPH assay was high with total flavonoid content ($r = 0.764$; $P < 0.001$; $r^2 = 0.583$; $t = 11.87$). This was in accordance with results of Roussos [40] which found a strong positive correlation between DPPH and flavonoids in blood orange juice. FRAP assay had an almost strong positive correlation with the

titratable acidity by ($r = 0.673$; $P < 0.001$; $r^2 = 0.453$; $t = 29.45$) and with Brutieridin in juice by ($r = 0.699$; $P < 0.001$; $r^2 = 0.488$; $t = 9.73$), and a moderate positive correlation with °Brix ($r = 0.441$; $P < 0.001$; $r^2 = 0.194$; $t = 41.07$) and neohesperidin in cloudy ($r = 0.385$; $P < 0.001$; $r^2 = 0.148$; $t = 8.97$). FRAP assay was also moderately correlated with melitidin in both juice ($r = 0.402$; $P < 0.001$; $r^2 = 0.161$; $t = 35.96$) and cloudy ($r = 0.406$; $P < 0.001$; $r^2 = 0.165$; $t = 44.75$) showing a very similar Pearson coefficient (Table 8). Lastly, vitamin C was found to be responsible of the antioxidant activity measured by FRAP assay ($r = 0.962$; $P < 0.001$; $r^2 = 0.926$; $t = 29.45$) (Table 8) similar to findings of other authors on citrus juices [41-43] but in partial disagreement with other results [44].

3.9. *Hierarchical Cluster Analysis and Principal Component Analysis*

The three cultivars were found to cluster into two clades (Figure 1). Clade 1 contained Fantastico and Femminello which showed a high similarity and were clustered at a distance of 1. The second cluster contained the Castagnaro cv alone, with the highest fruit and peel weights, in particular Castagnaro showed a peel weight double or more than double than Femminello. In Castagnaro the highest vertical and horizontal diameters were also found, the lowest Flavonoids content in juice and cloudy, the lowest titratable acidity. Principal Component Analysis (PCA) was performed on the three cultivars and 30 parameters were included in the test. (Figure 2). Two Eigen values were obtained and together accounted for 100% of the cumulative variability. The Eigen values and the percentage of total variance were 19.7481 (65.8%) and 10.2519 (34.2%). The visualization of the discrimination between the different orange cultivars on the plane of the first two functions led to a distinct separation. The cultivars were split between three sides of the plane which demonstrate the significant difference among the cultivars. The graphic also shows how the parameters are linked or separated from the cultivar factor. The Castagnaro cultivar located in the right corner of the plane, is linked to the neoeriocitrin cloudy, fruit weight/juice content ratio. The Fantastico cultivar located in the lower center of the plane, is more influenced by the brutieridin in juice and in cloudy. Finally, the Femminello cultivar, which is located in the left corner of the plane, is influenced by the °Brix % / titratable acidity ratio, turbidity, juice content/peel weight ratio. Some parameters were showing independency from the cultivar factor because of the their location in the plane, such as the naringin in cloudy, the naringin in juice, °Brix %, FRAP value, DPPH value, titratable acidity, neohesperidin, total flavonoids in juice and in cloudy. Also, some parameters are correlated negatively, such as neohesperidin and naringin, as these parameters are located in opposite directions in the plane.

4. Conclusions

Bergamot is a tree and fruit with a very strong geographical connotation, growing almost exclusively in Reggio Calabria province (South Italy). Three cultivars of this Citrus genus are known (Castagnaro, Fantastico and Femminello) and this study has shown a strong effect of both the cultivar (genotype) and the harvest date. These variables were found to influence the biometrics and the physico-chemical properties of fruits and fruit juice. Castagnaro is the cultivar producing the heaviest fruit, with the highest vertical and horizontal diameter and with the highest peel content during fruit ripening from October to March. Vitamin C content decreases during bergamot fruit ripening and it is close to the mean or in a higher quantity compared to other citrus fruit juice. The findings of this study have shown that bergamot fruit is a very good source of flavonoids which can be directly used in food and beverage preparation when obtained from fruit juice and pulp, or which can be extracted from cloudy for food, beverages and pharmaceutical purposes. Naringin, and neohesperidin were two flavonoids predominating in both the bergamot juice and in the bergamot cloudy with brutieridin as one of the two most represented flavonoid in bergamot juice and naringin as the most represented flavonoid in the bergamot cloudy. Brutieridin and melitidin are two flavonoids characterising the bergamot juice.

Acknowledgements

This research was conducted in the ambit of a Collaboration Agreement between the Herbal & Antioxidant Derivatives (H&AD s.r.l.) and the Department AGRARIA (Mediterranea University of Reggio Calabria, Italy), with Dr Angelo Maria Giuffrè as the Head of scientific research.

References

1. Strano, A.; Falcone, G.; Nicolò, B.F.; Stillitano, T.; De Luca, A.I.; Nesci, F.S.; Gulisano, G. Eco-profiles and economic performances of a high-value fruit crop in southern Italy: a case study of bergamot (*Citrus bergamia* Risso). *Agroecol. Sust. Food* **2017**, *41*, 1124-1145.
2. ISTAT, 2019. <http://agri.istat.it/jsp/dawinci.jsp?q=plC250000030000193200&an=2017&ig=1&ct=281&id=15A|21A|31A>. (accessed on 6 Dec 2018).
3. Giuffrè, A.M.; Zappia, C.; Capocasale, M.; Poiana, M.; Sidari, R.; Di Donna, L.; Bartella, L.; Sindona, G.; Corradini, G.; Giudici, G.; Caridi, A.. Vinegar production to valorise *Citrus bergamia* by-products. *Eur. Food Res. Tech.* **2017**, *245*, 667-675. doi: 10.1007/s00217-018-3189-y

4. Qiu, T.; Wu, D.; Yang, L.; Ye, H.; Wang, Q.; Cao, Z.; Tang, K. Exploring the mechanism of flavonoids through systematic bioinformatics analysis. *Front. Pharmacol.* **2018**, *9*, article No 918. doi: 10.3389/fphar.2018.00918

5. Martirosyan, D.M.; Singh, J. A new definition of functional food by FFC: what makes a new definition unique? *Functional Foods in Health and Disease* **2015**, *5*, 209–223.

6. Lee, S.C.; Foo, M.H. "Functional Foods and Its Biomarkers." Introduction to Functional Food Science: Textbook. 2nd ed. Richardson, TX: Functional Food Center, 2014.

7. Clydesdale, F. Functional foods: opportunities and challenges. Institute of Food Technologist 58.12: 35-40 (2004). Available at: http://www.ift.org/~media/Knowledge%20Center/Science%20Reports/Expert%20Reports/Functional%20Foods/Functionalfoods_expertreport_full.pdf

8. American Dietetic Association: Position of the American Dietetic Association: functional foods. *J. Am. Diet. Assoc.* **1999**, *99*, 1278–1285.

