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Recovery of Bioactive Compounds from Calabrian Bergamot Citrus Waste: Selection of Best Green Extraction

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Abstract: The purpose of this study was to select the best green extraction technique to recover the bioactive compounds in Calabrian Bergamot waste (Pomace). Different experimental variables such as solvent, time, and temperature were tested and the main physicochemical characteristics and antioxidant activity and constituents, such as total flavonoids, individual flavonoids, and limonoids (UHPLC-DAD) were analyzed. Later, the best extraction methodology was applied to characterize the individual portions that compose the bergamot pomace (albedo/pulp, seeds, and juice) of three different Calabrian cultivars: Fantastico, Femminello, and Castagnaro. Results of this study evidence that bergamot waste possesses a high antioxidant content that can be potentially used for further applications in the food industry.

Keywords: antioxidant activity; bergamot pomace; bioactive compounds; *Citrus bergamia Risso*; green extraction; microwave assisted; ultrasound assisted; waste

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1. Introduction

Scientific studies showed that the Mediterranean diet, mainly linked to the intake of antioxidant components contained in foods, leads to a lower risk of developing age-related vascular disease, with an increase in longevity. Citrus fruits are included among the foods that contain a high content of antioxidant compounds, particularly flavonoids. One of the most important citrus fruits grown in Calabria is Bergamot which represents a valuable source of active molecules that contribute to antioxidant, anti-inflammatory, and cholesterol reduction capacities [1,2].

Citrus bergamia Risso, commonly named Bergamot, is a hybrid plant of lemon and sour orange, belonging to the Rutaceae family. About 90% of the world's production is concentrated in Italy, in the province of Reggio Calabria [3,4], characterized by a microclimate suitable for their growth. Bergamot peel is used to extract a valuable essential oil (claimed DPI since 1999 from the European Union), obtained by rasping and cold pressing the fruit peel as defined by International Organization for Standardization [5]. Essential oil is widely employed in pharmaceutical, cosmetic, and food industries [6]; while its juice, obtained from the endocarp of the fruit, in the last few years has been used to formulate beverages mixed with other fruit juices, due to its bitter taste [7].

From the bergamot essential oil extraction process, about 50–65% become waste (peel, mesocarp, and juice) that needs to be managed by the manufacturing industry [8]. The high amount of annually generated waste, if not properly managed, can be a major environmental problem. For this reason, the recovery of these wastes and their use for the extraction of natural antioxidants can represent a valid sustainable alternative [9].

Bergamot fruits and their derivative products, called also "Pomace" have aroused much interest in the scientific community, because of their beneficial effects on human

health. These, represent a valuable source of bioactive molecules with a distinctive bitter taste, containing mainly the flavanones neohesperidin, naringin, neoeriocitrin, and minor amounts of flavones and furanocoumarins [1,3]. It has been shown that flavonoids have different health properties, especially determined by their antioxidant constituents [10]. Particularly, in recent years, some studies have focused on flavonoids of bergamot because they have important properties, such as protection against some types of cardiovascular diseases [11,12]. For example, neohesperidin and neoeriocitrin have efficient properties in osteoporosis treatment, while naringin possesses anti-inflammatory and anti-tumor characteristics [13–15]. Moreover, bergamot is a natural source of vitamins A, C, and B, sugars, pectin, fiber, and other compounds with biological properties [16–20].

Over the years, several researchers have proposed different extraction methods for bioactive compounds, but nowadays, is essential that these extraction techniques are environmentally friendly, safe, and non-toxic, namely, "green". Green technology can be defined as a method useful to improve not only the production processes but also the environmental impact, for this reason, the application of solvents with improved characteristics such as pressurized and supercritical fluids, ionic liquids, deep eutectic solvents or high static pressure, were widely studied [21,22]. (Rodríguez-Rojo, Choi et al., 2019). Other applied techniques, instead, use nonconventional energies, such as ultrasound and microwave-assisted extraction, and are very versatile and applicable to different matrices, with ease of application and low consumption [23–25]. The adoption of alternate extraction systems is helpful to reduce the drawbacks connected to the use of methodologies too expensive and not economically convenient and to increase the extraction yields.

The present study aimed to select the best green extraction useful to maximize the recovery of phytochemical compounds present in bergamot pomace. In this regard, several extraction procedures were tested, and the obtained extracts were investigated for their antioxidant properties both spectrophotometrically and through ultrahigh-performance liquid chromatography (UHPLC). Subsequently, the best extraction technique was selected to analyze the individual portions that constitute the fruit in the three Calabrian cultivars of bergamot: Castagnaro, Femminello, and Fantastico. Castagnaro is a cultivar that allows the harvest of the fruits for a longer time, but the quality of essential oil (EO) is the least valuable and the production is not constant in different production years; Femminello is characterized by a low yield of EO, but excellent quality; Fantastico represents a hybrid of the first two varieties and it is the most cultivated [26].

Despite bergamot pomace representing industrial waste, at the same time, it is very rich in antioxidant compounds that could later be reused as natural additives in the food industry. The obtained results will provide information to guide future utilization of bergamot, and in general citrus waste as an important source of bioactive compounds to reuse in the food field as nutritional and/or preservative compounds.

2. Materials and Methods

2.1. Raw Materials

Bergamot (*Citrus bergamia Risso*) pomace (BP) represented by skins, pulp, and seeds, has been found at a citrus farm processing situated in Reggio Calabria (Italy). This company works the fruits mainly for the production of essential oil and in a small part of juice. After the transport to the Food Technology laboratory of the Mediterranea University of Reggio Calabria, BP was subjected to dehydration (at 50 °C) to reduce the moisture content (up to 12%) and powdered to facilitate the extraction process.

In the second phase, twenty bergamot fruits *(BF*) of three different cultivars (Castagnaro, Femminello, and Fantastico), harvested on a farm situated in the province of Reggio Calabria (Italy), were analyzed. After peel abrasion for essential oil extraction, the parts of bergamot fruits remaining, which included albedo/pulp, juice, and seeds, were individually separated and the antioxidant compounds were extracted using the best extraction process following the selected method (Section 2.3).

2.2. Chemicals and Apparatus

Ethanol (96% *v/v* (F.C.C.), Food grade, PanReac)) and drinking water were used for the extraction process. Folin–Ciocalteu's phenol reagent, 2,2'-azino-bis acid (3- ethylbenzothiazolin-6-sulfonic acid) (ABTS), 2,2- diphenyl-1-picrylhydrazyl (DPPH), and Trolox were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, ultrapure water, and acetonitrile (UHPLC grade, Carlo Erba, Italy) were used for chromatographic analysis. Eriocitrin, Neoeriocitrin, naringin, and neohesperidin were purchased from Merck (Darmstadt, Germany).

A heating magnetic stirrer (Velp Scientifica, Usmate Velate (MB), Italy); a Sonoplus Ultrasonic homogenizers (BANDELIN, Ultraschall seit 1955); and a Microwave Digestion System (ETHOS EASY, Millestone, Bergamo, Italy) were used.

For spectrophotometric analyses, a double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV-Vis λ2, Waltham, MA, USA) was used.

A UHPLC system (Knauer, Berlin, Germany), a Kinetex® 2.6 µm Biphenyl 100 Å column 100 × 2.1 mm (Torrance, CA, USA) coupled with a Photo Diode Array Detector (PLATINblue, Knauer) and Clarity software were used for the quali-quantitative analysis of individual bioactive compounds.

2.3. Extraction of Antioxidant Compounds

The experimental procedure was performed during the crop season of 2021 and a schematic overview is shown in Figure 1.

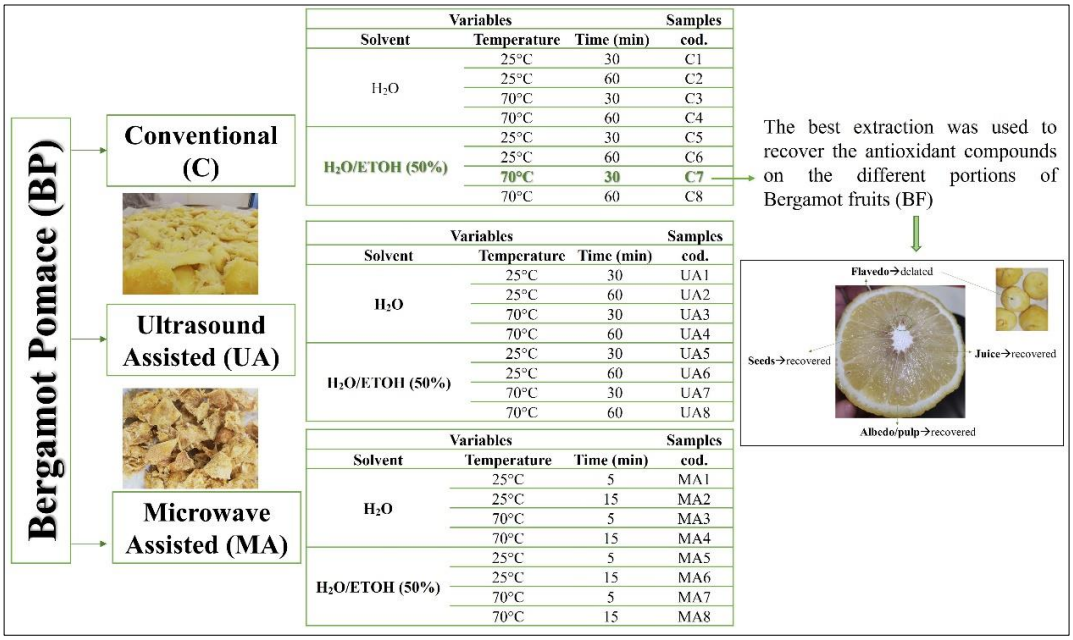

Figure 1. Schematic overview of the experimental procedure.

