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Phytochemical properties and antioxidant potential from *Citrus bergamia*, Risso (bergamot) juice extracted from three different cultivars

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Summary

In plant-derived products there are naturally occuring bioactive compounds: that is, substances with or without nutritional value, having biological activity in the prevention of disease development, thus protecting human health.

The following physical and nutritional properties of three bergamot cultivars (Castagnaro, Fantastico and Femminello) were determined and compared: pH, titratable acidity, vitamin C, total flavonoids, total polyphenols, antocianyn, bioactive molecules and antioxidant capacity (ABTS and DPPH assay). The comparison data, were found to be statistically different.

In all juice samples analyzed the highest antioxidant capacity was found in Castagnaro juice (64.21 % I of DPPH and 1.97 % I of ABTS) compared to Fantastico (44.48 % I of DPPH and 1.83 % I of ABTS) and Femminello (33.39 % I of DPPH and 1.13 % I of ABTS). These differences could be attributed to the individual characteristics of these cultivars.

Introduction

Bergamot "*Citrus bergamia* Risso", is a citrus fruit belonging to the family Rutaceae, of the genus Citrus.

The origin of bergamot still remains mysterious. This plant has been successfully cultivated in Calabria (Italy) since the eighteenth century in a small area with the ideal habitat. Production is mostly limited to the Ionian Sea coastal areas of the province of Reggio di Calabria in Italy, to such an extent that it is a symbol of the entire city. Most of the bergamot comes from a short stretch of land where the temperature is favourable. It is also grown in southern France and in the Côte d'Ivoire for the essential oil and in Antalya in southern Turkey for its marmalade. Only three cultivars are commercially cultivated: "Castagnaro", Femminello" and "Fantastico". Formerly, Femminello and Castagnaro made up virtually all commercial plantings but they have largely been replaced by Fantastico, a hybrid of Femminello and Castagnaro.

The fruit is not generally grown for juice consumption, which, in contrast to that from other citrus fruits, is considered a waste product of the essential oil production; it has not found a real use in the food industries until now because of its bitter taste.

In the past, bergamot was largely grown for the cosmetics and perfume industries, since its essential oil is rich in terpenes, esters and alcohols which have a characteristically intense fragrance.

In recent years, the beneficial properties of bergamot juice have been raising interest and have been the subject of several recent studies, which consider the potential of its health promoting substances (Kim et al., 2003; Kurowska and Manthey, 2004; Miceli et al., 2007; Pernice et al., 2009; Mollace et al., 2011; Impellizzeri et al., 2014; Ferlazzo et al., 2015; Filocamo et al., 2015).

Thanks to growing interest in bioactive, antioxidant compounds from alimentary source, bergamot juice has attracted the attention of the scientific research, due to its high flavonoid content.

Bergamot presents a unique profile of flavonoids and flavonoid glycosides in its juice, such as neoeriocitrin, neohesperidin, naringin, narirutin (Sicari et al., 2015). Diets rich in flavonoids reduce post-ischemic miocardiac damage in rats (Facino et al., 1999), coronaric damage and the incidence of heart attacks in elderly man (Hertog et al., 1993).

The major causes of cell damage following oxidative stress are reactive oxygen species (ROS). Reactive oxygen species (ROS) are produced as a normal product of plant cellular metabolism. Various environmental stresses lead to excessive production of ROS causing progressive oxidative damage and ultimately cell death (Sastre et al., 2000).

After ingestion as food the flavanone glycosides are metabolized by human intestinal bacterial microflora to the respective aglycone (Felgines et al., 2000; Matsumoto et al., 2004), which seem to possess antioxidant (Hirata et al., 2005), anticarcinogenic (Shen et al., 2004) and anti-inflammatory (Rotelli et al., 2003) properties.

Flavanoids are one of the most widely studied natural antioxidants, due to their anti-inflammatory and anti-atherosclerotic properties (Balestieri et al., 2003; Jung et al., 2003).

The wide variety and high quantity of flavonoids found in bergamot juice may offer new applications for the juice, which until now has been largely unexploited.

The aim of this study was to investigate and characterize the bioactive components of bergamot juices obtained from three cultivars Fantastico, Femminello and Castagnaro grown in Calabria (Italy) and their antioxidant potential.