9. DeFelice, S.L. 'The nutraceutical revolution, its impact on food industry research and development'. *Trends Food Sci. Tech.* **1995**, *6*, 59–61.

10. Giuffrè, A.M.; Zappia, C.; Capocasale, M. Physico-chemical stability of blood orange juice during frozen storage. *Int. J. Food Prop.* **2017**, *20*:sup2, 1930–1943.

11. IFUMA 30 International Fruit and Vegetable Juice Association (IFUMA). 2016. www.ifu-fruitjuice.com/ifu-methods.

12. Panuccio, M.R.; Papalia, T.; Attinà, E.; Giuffrè, A.M.; Muscolo, A. Use of digestate as an alternative to mineral fertilizer: effects on growth and crop quality. *Arch. Agron. Soil Sci.* **2019**, *65*, 700–711. <https://doi.org/10.1080/03650340.2018.1520980>

13. Sicari, V.; Dorato, G.; Giuffrè, A.M.; Rizzo, P.; Albunia, A.R. The effect of different packaging on nutritional properties and antioxidant activity of oranges during storage. *J. Food Process. Pres.* **2017**, *41*:e13168. <https://doi.org/10.1111/jfpp.13168>

14. Cserhalmi, Z.; Sass-Kiss, Á.; Tóth-Markus, M.; Lechner, N. Study of pulsed electric field treated citrus juices. *Innov. Food Sci. Emerg.* **2006**, *7*, 49 – 54.

15. Destani, F.; Cassano, A.; Fazio, A.; Vincken, J.P.; Gabriele, B. Recovery and concentration of phenolic compounds in blood orange juice by membrane operations. *J. Food Eng.* **2013**, *117*, 263–271. <http://dx.doi.org/10.1016/j.jfoodeng.2013.03.001>

16. Ryan, M.J.; Dudash, H.J.; Docherty, M.; Geronilla, K.B.; Baker, B.A.; Haff, G.G.; Cutlip, Always, S.E. Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes and positive muscle work in chronically loaded muscles of aged rats. *Exp. Gerontol.* **2010**, *45*, 882–895.
17. Asensi-Fabado, A.A.; Munné-Bosch, S. Vitamins in plants: occurrence, biosynthesis and antioxidant function. *Trends Plant Sci.* **2010**, *15*, 582–592. doi:10.1016/j.tplants.2010.07.003
18. Li, Y.; Schellhorn, H.E. Can ageing-related degenerative diseases be ameliorated through administration of vitamin C at pharmacological levels? *Med. Hypotheses* **2007**, *68*, 1315–1317. doi:10.1016/j.mehy.2006.10.035
19. Sauberlich, H.E. Bioavailability of vitamins. *Prog. Food Nut. Sci.* **1985**, *9*, 1–33.
20. Sicari, V.; Pellicanò, T.M.; Giuffrè, A.M.; Zappia, C.; Capocasale, M.; Poiana, M. Physical chemical properties and antioxidant capacities of grapefruit juice (*Citrus paradisi*) extracted from two different varieties. *Int. Food Res. J.* **2018**, *25*, 1978–1984.
21. Kang, J.H.; Ivey, K.L.; Boumenna, T.; Rosner, B.; Wiggs, J.L.; Pasquale, L.R. Prospective study of flavonoid intake and risk of primary open-angle glaucoma. *Acta Ophthalmol.* **2018**, *96*, e692–e700. doi: 10.1111/aos.13705
22. Khan, H.; Jawad, M.; Kamal, M.A.; Baldi, A.; Xiao, J.; Nabavi, S.M.; Daglia, S.M. Evidence and prospective of plant derived flavonoids as antiplatelet agents: Strong candidates to be drugs of future. *Food Chem. Toxicol.* **2018**, *119*, 355–367. <https://doi.org/10.1016/j.fct.2018.02.014>
23. Veríssimo, G.; Bast, A.; Weseler, A.R. Monomeric and oligomeric flavanols maintain the endogenous glucocorticoid response in human macrophages in pro-oxidant conditions in vitro. *Chem-Biol Interact.* **2018**, *291*, 237–244. <https://doi.org/10.1016/j.cbi.2018.06.024>
24. Zhang, D.; Du, M.; Wei, Y.; Wang, C.; Shen, L. A review on the structure–activity relationship of dietary flavonoids for protecting vascular endothelial function: Current understanding and future issues. *J. Food Biochem.* **2018**, *42*, e12557. <https://doi.org/10.1111/jfbc.12557>
25. Hughes, L.A.; Arts, I.C.; Ambergen, T.; Brants, H.A.; Dagnelie, P.C.; Goldbohm, R.A.; van den Brandt, P.A.; Weijenberg, M.P. Higher dietary flavone, flavonol, and catechin intakes are associated with less of an increase in BMI over time in women: a longitudinal analysis from the Netherlands Cohort Study. *Am J. Clin. Nut.* **2008**, *88*, 1341–1352.

26. Feliciano, R.P.; Pritzel, S.; Heiss, C.; Rodriguez-Mateos, A. Flavonoid intake and cardiovascular disease risk. *Curr. Opin. Food Sci.* **2015**, *2*, 92–99. doi: 10.1016/j.cofs.2015.02.006

27. Cushnie, T.P.T.; Lamb, A.J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Ag.* **2005**, *26*, 343–356.

28. Asres, K.; Seyoum, A.; Veeresham, C.; Bucar, F.; Gibbons, S. Naturally derived anti-HIV agents. *Phytother. Res.* **2005**, *19*, 557–581.

29. Kim, H.P.; Son, K.H.; Chang, H.W.; Kang, S.S. Anti-inflammatory plant flavonoids and cellular action mechanisms. *J. Pharmacol. Sci.* **2004**, *96*, 229–245.

30. Di Donna, L.; De Luca, G.; Mazzotti, F.; Napoli, A.; Salerno, R.; Taverna, D.; Sindona, G. Statin-like principles of Bergamot fruit (*Citrus bergamia*): Isolation of 3-hydroxymethylglutaryl flavonoid glycosides. *J. Nat. Prod.* **2009**, *72*, 1352–1354.