With the aim to obtain an extract with high antioxidant power, different techniques were carried out, following the methods reported in another of our recent works [25]. We applied: conventional extraction (C); ultrasound extraction (UA); and microwave extraction (MA) (Table 1).

Different food grade solvents/water (H_2O) and water/ethanol (H_2O:EtOH, 50%), different temperatures: 25 and 50 °C; and different times: 30 and 60 min were tested. Only for MA extraction the extraction time was different (5 and 15 min).

For all types of extraction was used 10 g of powdered BP and 50 mL of solvent (1:5, sample: solvent ratio). The samples were named with numbers from 1 to 8 matched by the initial related to the extraction system (C, UA, and MA), as shown in Figure 1.

The best-selected extraction technique was applied also for the different portions of BF (albedo/pulp, seeds, juice).

Table 1. Extraction technique performed.

Conventional solid–liquid extraction	Using a magnetic stirrer equipped with a temperature control device
Ultrasound-assisted extraction	The Ultrasonic were applied for 30 and 60 min (25 °C: ω = 10%, pulsation time on 1 s off 15 s and 70 °C: ω = 50%, pulsation time on 1 s off 1 s), frequency of 59 kHz
Microwave-assisted extraction	The applied microwave power (Watt) was: 250 W corresponds to 25 °C and 800 W corresponds to 70 °C.

2.4. Analytical Methods

2.4.1. Total Phenolic Content (TPC)

For TPC determination the method reported by González-Molina [27], (appropriately modified) was followed. 0.2 mL of each extract (BP and BF and Juice, diluted 1/5, *v/v*), 5 mL of deionized water and 1 mL of Folin-Ciocalteu reagent were placed in a volumetric flask (25 mL) and mixed. After 8 min, 10 mL of Na_2CO_3, 20% were added. The reaction mixture was left to dark for two hours first to read the absorbance at 765 nm. The results were compared with a gallic acid calibration curve and were expressed as mg of gallic acid g^{-1} (mg GAE g^{-1} dw) of the dry weight of pomace and mg of gallic acid 100 mL^{-1} for juice (mg GAE 100 mL^{-1}).

2.4.2. Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined following the method described by Papoutsis et al. [28]. In a volumetric flask (5 mL), 0.5 mL of each extract (BP and BF and Juice, diluted 1/5, *v/v*), 1 mL of water, and 0.15 mL of $NaNO_2$, 5% were mixed and incubated for 6 min. Then, 0.15 mL of AlCl3 10% was added and after 6 min, 2 mL of NaOH 4% and 0.7 mL of deionized water were mixed. The mixture was incubated in the dark for 15 min and then the absorbance was measured at 510 nm. The results were compared with a catechin calibration curve and were expressed as mg of catechin g^{-1} (mg CE g^{-1} dw) of the dry weight of pomace and mg of catechin 100 mL^{-1} for juice (mg CE 100 mL^{-1}).

2.4.3. Total Antioxidant Activity (DPPH and ABTS Assays)

For the determination of total antioxidant activity (TAA), DPPH and ABTS assays were performed following the methods reported by De Bruno et al. [29]. The DPPH assay involves the reaction between the radical (2,2-diphenyl-1-picrylhydrazyl) and the bioactive compounds present in the samples to analyze, generating a discoloration of the reaction solution [30]; 50 µL of the extract or filtered juice and 2950 µL of methanol solution of DPPH (6×10^{-5} M) were mixed in a cuvette and let us react for 30 min in darkness, and after the absorbance was measured at 515 nm.

For the ABTS assay, 25 µL of extract or filtered juice and 2975 µL of ABTS+ solution (7 mM) were mixed in a cuvette and let us react for 6 min, and after the absorbance was measured at 734 nm.

For both the assays the results were reported as mM Trolox equivalents g^{-1} (mM TE g^{-1} dw) of the dry weight of pomace and for juice as mM Trolox equivalents L^{-1} for juice (mM TE L^{-1}).

2.4.4. Chromatographic Conditions and Validation Methods for the Evaluation of Individual Bioactive Compounds

Chromatographic determination of bioactive components was performed following the method reported by Romeo et al. [31], using a UHPLC system. The chromatographic conditions are shown in Figure 2, whereas the following elution solvents were used: water (acidified with formic acid to pH 3.10, A) and acetonitrile (B) and 5 µL of the sample.

	Time (min)	A(%)	B(%)	Flow (mL/min)
1	Initial	95.0	5.0	0.400
2	3.00	95.0	5.0	0.400
3	17.00	40.0	60.0	0.400
4	15.50	0.0	100.0	0.400
5	20.00	95.0	5.0	0.400
6	21.00	95.0	5.0	0.400

Figure 2. The antioxidant's chromatographic conditions (elution gradient).

External standards (eriocitrin, neoeriocitrin, naringin, neohesperidin, melitidin, and brutieridin) were used for the quantification and the results were expressed as mg g^{-1} of dry weight (mg g^{-1} dw), and mg L^{-1} for juice.

For limonoids determination was followed the method described by Celano et al. [32], using a UHPLC-DAD system equipped with a Kinetex 2.6 µm Biphenyl 100 Å. The chromatographic conditions are shown in Figure 3, in which the mobile phase consisted of water (A) and acetonitrile (B).

	Time (min)	A(%)	B(%)	Flow (mL/min)
1	Initial	95.0	5.0	0.400
2	15.00	75.0	25.0	0.400
3	20.00	40.0	60.0	0.400
4	21.00	2.0	98.0	0.400
5	25.00	2.0	98.0	0.400
6	28.00	95.0	5.0	0.400
7	30.00	95.5	5.0	0.400

Figure 3. Limonoids' chromatographic conditions (elution gradient).

External standards (limonin and nomilin) were used for the quantification of limonoids and the results were reported as mg g^{-1} of dry weight (mg g^{-1} dw).

2.5. Statistical Analysis

The results obtained in this experimentation were reported as mean value ± standard deviation. For the statistical elaboration, One-way analysis of variance (ANOVA), and Multivariate analysis with Tukey's post hoc test at $p < 0.05$ were performed with the SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA). For the determination of correlation coefficients (r) among TPC, TFC, DPPH, and ABTS assays Pearson's correlation test was carried out.

3. Results and Discussion

3.1. Bergamot Pomace (BP): Antioxidant Characterization

Different Food grade solvents (H_2O and H_2O: EtOH), times, temperatures, and systems to assist the extractability were applied to optimize the extraction yield of bioactive compounds present in the BP. All the obtained extracts were analyzed by spectrophotometric methods, for the determination of total bioactive components, in particular: total polyphenol content (TPC), flavonoid content (TFC), and total antioxidant activity (DPPH and ABTS). Statistical differences were analyzed among all the studied variability (ANOVA). Multivariate analysis carried out on the antioxidant extracts, showed significant differences

($p < 0.01$) for extraction methods and used solvents, considering all the total antioxidant assays, and the results are reported in Table 2. However, significant differences were also highlighted for extraction * solvent, extraction * temperature, and extraction * solvent * temperature ($p < 0.01$).

Table 2. Multivariate statistical analysis of different extraction methods.

Dependent Variable	DPPH	ABTS	TPC	TFC
Extraction	**	**	**	**
Solvent	**	**	**	**
Temperature	n.s.	n.s.	**	**
Time	*	n.s.	**	**
Extractions* Solvent	**	**	**	**
Extraction * temperature	**	**	**	**
Solvent * temperature	n.s.	**	**	**
Extration * Solvent * temperature	**	**	**	**
Extration * Time	*	n.s.	**	**
Solvent * Time	n.s.	*	**	**
Extration * Solvent * Time	*	n.s.	**	**
Temperature * Time	n.s.	**	n.s.	n.s.
Extraction * Temperature * Time	n.s.	**	**	**
Solvent * Temperature * Time	n.s.	**	**	n.s.
Extraction * Solvent * TemperaturE * Time	n.s.	**	n.s.	**

n.s. not significant; ** Significance at $p < 0.01$; * Significance at $p < 0.05$.