Materials and methods

Chemicals

Folin Ciocalteu's reagent, ABTS [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonate)], 2,2-diphenyl-1-picryl hydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid, 97 % (Trolox) were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA).

Phenolic acids (gallic, vanillic, caffeic, ferulic, *p*-coumaric, and chlorogenic) were purchased from Sigma-Aldrich Chem. Co. (Milwaukee, WI, USA).

The other phenolic compounds, cyanidin 3-glucoside, narirutin, naringin, hesperidin, neohesperidin quercetin, rhamnetin and rutin (quercetin-3-O-rutinoside), were supplied by Extrasynthese (Genay-France). Acetonitrile, formic acid and water, all HPLC grade solvents, were obtained from Carlo Erba Reagents (Milano, Italia). All other reagents used were of analytical grade.

Samples and fruit juices extraction

Bergamot fruits of three different cultivars (Tab. 1) were obtained from a grower in the south of Italy (province of Reggio Calabria) in February 2015.

The fruits used in the experiments are all from the same area of Bova Marina on the Ionian coast of the province of Reggio Calabria, at 20 - 30 m.a.s.l. The trees were all between 15 - 20 years old and in

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Tab. 1: Bergamot cultivars of fruit juices analyzed.

Species	Variety	
Citrus × Aurantium Bergamia	Castagnaro	**C**
	Fantastico	**F**
	Femminello	**Fe**

full production at the time of harvest. All the trees were watered by drip-irrigation and subject to the same agricultural practices.
The fruits were harvested at optimum maturity and carefully selected according to uniformity of size and shape, and subsequently transported, in a plastic crate to the laboratory.
Harvesting was conducted at random on 10 trees per cultivar. Five (5) fruits per tree were collected, washed and wiped. Juices were extracted by cutting the fruit in half and careful hand-squeezing in a commercial kitchen juicer. The juices were passed through a strainer to remove pulp and seeds. Squeezed juices were centrifuged at 3000 rpm for 15 min. The supernatant was passed through a 0.45 µm filter (Millipore Corporation, Bedford, USA) and was used directly for the final samples. The obtained juices were kept at -18 °C until analysis.

Chemical parameters
Sugar concentration, in terms of °Brix, was measured by using a hand refractometer (Atago Co., Tokyo, Japan) with a scale range of 0 - 32 °Brix.
The suspended solid content was determined in relation to the total juice (%, w/w) by centrifuging 45 ml of a pre-weight sample at 2000 rpm for 20 min; the weight of settled solids was determined after removing the supernatant.
The pH was measured in sample juices by pH meter (Basic Model 20, Crison) and the total titratable acidity (TA) was assessed by titration with NaOH (0.1 N) to pH 8.1 and expressed as % citric acid.
The ascorbic acid content was evaluated using iodometric titration with a iodine 0,01 N solution. Results are expressed in mg/100 ml of juice.

Total phenols, flavonoids and anthocyanins spectrophotometric determination
Total phenols were estimated colorimetrically by using the Folin-Ciocalteau method (Singleton and Rossi, 1965) and results were expressed as gallic acid equivalents. An aliquot of 500 µl of juice was mixed with 1 ml of Folin-Ciocalteu reagent and 10 ml of 20% Na_2CO_3. The absorbance was measured at $\lambda = 760$ nm using a UV-Vis Agilent 8453 spectrophotometer (Agilent Technologies, Italy) after 2 h in the dark. The results were expressed as gallic acid equivalents (GAE) in mg/100 ml.
Flavonoid content was determined using $AlCl_3$ assay (Yoo et al., 2014). An aliquot of 500 µl of juice was mixed with 300 µl of 5% $NaNO_2$. After 5 min, 600 µl of 10% $AlCl_3$ was added and finally, after a further 5 min, 2 ml of 1 M NaOH was added to the mixture. The absorbance was measured at $\lambda = 510$ nm using a UV-Vis Agilent 8453 spectrophotometer (Agilent Technologies, Italy). The results were expressed as rutin equivalents in mg/100 ml. Anthocyanins were estimated by a pH-differential method (Nielsen et al., 1991). The extract was diluted in a pH 1.0 and pH 4.5 buffer solution. Absorbance was measured in a UV-Vis Agilent 8453 (Agilent Technologies, Italy) spectrophotometer at 520 and at 700 nm. The expression $A = [(A_{520}-A_{700})_{pH1} - (A_{520}-A_{700})_{pH4.5}]$ was used. Results were expressed as µg of cyanidin 3-glucoside/ml using 29.600 molar extinction coefficient (Fattahi et al., 2009).