31. Di Donna, L.; Gallucci, G.; Malaj, N.; Romano, E.; Tagarelli, A.; Sindona, G. Recycling of industrial essential oil waste: Brutieridin and Melitidin, two anticholesterolaemic active principles from bergamot albedo. *Food Chem.* **2011**, *125*, 438–441. doi:10.1016/j.foodchem.2010.09.025

32. Di Donna, L.; Taverna, D.; Mazzotti, F.; Benabdelkamel, H.; Attya, M.; Napoli, A.; Sindona, G. Comprehensive assay of flavanones in citrus juices and beverages by UHPLC–ESI-MS/MS and derivatization chemistry. *Food Chem.* **2018**, *141*, 2328–2333. <http://dx.doi.org/10.1016/j.foodchem.2013.05.034>

33. Di Donna, L.; Iacopetta, D.; Cappello, A.R.; Gallucci, G.; Martello, E.; Fiorillo, M.; Dolce, V.; Sindona, G. Hypocholesterolaemic activity of 3-hydroxy-3-methyl-glutaryl flavanones enriched fraction from bergamot fruit (*Citrus bergamia*): ‘In vivo’ studies. *J Funct. Foods* **2014**, *7*, 558–568. <http://dx.doi.org/10.1016/j.jff.2013.12.029>

34. Fiorillo, M.; Peiris-Pagès, M.; Sanchez-Alvarez, R.; Bartella, L.; Di Donna, L.; Dolce, V.; Sindona, G.; Sotgia, G.; Cappello, A.R.; Lisanti, M.P. Bergamot natural products eradicate cancer stem cells (CSCs) by targeting mevalonate, Rho-GDI-signalling and mitochondrial metabolism. *BBA - Bioenergetics* **2018**, *1859*, 984–996. <https://doi.org/10.1016/j.bbabi.2018.03.018>

35. Del Caro, A.; Piga, A.; Vacca, V.; Agabbio, M. Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. *Food Chem.* **2004**, *84*, 99–105. doi:10.1016/S0308-8146(03)00180-8

36. Giuffrè, A.M.; Capocasale, M.; Zappia, C.; Poiana, M. Influence of high temperature and duration of heating on the sunflower seed oil properties for food use and bio-diesel production. *J. Oleo Sci.* **2017**, *66*, 1193–1205. doi: 10.5650/jos.ess17109

37. Giuffrè, A.M.; Zappia, C.; Capocasale, M. Effects of high temperatures and duration of heating on olive oil properties for food use and biodiesel production. *J. Am. Oil Chem. Soc.* **2017**, *94*, 819–830. doi 10.1007/s11746-017-2988-9

38. Giuffrè, A.M.; Zappia, C.; Capocasale, M. Tomato seed oil for edible use: cold break, hot break and harvest year effects. *J. Food Process. Pres.* **2017**, *41*, e13309. <https://doi.org/10.1111/jfpp.13309>.

39. Szeto, Y.T.; Tomlinson, B.; Benzie, I.F. Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. *Brit. J. Nut.* **2002**, *87*, 55–59. doi: 10.1079/BJN2001483

40. Roussos, P.A. Phytochemicals and antioxidant capacity of orange (*Citrus sinensis* (L.) Osbeck cv. Salustiana) juice produced under organic and integrated farming system in Greece. *Sci. Hort.* **2011**, *129*, 253–258. doi:10.1016/j.scienta.2011.03.040

41. Gardner, P.T.; White, T.A.C.; McPhail, D.B.; Duthie, G.G. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem.* **2000**, *68*, 471–474.

42. Klimczak, I.; Małecka, M.; Szlachta, M.; Gliszczyńska-Świgło, A. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *J Food Compos. Anal.* **2007**, *20*, 313–322. doi:10.1016/j.jfca.2006.02.012

43. Sdiri, S.; Bermejo, A.; Aleza, P.; Navarro, P.; Salvador, A. Phenolic composition, organic acids, sugars, vitamin C and antioxidant activity in the juice of two new triploid late-season mandarins. *Food Res. Int.* **2012**, *49*, 462–468. <http://dx.doi.org/10.1016/j.foodres.2012.07.040>

44. Ramful, D.; Tarnus, E.; Aruoma, O.I.; Bourdon, E.; Bahorun, T. Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food Res. Int.* **2011**, *44*, 2088–2099. doi:10.1016/j.foodres.2011.03.056

Table 1. Biometrics of bergamot fruit. Results are presented as the mean value \pm standard deviation, n=8, (2016-2017 and 2017-2018 harvest years). ***significance at $P < 0.001$; ** significance at $P < 0.01$; * significance at $P < 0.05$. Means in the same line are distinguished by small letters. Means in the same column are distinguished by capital letters .

	<i>Cultivar</i>	October	November	December	January	February	March	<i>Sign.</i>
Vertical Diameter (cm)	Castagnaro	8.63 \pm 0.25 cA	8.67 \pm 0.10 bcA	9.02 \pm 0.10 abA	9.07 \pm 0.06 aA	9.04 \pm 0.09 aA	9.0 \pm 0.10 abA	**
	Fantastico	7.33 \pm 0.06 cB	7.3 \pm 0.10 cB	8.12 \pm 0.10 aB	8.11 \pm 0.09 aB	7.65 \pm 0.09 bB	7.15 \pm 0.09 cB	***
	Femminello	6.90 \pm 0.1 aC	6.53 \pm 0.25 abC	6.47 \pm 0.15 bC	6.83 \pm 0.06 abC	6.83 \pm 0.06 abC	6.5 \pm 0.17 abC	**
	<i>Sign.</i>	***	***	***	***	***	***	
Horizontal Diameter (cm)	Castagnaro	8.8 \pm 0.10 bA	8.7 \pm 0.20 bA	9.77 \pm 0.15 aA	9.60 \pm 0.10 aA	9.43 \pm 0.03 aA	9.77 \pm 0.12 aA	***
	Fantastico	7.13 \pm 0.06 cB	7.2 \pm 0.10 cB	8.40 \pm 0.05 aB	8.36 \pm 0.06 aB	7.98 \pm 0.08 bB	8.04 \pm 0.06 bB	*
	Femminello	7.07 \pm 0.25 aB	6.53 \pm 0.21 bC	6.67 \pm 0.12 a bC	7.1 \pm 0.10 aC	7.03 \pm 0.25 abC	6.77 \pm 0.15 abC	*
	<i>Sign.</i>	**	***	***	***	***	***	
Pulp in juice (%)	Castagnaro	10.17 \pm 0.29 bA	10.00 \pm 0.50 bB	7.00 \pm 0.50 cA	9.83 \pm 0.29 bA	10.33 \pm 0.58 bA	11.67 \pm 0.58 aAB	***
	Fantastico	10.33 \pm 0.58 aA	10.33 \pm 0.58 aA	7.33 \pm 0.58 bA	10.33 \pm 0.58 aA	10.33 \pm 0.58 aA	10.00 \pm 1.0 aB	***
	Femminello	10.00 \pm 0.0 bA	9.00 \pm 0.0 cB	8.00 \pm 0 dA	10.00 \pm 0.0 bA	7.03 \pm 0.06 eB	12.17 \pm 0.29 aA	***
	<i>Sign.</i>	n.s.	*	n.s.	n.s.	***	**	

Table 2. Biometrics of bergamot fruit. Results are presented as the mean value \pm standard deviation, n=8, (2016-2017 and 2017-2018 harvest years). ***significance at $P < 0.001$; ** significance at $P < 0.01$; * significance at $P < 0.05$. Means in the same line are distinguished by small letters. Means in the same column are distinguished by capital letters.