Significative differences in TPC ($p < 0.01$) were observed among the different extraction systems (C, UA, MA) and for the different studied variables within the same extraction system (Table 3).

Table 3. Total phenolic content and Total flavonoid content of different BP extracts.

TPC (mg GAE g^{-1} dw)	C	UA	MA	Sign.
1	12.00 ± 0.43^{eC}	22.72 ± 0.37^{abA}	13.61 ± 0.16^{cB}	**
2	19.60 ± 0.29^{cB}	21.76 ± 0.36^{abcA}	13.42 ± 0.22^{cdC}	**
3	15.59 ± 0.04^{dB}	18.63 ± 0.69^{dA}	12.27 ± 0.07^{deC}	**
4	23.66 ± 0.22^{bA}	20.79 ± 0.65^{bcB}	13.51 ± 0.43^{cdC}	**
5	19.24 ± 1.22^{cA}	23.64 ± 0.27^{aA}	9.53 ± 0.24^{fB}	**
6	21.97 ± 0.76^{bA}	23.28 ± 0.55^{aA}	11.82 ± 0.41^{eB}	**
7	26.30 ± 0.09^{aA}	20.52 ± 0.62^{cdB}	19.44 ± 0.07^{aB}	**
8	26.07 ± 0.62^{aA}	21.10 ± 0.15^{bcB}	17.73 ± 0.63^{C}	**
Sign.	**	**	**	
TFC (mg CAE g^{-1} dw)	C	UA	MA	Sign.
1	2.67 ± 0.32^{dC}	7.52 ± 0.00^{abA}	5.14 ± 0.06^{bB}	**
2	4.93 ± 0.35^{bcB}	7.81 ± 0.05^{aA}	4.47 ± 0.08^{cB}	**
3	5.58 ± 0.10^{abB}	6.43 ± 0.09^{cdA}	4.03 ± 0.09^{dC}	**
4	5.72 ± 0.39^{abB}	7.13 ± 0.00^{bA}	4.14 ± 0.03^{cdC}	**
5	4.16 ± 0.04^{cB}	6.59 ± 0.25^{cA}	2.92 ± 0.09^{eC}	**
6	4.51 ± 0.16^{cB}	6.24 ± 0.12^{cdA}	3.01 ± 0.10^{eC}	**
7	6.18 ± 0.12^{aA}	6.02 ± 0.04^{dAB}	5.58 ± 0.14^{aB}	*
8	5.61 ± 0.26^{abAB}	6.11 ± 0.17^{cdA}	5.27 ± 0.13^{abB}	*
Sign.	**	**	**	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$.

Conventional and Ultrasound assisted extraction has provided important results in terms of total phenolics recovery. The highest values were measured for the conventional extraction system (C), particularly for C7 and C8 samples (26.30 and 26.06 mg GAE g^{-1} dw,

respectively), also if the Ultrasound assisted system (UA) led to a good recovery of TPC (about 23.64 mg GAE g^{-1} dw in UA5 extract). The obtained values are higher than those reported by Gabriele et al. [8] (17.44 ± 0.40 mg GAE g^{-1} dw) on lyophilized bergamot fruits, and by Multari et al. [33] (410 ± 36.8 mg kg^{-1}) on bergamot pomace. Moreover, the content of total phenols was higher compared with other citrus (lemon and orange peel), as reported by Gorinstein et al. [34]. Among all the evaluated extracts, those obtained through microwave-assisted (MA), showed less extractability (between 10 and 19 mg GAE g^{-1} dw).

Regarding the TFC the obtained values were reported in Table 3. The amount of determined TFC showed great variability in accordance with the applied extraction procedure ($p < 0.01$). This spectrophotometric assay showed a different trend compared with the TPC assay; indeed, great extractability was obtained through the application of UA treatment, particularly in the samples prepared using water as an extraction solvent. As demonstrated by some authors, the application of ultrasound to facilitate the extraction of bioactive compounds is very useful, indeed, this technique adopts an acoustic cavitation system that combines ultrasound and traditional solvent extraction. The sound waves induce forces that can break the cell walls intensifying the release of constituents [25]. In comparison to water, ethanol is characterized by a higher heating efficiency when applied in an aqueous mixture and it is preferred thanks to its better capacity in solving the phenolic compounds. Higher values were estimated in the samples UA1 and UA2 (7.52 and 7.81 mg CE g^{-1} dw, respectively). In all the other situations was found a lower content of TFC. Nevertheless, a similar recovery of TFC was evidenced for the sample obtained with a hydroalcoholic mixture (7 and 8) for all the extraction systems (C, UA, and MA).

The antioxidant ability of the different obtained extracts was analyzed in vitro by applying two common spectrophotometric assays: DPPH, and ABTS (Figure 4a,b). These tests are based on the ability of an antioxidant matrix (represented by the antioxidant extract) to reduce a radical species. The antioxidant molecules present in the extracts react against the radicals and causing oxidation of the reacting molecule, with a related decrease in the solution absorbance (discoloration reaction).

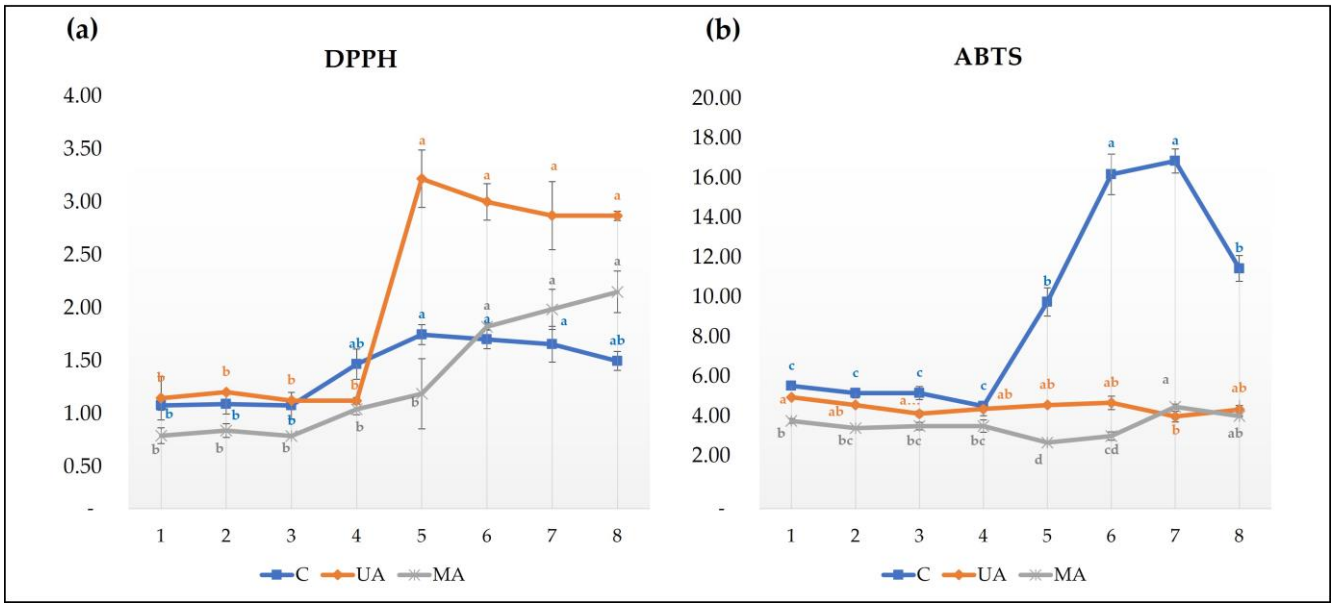

Figure 4. Results of total antioxidant activity ((**a**), DPPH; (**b**), ABTS) of different BP extracts (mM TE g^{-1} dw). Small letters show significant differences as assessed by Tukey's post hoc test.

The DPPH assay on the different obtained extracts showed highly significant differences among the samples ($p < 0.01$), with values that ranged between 0.71 and 3.21 mM TE g^{-1} dw. As clearly shown in Figure 4a, the antioxidant extracts recovered through the

use of a hydroalcoholic solvent (H_2O: EtOH, 50%) highlighted the highest total antioxidant activity ($p < 0.01$), particularly, the extracts recovered with ultrasound.

Concerning the ABTS assay, the results are shown in Figure 4b, where is possible to observe that the obtained values highlighted a higher antioxidant activity compared to the DPPH assay, with values that ranged between 3.22 and 16.93 mM TE g^{-1} dw. The best extraction in terms of total antioxidant activity was obtained by applying the hydroalcoholic solution (H_2O-EtOH, 50%) as extraction solvent and assisted by a conventional maceration (samples C6 and C7). The two different antioxidant assays, respond differently to extracts, this trend is probably due to the different interaction of the two free radicals with antioxidant molecules.