Liquid chromatographic analysis of antioxidant compounds
Antioxidant compounds in the different bergamot varieties were determined by HPLC/DAD. Analyses were performed on a Knauer (Asi Advanced Scientific Instruments, Berlin) system equipped with two pumps Smartiline Pump 1000, a Rheodyne injection valve (20 µl), a photodiode array detector UV/VIS equipped with a semi micro-cell. Data processing data were carried out using Clarity Software (Chromatography Station for windows). Compounds were separated in a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm). As La Torre et al. (2006) reports, the mobile phase was a gradient prepared from formic acid in water (pH = 3, solvent A) and formic acid in acetonitrile (pH = 3, solvent B): 0.01 - 20.00 min 5% B isocratic; 20.01 - 50.00 min, 5 - 40% B; 50.01 - 55.00 min, 40 - 95% B; 55.01 - 60.00 min 95% B isocratic. The column temperature was 30 °C and the flow rate was 1.0 ml/min. Samples were filtered through a 0.45 mm membrane filter before injection. The injection volume was 20 µl.
Peaks were monitored at 280, 254 and 365 nm. The identification and quantification of antioxidant compounds were carried out from the retention times in comparison with authentic standards. Analyses were performed in triplicate.

DPPH test
The antioxidant activity was determined using DPPH free radical assay (Brand-Williams et al., 1995). An aliquot of 2.5 ml of 0.06 mM DPPH methanolic solution was added to 50 µl of citrus juices. The absorbances at t_0 and t_5 were measured at $\lambda = 515$ nm using a UV-Vis Agilent 8453 spectrophotometer (Agilent Technologies, Italy). The results were expressed according to the following equation:

$$(\%)\ Inhibition = (1-A_f/A_0) \times 100$$

where A_f = absorbance DPPH fruit juice at t = 5 min and A_0 = absorbance DPPH (control) at t = 0 min. All tests were run in triplicate and the results expressed as means ± standard deviation (SD). Trolox was used as a standard antioxidant and juice activity was expressed in mM of Trolox equivalents.

ABTS radical-scavenging assay
The radical scavenging capacity of the samples for the ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonate) radical cation was determined as described by Re et al. (1999). ABTS was generated by mixing 7 mM of ABTS and $K_2S_2O_8$ (potassium persulfate) 140 mM. Followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted (1:80) with ethanol to give an absorbance at 734 nm using the spectrophotometer. Each sample was diluted, was allowed to react with fresh ABTS solution (900 µL), and then the absorbance was measured 6 min after initial mixing. All measurements were performed in triplicate.

Statistical analysis
Each value is the mean of three replicates. Analysis of variance (one-way ANOVA) was conducted using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL) and the Tukey's test was used to determine any significant difference among all treatments at $P < 0.05$.

Results and discussion

Significant differences ($P < 0.05$) were determined among means of the three cultivars investigated.
The quality parameters, including pH, Total Soluble Solids (TSS),

Total Acidity (TA) and TSS/TA ratio of bergamot juices were shown in Tab. 2. These parameters are known to be important for the sensory characteristics of fruit (HUMAYUN et al., 2014).

Tab. 2: TA, TSS, TSS/TA ratio and pH of bergamot juice. Data are expressed as mean ±RSD (n = 3). Letters represent significant differences between samples ($p < 0.05$); TA = Total Acidity; TSS = Total Soluble Solids.

Citrus fruit	TSS	TA (g 100 mL^{-1})	pH	TSS/TA
C	10.70 ± 0.62^{b}	1.03 ± 0.03^{a}	2.84 ± 0.07^{a}	10.38 ± 0.32^{c}
F	9.40 ± 0.00^{c}	0.76 ± 0.05^{c}	2.77 ± 0.03^{a}	12.36 ± 0.15^{b}
Fe	11.27 ± 0.76^{a}	0.87 ± 0.04^{b}	2.79 ± 0.03^{a}	12.95 ± 0.27^{a}
	**	**	*	**

The pH of the juice samples were between 2.77 and 2.84. Femminello had the highest TSS value (11.27%) whereas Fantastico had the lowest value (9.40%). Castagnaro had the highest TA value (1.03 g/100 mL) followed by Femminello (0.87 g/100 mL), while Fantastico had the lowest value (0.76 g/100 mL). It might be presumed that the different pH values of different bergamot cultivars might be related to the variation of organic acids. The TSS/TA ratio was also an important parameter, related to quality characteristics of citrus fruits, where Femminello had the highest value of TSS/TA (12.95), and Castagnaro had the lowest value (10.38).