	Cultivar	October	November	December	January	February	March	Sign.
Fruit weight (g)	Castagnaro	245 \pm 6 eA	277 \pm 8 dA	354 \pm 14 cA	363 \pm 5 cA	397 \pm 5 bA	421 \pm 7 aA	***
	Fantastico	173 \pm 3 dB	201 \pm 2 cB	241 \pm 4 bB	194 \pm 5 cB	266 \pm 3 aB	258 \pm 12 aB	***
	Femminello	150 \pm 2 cC	174 \pm 1 bC	131 \pm 2 dC	170 \pm 2 bC	185 \pm 4 aC	146 \pm 7 cC	***
	Sign.	***	***	***	***	***	***	
Peel weight (g)	Castagnaro	44.01 \pm 0.26 cdA	49.77 \pm 0.15 bcA	55.01 \pm 0.25 bA	55.03 \pm 0.67 bA	74.02 \pm 5.29 aA	41.97 \pm 0.65 dA	***
	Fantastico	31.43 \pm 0.21 dB	34.81 \pm 0.08 cB	41.13 \pm 0.86 bB	42.23 \pm 0.67 bB	51.58 \pm 0.10 aB	26.53 \pm 0.12 eB	***
	Femminello	25.83 \pm 0.12 bC	22.8 \pm 0.17 cC	20.97 \pm 0.06 dC	20.87 \pm 0.15 dC	26.90 \pm 0.10 aC	19.53 \pm 0.50 eC	***
	Sign.	***	***	***	***	***	***	
Juice Content (%)	Castagnaro	19.73 \pm 0.68 fC	21.73 \pm 0.21 eB	28.77 \pm 0.16 dC	40.08 \pm 0.16 aA	36.07 \pm 0.49 bB	34.73 \pm 0.17 cB	***
	Fantastico	29.33 \pm 0.25 eA	30.50 \pm 0.36 dA	31.93 \pm 0.23 cB	33.57 \pm 0.21 bB	30.12 \pm 0.03 dC	39.97 \pm 0.42 aA	***
	Femminello	21.00 \pm 0.10 eB	22.13 \pm 0.15 dB	39.03 \pm 0.42 bA	33.73 \pm 0.21 cB	39.07 \pm 0.12 bA	40.01 \pm 0.46 aA	***
	Sign.	***	***	***	**	***	***	
Fruit weight / Peel weight	Castagnaro	5.58 \pm 0.13 cAB	5.57 \pm 0.18 cB	6.44 \pm 0.22 bA	6.60 \pm 0.07 bA	5.38 \pm 0.32 cB	10.04 \pm 0.26 aB	***
	Fantastico	5.50 \pm 0.09 aB	5.78 \pm 0.06 aB	5.85 \pm 0.13 aB	4.59 \pm 0.14 cC	5.16 \pm 0.05 bB	9.73 \pm 0.51 aA	***
	Femminello	5.81 \pm 0.05 dA	7.64 \pm 0.07 dA	6.26 \pm 0.08 cA	8.16 \pm 0.08 bB	6.87 \pm 0.17 cdA	7.49 \pm 0.32 aA	***
	Sign.	*	***	**	***	***	***	
Fruit weight / Juice content	Castagnaro	12.44 \pm 0.17 bcA	12.76 \pm 0.29 bA	12.32 \pm 0.52 bA	9.07 \pm 0.14 dA	11.01 \pm 0.25 cdA	12.13 \pm 0.15 aA	***
	Fantastico	5.90 \pm 0.14 dC	6.60 \pm 0.15 cC	7.54 \pm 0.15 bB	5.78 \pm 0.18 dB	8.83 \pm 0.10 aB	6.46 \pm 0.31 cB	***
	Femminello	7.14 \pm 0.10 bB	7.87 \pm 0.04 cB	3.37 \pm 0.09 dC	5.05 \pm 0.10 dC	4.73 \pm 0.12 eC	3.66 \pm 0.18 aC	
	Sign.	***	***	***	***	***	***	
Juice content / Peel weight	Castagnaro	0.45 \pm 0.02 eC	0.44 \pm 0.01 bC	0.52 \pm 0.00 dC	0.73 \pm 0.01 aC	0.49 \pm 0.04 cC	0.83 \pm 0.02 bC	***
	Fantastico	0.93 \pm 0.01 bA	0.88 \pm 0.01 aB	0.78 \pm 0.01 eB	0.79 \pm 0.01 cB	0.58 \pm 0.00 dB	1.51 \pm 0.02 eB	***
	Femminello	0.81 \pm 0.01 fB	0.97 \pm 0.00 eA	1.86 \pm 0.02 bA	1.62 \pm 0.02 cA	1.45 \pm 0.00 dA	2.05 \pm 0.03 aA	***
	Sign.	***	***	***	***	***	***	

Table 3. Physico-chemical properties of bergamot juice. Results are presented as the mean value \pm standard deviation, n=8, (2016-2017 and 2017-2018 harvest years). ***significance at $P < 0.001$; ** significance at $P < 0.01$; * significance at $P < 0.05$. Means in the same line are distinguished by small letters. Means in the same column are distinguished by capital letters .