For all the analyzed extracts, the two total antioxidant assays showed different results, with higher concentrations of total antioxidants highlighted by the ABTS assay. It could be dependent on the fact that ABTS assay generally is a more sensitive hydrophilic and lipophilic antioxidant, while DPPH assay is more sensitive to lipophilic antioxidants [35]. This affinity was also described by Zacarías-García [36] which highlighted the highest value for ABTS compared to DPPH in the hydrophilic fraction.

As already reported in other works, the extractability of bioactive compounds is strongly dependent on different applied extraction variables, such as solvent, time, temperature, etc. [37] as also evidenced in this work.

In addition, positive correlations were found between TPC/DPPH, particularly for the conventional extraction method ($r > 0.7$), while the ABTS assay was related to both TPC and TFC ($r > 0.95$) especially for microwave extraction.

3.2. Quantification of Individual Bioactive Compounds through Liquid Chromatography

The quali-quantitative analysis of the prevalent neohesperidose flavanones (eriocitrin, neoeriocitrin, naringin; neohesperidin, metilidin, and brutieridin) present in bergamot pomace extracts was performed by UHPLC system.

In Table 4, the validation of chromatographic analysis was reported. The applied methodology was developed by the injection of different concentrations of standard solutions. Particularly, limits of detection (LOD) and quantification (LOQ); the correlation coefficient (R2) and regression equations for each antioxidant standard were studied.

Table 4. UHPLC validation method.

Compounds	Regression Equation	R^2	LOD μg g^{-1}	LOQ μg g^{-1}
Eriocitrin	y = 41.62x − 15.40	0.9999	0.0625	0.2245
Neoeriocitrin	y = 37.66x − 17.39	0.9999	0.0536	0.2777
Naringin	y = 42.08x − 13.67	0.9993	0.0635	0.2326
Neohesperidin	y = 51.28x + 8.76	0.9997	0.1290	0.2129
Limonin	y = 41.28x + 47.93	0.9998	0.0361	0.9984
Nomilin	y = 38.40x − 19.25	0.9996	0.0932	0.2465

While for Metilidin and Brutieridin compounds the Naringin was used as standard, and the results are expressed as naringin equivalent (mg g^{-1} dw).

Figure 5 shows the chromatographic profiles of the obtained extracts and the main identified compounds. Particularly, seven phenolic compounds were detected: eriocitrin, neoeriocitrin, narirutin, naringin, neohesperidin, melitidin and brutieridin.

Neoeriocitrin, naringin, neohesperidin, and brutieridin were the main flavonoids detected in BP (Table 5), as also reported by other authors [7,38] and represent the typical compounds that determine the bitterness in the fruit [39].

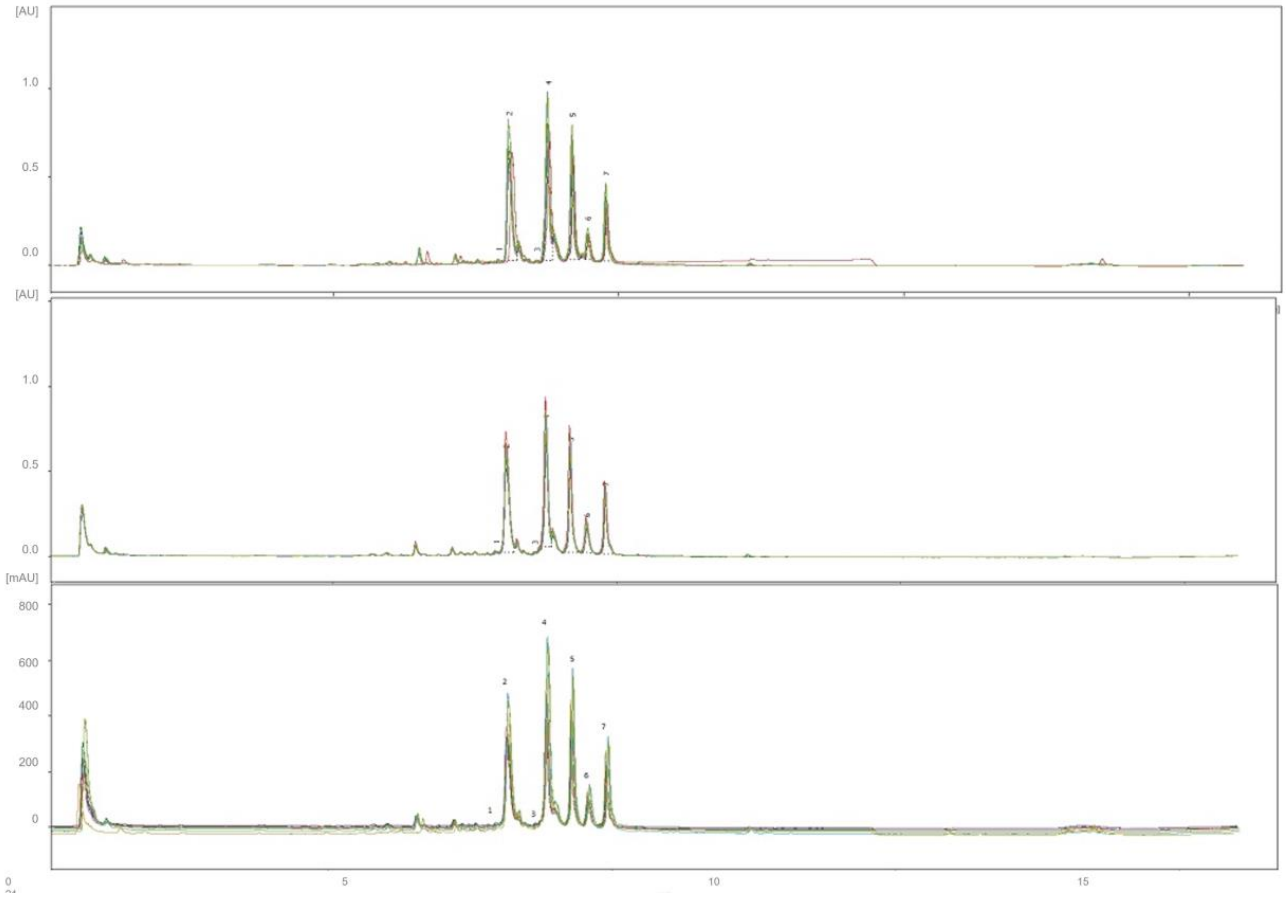

Figure 5. Example of chromatographic profiles of different BPs injected by UHPLC. (1) eriocitrin; (2) neoeriocitrin; (3) narirutin; (4) naringin; (5) neohesperidin; (6) melitidin and (7) brutieridin.

Among these, neoeriocitrin was the most abundant in BP extract ranging from 5.03 to 14.14 mg g^{-1} dw; in particular, it showed its highest amounts (13.95 and 14.14 mg g^{-1} dw) in the C7 and C8 samples, obtained by conventional extraction at 70 °C for 30 and 60 min. Regarding the other prevailing compounds, the range were 4.69–12.87 mg g^{-1} dw for naringin; 2.84–7.35 mg g^{-1} dw for neohesperidin, 2.05–5.67 mg g^{-1} dw for brutieridin, and 1.12–2.67 mg g^{-1} dw for melitidin. Lesser quantities of eriocitrin and narirutin were instead determined in all samples.

All the assayed extracts were significantly different for the phenolic content, related to the extraction system (C, UAE, and MA, $p < 0.01$) and variables of extraction (times and temperatures, $p < 0.01$). The conventional extraction system produced the greatest extractability of this class of antioxidants with similar values in C7 and C8 samples. As observed previously in other food matrices, the hydroalcoholic solvent is the best choice to obtain the maximum yield of antioxidant compounds [25,37], when combined with 70 °C temperature [25].

Additionally, the limonoid content was evaluated on Bergamot pomace extract, as it represents an interesting index to evaluate, indeed, its contribution to the bitter taste of citrus fruits, as also reported by Shi et al. [40]. In addition, limonoids from Citrus are getting a lot of scientific interest for their bioactive properties in vitro and in vivo such as antitumor, antioxidative, and antibacterial compounds [41]. The chromatographic analysis showed that also the highest yield of the two mains determined limonoids (limonin and nomilin) was obtained through conventional extraction technique C7 (Figure 6).

Table 5. Results of the prevalent flavanones on the different BP extracts (mg g^{-1} dw).