Bergamot juice is a rich source of vitamin C, which is an important antioxidant (MILLER and RICE-EVANS, 1997) and the concentration of vitamin C is also a significant indicator of bergamot juice quality. Changes in vitamin C could be a good indicator of enzymatic or non-enzymatic degradative reactions taking place during processing or storage of the fruit (SKREDE, 1996).

As can be seen, Castagnaro juice showed a higher concentration of total acidity and ascorbic acid compared to Femminello and Fantastico. Concentration of ascorbic acid in cultivars Castagnaro, Fantastico and Femminello were found to be 0.92, 0.74 and 0.61 mg/mL respectively (Tab. 3).

The total anthocyanin (TAC), polyphenol (TPC) and flavonoid (TFC) content among the different bergamot cultivars were statistically significant, as given in Tab. 3.

Total flavonoids were determined using $AlCl_3$ assay (expressed as rutin equivalents), total phenolics were measured by Folin Ciocalteau method (expressed as gallic acid equivalents) and total antocianyns were estimated by a pH-differential method (expressed as μg of cyanidin 3-glucoside/ml). Total flavonoids ranged from 0.19 mg/mL to 0.34 mg/mL, Castagnaro cultivar had a much higher content of total flavonoids than Fantastico or Femminello. Total phenolics in bergamot juice ranged between 0.81 and 1.35 mg/mL, Fantastico cultivar presents the highest value (1.35 mg/mL), while Femminello had the lowest value (0.81 mg/mL).

Tab. 3: Flavonoids, polyphenols, anthocyanins and ascorbic acid contents of bergamot juice. Data are expressed as mean ±RSD (n = 3). Letters represent significant differences between samples ($p < 0.05$).

Citrus fruit	Flavonoid Total mg mL^{-1}	Polyphenol Total mg mL^{-1}	Antocianyn Total mg mL^{-1}	Ascorbic Acid mg mL^{-1}
C	0.34 ± 0.02^{a}	1.53 ± 0.07^{a}	0.95 ± 0.03^{a}	0.92 ± 0.05^{a}
F	0.21 ± 0.03^{b}	1.35 ± 0.04^{b}	0.68 ± 0.07^{b}	0.74 ± 0.06^{b}
Fe	0.19 ± 0.02^{b}	0.81 ± 0.02^{c}	0.65 ± 0.03^{b}	0.61 ± 0.04^{c}
	**	**	**	**

The total anthocyanin contents were from 0.95 μg/mL (Castagnaro) to 0.65 μg/100g (Femminello) and 0.67 μg/mL (Fantastico). Castagnaro was observed to be higher in anthocyanin content compared to other cultivars.

The amounts of total phenols in analyzed juices show significant differences between different cultivars (Tab. 3). The highest content of this fraction was detected in Castagnaro juice (1.53 mg/mL), while the lowest values were detected in Femminello (0.81 mg/mL).

Tab. 4 shows the phenolic acid contents of the Castagnaro, Fantastico and Femminello bergamot juices, expressed by mean (mg/mL). Bergamot juice includes gallic acid, caffeic acid, vanillic acid and chlorogenic acid. Castagnaro juice showed a higher concentration of phenolic acid compared to the other cultivars (9.37 mg/mL of chlorogenic acid, 1.86 mg/mL of gallic acid, 1.70 mg/mL of vanillic acid and 1.51 mg/mL of caffeic acid). The variation of phenolic compounds in the fruits depends on many factors such as the degree of maturity at harvest, genetic differences, and environmental conditions, etc.

Tab. 4: Phenolic acid contents of bergamot juice (mg mL^{-1}). Data are expressed as mean ±RSD (*n* = 3). Letters represent significant differences between samples ($p < 0.05$); nd means not detected.