	Cultivar	October	November	December	January	February	March	Sign.
Turbidity (%)	Castagnaro	33.67 \pm 0.25 eB	36.55 \pm 0.13 cA	34.48 \pm 0.28 dB	48.78 \pm 0.30 aB	36.37 \pm 0.15 cB	39.50 \pm 0.17 bB	***
	Fantastico	34.20 \pm 0.10 eB	32.28 \pm 0.16 fC	36.14 \pm 0.09 cA	49.12 \pm 0.08 ab	35.27 \pm 0.06 dC	39.23 \pm 0.21 bB	***
	Femminello	35.27 \pm 0.31 dA	35.33 \pm 0.23 dB	35.7 \pm 0.46 dA	54.03 \pm 0.45 aa	38.50 \pm 0.26 cA	40.03 \pm 0.15 bA	***
	Sign.	**	n.s.	*	*	n.s.	n.s.	
pH	Castagnaro	2.4 \pm 0.06 cA	2.4 \pm 0.06 cA	2.5 \pm 0.06 bcA	2.7 \pm 0.06 abA	2.7 \pm 0.06 abA	2.8 \pm 0.06 aA	***
	Fantastico	2.4 \pm 0.0 ba	2.4 \pm 0.06 bA	2.5 \pm 0.10 abAB	2.5 \pm 0.10 abAB	2.6 \pm 0.12 abA	2.7 \pm 0.10 aA	**
	Femminello	2.2 \pm 0.12 dB	2.3 \pm 0.06 dA	2.3 \pm 0.06 dA	2.4 \pm 0.06 bcB	2.6 \pm 0.0 abA	2.7 \pm 0.0 aA	***
	Sign.	**	n.s.	*	*	n.s.	n.s.	
°Brix	Castagnaro	9.4 \pm 0.06 aA	9.5 \pm 0.06 aB	8.3 \pm 0.06 dB	8.7 \pm 0.10 bB	8.5 \pm 0.06 cA	8.3 \pm 0.06 cdA	***
	Fantastico	9.1 \pm 0.06 bB	10.0 \pm 0.10 aA	8.7 \pm 0.10 cA	9.1 \pm 0.10 bA	8.2 \pm 0.20 dAB	7.9 \pm 0.60 eB	***
	Femminello	9.1 \pm 0.12 aB	8.6 \pm 0.06 bC	8.6 \pm 0.12 bA	9.3 \pm 0.06 aA	8.0 \pm 0.06 cB	8.2 \pm 0.06 cA	***
	Sign.	**	***	**	***	*	***	
Titratable Acidity (g/L)	Castagnaro	53.86 \pm 0.29 aB	51.77 \pm 0.06 bB	49.74 \pm 0.26 cA	42.2 \pm 0.26 dC	40.67 \pm 1.42 dC	34.98 \pm 0.2 eC	***
	Fantastico	58.67 \pm 0.06 bA	59.50 \pm 0.10 aA	47.58 \pm 0.06 cB	46.31 \pm 0.06 dB	46.23 \pm 0.13 dB	39.83 \pm 0.15 eB	***
	Femminello	54.28 \pm 0.32 aB	49.87 \pm 0.85 bC	46.63 \pm 0.81 cB	55.37 \pm 0.32 aA	49.0 \pm 0.36 bA	41.90 \pm 0.25 dA	***
	Sign.	***	***	***	***	***	***	
(Maturity Index)	Castagnaro	1.75 \pm 0.02 bcB	1.84 \pm 0.01 bcB	1.66 \pm 0.01 bAB	2.06 \pm 0.02 bB	2.08 \pm 0.07 cB	2.38 \pm 0.01 aA	***
	Fantastico	1.56 \pm 0.01 bcB	1.68 \pm 0.02 bB	1.83 \pm 0.02 bB	1.97 \pm 0.02 dC	1.77 \pm 0.05 cdB	1.97 \pm 0.02 aA	***
	Femminello	1.68 \pm 0.03 bA	1.72 \pm 0.02 aA	1.84 \pm 0.01 bA	1.67 \pm 0.00 aA	1.64 \pm 0.00 bA	1.97 \pm 0.02 bB	***
	Sign.	**	***	*	***	***	***	
(mL NaOH)	Castagnaro	2.07 \pm 0.06 aA	1.87 \pm 0.06 bB	1.43 \pm 0.06 cC	2.1 \pm 0.10 aA	2.13 \pm 0.06 aA	2.03 \pm 0.06 abB	***
	Fantastico	2.07 \pm 0.06 aA	2.10 \pm 0.10 aA	1.97 \pm 0.12 aA	1.90 \pm 0.10 aA	1.53 \pm 0.06 bB	1.60 \pm 0.10 bC	***
	Femminello	2.03 \pm 0.15 bA	1.47 \pm 0.06 cdC	1.67 \pm 0.06 cB	1.47 \pm 0.06 cdB	1.43 \pm 0.06 dB	2.37 \pm 0.06 aA	***
	Sign.	n.s.	***	***	***	***	***	
(mg/L)	Castagnaro	831 \pm 7 aB	593 \pm 7 cB	672 \pm 13 bA	474 \pm 12 dC	498 \pm 6 dA	341 \pm 4 eB	***
	Fantastico	867 \pm 6 aA	582 \pm 7 bB	566 \pm 3 bB	571 \pm 9 bA	504 \pm 4 cA	457 \pm 5 dA	***
	Femminello	669 \pm 4 aC	635 \pm 7 bA	556 \pm 13 cB	543 \pm 6 cB	492 \pm 4 dA	349 \pm 9 eB	***
	Sign.	***	***	***	***	n.s.	***	

Table 4. Main Flavonoids in bergamot juice. Results are presented as the mean value \pm standard deviation, n=8, (2016-2017 and 2017-2018 harvest years). ***significance at $P < 0.001$; ** significance at $P < 0.01$; * significance at $P < 0.05$. Means in the same line are distinguished by small letters. Means in the same column are distinguished by capital letters.

	Cultivar	October	November	December	January	February	March	Sign.
Neoeriocitrin (%)	Castagnaro	10.16 \pm 0.08 eC	13.85 \pm 0.11 cB	10.14 \pm 0.05 eC	12.64 \pm 0.17 dA	20.58 \pm 0.19 aB	17.95 \pm 0.12 bA	***
	Fantastico	12.84 \pm 0.07 cB	12.35 \pm 0.05 dC	12.82 \pm 0.08 cB	11.72 \pm 0.09 eB	22.75 \pm 0.05 aA	16.67 \pm 0.06 bC	***
	Femminello	15.26 \pm 0.19 cA	15.98 \pm 0.29 bA	15.35 \pm 0.11 cA	10.30 \pm 0.05 dC	15.23 \pm 0.03 cC	17.41 \pm 0.15 aB	***
	Sign.	***	***	***	***	***	***	
Naringin (%)	Castagnaro	29.13 \pm 0.06 dB	28.62 \pm 0.26 dA	29.01 \pm 0.15 dB	39.52 \pm 0.47 bA	33.36 \pm 0.18 cA	42.61 \pm 0.13 aA	***
	Fantastico	25.15 \pm 0.08 dC	26.35 \pm 0.05 cB	26.11 \pm 0.06 cC	28.42 \pm 0.09 aC	27.21 \pm 0.16 bB	28.63 \pm 0.12 aB	***
	Femminello	30.69 \pm 0.19 cA	23.92 \pm 0.20 eC	30.8 \pm 0.05 cA	37.18 \pm 0.03 bB	26.65 \pm 0.03 dC	42.3 \pm 0.26 aA	***
	Sign.	***	***	***	***	***	***	
Neohesperidin (%)	Castagnaro	17.87 \pm 0.06 bC	16.75 \pm 0.20 cC	17.75 \pm 0.13 bB	33.11 \pm 0.10 aA	13.82 \pm 0.12 dC	17.92 \pm 0.07 bC	***
	Fantastico	21.05 \pm 0.23 bA	20.51 \pm 0.12 cA	20.06 \pm 0.23 cA	29.16 \pm 0.08 aC	18.51 \pm 0.11 dB	29.34 \pm 0.24 aA	***
	Femminello	18.35 \pm 0.18 eB	19.51 \pm 0.17 cB	18.01 \pm 0.10 eB	32.62 \pm 0.15 aB	22.91 \pm 0.02 bA	18.78 \pm 0.03 dB	***
	Sign.	***	***	***	***	***	***	
Melitidin (%)	Castagnaro	11.65 \pm 0.29 bA	13.32 \pm 0.21 aA	11.51 \pm 0.13 bA	5.21 \pm 0.08 dC	13.68 \pm 0.11 aA	7.13 \pm 0.11 cA	***
	Fantastico	8.13 \pm 0.09 cB	8.36 \pm 0.08 bC	8.19 \pm 0.10 b cC	7.23 \pm 0.06 dA	9.23 \pm 0.07 aB	3.30 \pm 0.10 eB	***
	Femminello	10.56 \pm 0.06 aB	10.43 \pm 0.15 aB	10.51 \pm 0.03 aB	6.91 \pm 0.03 dB	8.27 \pm 0.03 bC	7.22 \pm 0.08 cA	***
	Sign.	***	***	***	***	***	***	
Brutieridin (%)	Castagnaro	31.49 \pm 0.05 aB	27.46 \pm 0.12 bC	31.47 \pm 0.15 aB	9.52 \pm 0.08 eC	18.56 \pm 0.07 cC	14.39 \pm 0.16 dB	***
	Fantastico	32.83 \pm 0.07 aA	32.43 \pm 0.16 bA	32.82 \pm 0.08 aA	23.47 \pm 0.16 cA	22.3 \pm 0.13 dB	22.06 \pm 0.07 dA	***
	Femminello	25.50 \pm 0.11 cC	30.16 \pm 0.05 aB	25.33 \pm 0.05 cC	12.99 \pm 0.12 eB	26.94 \pm 0.14 bA	14.29 \pm 0.01 dB	***
	Sign.	***	***	***	***	***	***	
Total Flavonoids in juice (g/L)	Castagnaro	276 \pm 3 dC	212 \pm 7 eC	488 \pm 11 aA	425 \pm 7 bB	419 \pm 8 bC	348 \pm 18 cC	***
	Fantastico	361 \pm 9 cA	303 \pm 6 dA	233 \pm 17 eC	509 \pm 8 bA	675 \pm 10 aB	678 \pm 4 aB	***
	Femminello	287 \pm 1 eB	408 \pm 4 cB	364 \pm 4 dB	390 \pm 12 cC	845 \pm 11 aA	824 \pm 7 bA	***
	Sign.	***	***	***	***	***	***	