	Eriocitrin				Neoeriocitrin				Naringin			
Samples	C	UA	MA	Sign.	C	UA	MA		C	UA	MA	
1	0.19 ^{efB}	0.26 ^{aA}	0.10 ^{aC}	**	9.83 ^{dB}	11.58 ^{bA}	7.31 ^{cC}	**	8.54 ^{fB}	9.49 ^{cA}	7.32 ^{cC}	**
2	0.19 ^{eB}	0.26 ^{aA}	0.09 ^{aC}	**	9.64 ^{dB}	11.28 ^{bcA}	6.08 ^{deC}	**	8.61 ^{fB}	9.67 ^{cA}	5.85 ^{dC}	**
3	0.22 ^{cA}	0.18 ^{bB}	0.05 ^{bC}	**	11.08 ^{cA}	9.26 ^{eB}	5.03 ^{fC}	**	10.02 ^{eA}	7.76 ^{eB}	5.13 ^{fC}	**
4	0.23 ^{bcA}	0.13 ^{eB}	0.05 ^{bC}	**	11.85 ^{bA}	10.09 ^{dB}	6.42 ^{dC}	**	10.47 ^{dA}	8.60 ^{dB}	5.63 ^{eC}	**
5	0.19 ^{fA}	0.18 ^{bB}	0.04 ^{bC}	**	10.16 ^{dB}	13.10 ^{aA}	5.04 ^{fC}	**	9.73 ^{eB}	11.03 ^{aA}	4.69 ^{gC}	**
6	0.24 ^{bA}	0.15 ^{cB}	0.04 ^{bC}	**	12.15 ^{bA}	11.30 ^{bcB}	5.93 ^{eC}	**	11.00 ^{cA}	10.18 ^{bB}	5.31 ^{fC}	**
7	0.25 ^{aA}	0.14 ^{dB}	0.04 ^{bC}	**	13.95 ^{aA}	11.04 ^{cB}	9.72 ^{aC}	**	12.47 ^{bA}	9.45 ^{cB}	8.32 ^{aC}	**
8	0.21 ^{dA}	0.11 ^{fB}	0.04 ^{bC}	**	14.14 ^{aA}	10.43 ^{dB}	9.01 ^{bC}	**	12.87 ^{aA}	8.71 ^{dB}	7.99 ^{bC}	**
Sign.	**	**	**		**	**	**		**	**	**	
	Neohesperidin				Melitidin				Brutieridin			
Samples	C	UA	MA		C	UA	MA		C	UA	MA	
1	5.13 ^{eB}	5.98 ^{bA}	3.98 ^{cC}	**	1.65 ^{eB}	2.46 ^{bA}	1.60 ^{bC}	**	3.59 ^{fB}	4.60 ^{bA}	2.97 ^{cC}	**
2	4.29 ^{fB}	5.61 ^{cA}	3.58 ^{dC}	**	1.49 ^{fB}	2.59 ^{abA}	1.41 ^{cC}	**	3.63 ^{fB}	4.73 ^{bA}	2.69 ^{dC}	**
3	5.81 ^{cdA}	4.80 ^{eB}	2.84 ^{fC}	**	1.83 ^{dA}	1.82 ^{eA}	1.22 ^{efB}	**	4.38 ^{dA}	3.52 ^{fB}	2.21 ^{fC}	**
4	6.03 ^{bcA}	5.21 ^{dB}	3.56 ^{dC}	**	1.99 ^{cA}	1.10 ^{dA}	1.38 ^{cdB}	**	4.50 ^{dA}	3.98 ^{eB}	2.71 ^{dC}	**
5	5.43 ^{deB}	6.78 ^{aA}	2.77 ^{fC}	**	1.87 ^{cdB}	2.67 ^{aA}	1.12 ^{fC}	**	3.88 ^{eB}	5.00 ^{aA}	2.05 ^{fC}	**
6	6.28 ^{bA}	6.03 ^{bA}	3.28 ^{eB}	**	2.12 ^{bB}	2.54 ^{abA}	1.28 ^{deC}	**	4.71 ^{cB}	4.95 ^{aA}	2.47 ^{eC}	**
7	7.35 ^{aA}	5.65 ^{cB}	5.03 ^{aC}	**	2.29 ^{aA}	2.29 ^{cA}	2.00 ^{aB}	**	5.67 ^{aA}	4.35 ^{cB}	3.95 ^{aC}	**
8	7.11 ^{aA}	5.49 ^{cB}	4.78 ^{bC}	**	2.28 ^{aA}	2.12 ^{dB}	1.94 ^{aC}	**	5.12 ^{bA}	4.13 ^{dB}	3.57 ^{bC}	**
Sign.	**	**	**		**	**	**		**	**	**	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. Abbreviations: **, significance at $p < 0.01$.

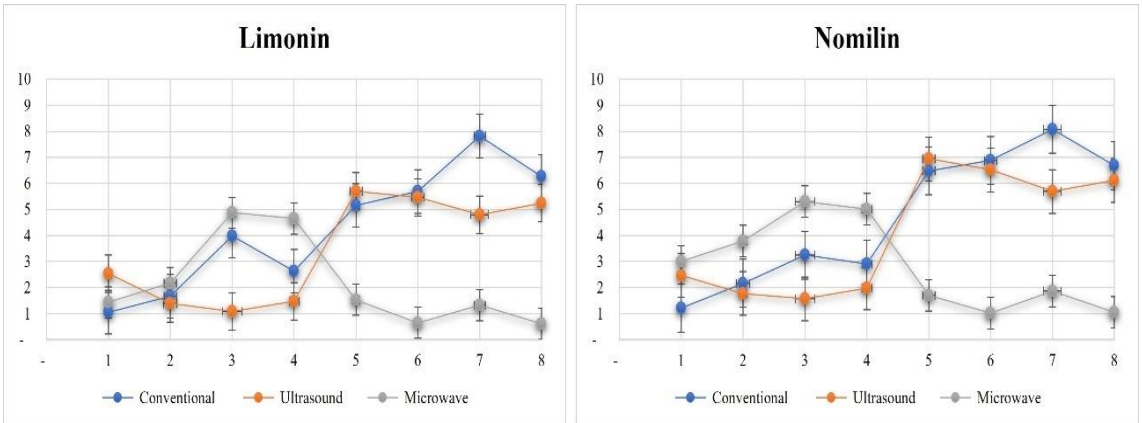

Figure 6. Concentration values (mg g^{-1}) of Limonin and Nomilin content in bergamot pomace extracts.

The statistical analysis carried out on the obtained results for limonoid content showed significant differences ($p < 0.01$) among all the studied variables (Table 6).

After identifying the best extraction system, namely conventional maceration for 30 min at 70 °C and using H_2O/EtOH 50% as extraction solvent, a correlated study on the antioxidant properties of the different portions of bergamot fruits was carried out. Specifically, this extraction technique was applied to test separately the individual portions of each fruit (albedo/pulp, seeds, and juice) belonging to the three cultivars Fantastico, Femminello, and Castagnaro.

The amounts of total flavonoid content quantified in the albedo/pulp portion showed significant differences ($p < 0.01$) among the three cultivars as shown in Figure 7. The highest content was 46.61 mg CE g^{-1} in Fantastico, and the lowest one in Castagnaro (19.11 mg CE g^{-1}). The same trend was observed for TPC with values that ranged between 61.76 mg GAE g^{-1} (Castagnaro) and 129.44 mg GAE g^{-1} (Fantastico).

Table 6. ANOVA limonoids content.

	Limonin				Nomilin			
	C	UA	MA	Sign.	C	UA	MA	Sign.
1	hC	eA	deB	**	gC	cB	dA	**
2	gB	fC	cA	**	fB	cC	cA	**
3	eB	gC	aA	**	dB	cC	aA	**
4	fB	fC	bA	**	eB	cC	bA	**
5	dB	aA	deC	**	cB	aA	fC	**
6	cA	bB	fC	**	bA	bA	gB	**
7	aA	dB	eC	**	aA	bB	eC	**
8	bA	cB	fC	**	bA	abB	gC	**
Sign.	**	**	**		**	**	**	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. Abbreviations: **, significance at $p < 0.01$.