Citrus fruit	Gallic acid	Vanillic acid	Caffeic acid	Chlorogenic acid
C	1.86 ± 0.02^{a}	1.70 ± 0.03^{a}	1.51 ± 0.07^{a}	9.37 ± 0.09^{a}
F	1.38 ± 0.06^{b}	1.13 ± 0.04^{b}	1.38 ± 0.06^{b}	4.13 ± 0.07^{b}
Fe	1.10 ± 0.04^{c}	0.85 ± 0.04^{c}	0.65 ± 0.1^{c}	3.21 ± 0.06^{c}
	**	**	**	**

Data expressed as mean ±RSD (*n* = 3). Letters represent significant differences between samples ($p < 0.05$).
nd: not detected

Flavanones are the major flavonoid in bergamot juice. Five flavanones: narirutin, naringin, hesperidin, neohesperedin and melitidin were identified in Castagnaro, Fantastico and Femminello juices. Tab. 5 shows that, of the five flavanones, naringin and neohesperedin were the most abundant in all cultivars. This agrees with the literature, which report that the most abundant components in bergamot juices are naringin, neoeriocitrin and neohesperedin (GIONFRIDDO et al., 1996; KAWAII et al., 2004; DUGO et al., 2005; GATTUSO et al., 2006; NOGATA et al., 2006). GATTUSO et al., 2007 in a similar study, found that the cultivar with the greatest antioxidant activity was Femminello, followed by Castagnaro, and lastly Fantastico. These results differ from those found in the present study, where the greatest antioxidant activity was found in Castagnaro, followed by Fantastico and then finally by Femminello. These differences might be due to a number of factors, including the area from which the fruits were harvested, the age of the trees from which the fruits were chosen, the degree of ripeness at the time of harvest, the climatic conditions during the harvest year, agricultural treatments that the trees had undergone, and the storage of the fruits after harvesting.

The concentration of flavanones was higher in Castagnaro juice compared to Fantastico and Femminello.

The flavones identified in all bergamot juices were rutin and diosmetin 6,8-glycoside. Fantastico bergamot juice contained more flavone compounds than Castagnaro and Femminello. The major flavone was diosmetin 6,8-glycoside in all cultivars. The lowest concentration was detected in the Femminello (313.4 mg/L), while the highest was detected in the Fantastico (424.6 mg/L).

Flavanone glycosides account for 95% of total flavonoids present in bergamot juice, while flavones can be found in the remaining 5%.

Reactive oxygen species (ROS) are closely connected to many

Tab. 5: Flavonoid contents of bergamot juice (mg L^{-1}). Data are expressed as mean ±rsd (n = 3). Letters represent significant differences between samples ($p < 0.05$), nd means not detected.

	Flavones		Flavanones					
Citrus fruit	**Rutin**	**Diosmetin 6,8-glycoside**	**Narirutin**	**Naringin**	**Neo-hesperidin**	**Hesperidin**	**Melitidin**	**Narirutin**
C	28.7±0.3^a	412.3±0.4^a	3.41±0.16^a	528.2±0.43^a	554.5±0.22^a	33.5±0.20^b	12.4±0.1^a	182±0.6^a
F	30.6±0.2^a	424.6 ±0.3^a	2.16±0.11^b	370.7±0.12^b	362.4±0.08^b	37.1± 0.15^{ab}	10.8±0.2^b	148±0.7^b
Fe	20.1±0.1^b	313.4±0.1^b	1.33±0.06^c	327.4±0.09^c	231.2±0.06^c	37.5±0.07^a	11.2±0.1^b	156±0.3^b
	**	**	**	**	**	**	**	**

pathological conditions such as inflammation, tumours, cardiovascular disease, cerebral ischemia and diabetes. DPPH˙ and ABTS˙ are stable free radicals, which have been widely accepted as a tool for estimating free radical scavenging activities of antioxidants (KRISHNAIAH et al., 2010). Both colorimetric methods are based on the prooxidant capacity of free radicals towards easily oxidizable substrates. Both methods are direct, stable, easily reproduced and widely used to determine total antioxidant activity on vegetable matrices.
All samples exhibited a radical scavenging activity against both radicals in a concentration-dependent manner (Tab. 6).