Table 5. Main Flavonoids in the aqueous extract: *Cloudy*. Results are presented as the mean value \pm standard deviation, n=8, (2016-2017 and 2017-2018 harvest years). ***significance at $P < 0.001$; ** significance at $P < 0.01$; * significance at $P < 0.05$. Means in the same line are distinguished by small letters. Means in the same column are distinguished by capital letters .

Table 6. Antioxidant activity of bergamot juice. AAE = Ascorbic Acid Equivalent. Results are presented as the mean value \pm standard deviation, n=8, (2016-2017 and 2017-2018 harvest years). ***significance at $P < 0.001$; ** significance at $P < 0.01$; * significance at $P < 0.05$. Means in the same line are distinguished by small letters. Means in the same column are distinguished by capital letters .

	Cultivar	October	November	December	January	February	March	Sign.
DPPH assay - juice (mg AAE/100mL)	Castagnaro	380.8 \pm 1.16dC	320.3 \pm 2.30eC	494.9 \pm 2.17aA	431.2 \pm 1.11bA	386.6 \pm 2.21cC	305.6 \pm 1.85fC	***
	Fantastico	423.4 \pm 2.17bA	361.7 \pm 3.79cB	340.7 \pm 1.70dB	416.8 \pm 2.41bB	453.3 \pm 4.62aB	450.9 \pm 4.23aB	***
	Femminello	394.3 \pm 4.01cB	420.6 \pm 5.11bA	330.8 \pm 3.53dC	340.0 \pm 7.71dC	478.4 \pm 6.59aA	473.9 \pm 4.10aA	***
	Sign.	***	***	***	***	***	***	
FRAP assay - juice	Castagnaro	45.21 \pm 0.96aA	34.44 \pm 1.93cdB	40.67 \pm 0.56bA	32.66 \pm 0.10dB	35.77 \pm 0.47cA	28.41 \pm 0.08eB	***
	Fantastico	45.84 \pm 0.39aA	36.93 \pm 0.64bAB	35.91 \pm 0.64bcB	35.05 \pm 0.64cdA	34.17 \pm 0.75dA	33.65 \pm 0.57dA	***
	Femminello	39.67 \pm 0.28aB	38.04 \pm 0.58aA	35.24 \pm 0.66bB	33.89 \pm 0.78AbcB	31.82 \pm 0.76cB	26.26 \pm 1.35dC	***
	Sign.	***	*	***	**	***	***	

Table 7. The correlation matrix of biometrics is built on the basis of 48 values for each parameter (4 replicates x 2 harvest years x 3 cultivars). In the South-West section of the matrix there are the *r* value (above) and the significance level (below) with $P < 0.05$, *. In the North-East section of the matrix there is the *t* value (in italics) with the significance of the *t*-Test calculated at 95% confidence interval and the R^2 value (underlined).

	Vertical Diameter	Horizontal Diameter	Pulp in juice	Fruit Weight	Peel Weight	Juice content	Fruit Weight/ Peel weight	Fruit Weight/ Juice content	Juice content/ Peel Weight	Turbidity
Vertical Diameter	1 1.38 0.918	1 0.002	7.99 0.766	19.33 0.774	14.76 0.013	26.50 0.028	4.59 0.028	0.28 0.702	45.50 0.587	37.96 0.000
Horizontal Diameter	0.958 n.s.	1 0.001	6.50 0.846	19.25 0.689	14.62 0.002	26.10 0.000	5.47 0.000	0.37 0.638	41.89 0.426	37.44 0.009
Pulp in juice	0.048 ***	0.024 ***	1 0.001	19.12 0.001	13.78 0.000	24.05 0.000	10.60 0.031	3.98 0.003	40.54 0.001	34.96 0.034
Fruit Weight	0.875 ***	0.920 ***	0.090 ***	1 0.664	16.56 0.013	17.23 0.034	19.37 0.034	19.26 0.619	19.84 0.345	16.69 0.000
Peel Weight	0.880 ***	0.830 ***	0.000 ***	0.815 ***	1 0.006	2.65 0.158	15.27 0.552	14.45 0.619	18.08 0.619	0.35 0.009
Juice content	-0.114 ***	0.042 ***	0.001 ***	0.112 ***	-0.075 ***	1 0.129	27.33 0.242	24.29 0.360	34.11 0.186	5.52 0.186
Fruit Weight/Peel Weight	-0.166 ***	0.020 ***	0.175 ***	0.185 ***	-0.397 ***	0.359 ***	1 0.003	2.70 0.220	26.13 0.056	38.63 0.056
Fruit Weight/Juice content	0.838 n.s.	0.799 n.s.	0.057 ***	0.787 ***	0.743 ***	-0.492 ***	-0.054 ***	1 0.682	16.53 0.071	34.16 0.071
Juice content / Peel Weight	-0.766 ***	-0.653 ***	0.032 ***	-0.587 ***	-0.787 ***	0.600 ***	0.469 ***	-0.826 ***	1 0.081	46.66 0.081
Turbidity	0.022 ***	0.093 ***	0.185 ***	0.010 ***	-0.093 n.s.	0.431 ***	0.236 ***	-0.266 ***	0.284 ***	1

Table 8. The correlation matrix of the physico-chemical properties of bergamot juice and bergamot *Cloudy* is built on the basis of 48 values for each parameter (4 replicates x 2 harvest years x 3 cultivars). In the South-West section of the matrix there are the r value (above) and the significance level (below) with $P < 0.05$, *. In the North-East section of the matrix there is the t value (in italics) with the significance of the t -Test calculated at 95% confidence interval and the R^2 value (underlined).