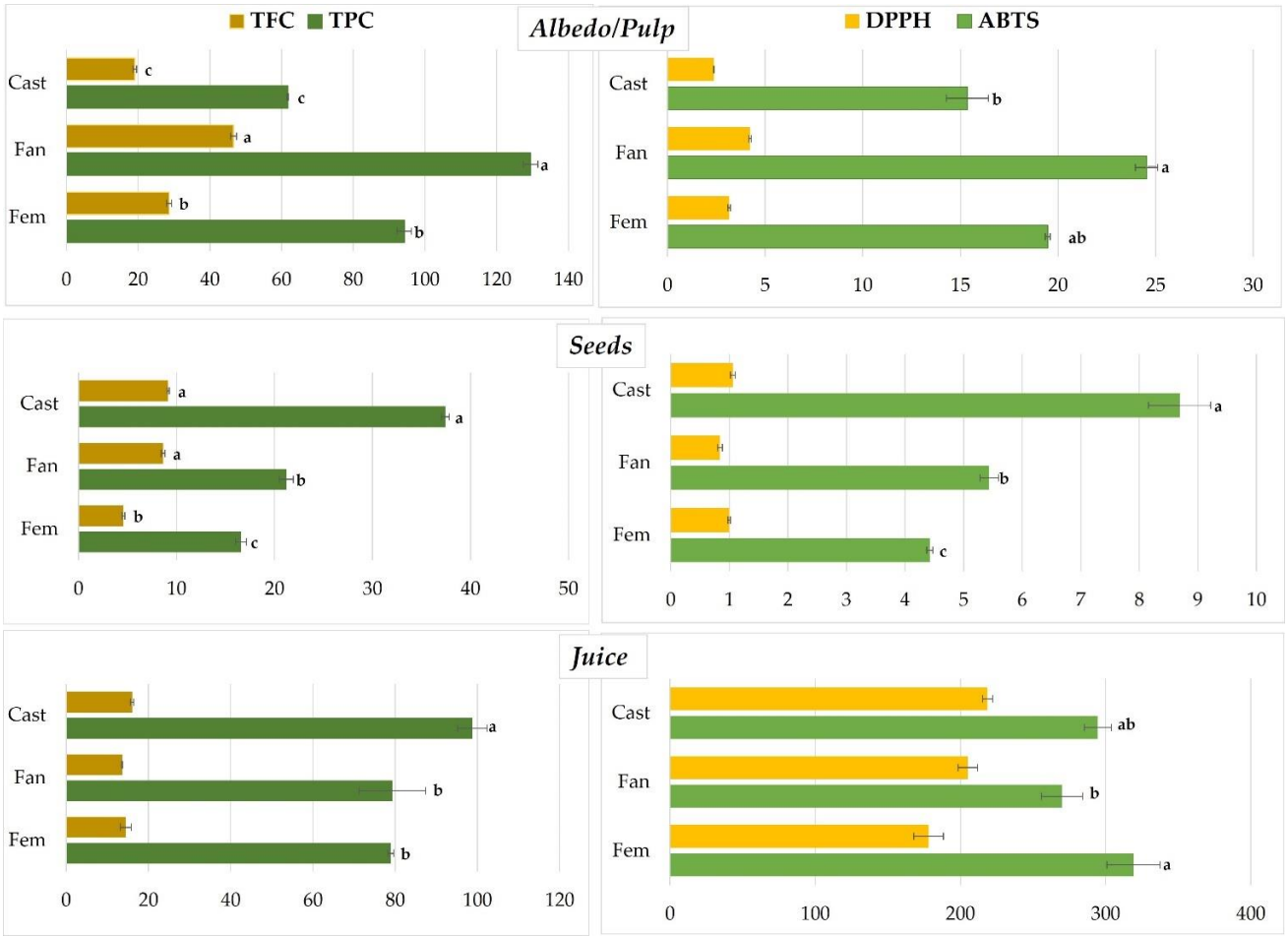

Figure 7. Total antioxidant activity of the different bergamot portions (Albedo/pulp and seeds: TFC: mg CE g^{-1} dw, TPC: mg GAE g^{-1} dw; ABTS and DPPH: mM TE g^{-1} dw; Juice: TFC: mg CE 100 mL^{-1}, TPC: mg 100 mL^{-1}, ABTS and DPPH: mM TE L^{-1}). Small letters show significant differences as assessed by Tukey's post hoc test.

The antioxidant activity of the albedo/pulp portion was studied by DPPH and ABTS radical scavenging assays. Both assays revealed a higher activity than those shown by BP sample. Higher results were expressed by ABTS assay, with statistical differences ($p < 0.05$) among the samples with ranges from 15.35 mM TE g^{-1} dw (Castagnaro) and 24.54 mM TE g^{-1} dw (Fantastico). Lower values were found in the DPPH (2.38 mM TE g^{-1} dw in Castagnaro and 4.22 mM TE g^{-1} dw in Fantastico) without statistical differences.

The antioxidant determinations on seeds revealed significant differences for TFC, with higher amounts in Castagnaro and Fantastico (9.16 and 8.64 mg CE g^{-1}, respectively) than in Femminello cultivars (4.59 mg CE g^{-1}).

The distribution of TPC among the cultivars' seeds was different compared to the albedo/pulp portion. TPC was quantified in the range of 16.57, 21.20, and 37.40 mg GAE g^{-1}, respectively on Femminello, Fantastico, and Castagnaro seeds. TPC in seeds was comparable with those reported by Al et al. [42].

The free radical scavenging activity measured with DPPH assay for seed extracts did not show differences with values of about 1 mM TE g^{-1} dw.

The highest level of scavenging activity based on ABTS assay was determined in Castagnaro seed extract (8.69 mM TE g^{-1} dw), and the lowest (4.42 mM TE g^{-1} dw) in Femminello, exhibiting the same trend of TPC, with a high correlation (r = 0.999).

Figure 7 also showed the different amounts of total flavonoid content in tested juices. Although the highest value was found in Castagnaro (160.08 mg CE L^{-1}), the replications conducted, and the statistical analysis suggested that there are no statistical differences among the analyzed samples.

The phenolic content in the juice was in accordance with the amounts detected by Xu et al. [43] and Chen et al. [44], who analyzed respectively the juice of fifteen and twenty-seven different varieties of citrus fruit. Their contents were 78.94 mg GAE 100 mL^{-1} in Femminello, 79.33 mg GAE 100 mL^{-1} in Fantastico, and 98.73 mg GAE 100 mL^{-1} in Castagnaro. The antioxidant activity, evaluated with the DPPH assay was more correlated to the TPC assay (r = 0.768) compared with the other assays. Cautela et al. [45], in fact, reported relevant differences in the chemical composition and functional properties of juices obtained with different processing methods.

Bergamot portions (albedo/flavedo, seeds, juice) showed high concentrations of flavanones, including eriocitrin, neoeriocitrin, naringin, neohesperidin, melitidin, and brutieridin (Table 7), as also reported by Walker et al. [46].

Table 7. Individual phenolic content in the different bergamot portions (Albedo/pulp and seeds mg g^{-1} dw; Juice mg L^{-1}).

		Eriocitrin	Neoeriocitrin	Naringin	Neohesperidin	Melitidin	Brutieridin
Albedo/pulp	FA	1.06 ± 0.04 ^{a}	88.91 ± 1.34 ^{a}	58.02 ± 0.58 ^{a}	27.67 ± 1.14 ^{a}	13.61 ± 0.99 ^{a}	26.09 ± 0.87 ^{a}
	FE	0.70 ± 0.03 ^{b}	58.37 ± 0.40 ^{b}	32.42 ± 1.92 ^{b}	11.41 ± 1.20 ^{b}	10.01 ± 0.64 ^{b}	18.30 ± 0.67 ^{b}
	CA	0.61 ± 0.03 ^{b}	33.19 ± 0.76 ^{b}	31.72 ± 0.39 ^{b}	13.85 ± 0.45 ^{b}	8.17 ± 0.34 ^{c}	12.45 ± 0.72 ^{c}
	Sign.	**	**	**	**	**	**
Seeds	FA	0.10 ± 0.01 ^{b}	1.96 ± 0.08 ^{c}	2.37 ± 0.10 ^{c}	0.91 ± 0.04 ^{c}	0.82 ± 0.05 ^{c}	1.5 ± 0.09 ^{c}
	FE	0.09 ± 0.01 ^{b}	4.27 ± 0.09 ^{b}	3.94 ± 0.08 ^{b}	1.71 ± 0.06 ^{b}	1.47 ± 0.09 ^{b}	2.76 ± 0.10 ^{b}
	CA	0.16 ± 0.02 ^{a}	9.04 ± 0.22 ^{a}	8.16 ± 0.18 ^{a}	3.71 ± 0.18 ^{a}	2.90 ± 0.23 ^{a}	5.32 ± 0.46 ^{a}
	Sign.	**	**	**	**	**	**
Juice	FE	5.43 ± 0.42 ^{a}	167.80 ± 6.03 ^{b}	186.70 ± 16.34 ^{a}	101.39 ± 6.78 ^{a}	57.59 ± 8.31	171.45 ± 8.33
	FA	4.59 ± 0.74 ^{b}	196.02 ± 19.13 ^{a}	141.83 ± 23.54 ^{b}	79.45 ± 10.84 ^{b}	47.70 ± 6.14	165.98 ± 7.39
	CA	4.46 ± 0.56 ^{b}	168.66 ± 17.01 ^{b}	161.48 ± 22.09 ^{ab}	81.88 ± 7.29 ^{ab}	48.56 ± 4.98	164.59 ± 4.96
	Sign.	**	**	*	**	ns	ns

Small letters within a column show significant differences as assessed by Tukey's post hoc test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; ns, not significant; FA: Fantastico; FE: Femminello; CA: Castagnaro.

Neoeriocitrin and naringin represent the prevalent flavonoid compounds in the albedo/pulp portion, with values that ranged between 33.19–88.91 and 31.72–58.02 mg g^{-1} dw, respectively. Lower content of this class of compounds was found in the seeds.