Tab. 6: Antioxidant capacity of bergamot juice. Data are expressed as mean ±RSD (n = 3). Letters represent significant differences between samples ($p < 0.05$)

Citrus fruit	DPPH (% I)	ABTS (% I)
C	64.21 ± 6.57^a	1.97 ± 0.73^a
F	44.48 ± 9.03^b	1.83 ± 0.32^b
Fe	33.39 ± 5.99^c	1.13 ± 0.11^c
	**	**

Phenolic compounds including anthocyanins, flavonoids, and phenolic acids are known to be responsible for antioxidant activities in fruits, and fruits with higher phenolic contents generally show stronger antioxidant activities.
The cultivar influenced the DPPH radical scavenging activity with a range of % I values from 64.21 to 33.39 and 44.48% (Castagnaro, Femminello and Fantastico samples, respectively). Castagnaro was also the most effective in the ABTS test with % I value of 1.97% followed by the Femminello sample (% I value of 1.13%).
The results showed that Castagnaro exhibited the highest antioxidant activity, while the lowest was found in Femminello, which showed the lowest values for both DPPH and ABTS radical scavenging assays (33.39% and 1.83%, respectively).
The ABTS and DPPH assays were used to evaluate the antioxidant capacity of fruits of the three bergamot cultivars, and the obtained values were compared with the amounts of total polyphenol, flavonoid, and anthocyanin determined in each cultivar. The results revealed a good correlation between total polyphenol, flavonoid, and anthocyanin contents and the antioxidant capacities, which were statistically significant ($p < 0.01$). The DPPH assay values were good correlated with total polyphenol (TP), total flavonoid (TF) and total anthocyanin (TAn) ($r = 0.904$ for TP, $r = 0.971$ for TF and $r = 0.963$ for TAn), and the ABTS assay values also were correlated with TP, TF, TAn ($r = 0.996$ for TP, $r = 0.719$ for TF and $r = 0.696$ for TAn. TP had a highest correlation with the antioxidant capacities. The above data showed that a high content of phytochemicals was in bergamot fruits resulted in a higher antioxidant activity. The antioxidant potential of bergamot juice has been previously investigated by TROVATO et al., 2010, who found a noticeable effect on scavenging DPPH radicals with IC_{50} value of 25.01 mL.

Conclusion

The results of this study indicate the main chemical parameters content of the cultivars (Castagnaro, Fantastico and Femminello) of bergamot fruit.
Bergamot juice contains several different compounds that can exert beneficial effects on human health. The aim of this study was to evaluate the qualitative and quantitative composition of a bergamot juice obtained from fruit of different cultivars harvested in province of Reggio Calabria (Southern Italy).
Significant differences regarding the chemical composition, nutritional value, and antioxidant activities among the bergamot cultivars were observed.
From the values obtained, it can be seen that Castagnaro shows a higher content of bioactive compounds and a greater antioxidant activity compared to the other two cultivars. These results may depend on the condition and treatment of the trees at the time the fruits used in the experiment were harvested.
Bergamot juice can be considered to be a good dietary source of nutrients and antioxidant compounds, especially flavonoids, anthocyanins and polyphenols. The correlation analysis indicated that total polyphenols contribute significantly to the antioxidant activity. The results indicated that bergamot juice has the potential to be further developed into a nutritionally interesting raw material for food and beverage applications. In particular, the Castagnaro cultivar showed a considerably high nutritive value and antioxidant activity and could be chosen for functional food development that benefits human health.

References

BALESTRIERI, M.L., CASTALDO, D., BALESTRIERI, C., QUAGLIUOLO, L., GIOVANE, A., SERVILLO, L., 2003: Modulation by flavonoids of PAF and related phospholipids in endothelial cells during oxidative stress. J. Lipid Res. 44, 380-387.

CALABRO, M.L., GALTIERI, V., CUTRONEO, P., TOMMASINI, S., FICARRA, P., FICARRA, R., 2004: Study of the extraction procedure by experimental design and validation of a LC method for determination of flavonoids in *Citrus bergamia* juice. J. Pharmaceut. Biomed. 35, 349-363.

CHENG, G.W., BREEN, P.J., 1991: Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. J. Am. Soc. Hort. Sci. 116, 865-869.

FATTAHI, J., FOTOUHI, R., BAKHSHI, D., AGHAJANZADEH, S., 2009: Fruit quality, anthocyanin, and cyanidin 3-glucoside concentrations of several blood orange varieties grown in different areas of Iran. Hort. Environ. Biotech. 50, 1-5.