Table 8

		pH juice	°Brix juice	TA juice	°Brix(%) / TA(%) juice	Vit. C juice	Formol N juice	Neocitrioc juice	Naringin juice	Neohesp juice	Melitidin juice	Brutieridin juice	Tot. Floids juice	Neocitrioc Cloudy	Naringin Cloudy	Neohesp Cloudy	Melitidin Cloudy	Brutieridin Cloudy	Tot. Floids Cloudy	DPPH assay juice	FRAP assay juice
Juice	pH	1 76.65 0.326	50.76 0.548	32.83 0.169	31.32 0.413	14.31 0.015	26.24 0.120	36.48 0.251	24.92 0.023	17.65 0.146	21.45 0.285	17.55 0.249	28.87 0.378	53.78 0.048	34.16 0.149	10.13 0.046	24.26 0.047	28.44 0.072	50.78 0.060	50.94 0.330	
	°Brix	-0.571 ***	1 43.67 0.529	24.10 0.153	30.98 0.413	78.41 0.119	12.73 0.309	28.34 0.036	16.67 0.002	0.40 0.072	15.18 0.094	17.30 0.490	19.42 0.184	43.33 0.002	25.30 0.011	16.34 0.180	8.36 0.008	28.41 0.021	49.98 0.272	41.07 0.194	
	TA	-0.740 ***	0.727 ***	1 18.93 0.432	28.73 0.588	51.46 0.010	32.97 0.317	14.50 0.309	22.54 0.001	40.53 0.078	17.97 0.377	15.75 0.147	23.51 0.446	11.54 0.003	18.49 0.177	46.73 0.135	40.02 0.016	28.24 0.025	44.67 0.006	11.34 0.453	
	°Brix(%) / TA(%)	0.411 ***	-0.391 ***	-0.657 ***	1 29.97 0.008	33.71 0.076	13.39 0.003	4.34 0.046	4.54 0.306	21.51 0.252	1.88 0.003	16.61 0.173	4.55 0.173	9.78 0.006	0.62 0.278	28.29 0.209	20.18 0.004	25.07 0.846	47.54 0.017	9.55 0.109	
	Vit. C	-0.643 ***	-0.643 ***	0.767 ***	0.089 ***	1 0.002	31.62 0.280	30.63 0.392	29.71 0.028	30.23 0.168	30.96 0.513	3.58 0.236	30.22 0.415	29.46 0.002	31.19 0.166	30.85 0.124	25.98 0.016	8.27 0.000	29.45 0.926		
	Formol Number	0.124 ***	0.345 ***	-0.098 ***	0.276 ***	-0.042 ***	1 0.001	27.60 0.161	37.30 0.039	25.77 0.000	19.40 0.030	22.10 0.064	17.57 0.040	29.82 0.078	54.78 0.208	35.06 0.208	12.91 0.001	36.64 0.001	28.44 0.055	50.86 0.048	51.90 0.002
	Neocitrioc	0.346 ***	-0.556 ***	-0.563 ***	0.054 ***	-0.529 ***	0.032 ***	1 0.020	17.80 0.150	7.56 0.004	9.96 0.096	8.35 0.166	17.07 0.303	8.65 0.232	26.99 0.002	14.26 0.232	19.67 0.003	7.38 0.002	28.39 0.016	49.13 0.014	26.06 0.196
	Naringin	0.501 ***	-0.190 ***	-0.556 ***	0.214 ***	-0.626 ***	0.401 ***	0.141 ***	1 0.032	8.62 0.080	25.67 0.759	5.47 0.019	16.43 0.202	8.67 0.100	4.71 0.201	3.77 0.121	32.18 0.162	24.57 0.005	28.32 0.023	46.93 0.390	
	Neohesp	0.153 ***	0.045 ***	-0.024 ***	-0.553 ***	-0.166 ***	-0.197 ***	-0.387 ***	0.179 ***	1 0.599	14.97 0.191	1.95 0.042	16.80 0.042	0.29 0.006	14.36 0.218	5.17 0.316	20.99 0.100	13.30 0.002	28.36 0.194	48.13 0.022	14.05 0.039
	Melitidin	-0.382 ***	0.269 n.s.	0.280 ***	0.502 ***	0.410 ***	0.000 ***	0.066 ***	-0.282 ***	-0.774 ***	1 0.174	14.19 0.163	17.30 0.032	17.01 0.077	37.46 0.085	22.54 0.339	9.54 0.008	4.57 0.182	28.41 0.045	49.91 0.161	
	Brutier.	-0.534 ***	0.306 ***	0.614 ***	0.050 n.s.	0.716 ***	-0.173 ***	-0.310 ***	-0.871 ***	-0.437 n.s.	0.417 ***	1 0.097	16.69 0.236	1.80 0.001	9.88 0.063	2.40 0.104	18.67 0.047	12.72 0.004	28.35 0.001	47.66 0.488	
	Total Floids	0.499 ***	-0.700 ***	-0.384 ***	-0.416 ***	-0.486 ***	-0.252 ***	0.407 ***	0.137 ***	0.205 ***	-0.404 ***	-0.311 ***	1 0.236	16.79 0.236	16.25 0.111	16.58 0.010	17.46 0.126	17.22 0.016	26.37 0.125	11.85 0.583	16.25 0.242
Cloudy	Neocitrioc	0.615 ***	-0.429 ***	-0.668 ***	0.080 ***	-0.644 ***	0.200 ***	0.550 ***	0.449 ***	-0.077 n.s.	-0.178 ***	-0.486 ***	0.486 ***	1 0.071	15.17 0.370	5.23 0.195	24.07 0.013	15.37 0.026	28.36 0.021	48.14 0.435	
	Naringin	-0.219 ***	-0.048 ***	0.054 ***	0.527 ***	0.048 ***	0.280 ***	-0.040 ***	0.316 ***	-0.467 ***	0.278 ***	0.035 ***	-0.333 ***	-0.266 ***	1 0.187	9.19 0.031	46.96 0.497	37.38 0.045	28.30 0.068	46.42 0.003	
	Neohesp	-0.386 ***	0.105 ***	0.421 ***	-0.657 n.s.	0.408 ***	-0.456 ***	-0.482 ***	-0.448 ***	0.562 ***	-0.291 ***	0.251 ***	-0.099 ***	-0.608 ***	-0.432 ***	1 0.038	29.49 0.001	21.27 0.358	28.33 0.000	47.47 0.148	
	Melitidin	-0.214 ***	0.424 ***	0.367 ***	0.457 ***	0.352 ***	0.032 ***	-0.052 ***	-0.348 ***	-0.316 ***	0.582 ***	0.323 ***	-0.355 ***	-0.442 ***	0.175 ***	-0.195 ***	1 0.171	18.21 0.282	28.43 0.002	50.46 0.165	
	Brutier.	0.217 ***	0.091 ***	0.125 ***	-0.062 ***	0.127 ***	0.022 ***	0.044 ***	-0.403 ***	0.047 ***	0.092 ***	0.217 ***	0.128 ***	0.116 ***	-0.705 ***	0.032 ***	0.414 ***	1 0.073	28.40 0.065	49.69 0.029	
	Total	-0.268 ***	-0.146 ***	0.158 ***	-0.920 ***	-0.114 ***	-0.235 ***	-0.126 ***	-0.071 ***	0.441 ***	-0.427 ***	-0.066 ***	0.353 ***	-0.161 ***	-0.213 ***	0.598 ***	-0.531 ***	-0.271 ***	1 0.040	26.73 0.040	28.30 0.043