The chromatographic analysis of Bergamot Juice resulted in a high content of neoeriocitrin, naringin, and neohesperidin. They showed values different from those reported by Leporini et al. [47], but more or less comparable with those reported by Walker et al. [46], Da Pozzo et al. [1], and Baron et al. [48]. The juice of Fantastico cv. showed the highest content in neoriocitrin (196.02 ± 19.13 mg L^{-1}) while naringin and neohesperidin were higher in

Femminello cv. Brutieridin ((hesperetin 7-(200-R-rhamnosyl-600-(30000-hydroxy-30000-methylglutaryl)-β-glucoside)) and melitidin (naringenin7-(200-R-rhamnosyl-600-(30000-hydroxy-30000-methylglutaryl)-β-glucoside)), which have anticholesterolemic activity, exhibiting statin-like properties [49,50], were found either in the albedo/pulp, seeds and juice of the three different bergamot cultivars showing higher concentration for brutieridin than melitidin as reported by Di Donna et al. [51].

The two major limonoid aglycones, limonin, and nomilin, were also detected in the different parts of bergamot fruits. They are responsible for bitterness in citrus fruits. Concerning the limonoids content evaluated on individual bergamot portions, Femminello cv showed a high amount of limonin and nomilin on albedo/pulp (6.77 and 8.79 mg g^{-1} dw, respectively). Bergamot seeds possess a higher content of limonin, particularly those of Castagnaro cv (13.29 mg g^{-1} dw). The different content of limonin is explained by the variability due to the state of ripeness of the fruit, the variety, and the part of the fruit [52].

4. Conclusions

This work reports a careful study carried out on the recovery of antioxidant compounds by bergamot fruits and their by-products. The evaluation of data is helpful for the selection of food-grade solvent extraction in bergamot by-products also related to the different parts of fruit and to the three varieties. Overall, the present work has shown the possibility to valorize this waste with sustainable methods minimizing environmental impact offering an important source of bioactive compounds such as polyphenols, flavonoids, and limonoids that could be recovered.

The discovery of the beneficial effect and antioxidant activity of these extracts obtained from the portions of fruit or bergamot pomace could lead to the development of new products such as nutraceuticals and/or natural preservatives to apply in the food industry to extend the shelf life of products.

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References

1. Da Pozzo, E.; De Leo, M.; Faraone, I.; Milella, L.; Cavallini, C.; Piragine, E.; Testai, L.; Calderone, V.; Pistelli, L.; Braca, A.; et al. Antioxidant and Antisenescence Effects of Bergamot Juice. *Oxidative Med. Cell. Longev.* **2018**, *9395804*, 1–14. [CrossRef] [PubMed]
2. Schwingshackl, L.; Morze, J.; Hoffmann, G. Mediterranean diet and health status: Active ingredients and pharmacological mechanisms. *Br. J. Pharmacol.* **2020**, *177*, 1241–1257. [CrossRef] [PubMed]
3. Tsiokanos, E.; Tsafantakis, N.; Termentzi, A.; Aligiannis, N.; Skaltsounis, L.A.; Fokialakis, N. Phytochemical characteristics of bergamot oranges from the Ionian islands of Greece: A multi-analytical approach with emphasis in the distribution of neohesperidose flavanones. *Food Chem.* **2021**, *343*, 128400. [CrossRef] [PubMed]
4. 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. Sustain. Food Syst.* **2017**, *41*, 1124–1145.
5. International Organization for Standardization, ISO 9235:2021. Aromatic Natural Raw Materials—Vocabulary. Available online: https://standards.iteh.ai/catalog/standards/sist/599cef4e-41ea-48e7-8dd1-2039e0ec6fbf/iso-9235-2021 (accessed on 17 May 2023).

6. Di Donna, L.; Bartella, L.; De Vero, L.; Gullo, M.; Giuffrè, A.M.; Zappia, C.; Capocasale, M.; Poiana, M.; D'Urso, S.; Caridi, A. Vinegar production from *Citrus bergamia* by products and preservation of bioactive compounds. *Eur. Food Res. Technol.* **2020**, *246*, 1981–1990. [CrossRef]
7. Giuffrè, A.M. Bergamot (*Citrus bergamia*, Risso): The Effects of Cultivar and Harvest Date on Functional Properties of Juice and Cloudy Juice. *Antioxidants* **2019**, *8*, 221. [CrossRef]
8. Gabriele, M.; Frassinetti, S.; Caltavuturo, L.; Montero, L.; Dinelli, G.; Longo, V.; Di Gioia, D.; Pucci, L. *Citrus bergamia* powder: Antioxidant, antimicrobial and anti-inflammatory properties. *J. Funct. Foods* **2017**, *31*, 255–265. [CrossRef]
9. Imeneo, V.; De Bruno, A.; Piscopo, A.; Romeo, R.; Poiana, M. Valorization of 'Rossa di Tropea' Onion Waste through Green Recovery Techniques of Antioxidant Compounds. *Sustainability* **2022**, *14*, 4387. [CrossRef]
10. Mandalari, G.; Nueno Palop, C.; Tuohy, K.; Gibson, G.R.; Bennett, R.N.; Waldron, K.W.; Bisignano, G.; Narbad, A.; Faulds, C.B. In vitro evaluation of the prebiotic activity of a pectic oligosaccharide-rich extract enzymatically derived from bergamot peel. *Appl. Microbiol. Biotechnol.* **2007**, *73*, 1173–1179. [CrossRef]
11. Cappello, A.R.; Dolce, V.; Iacopetta, D.; Martello, M.; Fiorillo, M.; Curcio, R.; Muto, L.; Dhanyalayam, D. Bergamot (*Citrus bergamia* Risso) Flavonoids and Their Potential Benefits in Human Hyperlipidemia and Atherosclerosis: An Overview. *Mini Rev. Med. Chem.* **2015**, *15*, 619–629. [CrossRef]
12. Benavente-Garcia, O.; Castillo, J. Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular, and anti-inflammatory activity. *J. Agric. Food Chem.* **2008**, *56*, 6185–6205. [CrossRef] [PubMed]
13. Liu, L.; Zuo, Z.; Lu, S.; Liu, A.; Liu, X. Naringin attenuates diabetic retinopathy by inhibiting inflammation, oxidative stress and NF-κB activation in vivo and in vitro. *Iran. J. Basic Med. Sci.* **2017**, *20*, 813–821. [PubMed]
14. Tan, Z.; Cheng, J.; Liu, Q.; Zhou, L.; Kenny, J.; Wang, T.; Lin, X.; Yuan, J.; Quinn, J.M.W.; Tickner, J.; et al. Neohesperidin suppresses osteoclast differentiation, bone resorption and ovariectomised-induced osteoporosis in mice. *Mol. Cell. Endocrinol.* **2017**, *439* (Suppl. C), 369–378. [CrossRef] [PubMed]
15. Li, L.; Zeng, Z.; Cai, G. Comparison of neoeriocitrin and naringin on proliferation and osteogenic differentiation in MC3T3-E1. *Phytomedicine* **2011**, *18*, 985–989. [CrossRef] [PubMed]
16. Postorino, E.; Poiana, M.; Pirrello, A.; Castaldo, D. Studio dell'influenza della tecnologia di estrazione sulla composizione del succo di bergamotto. *Essenze Deriv. Agrum.* **2001**, *71*, 57–66.
17. Laratta, B.; De Masi, L.; Minasi, P.; Giovane, A. Pectin methylesterase in *Citrus bergamia* R.: Purification, biochemical characterisation and sequence of the exon related to the enzyme active site. *Food Chem.* **2008**, *110*, 829–837. [CrossRef]
18. Ferro, Y.; Montalcini, T.; Mazza, E.; Foti, D.; Angotti, E.; Gliozzi, M.; Nucera, S.; Paone, S.; Bombardelli, E.; Aversa, I.; et al. Randomized Clinical Trial: Bergamot Citrus and Wild Cardoon Reduce Liver Steatosis and Body Weight in Non-diabetic Individuals Aged Over 50 Years. *Front. Endocrinol.* **2020**, *11*, 494. [CrossRef]
19. Mandalari, G.; Bennett, R.N.; Bisignano, G.; Saija, A.; Dugo, G.; Lo Curto, R.B.; Faulds, C.B.; Waldron, K.W. Characterization of Flavonoids and Pectins from Bergamot (*Citrus bergamia* Risso) Peel, a Major Byproduct of Essential Oil Extraction. *J. Agric. Food Chem.* **2016**, *54*, 197–203. [CrossRef]
20. Abeysinghe, D.C.; Li, X.; Sun, C.D.; Zhang, W.S.; Zhou, C.H.; Chen, K.S. Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. *Food Chem.* **2007**, *104*, 1338–1344. [CrossRef]
21. Rodríguez-Rojo, S. Intensification Technologies to Efficiently Extract Antioxidants from Agro-Food Residues. *Antioxidants* **2021**, *10*, 1568. [CrossRef]
22. Choi, Y.H.; Verpoorte, R. Green solvents for the extraction of bioactive compounds from natural products using ionic liquids and deep eutectic solvents. *Curr. Opin. Food Sci.* **2019**, *26*, 87–93. [CrossRef]
23. Banerjee, J.; Singh, R.; Vijayaraghavan, R.; MacFarlane, D.; Patti, A.F.; Arora, A. Bioactives from fruit processing wastes: Green approaches to valuable chemicals. *Food Chem.* **2017**, *225*, 10–22. [CrossRef]
24. Chemat, F.; Rombaut, N.; Sicaire, A.-G.; Meullemiestre, A.; Fabiano-Tixier, A.-S.; Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochem.* **2017**, *34*, 540–560. [CrossRef] [PubMed]
25. Imeneo, V.; Romeo, R.; De Bruno, A.; Piscopo, A. Green-sustainable extraction techniques for the recovery of antioxidant compounds from "citrus Limon" by-products. *J. Environ. Sci. Health B* **2022**, *57*, 220–232. [CrossRef]
26. Valussi, M.; Donelli, D.; Firenzuoli, F.; Antonelli, M. Bergamot Oil: Botany, Production, Pharmacology. *Encyclopedia* **2021**, *1*, 152–176. [CrossRef]
27. González-Molina, E.; Moreno, D.A.; García-Viguera, C. A new drink rich in healthy bioactives combining lemon and pomegranate juices. *Food Chem.* **2009**, *115*, 1364–1372. [CrossRef]
28. Papoutsis, K.; Pristijono, P.; Golding, J.B.; Stathopoulos, C.E.; Bowyer, M.C.; Scarlett, C.J.; Vuong, Q.V. Optimizing a sustainable ultrasound-assisted extraction method for the recovery of polyphenols from lemon by-products: Comparison with hot water and organic solvent extractions. *Eur. Food Res. Technol.* **2018**, *244*, 1353–1365. [CrossRef]
29. De Bruno, A.; Romeo, R.; Piscopo, A.; Poiana, M. Antioxidant quantification in different portions obtained during olive oil extraction process in an olive oil press mill. *J. Sci. Food Agric.* **2021**, *101*, 1119–1126. [CrossRef] [PubMed]
30. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]