FELGINES, C., TEXIER, O., MORAND, C., MANACH, C., SCALBERT, A., RÉGÉ-

RAT, F., RÉMÉSY, C., 2000: Bioavailability of the flavanone naringenin and its glycosides in rats. A. J. Physiol.-Gastr. L. 279, 1148-1154

FERLAZZO, N., VISALLI, G., SMERIGLIO, A., CIRMI, S., LOMBARDO, G.E., CAMPIGLIA, P., DI PIETRO, A., NAVARRA, M., 2015: Flavonoid fraction of orange and bergamot juices protect human lung epithelial cells from hydrogen peroxide-induced oxidative stress. Evid.-Based Compl. Alt. doi: 10.1155/2015/957031.

FILOCAMO, A., BISIGNANO, C., FERLAZZO, N., CIRMI, S., MANDALARI, G., NAVARRA, M., 2015: In vitro effect of bergamot (*Citrus bergamia*) juice against cagA-positive and-negative clinical isolates of *Helicobacter pylori*. BMC Complem. Altern. Med. 30, 256.

GATTUSO, G., CARISTI, C., GARGIULLI, C., BELLOCCO, E., TOSCANO, G., LEUZZI, U., 2006: Flavonoid glycosides in bergamot juice (*Citrus bergamia* Risso). J. Agr. Food Chem. 54, 3929-3935.

GATTUSO, G., BARRECA, D., CARISTI, C., GARGIULLI,C., LEUZZI, U., 2007: Distribution of flavonoids and furocoumarins in juices from cultivars of *Citrus bergamia* Risso. J. Agr. Food Chem. 55, 9921-9927.

GIONFRIDDO, F., POSTORINO, E., BOVALO, F., 1996: I flavanoni glucosidici nel succo di bergamoto. Essenza e Derivati Agrumari 4, 404-416.

HERTOG, M.G.L., FESENS, E.J.M., HOLLMAN, P.C.H., KATAN, M.B., KROMHOUT, D., 1993: Dietary antioxidant flavonoids and the risk of coronary heart disease: The Zutphen elderly study. Lancet 342, 1007-1011.

HIRATA, A., MURAKAMI, Y., SHOJI, M., KADOMA, Y., FUJISAWA, S., 2005: Kinetics of radical-scavenging activity of hesperetin and hesperidin and their inhibitory activity on COX-2 expression. Anticancer Res. 25, 3367-3374.

HUMAYUN, A., GAUTAM, C.K., MADHAV, M., SOURAV, S., RAMALINGAM, C., 2014: Effect of citric and malic acid on shelf life and sensory characteristics of orange juice. Int. J. Pharm. Pharm. Sci. 6, 117-119.

IMPELLIZZERI, D., BRUSCHETTA, G., DI PAOLA, R., AHMAD, A., CAMPOLO, M., CUZZOCREA, S., ESPOSITO, E., NAVARRA, M., 2015: The anti-inflammatory and antioxidant effects of bergamot juice extract (BJe) in an experimental model of inflammatory bowel disease. Clin. Nutr. 34, 1146-1154.

JUNG, U.J., KIM, H.J., LEE, J.S., LEE, M.K., KIM, H.O., PARK, E.J., KIM, H.K., JEONG, T.S., CHOI, M.S., 2003: Naringin supplementation lowers plasma lipids and enhances erythrocyte antioxidant enzyme activities in hypercholesterolemic subjects. Clin. Nutr. 22, 561-568.

KIM, H.K., JEONG, T.S., LEE, M.K., PARK, Y.B., CHOI, M.S., 2003: Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. Clin. Chim. Acta 327, 129-137.

KRISHNAIAH, D., SARBATLY, R., NITHYANANDAM, R., 2010: A review of the antioxidant potential of medicinal plant species. Food Bioprod. Process. 89, 217-233.

KUROWSKA, E.M., MANTHEY, J.A., 2004: Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hyper-cholesterolemia. J. Agr. Food Chem. 52, 2879-2886.

LA TORRE, G.L., SAITTA, M., VILASI, F., PELLICANÒ, T., DUGO, G., 2006: Direct determination of phenolic compounds in Sicilian wines by liquid chromatography with PDA and MS detection. Food Chem. 94, 640-650.