Floods																				
Juice		DPPH assay	0.245 ***	-0.522 ***	-0.079 ***	-0.128 ***	0.000 ***	-0.220 ***	0.116 ***	-0.151 ***	0.149 ***	0.211 ***	0.024 ***	0.764 ***	0.143 ***	-0.260 ***	0.017 ***	-0.048 ***	0.256 ***	0.199 ***
		FRAP assay	-0.574 ***	0.441 ***	0.673 ***	-0.330 ***	0.962 ***	0.044 ***	-0.309 ***	-0.624 ***	-0.196 ***	0.402 ***	0.699 ***	-0.492 ***	-0.659 ***	0.054 n.s.	0.385 ***	0.406 ***	0.171 ***	-0.206 ***

1

1

* * * * * H I E R A R C H I C A L C L U S T E R A N A L Y S I S * * * * *

Dendrogram using Complete Linkage

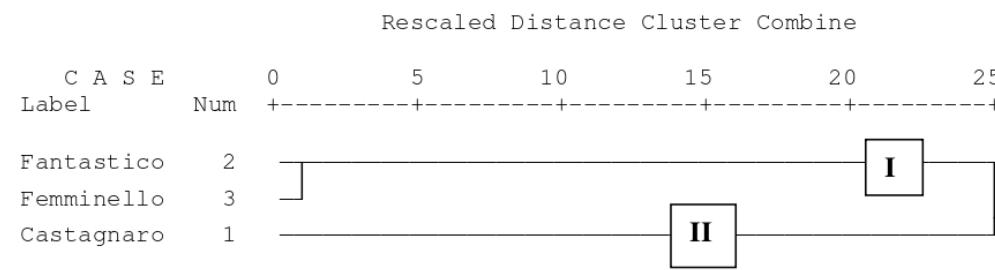


Figure 1. Two-dimensional dendrogram obtained from the cluster analysis of the fruit biometrics and of the physico-chemical properties of juice and cloudy of bergamot fruit (*Citrus bergamia*, Risso).

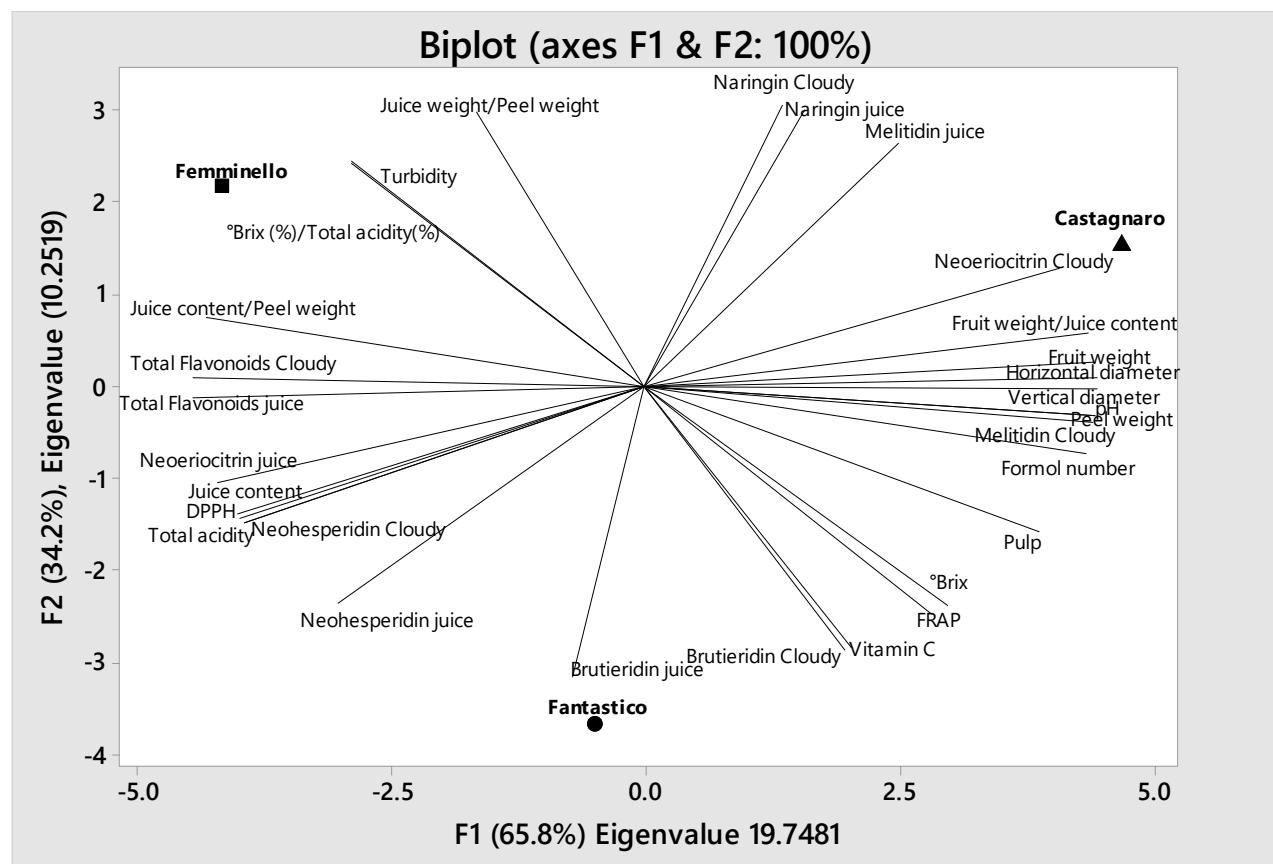


Figure 2. Score plot of the PCA performed on the biometrics and the physico-chemical properties of juice and cloudy of the three cultivars (*Castagnaro*, *Fantastico* and *Femminello*) of bergamot fruit (*Citrus bergamia*, Risso).