31. Romeo, R.; De Bruno, A.; Imeneo, V.; Piscopo, A.; Poiana, M. Evaluation of enrichment with antioxidants from olive oil mill wastes in hydrophilic model system. *J. Food Process. Preserv.* **2019**, *43*, 1–9. [CrossRef]
32. Celano, R.; Campone, L.; Pagano, I.; Carabetta, S.; Di Sanzo, R.; Rastrelli, L.; Piccinelli, A.L.; Russo, M. Characterisation of nutraceutical compounds from different parts of particular species of *Citrus sinensis* 'Ovale Calabrese' by UHPLC-UV-ESI-HRMS. *Nat. Prod. Res.* **2019**, *33*, 244–251. [CrossRef]
33. Multari, S.; Carlin, S.; Sicari, V.; Martens, S. Differences in the composition of phenolic compounds, carotenoids, and volatiles between juice and pomace of four citrus fruits from Southern Italy. *Eur. Food Res. Technol.* **2020**, *246*, 1991–2005. [CrossRef]
34. Gorinstein, S.; Martín-Belloso, O.; Park, J.S.; Haruenkit, R.; Lojek, A.; Číž, M.; Caspi, A.; Libman, I.; Trakhtenberg, S. Comparison of some biochemical characteristics of different citrus fruits. *Food Chem.* **2001**, *74*, 309–315. [CrossRef]
35. Kim, D.-O.; Lee, K.W.; Lee, H.J.; Lee, C.Y. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J. Agric. Food Chem.* **2002**, *50*, 3713–3717. [CrossRef] [PubMed]
36. Zacarías-García, J.; Rey, F.; Gil, J.V.; Rodrigo, M.J.; Zacarías, L. Antioxidant capacity in fruit of Citrus cultivars with marked differences in pulp coloration: Contribution of carotenoids and vitamin C. *Food Sci. Technol. Int.* **2021**, *27*, 210–222. [CrossRef] [PubMed]
37. De Bruno, A.; Romeo, R.; Fedele, F.L.; Sicari, A.; Piscopo, A.; Poiana, M. Antioxidant activity shown by olive pomace extracts. *J. Environ. Sci. Health B* **2018**, *53*, 526–533. [CrossRef] [PubMed]
38. Bartella, L.; Mazzotti, F.; Talarico, I.R.; De Luca, G.; Santoro, I.; Prejanò, M.; Riccioni, C.; Marino, T.; Di Donna, L. Structural Characterization of Peripolin and Study of Antioxidant Activity of HMG Flavonoids from Bergamot Fruit. *Antioxidants* **2022**, *11*, 1847. [CrossRef]
39. Russo, M.; Arigò, A.; Calabrò, M.L.; Farnetti, S.; Mondello, L.; Dugo, P. Bergamot (*Citrus bergamia* Risso) as a source of nutraceuticals: Limonoids and flavonoids. *J. Funct. Foods* **2016**, *20*, 10–19. [CrossRef]
40. Shi, Y.S.; Zhang, Y.; Li, H.T.; Wu, C.H.; El-Seedi, H.R.; Ye, W.K.; Wang, Z.W.; Li, C.B.; Zhang, X.F.; Kai, G.Y. Limonoids from Citrus: Chemistry, anti-tumor potential, and other bioactivities. *J. Funct. Foods* **2020**, *75*, 104213. [CrossRef]
41. Zhang, Y.; Wang, J.S.; Wang, X.B.; Gu, Y.C.; Wei, D.D.; Guo, C.; Kong, L.Y. Limonoids from the fruits of *Aphanamixis polystachya* (Meliaceae) and their biological activities. *J. Agric. Food Chem.* **2013**, *61*, 2171–2182. [CrossRef]
42. Al, N.A.; Ibrahim, N.S.; Kadhom, I.M. Estimation of Phenolic Compounds and Evaluation of Their Antioxidant Activity of Some Parts of the Orange Plant (*Citrus Sinensis* L.). *Eur. J. Mol. Clin. Med.* **2020**, *7*, 4811–4822.
43. Xu, G.; Liu, D.; Chen, J.; Ye, X.; Ma, Y.; Shi, J. Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food Chem.* **2008**, *106*, 545–551. [CrossRef]
44. Chen, Q.; Wang, D.; Tan, C.; Hu, Y.; Sundararajan, B.; Zhou, Z. Profiling of Flavonoid and Antioxidant Activity of Fruit Tissues from 27 Chinese Local Citrus Cultivars. *Plants* **2020**, *9*, 196. [CrossRef]
45. Cautela, D.; Vella, F.M.; Laratta, B. The Effect of Processing Methods on Phytochemical Composition in Bergamot Juice. *Foods* **2019**, *8*, 474. [CrossRef]
46. Walker, R.; Janda, E.; Mollace, V. Chapter 84—The Use of Bergamot-derived Polyphenol Fraction in Cardiometabolic Risk Prevention and its Possible Mechanisms of Action. *Polyphen. Hum. Health Dis.* **2014**, *2*, 1087–1105. [CrossRef]
47. Leporini, M.; Tundis, R.; Sicari, S.; Loizzo, M.R. Citrus species: Modern functional food and nutraceutical-based product ingredient. *Ital. J. Food Sci.* **2021**, *33*, 63–107. [CrossRef]
48. Baron, G.; Altomare, A.; Mol, M.; Garcia, J.L.; Correa, C.; Raucci, A.; Mancinelli, L.; Mazzotta, S.; Fumagalli, L.; Trunfio, G.; et al. Analytical Profile and Antioxidant and Anti-Inflammatory Activities of the Enriched Polyphenol Fractions Isolated from Bergamot Fruit and Leave. *Antioxidants* **2021**, *10*, 141. [CrossRef]
49. Cai, Y.; Xing, G.; Shen, T.; Zhang, S.; Rao, J.; Shi, R. Effects of 12-week supplementation of *Citrus bergamia* extracts-based formulation CitriCholess on cholesterol and body weight in older adults with dyslipidemia: A randomized, double-blind, placebo-controlled trial. *Lipids Health Dis.* **2017**, *16*, 251. [CrossRef] [PubMed]
50. Fiorillo, M.; Peiris-Pagès, M.; Sanchez-Alvarez, R.; Bartella, L.; Di Donna, L.; Dolce, V.; Sindona, G.; Sotgia, F.; Cappello, A.R.; Lisanti, M.P. Bergamot natural products eradicate cancer stem cells (CSCs) by targeting mevalonate, Rho-GDI-signaling and mitochondrial metabolism. *BBA Bioenergy* **2018**, *1859*, 984–996. [CrossRef] [PubMed]
51. 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. [CrossRef]
52. Sharma, K.; Mahato, N.; Cho, M.H.; Lee, Y.R. Converting citrus wastes into value-added products: Economic and environmently friendly approaches. *Nutrition* **2017**, *34*, 29–46. [CrossRef] [PubMed]