LEONTOWICZ, M., JESIONA, I., LEONTOWICZ, H., PARK, Y.S., NAMIESNIK, J., JASTRZEBSKI, Z., KATRICH, E., TASHMA, Z., GORINSTEIN, S., 2014: Bioactivity and bioavailability of minerals in rats loaded with cholesterol and kiwi fruit. Microchemic. J. 114, 148-154.

MATSUMOTO, H., IKOMA, Y., SUGIURA, M., YANO, M., HASEGAWA, Y., 2004: Identification and quantification of the conjugated metabolites derived from orally administered hesperidin in rat plasma. J. Agr. Food Chem. 52, 6653-6659.

MICELI, N., MONDELLO, M.R., MONFORTE, M.T., SDRAFKAKIS, V., DUGO, P., CRUPI, M.L., TRAVIANO, M.F., DE PASQUALE, R., TROVATO, A., 2007: Hypolipidemic effect of citrus bergamia Risso et poiteau juice in rats fed a hypercholesterolemic diet. J. Agr. Food Chem. 55, 10671-10677.

MILLER, N.J., RICE-EVANS, C.A., 1997: The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chem. 60, 331-337.

MOLLACE, M., SACCO, I., JANDA, E., MALARA, C., VENTRICE, D., COLICA, C., VISALLI, V., MUSCOLI, S., RAGUSA, S., MUSCOLI, A., ROTIROTI, D., ROMEO, F., 2011: Hypolipedemic and hypoglycaemic activity of bergamot polyphenols: from animal models to human studies. Fitoterapia 82, 309-316.

NIELSEN, I.L.F., HAREN, G.R., MAGNUSSEN, E.L., DRAGSTED, L.O., RASMUSSEN, S.E., 2003: Quantification of anthocyanins in commercial black currant juices by simple high-performance liquid chromatography. Investigation of their pH stability and antioxidative potency. J. Agr. Food Chem. 51, 5861-5866.

NOGATA, Y., SAKAMOTO, K., SCHIRATSUCHI, H., ISHII, T., YANO, M., OHTA, H., 2006: Flavonoid composition of fruit tissues of citrus species. Biosci. Biotech. Biochem. 70, 178-192.

PERNICE, R., BORRIELLO, G., FERRACANE, R., BORRELLI, R., CENNAMO, F., RITIENI, A., 2009: Bergamot: a source of natural antioxidants for functionalised fruit juices. Food Chem. 112, 545-550.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M., RICE-EVANS, C., 1999: Antioxidant activity applying an improved ABTS• radical cation decolarization assay. Free Radical Bio. Med. 26, 1231-1237.

ROTELLI A.E., GUARDIA, T., JUÁREZ, A.O., DE LA ROCHA, N.E., PELZER, L.E., 2003: Comparative study of flavonoids in experimental models of inflammation. Pharmacology Research 48, 601-606.

SASTRE, J., PALLARDO, V., VINA, J., 2000: Mitochondrial oxidative stress plays a key role in aging and apoptosis. IUBMB Life 49, 427-435.

SICARI, V., LOIZZO, M.R., BRANCA, V., PELLICANÒ, T.M., 2015: Bioactive and antioxidant activity from *citrus bergamia* risso (bergamot) juice collected in different areas of Reggio Calabria province, Italy. Int. J. Food Prop. http://dx.doi.org/10.1080/10942912.2015.1089893.

SHEN, S.C., KO, C.H., TSENG, S.W., TSAI, S.H., CHEN, Y.C., 2004: Structurally related antitumor effects of flavanones in vitro and in vivo: involvement of caspase 3 activation, p21 gene expression, and reactive oxygen species production. Toxicology Appl. Pharm. 197, 84-95.

SINGLETON, V.L., ROSSI, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16, 144-158.

SKREDE, G., 1996: Fruits. In: Jeremiah, L.E. (ed.), Freezing effects on food quality. Dekker, New York.

TROVATO, A., TAVIANO, M.F., PERGOLIZZI, S., CAMPOLO, L., DE PASQUALE, R., MICELI, N., 2010: Citrus bergamia Risso and Poiteau juice protects against renal injury of diet-induced hypercholesterolemia in rats. Phytother. Res. 24, 514-519.

YOO, K.M., LEE, C.H., LEE, H., MOON, B., LEE, C.Y., 2008: Relative antioxidant and cytoprotective activities of common herbs. Food Chem. 106, 929-9369.

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